Sustainable Urban Renewal and Wastewater Treatment

No. 32 2003

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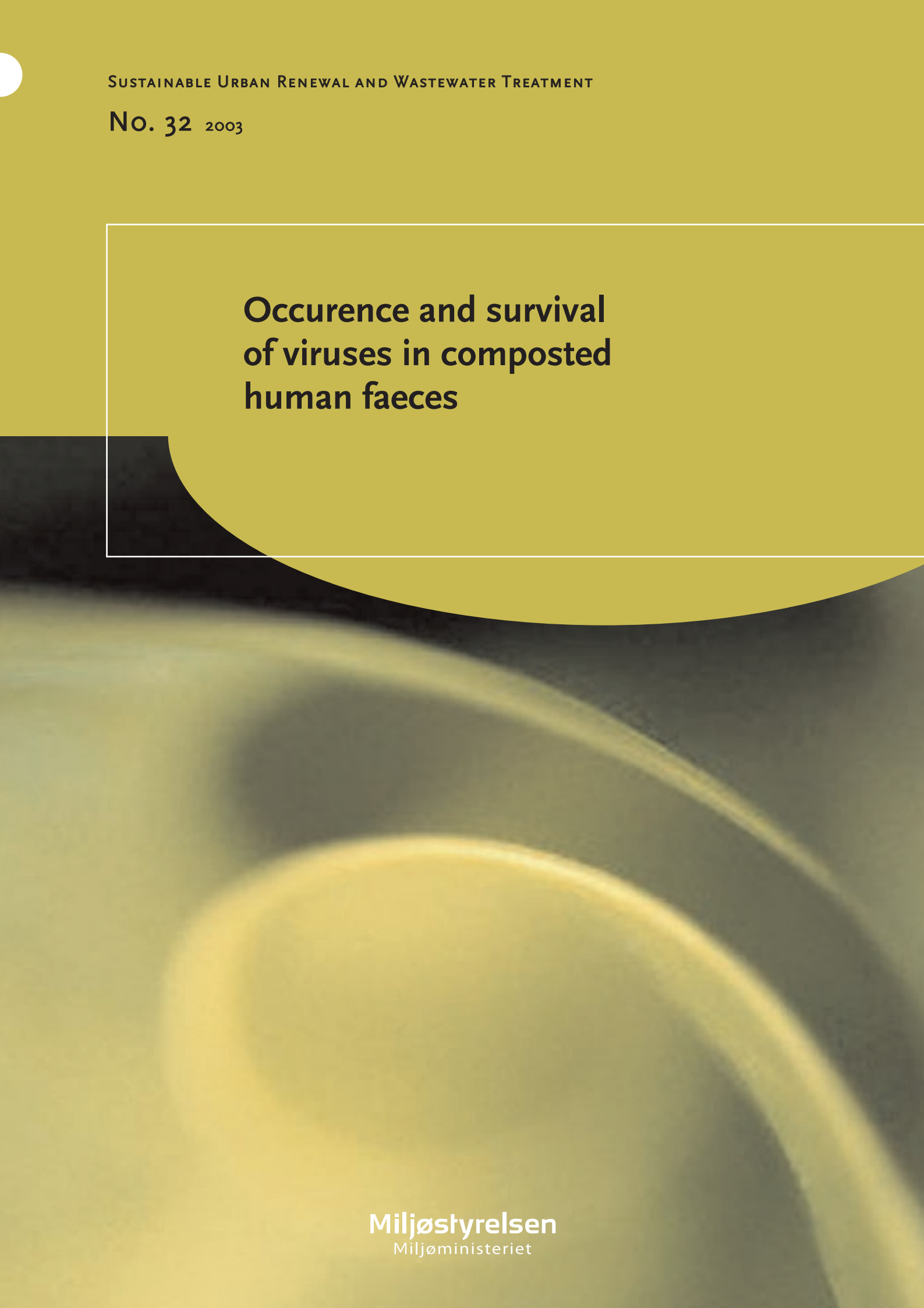

Miljøstyrelsen
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Occurence and survival of viruses in composted human faeces

Luca Guardabassi and Anders Dalsgaard
The Royal Veterinary and Agricultural University,
Department of Veterinary Microbiology

Mark Sobsey
University of North Carolina, Department of
Environmental Sciences and Engineering

Danish Environmental Protection Agency
Danish Ministry of the Environment

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Preface

The Danish Environmental Protection Agency (Miljøstyrelsen) has recently developed a number of projects on recycling of faeces and urine of human origin. Particular attention has been given to the environmental and public health aspects concerning the fate of bacteria and parasites during the process of recycling. However, little is known about the occurrence and survival of viruses in composted human faeces. The available data are scattered over the literature and often published in books, journals and reports covering different areas and targeted at different readers (e.g. virologists, engineers and physicians).

The present works summarizes the existing knowledge on the occurrence of pathogenic viruses in human faeces and their survival in compost products derived from faeces, sewage sludge and other organic waste. The review is composed by three chapters: the first two chapters are introductory chapters describing the process of composting (chapter 1) and relevant biological, clinical and epidemiological aspects on viruses excreted in human faeces (chapter 2). Chapter 3 describes the survival of viruses during storage, production and utilization of composted organic waste.

In the preparation of this report, the authors have brought together data from books, journals and reports covering different areas and disciplines (i.e. microbiology, compost technology and human health), including grey literature and unpublished material available on the Internet. The selected literature was primarily identified using the ISI Web of Science (http://wos.isiglobalnet.com), a database covering over 16,000 international journals, books and proceedings. Additional references were identified through reading of the selected literature and subsequent search on cited authors. Internet search engines were used to obtain relevant information from the web.

Due to the scarcity of literature on survival of viruses during composting of human faeces and the similarity to virus survival during composting of other types of organic waste, the search was extended to obtain information on composting of sewage sludge, liquid organic waste and domestic solid waste. Studies on virus survival during composting of manure or animal carcasses were also included as certain animal viruses are closely related human viruses.

The members of the project steering group were:

- Linda Bagge, Miljøstyrelsen.
- Line W. Hollesen, Miljøstyrelsen
- Anders Dalsgaard, Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University.
- Representative from Sundhedsstyrelsen
- Representative from Fødevaredirektoratet

We thank Dr. Peter Henrik Andersen (Department of Epidemiology, Statens Serum Institut) and Dr. Francois-Xavier Hanon (Department of

Epidemiology Research, Statens Serum Institut) for providing us with data on the occurrence of enteric viruses in Denmark.

Summary and conclusions

Recycling of organic waste reduces the environmental and economical problems associated with waste disposal. Any type of organic waste, including human faeces and urine, can be recycled and utilized for agricultural or other purposes, avoiding recourse to waste disposal and allowing return of nutrients to soils and plants. In Denmark, there is an increased interest in recycling of human excreta. Compared with traditional sewerage-based sanitation systems, this practice regards human excreta as a resource to be used rather than as a waste to be disposed. Recycling of human excreta benefits the environment and may contribute to production of safe agricultural products by avoiding the use of chemical fertilisers.

The reuse of excreta requires a process of stabilization, which consists in the degradation of organic matter accompanied by elimination of pathogenic organisms, reduction of the total mass and removal of undesirable odours. Composting represents a suitable method for stabilization of faecal material. The process of composting is based on the decomposition of organic matter by microorganisms under aerobic conditions. Aeration can be provided by means of blowers and air diffusers in aerated static pile and enclosed (in-vessel) systems, or by periodical turning of the composting heap in windrow systems. This report only addresses survival of viruses during centralised controlled composting, as decentralised composting, e.g. at the household level, is less likely to generate the adequate heat required for virus inactivation. Also, control of temperature and other parameters are often not possible in decentralised composting systems.

decentralized compost systems not likely to attain high temperatures

The aim of this report is to provide the knowledge necessary to evaluate the possible occurrence of pathogenic viruses in human composted faeces, with particular regard to the conditions existing in Denmark. The report contains a review of the literature on the occurrence of pathogenic viruses in human faeces and their survival during storage, treatment and utilization of composted faeces. The information available on this subject was scarce and scattered over the literature. Therefore, the Danish Environmental Protection Agency identified the need to collect such information and make it available for decision-making and risk assessment in relation to future recycling of human excreta.

Based on this review, none of the presently known viral pathogens occurring in human faeces appears to be able to survive composting if the process is properly managed according to the current legislation on composting in Denmark and other European countries. In particular, exposure to 55°C for two weeks (i.e. controlled composting) or 70°C for one hour (i.e. controlled sanitation), as required by the Danish legislation, appears to ensure a complete inactivation of all pathogenic viruses occurring in human faeces.

What needs to happen in a compost operation... high temp process

Viral inactivation during composting is mainly due to the heat generated by microbial degradation, which determines irreversible damage of the viral structure To a lesser extent, viruses are also inactivated by the antiviral activity of proteolytic enzymes produced by bacteria and by ammonia released as a consequence of protein degradation.

The efficiency of composting in viral inactivation has been demonstrated by several studies on composting of sewage sludge, animal faeces and other types of organic waste. Nevertheless, some studies have pointed out the difficulty in maintaining the necessary parameters (i.e. temperature, aeration and moisture content) and controlling them in the routine practical operation, in particular for windrow systems.

The monitoring and documentation of key parameters like temperature, aeration and moisture are essential to ensure a complete inactivation of viruses during composting. Enclosed systems are the safest systems for composting of human faeces, as they ensure a better control of such parameters. In contrast, windrow systems do not guarantee efficient heat exposure and virus elimination due to the influence of ambient temperature on the surface of the windrows. Static aerated piles have an intermediate efficiency in pathogen removal and their use is suitable for situations where the use of close reactors is not affordable.

There are failures even with the high temperature process

Independent of the system used, viruses can survive the composting process only if certain zones of the heap are not exposed or exposed for too short times to the temperatures required for viral inactivation. In order to ensure the safety of the final product, it is necessary to monitor the efficiency of composting plants in pathogen reduction. Different systems are available for this scope, including direct process validation (i.e. "germ-carriers" inoculated with viral indicators are inserted into the heap and analyzed at the end of the composting process), indirect process supervision (i.e. control of temperature, dry matter and pH within the heap) and end-product analysis.

Virus survival in composted human faeces depends not only on the conditions used for composting, but also on the storage conditions. Virus numbers decrease during storage of faeces, due to their incapability to replicate outside of the host and susceptibility to environmental conditions. However, the die-off rate of viruses present in human faeces is extremely variable depending on both the type of virus and the storage conditions. While 100 days of storage appear to be sufficient to determine complete inactivation of enteroviruses, hepatitis A virus (HAV) is reduced only 1-2 log units when contaminated manure is stored for 70 days at low ambient temperature.

The presence of enteric viruses can be substantially reduced during storage by enhancing high pH and low moisture conditions. Alkaline conditions can be achieved by the addition of wood ash, lime or other alkaline substances. Faecal dehydration may be enhanced by the addition of different substances, e.g. soil. The effect of aeration on virus survival in faeces should be further studied with the scope to develop possible applications in the design and construction of urine-diverting toilets.

The most important pathogenic viruses shed in human faeces are adenoviruses, astroviruses, caliciviruses, enteroviruses, HAV, hepatitis E virus (HEV) and rotaviruses. All these enteric viruses lack an envelope, which make them relative resistant to heat and acid pH conditions compared with enveloped viruses. The most heat-resistant human pathogenic virus is HAV, for which temperatures of 60°C for 30 min or 80°C for 10 min have been reported not to be sufficient for complete inactivation. However, even HAV is inactivated under temperature conditions comparable to those used for composting (e.g. 60°C for 10 hours).

In Denmark, all the major groups of enteric viruses are present in the population except for the HEV, the presence of which is mainly limited to developing countries. The incidence of HAV was estimated to be about 1.5 cases per 100.000 inhabitants in the year 2000. Rotaviruses are more common than other viruses causing diarrhoea in young children (adenoviruses, astroviruses and caliciviruses). The occurrence of enteroviruses in faecal specimens from symptomatic patients is around 13-23%. Caliciviruses have been found to be implicated in approximately 40% of food-borne disease outbreaks.

Based on data on the occurrence of viruses in sewage in Denmark which has been estimated to 10^3 to 10^5 PFU/100 g for enteroviruses (including HAV) and 2 to 10^2 PFU (Plaque Forming Units)/100 g for rotaviruses, the concentration of viral particles in human faeces collected for composting would be expected to exceed these numbers. Adenovirus, astrovirus and rotavirus should be more frequent from late autumn to early spring, whereas enteroviruses are likely to be more common during the summer period.

Enteric viruses can persist for a long time after application of contaminated sludge to soil, as demonstrated by their recovery in sludge burials 6 months after the last sludge disposal. High temperature and low moisture are the two main factors affecting the persistence of viruses in soil. Accordingly, viruses seem to persist longer during the winter than during the summer. The persistence of viruses in soil is also influenced by the type of soil, with clay soils allowing a longer survival of viruses compared with sandy and muck soils. HAV has appears to survive in soil longer than other enteric viruses.

No studies are available on the risks posed to human health by the use of composted human faeces. A recent risk assessment study has estimated that urban waste compost does not contain adequate levels of *Salmonella* and parasites to represent a risk for human health. It should be noted that viruses are generally characterised by lower infective doses (1 to 10^2) compared with bacteria (10^2 to 10^6). However, differently from bacteria, viruses are not capable of replicating outside of the human body. Consequently, low numbers of viruses accidentally survived to composting cannot multiply during storage of the compost product or after its application to land.

Sammenfatning og konklusioner

Recirkulering af organisk affald reducerer de miljømæssige og økonomiske problemer ved affaldshåndtering. Forskellige typer organisk affald, herunder fækalier og urin fra mennesker, kan recirkuleres og anvendes som gødning i landbruget eller til andre formål. Herved reduceres udgifter til affaldsdeponering og næringsstoffer føres tilbage til jord og planter. Der er en øget interesse i Danmark for at recirkulerer menneskets fækalier. I modsætning til udledning og transport af fækalier i kloaksystemet, så betragtes menneskets fækalier i recirkuleringssystemer som en ressource og ikke som affald der skal ledes bort. Recirkulering af menneskets fækalier kan således være gavnligt for miljøet og bidrage til en øget landbrugsproduktion med en samtidig nedsat brug af uorganisk kunstgødning.

Menneskets fækalier skal behandles og stabiliseres inden de kan recirkuleres. Denne behandling består i en nedbrydning af det organiske materiale, en reduktion og/eller eliminering af smitstoffer, en reduktion i volumen og vægt, samt fjernelse af dårlig lugt. Kompostering er en egnet metode til en sådan behandling af menneskets fækalier og er baseret på mikroorganismers nedbrydning af organisk stof under tilstedeværelse af ilt (aerobe forhold). Ilt tilføres ofte ved kompostering af affald placeret i rækker/stakke og i lukkede kompostsystemer, samt ved periodevis at vende kompostmaterialet.

Rapporten indeholder kun oplysninger om virus overlevelse ved centraliseret kontrolleret kompostering, da der i decentral kompostering, eksempelvis i husholdninger, kun sjældent kan forventes den nødvendige temperatur udvikling til inaktivering af virus. Det er endvidere ofte ikke muligt at kontrollere temperatur og andre parameter i decentrale kompostsystemer.

Med udgangspunkt i danske forhold, er rapportens formål at videregive den nødvendige viden til en evaluering af forekomsten af sygdomsfremkaldende virus i komposterede fækalier fra mennesker. Rapporten er baseret på et studium af tilgængelig litteratur om forekomst af smitsomme virus i menneskets fækalier og overlevelse af virus under opbevaring, behandling og anvendelse af komposterede fækalier. Mængden af litteratur var begrænset og spredt og Miljøstyrelsen havde derfor identificeret et behov for at indsamle og formidle den eksisterende viden til anvendelse af myndigheder, beslutningstagere og andre til udarbejdning af risikovurderinger, samt vejledninger og regler for recirkulering af menneskets fækalier.

Litteraturstudiet viste, at de kendte smitsomme virus, som kan forekomme i fækalier fra mennesker, ikke kan overleve kompostering, hvis denne er udført korrekt efter gældende bekendtgørelser og regler i Danmark og andre europæiske lande. Eksponering af virus til temperaturer over 55°C i mere end 2 uger (kontrolleret kompostering) eller til 70°C i én time (kontrolleret hygiejnisering), som påkrævet i Danmark, vil sikre en fuldstændig inaktivering af alle smitsomme virus som forekommer i fækalier fra mennesker.

Inaktivering af virus under kompostering skyldes især en irreversibel beskadigelse af strukturerne i virus forårsaget af varme udviklet ved mikrobiel

aktivitet. Virus kan også i mindre omfang inaktiveres af proteolytiske enzymer produceret af bakterier og af ammoniak frigjort ved omsætning af proteiner.

Selvom adskillige studier har vist, at kompostering er i stand til at inaktivere virus i spildevandsslam, husdyrgødning, og andre typer organisk affald, har andre studier understreget hvor svært det er at opretholde et konstant niveau af vigtige kompostparametre (eksempelvis temperatur, lufttilførsel, og vandindhold). Det er endvidere svært, at kontrollere disse parameter under den daglige drift af kompostanlæg, især i kompost placeret i rækker.

Overvågning og dokumentation af særlige parameter såsom temperatur, beluftning, og fugtighed er essentielle til sikring af en komplet inaktivering af virus under kompostering. Lukkede systemer er de mest sikre til kompostering af fækalier, da det er lettere at kontrollerer de essentielle parametre og opnå en ensartet temperatur overalt i komposten. Det er derimod vanskeligt at sikre en ensartet og tilstrækkelig temperatur og virus inaktivering i åbne kompostsystemer, eksempelvis i rækker, på grund af omgivelsernes indvirkning på temperaturen i de ydre/øvre kompostlag. Det er noget nemmere at holde en konstant temperatur i beluftede kompoststakke, og disse kan derfor anvendes hvis der ikke er mulighed for at komposterer fækalier i lukkede systemer.

Uafhængig af kompostsystemet, kan virus kun overleve hvis dele eller områder af komposten ikke eksponeres for eller eksponeres i for kort tid til de temperaturer som er nødvendige til inaktivering af virus. For at kunne garanterer sikkerheden ved slutproduktet, er det nødvendigt at kontrollere hvor effektive kompostanlæg er til at reducere antallet af smitstoffer. Der findes forskellige af sådanne kontrolmetoder, bl.a. ved direkte målinger af kompostprocessen og dens reduktion af virusindikatorer tilsat i særlige beholdere ved påbegyndelse af komposteringen. Kompostprocessen kan også kontrolleres ved måling af temperaturer, tørstofindhold og pH i komposten og ved analyse af slutproduktet (indirekte kontrol).

Overlevelse af virus i fækalier fra mennesker afhænger ikke kun af forholdene ved komposteringen, men også af opbevaringsforholdene inden kompostering. Antallet af virus reduceres under opbevaring af fækalier, da virus ikke er i stand til at opformere sig uden for en vært og samtidig påvirkes at forskellige miljøforhold. Henfaldsraten for virus i fækalier fra mennesker er dog yderst variabel og afhænger især af virustypen og opbevaringsforholdene. Som eksempel er cirka 100 dages opbevaring tilstrækkelig til en komplet inaktivering af enterovirus, mens hepatitis A virus (HAV) kun reduceres med 1-2 log enheder ved opbevaring af virusholdigt fækalier i 70 dage ved lave udendørstemperaturer.

Overlevelse af virus kan reduceres mærkbart ved at øge pH og holde en lav luftfugtighed. Basiske forhold kan opnås ved tilsætning af aske fra træ, kalk eller andre alkaliske stoffer. Udtørring af fækalier kan øges ved tilsætning af forskellige stoffer, eksempelvis jord. Påvirkning af virus ved tilførsel af luft er usikker og bør undersøges nærmere, eksempelvis i forbindelse med udvikling og konstruktion af urinseparerende toiletter.

De vigtigste virus som udskilles gennem fækalier fra mennesker er adenovirus, astrovirus, calicivirus, enterovirus, HAV, hepatitis E virus (HEV) og rotavirus. Alle disse virus mangler en kappe og er derved relative resistente overfor varme og lave pH forhold sammenlignet med virus som er omsluttet af en

kappe. Det mest varme resistente virus hos mennesker er HAV, som selv ved eksponering til 60°C i 30 min eller til 80°C i 10 min ikke undergår en komplet inaktivering. HAV inaktiveres dog ved de temperaturer som opnås ved en kontrolleret kompostering (eksempelvis 60°C i 10 timer).

Undtaget HEV, findes de nævnte hovedgrupper af mavetarmvirus hos mennesker i Danmark. HEV optræder især hos mennesker i udviklingslande. Incidensen af HAV infektion i Danmark blev i år 2000 beregnet til cirka 1,5 tilfælde per 100.000 personer. Rotavirus optræder hyppigere end andre virus som også forårsager børnediarré (adenovirus, astrovirus og calicivirus). Forekomsten af enterovirus i fækalieprøver fra personer med klinisk sygdom er omkring 13-23 %. Calicivirus anslåes at være involveret i omkring 40 % af alle fødevarebårne sygdomsudbrud i Danmark.

Koncentrationen af virus i spildevand i Danmark er anslået til omkring 10^3 to 10^5 PFU/100 ml for enterovirus (inklusiv HAV) og 2 to 10^2 PFU (Plaque Forming Units)/100 ml for rotavirus. Koncentrationen af viruspartikler i fækalier fra mennesker indsamlet til kompostering kan derfor forventes at være højere end de anslåede værdier for spildevand. Adenovirus, astrovirus og rotavirus vil normalt optræde hyppigere fra sidst på efteråret til det tidlige forår, hvorimod enterovirus forventes at optræde hyppigere om sommeren.

Påvisning af mavetarmvirus 6 måneder efter udbringning af forurenet slam, viser at virus kan overleve i lange perioder efter udbringning af forurenet slam på landbrugsjord. En høj temperatur og lav fugtighed er de vigtigste faktorer som påvirker virus overlevelse i jord. Virus overlever derfor længere om vinteren end om sommeren. Overlevelsen af virus i jord påvirkes også af jordtypen, hvor lerjord tillader en længere overlevelse sammenlignet med sandjord. HAV viser en øget overlevelse i jord sammenlignet med andre mavetarmvirus.

Der eksisterer ingen vurderinger af risici for mennesker ved anvendelse og udbringning af komposterede fækalier fra mennesker. En risikovurdering af anvendelsen af komposteret byaffald har fornyelig vist, at denne type affald ikke indeholdt et tilstrækkeligt antal *Salmonella* og parasitter til at udgøre en risiko for mennesker sundhed. Det skal dog bemærkes, at virusinfektioner generelt er karakteriseret ved en lav infektionsdosis (1 til 100) sammenlignet med infektionsdosis ved bakterielle infektioner (10^2 til 10^6). Virus er dog i modsætning til bakterier ikke i stand til at formere sig uden for værten. Et lavt antal virus som eventuel ved en fejl overlever en kompostering kan således ikke opformerer sig under opbevaring af komposten eller efter udbringning af komposten på landbrugsjord.

1 The composting process

1.1 Introduction

An important problem that the modern society has to face is the disposal of increasing quantities of waste. The European Union alone produces approximately 1,300 million tonnes of waste per year (1). Due to the environmental and economical concerns associated with sanitary landfilling and incineration, increasing attention has been given to the practice of recycling and to the use of organic waste for agricultural purposes.

Organic waste products are rich in organic carbon and relatively poor in inorganic nitrogen, which is more bioavailable for plants compared with organic nitrogen. The reuse of organic waste in agriculture requires a process of *stabilization* ("maturation"), which consists in the degradation of organic matter accompanied by a transformation of organic forms of nitrogen into inorganic forms, reduction of the total mass, elimination of pathogenic organisms and removal of undesirable odours.

Composting is one method for stabilization of organic waste products. Other methods include aerobic digestion, anaerobic digestion, lime stabilization and heat treatment (2). Composting is based on the decomposition of organic matter by microorganisms under aerobic conditions. The types of waste materials most commonly used for production of compost are yard waste, sewage sludge, municipal solid waste, household waste, industrial and agricultural by-products (wood, animal droppings, etc.).

Composting is regarded as a fully sustainable practice, since it aims at both conservation of the environment, human safety and economically convenient production (3). The use of compost contributes to conservation of the environment by reducing both utilization of non-renewable resources and consumption of energy for waste treatment and production of chemical fertilizers. Composting indirectly also contributes to human safety by avoiding an improper fate or disposal of organic wastes. Furthermore, due its low cost, compost is convenient to the farmer, but even more to the society by avoiding the use of expensive solutions for waste disposal.

1.2 Process description

Composting is an aerobic process during which microorganisms convert an organic substrate into stabilized organic matter with production of heat. The process generally starts by mixing dewatered organic waste with a *bulking agent*, such as wood chips, yard trimmings, bark, rice hulls, municipal solid waste or previously composted material. Bulking agents are used to add a source of carbon, lower moisture content, provide structural support, increase porosity and favour aeration. The mixture is then composted in the presence of air for a period of 2-4 weeks depending on the type of system used, followed by a maturation phase (curing) of approximately the same duration. Finally, the compost product can be screened to remove unwanted

components or to recover the bulking agent, and prepared for a particular market and purpose.

The composting mass is a dynamic microbial ecosystem, in which different groups of microorganisms develop and become predominant during the different phases of composting. The process consists of at least three different phases: the mesophilic phase, thermophilic phase and the maturation (curing) phase.

During the mesophilic phase, mesophilic bacteria utilize readily available organic matter, determining a rapid increase of temperature. The temperature can reach 55°C in a few days and go up to 80°C if the system is not properly controlled (4). The increase of temperature is accompanied with a radical change in the physical and chemical characteristics of the initial waste material.

The thermophilic phase is characterized by the growth of thermophilic bacteria, fungi and actinomycetes. Among the thermophilic organisms found in compost, there are various species of fungi, actinomycetes and endospore-forming bacteria (mainly *Bacillus* spp.)(5-7). Thermophilic bacteria play an important role in the degradation of proteins and carbohydrates, whereas actinomycetes and fungi contribute to the degradation of more complex compounds like cellulose and lignin (2).

During the maturation phase, the low amount of readily available nutrients determines a reduction in the microbial activity and consequently in the production of heat. This phase allows further stabilization, reduction of pathogens and decomposition of cellulose and lignin. Particularly important during this phase is the formation of humic and fulvic acids, which confer valuable fertilization properties to the compost product.

1.3 Systems of composting

Although there exists a number of different composting methods and technologies, there are three main systems of centralised composting: aerated static pile process, windrow process and enclosed systems. The following paragraphs provide a short description of each system. Small-scale technologies for decentralised composting at the household level are also briefly described.

1.3.1 Aerated static pile systems

This system consists in the formation of piles of dewatered organic waste mixed with a bulking agent. The piles can be covered with screened compost to reduce odours and to maintain a high temperature inside the pile. Aeration is provided by means of blowers and air diffusers (Fig. 1.1). Aerated static piles are most commonly used for homogeneous materials (e.g. sludge) and are not appropriate for heterogeneous materials that need to be mixed during composting (e.g. municipal solid waste).

1.3.2 Windrow systems

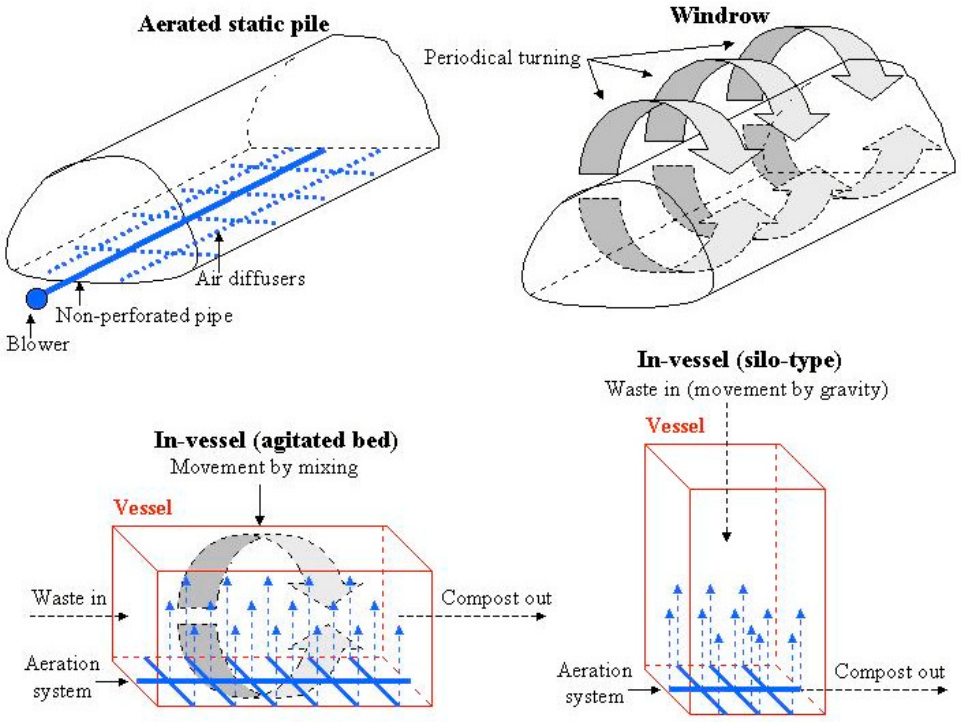
The windrow system is the least expensive and most common approach. The mixture of dewatered organic waste and bulking agent is stacked in rows called windrows and the composting mass is aerated by turning the windrows either manually or mechanically (Fig. 1.1). Turning programs are generally

designed to ensure the different aeration rates required during the different phases of the composting process. Alternatively, temperature can be used as a turning indicator, so that windrows are turned when a certain temperature (usually 55 or 60°C) is reached.

1.3.3 Enclosed (in-vessel) systems

These systems are enclosed into containers (i.e. vessels) to ensure control of temperature, oxygen concentration and odours. Due to their high cost, enclosed systems are particularly appropriate when a high quality of the compost product is required. The vessel can be anything from a silo to a concrete-lined trench. The silo-type systems rely on gravity to move the composting material through the vessel, whereas in other enclosed systems (e.g. agitated bed system), the material is moved through the vessel by mixing, combining the advantages of windrow and aerated static pile systems (Fig. 1.1).

Fig. 1.1. Schematic description of the main systems of centralised composting.



1.3.4. Decentralised systems of composting

A number of different containers are used to collect and store human faeces at the household level. Often such containers or vaults are simple plastic units located beneath the toilets, i.e. urine-separating toilets. The containers often have a lid with an inserted ventilator for removal of malodour. In addition to faecal matter, containers will contain toilet paper, various amounts of urine and the users may also add saw dust or other substances to maintain a relative dry environment and enhance the composting process.

what's happening in decentralized compost toilet environments

Little composting and temperature development seems to be occurring when faecal matter is collected and stored at the household level. This is mainly caused by nearly anaerobic conditions and a low content of organic matter. Thus, only minor reductions in pathogen numbers can be expected in the majority of such decentralised systems for collection and storage of faeces.

Danish legislation on managing waste from composting toilet

Faeces collected in decentralised systems will therefore most often need to be transported and composted in centralised systems if reductions in pathogens are to be obtained according to current legislation (sections 1.3.1-1.3.3).

1.4 Factors to be controlled during composting

Several physical and chemical parameters influence the activity of microorganisms during composting. Temperature, aeration and moisture are the most important parameters (8,9). Their control is essential to ensure both stability and hygienic safety of the final product.

1.4.1 Temperature

Temperature is probably the most important factor to be controlled during composting. While temperatures between 45 and 55°C ensure the best degradation rates of the compost material, temperatures above 55°C maximize pathogen reduction (9). Above 60°C, the degree of microbial diversity is markedly decreased, with negative consequences on the degradation process (10). Accordingly, the choice of the operational temperature will influence both the stability and the hygienic quality of the final product.

A wide variation of temperature can exist within the composting mass. For example, in aerated static pile systems the core temperature can reach 70°C, while the outer zone remains near ambient temperature (11). Temperature is controlled by adjusting the aeration levels in the case of aerated systems or by turning the windrows in the case of windrows systems. Particular systems have been developed to ensure that the air supply rate is appropriate to the composting requirements of the local mass (9). This is unlike traditional systems, where the same supply rate is applied to the whole of the mass regardless of the degradation stage.

1.4.2 Aeration

Aeration provides oxygen to the aerobic organisms necessary for composting. In general, anaerobic conditions prolong the duration of the composting process and, above all, lead to relatively lower temperatures with adverse effects on sanitation. Oxygen is not only necessary for aerobic metabolism of microorganisms, but also for oxidizing the various organic molecules present in the composting mass (8). Aeration has also the important function to control temperature as well as to remove the excess of moisture and gases.

The air requirements for composting depend on the type of waste, the process temperature to be reached, the stage of the process (i.e. higher requirements in the early stages) and the moisture content. Air can be supplied by agitation in the windrows process, by forced aeration in the aerated static piles or by a combination of the two in aerated/agitated systems.

1.4.3 Moisture

Moisture control is also an important factor to be controlled during composting as it influences structural and thermal properties of the material, as well as the rate of biodegradation. The initial moisture content generally varies between 55 and 65% depending on the material used (9). During

composting, a loss of water occurs as a consequence of evaporation. Reduction of the moisture content below 30-35% must be avoided since it causes a marked reduction of the microbiological activity and a premature end of the process due to exsiccation. Too much moisture should be also avoided as it interferes with aeration by clogging the pores in the composting heap (8).

There is not a target value for the final moisture content. Most composting processes operate with moisture contents between 40-60%. In agitated or turned systems, a trained operator is able to evaluate whether the material is too dry or too wet and make appropriate adjustments. This is in contrast to aerated static pile systems, where a measurement of the moisture content is needed. However, the wide variation in the moisture contents throughout the pile mass makes such measurement difficult (9). Moisture can be controlled either directly by adding water or indirectly by changing the operating temperature or the aeration regime.

1.4.4 Carbon/nitrogen ratio

The control of the ratio of carbon to nitrogen (C/N ratio) is important because the microbes responsible for the degradation process need adequate levels of nitrogen. The optimal carbon/nitrogen (C/N) ratio in the starting material is around 25 (8). A too low C/N ratio slows decomposition and increase nitrogen loss through ammonia volatilisation, especially at high pH and temperatures values. A to high C/N ratio (>35) delays the process, since microorganisms must oxidize the excess of carbon until a more convenient C/N ratio for their metabolism is reached (8). The C/N ratio of the starting material can be adjusted prior to composting. In the case of human faeces, the addition of a carbon source (e.g. bark, wood shavings or straw) is needed due to their low C/N ratio (5-10)(12).

1.5 Properties and use of compost

The compost product is a humus-like material with excellent properties as a fertilizer. Compost enhances plant growth by providing nutrients, improving root penetration and in some cases reducing plant disease. The positive effect of certain compost products on plant disease control has been attributed to competition for nutrients, antibiotic production and predation by beneficial microorganisms, as well as activation of disease resistance genes in plants (13). Depending on the degree of maturity and quality, compost can be used in vine yards, mushroom farming, agri- and horticulture, reforestation, preparation of sport fields, maintenance of parks, gardens and motor-way embankments, and rehabilitation of mines and sand pits.

Compost, especially when derived from biosolids, contains less nitrogen and nutrients compared with other types of treated organic waste as a consequence of dewatering, dilution of nutrients by addition bulking material and loss of ammonia during the composting process. However, nutrients are released more slowly and are available to plants over a longer period of time (14). The slow release of nutrients is more consistent with plant uptake needs and reduces leaching of nitrogen, which is an important environmental concern associated with the use of other types of conventional fertilizers and soil conditioners (15).

Compost is not only a good fertilizer but also an excellent soil conditioner. Application of compost to land improves aeration, water-holding capacity, nutrient content and structure of the soil. Due to these properties, compost is

also effectively used for landscaping, erosion control, landfill cover, turf remediation, alleviation of compacted soil, and wetland restoration (16,17).

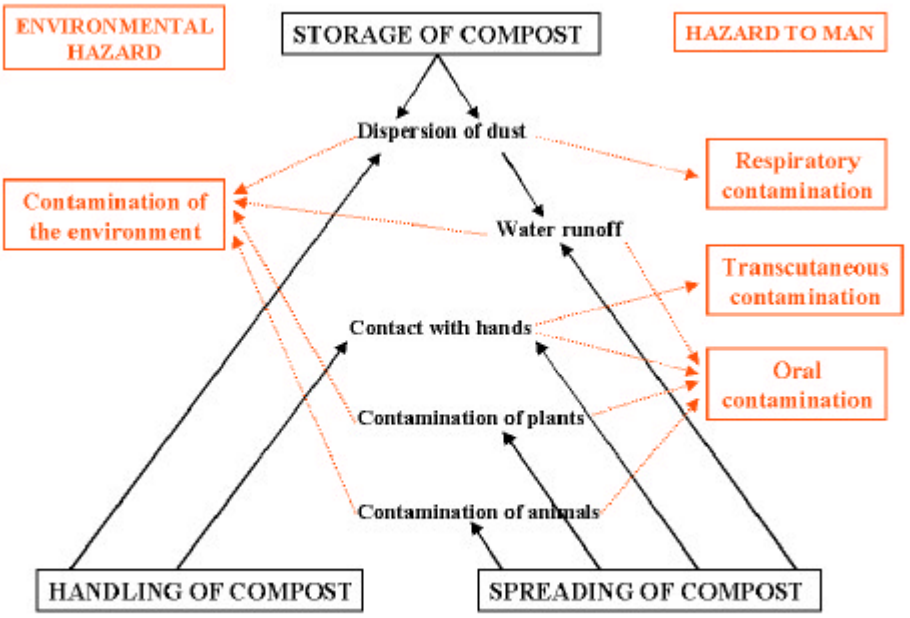
Compost used for a specific purpose is more efficient when specially designed (i.e. tailored compost). For example, compost intended to prevent erosion may not give the best results when used to alleviate soil compaction. Tailored compost is adjusted to fit a specific application and soil type by controlling the maturity, pH, density, particle size, moisture, salinity and organic content of the final product.

Innovative uses of compost are bioremediation and biofiltration (18). Compost bioremediation refers to the use of microorganisms in a mature, cured compost to sequester or breakdown contaminants in water or soil. This practice has proven effectiveness in degrading or altering many types of contaminants, such as chlorinated and non-chlorinated hydrocarbons, wood-preserving chemicals, heavy metals, pesticides, petroleum products and explosives (18,19). Compost biofiltration implies the physical removal of contaminants in water or air by filters composed by multiple layers of tailored compost. This practice has been successfully used for separation of physical debris, surface scum and chemical contaminants from stormwater (i.e. stormwater management), as well as for disposal of volatile organic compounds and odour control (18).

1.6 Hazard to man and the environment

The use of compost may be associated with hazards to man and the environment. Depending on the original raw material, compost products may contain various chemical and microbiological contaminants causing health and environmental risks. Man and the environment are exposed to contaminants during production, storage and utilization of compost. Fig. 1.2 summarizes the different means and routes of contamination and their risk implication.

Fig. 1.2. Pathways of contamination with chemical and microbiological pollutants during production and utilization of compost. Adapted from Déportes et al. (20).



1.6.1 Chemical risk

Composts can contain a wide range of toxic substances, including inorganic and organic compounds. The contamination of the finished product generally originates from the primary material. Composts derived from municipal solid wastes, sewage sludge and yard wastes may contain relative high concentrations of toxic chemicals. In the case of composted human faeces, contamination with toxic chemicals seems to represent a relative minor hazard also when composted human faeces are used as fertilizers in agriculture.

The most important class of toxic compounds that can occur in compost is represented by heavy metals. These elements have the characteristics of being highly toxic and scarcely biodegradable. According to a recent risk assessment study (20), the highest risk is associated with hand-mouth contact and ingestion by children of compost heavily contaminated with cadmium, chromium, lead or mercury. The risk that humans are seriously exposed to metals through the food chain appears to be lower, since most compounds do not accumulate in vegetables, and accumulate in offal rather than in meat of animals (20).

Although the chemical risk associated with the application of compost to soil appears to be of little importance, heavy metals persist in soil for many years and repeated application might lead to an accumulation of these pollutants. For this reason, both European and American regulations define not only concentration limits in compost, but also concentration limits in soil and maximum annual loads (21,22).

1.6.2 Microbiological risk

The microbiological risk associated with the production and the use of compost is the possible infection of humans, animals or plants by pathogens occurring in raw materials used for composting as well as by pathogens developing during the composting process.

1.6.2.1 Pathogens present in raw material

Human and animal pathogens are particularly frequent in raw material when such material contains faecal matter, food residuals or animal wastes. The principal pathogenic bacteria, helminth and protozoan parasites occurring in human faeces are listed in Table 1.1. The occurrence of pathogenic viruses in human faeces is presented and discussed in chapter 2.

Table 1.1. Main pathogenic bacteria, helminths and protozoa excreted in human faeces. Adapted from Feachem et al. (23) and U.S. Environmental Protection Agency (24).

Bacteria	Helminths	Protozoa
Salmonella spp.	*Taenia saginata* (cestode)	*Cryptosporidium parvum*
Pathogenic *Eschericha coli*	*Taenia solium* (cestode)	*Entamoeba histolytica*
Campylobacter jejuni/coli	*Hymenolepsis nana* (cestode)	*Giardia lamblia*
Shigella spp.	*Ascaris lumbricoides* (nematode)	*Balantidium coli*
Vibrio cholerae	*Stongyloides stercoralis* (nematode)	
Yersinia enterocolitica	*Enterobius vermicularis* (nematode)	
Leptospira icterohaemorrhagiae	*Trichuris trichiura* (nematode)	
	Ancylostoma duodenale (nematode)	
	Necator americanus (nematode)	

The microbiological hazard to humans arising from spreading of compost appears to be low if adequate temperatures and exposure times have been obtained during composting (25). The heat generated during the oxidation process destroys most pathogens present in raw materials. Furthermore, the composting mass turns to be an unsuitable substrate for the growth of some pathogens due to loss of moisture, depletion of nutrients and microbial antagonism (25). A recent risk assessment study has estimated that compost does not contain adequate levels of *Salmonella* and parasites to represent a risk for human health (20). According to this study, the infective dose of *E. coli* (10^6) is not likely to be reached after ingesting a mixture of soil and compost, whereas the infective dose of enterococci (10^9) may be attained only in cases of pica.

Plant pathogens may be present in residues of infested plants used for composting (e.g. composting of yard waste). The risks of plant infection are very low for most soil-born viruses, as the vectors (nematodes or fungi) needed for plant infection are destroyed during composting (26). Higher risk are associated with the presence in raw materials of the tobacco mosaic virus (TMV), as this virus is not completely inactivated during composting and can be directly transmitted to plant roots without a vector. Accordingly, compost from plant residues infested by the TMV should not be used in susceptible crops (26).

1.6.2.2 Pathogens developing during composting

Some fungi and bacteria developing during the process of composting constitute a potential risk for both workers at composting plants and users of compost (15,20). These organisms are bound to dust produced during composting and can heavily contaminate the atmosphere of composting plants (27). The most frequently studied organism is the fungus *Aspergillus fumigatus*, an opportunist pathogen causing allergies, asthma and respiratory infections. Risks derive not only from living organisms, but also from spore and endotoxins of bacterial origin (15,20).

The consequences of this form of air pollution (i.e. bioareosol) on occupational health are not clear. The available epidemiological studies are inadequate to determine whether exposure to bioareosol have a significant impact on human health (28).

1.7 Legislation

This section introduces the current legislation on production and use of compost in Denmark, in the EU and in the USA. None of the current regulations specifically address the sanitary aspects regarding composted human faeces. The production and the use of compost originating from human faeces are generally regulated by the legislation on sewage sludge.

1.7.1 In Denmark

According to the Danish legislation (29,30), compost derived from sewage sludge can be used without sanitary restrictions only if it has been processed in a closed reactor and exposed to minimum 70°C for at least one hour (controlled sanitation). The compost product has to be free of *Salmonella* spp. and may contain less than 100 CFU of faecal streptococci per g of finished product. Composted sewage sludge derived from open-air systems cannot be used as a fertilizer in edible crops or parks. When applied to forest soils, the forests must be closed to the public for a period of 6 months. The application of sewage sludge on grassland is prohibited for one year before grazing, and on forage crops before harvesting.

Organic waste other than sewage sludge can be treated by controlled composting (≥55°C in all material for at least two weeks) without any sanitary restriction. The only exception is given by composted household waste, which has to be plough in when applied to areas used for cloven-footed animals. Details about the Danish guidelines can be seen elsewhere (29,30)(http://mst.dk/).

1.7.2 In the EU

The EU strategy for waste management is based on minimization, reuse, material recycling, energy recovery and safe disposal. Since 1975, the Member States have to take appropriate steps to encourage the prevention, recycling and processing of waste, and to ensure that waste is disposed without endangering human health or without harming the environment (31).

The EU directive of 1986 on sewage sludge invited the Member States to prohibit the use of untreated sludge in agriculture and the use of any type of sludge in grassland to be grazed, in forage crops to be harvested within a short period and in grounds intended for the cultivation of fruit and vegetables which are in direct contact with soil and normally eaten raw (21). Furthermore, the directive established the concentration limits of heavy metals in soil and sludge, and defined the maximum quantities of heavy metals that can be introduce into soil per unit of area and time.

In 2001, the Commission communicated to the Council and the European Parliament its intention to broaden the sewage sludge directive to cover all types of sludge and all land spreading operations (not only use in agriculture) (32). In the same year, a working document was draft by the EU Directorate of General Environment as a basis for preliminary discussions to improve the present legislation for biodegradable waste management (33).

1.7.3 In the USA

The production and use of compost is regulated in the Part 503 rule of the Environmental Protection Agency (EPA)(34). The rule is based on 3 parameters for determining the quality of biosolids, i.e. sewage sludge which has been treated and meets state and federal standards for land application: presence of pollutants, presence of pathogens and attractiveness to vectors (e.g. rodents, birds, flies or mosquitoes). Biosolids that meet the most stringent limits for all 3 parameters are designated as Exceptionally Quality (EQ). These products are exempted from the general requirements, management practices and site restrictions defined in the EPA Part 503 rule. They can therefore be used with no more restrictions than any other fertilizer or soil amendment product.

The first parameter that must be assessed to determine the overall quality of biosolids is the levels of pollutants. EPA chose to determine the pollutant limits based upon a scientific risk assessment. The risk assessment process considered 14 representative pathways by which humans, animals and plants are exposed to pollutants present in biosolids (35). Details on pollutant limits can be found in the EPA website (http://www.epa.gov/).

The second parameter in determining biosolids quality is the presence of pathogens. The EPA Part 503 rule states that the producer is responsible for monitoring and certifying pathogen reduction. Two classes of biosolids (A and B) are defined based on pathogen density limits (Table 1.2). In addition to the requirements on pathogen density, class A biosolids must be treated by one of the Processes to Further Reduce Pathogens (PFRP), such as composting, heat drying and high-temperature aerobic digestion. For class B biosolids, producers may document compliance by analysing the material for faecal coliform levels (Table 1.2). Alternatively, class B biosolids must be treated by one of the Processes to Significantly Reduce Pathogens (PSRP), such as aerobic digestion, anaerobic digestion, air drying and lime stabilization (34).

Table 1.2. Pathogen density limits for land application of biosolids in the USA (34).

Pathogen or indicator	Standard density limits (dry wt)[1]	
	Class A	Class B
Faecal coliforms	<1000 MPN per g or	<2.000.000 MPN / g
Salmonella	<3 MPN / 4 g and	-
Enteric viruses	< 1PFU / 4 g and	-
Viable helminth ova	< ¼ g	-

[1]The geometric mean of seven samples must be comprised in these limits.

While class A biosolids are virtually pathogen free and do not pose a risk of infectious disease transmission through casual contact or ingestion, in class B biosolids pathogens are reduced to levels that are unlikely to pose a threat to public health and the environment under specified use conditions. Consequently, site restrictions are imposed to minimize the potential of human and animal contact with class B biosolids following their application to land. Furthermore, class B biosolids cannot be sold or given away in bags or other containers.

The degree of attractiveness of sewage sludge to vectors is the third parameter of biosolids quality. Vectors are animal and insects that can transmit pathogens to humans, animals or plants. Reducing the attractiveness of biosolids to vectors reduces the potential for spreading diseases from

pathogens present in biosolids. The Part 503 rule contains 12 options to either reduce the attractiveness of biosolids to vectors (options 1 to 8 and option 12) or prevent vectors from coming in contact with the biosolids (options 9 to 11)(Table 1.3). One of these options must be met if biosolids are to be applied to land (34).

Table 1.3. The 12 options to reduce vector attraction in biosolids to be land applied (34).

Option	Description
1	Meet 38% reduction in volatile solids content
2	Demonstrate vector attraction reduction with additional anaerobic digestion in a bench-scale unit
3	Demonstrate vector attraction reduction with additional anaerobic digestion in a bench-scale unit
4	Meet a Specific Oxygen Uptake Rate (SOUR≤1.5 mg of oxygen per hour per g of total biosolids)
5	Use aerobic processes at greater than 40° for 14 days or longer
6	Add alkaline material to raise pH to 12, maintain pH at 12 for 2 h and at 11.5 for other 22 h
7	Dry biosolids with no unstabilized solids to at least 75% solids
8	Dry biosolids with unstabilized solids to at least 90% solids
9	Inject biosolids beneath the soil surface
10	Incorporate biosolids into the soil within 6 h of application to or placement on the land
11	Cover biosolids placed on a surface disposal site with soil or other material at the end of the day
12	Alkaline treatment of domestic septage to at least pH 12 for 30 min

2 Pathogenic viruses in human faeces

2.1 Introduction

Over 130 different types of pathogenic viruses are shed in human faeces (Table 2.1). Based on their pathogenesis, such viruses can be classified as enteropathogenic viruses, for which the gastrointestinal system is the principal site of infection (e.g. astroviruses, caliciviruses and rotaviruses) and non-enteropathogenic viruses, which can occur in the intestinal tract but not in association with gastroenteritis (e.g. most adenoviruses, enteroviruses and hepatitis A/E viruses). Other viruses (e.g. enteric coronaviruses, certain "small round viruses" and "parvovirus-like" agents) have been detected in faeces of patients affected by gastroenteritis, but their pathogenicity has not been proved yet (36).

Contamination with urine or blood may lead to the occurrence in faeces of other important human pathogenic viruses, like the human immunodeficiency virus (HIV) and the hepatitis B virus (HBV). However, such viruses are not of particular relevance in relation to composting of human faeces, due to their sporadic occurrence in human faeces, their poor survival in the environment, and their route of transmission (i.e. parenteral). These factors minimize the risks of human exposure to these viruses via the production and usage of composted human faeces.

This chapter describes the most important viral pathogens occurring in human faeces (section 2.2). Particular emphasis was given to review aspects relevant to composting of human faeces, like their occurrence in human faeces (section 2.3), resistance to physical-chemical factors (section 2.4) and infective dose for humans (section 2.5).

Table 2.1. Main pathogenic viruses occurring in human faeces. Modified from Hurst (37).

Virus group		*No. of serotypes*	*Disease*
Adenovirus		*47*	*Respiratory disease, conjunctivitis, gastroenteritis*
Astrovirus		*8*	*Gastroenteritis*
Calicivirus		*2*	*Gastroenteritis*
Enterovirus	Poliovirus	*3*	*Paralysis, meningitis*
	Coxsackievirus A	*24*	*Herpangina, respiratory disease, meningitis, paralysis*
	Coxsackievirus B	*6*	*Myocarditis, pericarditis, congenital heart anomalies, rash, diarrhoea, meningitis, respiratory disease, pleurodynia*
	Echovirus	*31*	*Meningitis, respiratory disease, pericarditis, myocarditis, rash, diarrhoea, fever*
	Enterovirus 68-71	*4*	*Meningitis, respiratory disease*
Hepatitis A virus		*1*	*Infectious Hepatitis*
Hepatitis E virus		*1*	*Infectious Hepatitis*
Rotavirus		*4 common 10 total*	*Gastroenteritis*

2.2 Major pathogenic viruses occurring in human faeces

This section reviews the basic morphological, biological, clinical and epidemiological traits of the principal viral pathogens occurring in human faeces. Data were obtained from relevant books (23,38), review articles in scientific journals (37), websites (39,40) and WHO documents (41,42).

2.2.1 Adenovirus

Adenoviruses (family *Adenoviridae)* are non-enveloped double-stranded DNA viruses with an icosahedral shape, a diameter between 80 and 110 nm, and fiber appendages protruding from the vertices of the icosahedral viral capsid. These viruses are unusually stable to physical and chemical agents, and adverse pH conditions. They tolerate a pH range of 5.0-9.5 and temperatures between 4 and 36EC. Heating above 56ºC disrupts the virus capsid, causing inactivation. These resistance properties confer to adenoviruses the ability to survive for long periods outside of the host cells. Furthermore, these viruses are among the most persistent in sewage treatment systems.

Adenoviruses are divided into subgroups A-F, with members of the A-E subgroups causing respiratory infections and members of the F sub-group causing enteric infections. Infections are common and affect primarily children. Symptoms of infections and epidemiological patterns vary between sub-groups and even for different species. Although some adenoviruses can cause gastroenteritis in young children (i.e. serotypes 40 and 41) or genital infections (i.e. serotypes 19 and 37), these organisms are most frequently observed to cause respiratory and eye infections. The duration of the illness is generally 7-8 days. The predominant symptoms include fever, throat pain, headache, abdominal pain and conjunctivitis. Asymptomatic infections occur with long-term virus shedding from the respiratory or enteric tracts.

Adenoviruses are endemic worldwide. In temperate regions, they show a seasonal incidence, with highest incidences in the fall, winter and early spring. Transmission generally occurs by the respiratory route (inhalation of aerosols) and sometimes by the faecal-oral route. Transmission by recreational water (e.g. swimming-pools or other recreational waters) has been documented. An infected person can excrete the virus from the respiratory tract. However, the virus can disappear from respiratory secretions after a short time and can be found in faecal specimens, sometimes for extended periods.

2.2.2 Astrovirus

Members of the family *Astroviridae* are small (26-32 nm of diameter), non-enveloped RNA viruses. The name of this group of viruses derives from their star-like appearance observed by transmission electron microscopy after negative staining. These viruses are resistant to pH 3 and can survive at 60°C for 5 min.

Astroviruses are primarily associated with mild gastroenteritis in infants and young children, although elderly, hospital patients and immunocompromised individuals can also be affected. These viruses display many of the epidemiological and clinical features of rotaviruses, but are not as common and not as virulent. The illness has a normal duration of 2-3 days. Viral shedding may begin a day before symptoms are seen and continue for several days after the diarrhoea has stopped.

Astrovirus infection occurs worldwide and accounts for 2-8% of cases of diarrhoea in infants, second only to rotavirus as a cause of childhood diarrhoea. Like rotaviruses, astrovirus infections occur throughout the year with peaks in the winter months. Person to person spread by the faecal-oral route is the main route of transmission. Outbreaks tend to occur where children are in close contact, as in day-care centres, kindergartens and paediatric wards.

2.2.3 Enteroviruses

Enteroviruses form a genus within the family *Picornaviridae*, which includes important animal (e.g. foot and mouth disease) and human (e.g. hepatitis A) pathogenic viruses. The genus *Enterovirus* was traditionally divided into three groups (polioviruses, coxsackieviruses and echoviruses). Enteroviruses are small (22-30 nm), non-enveloped, RNA viruses that are highly resistant to the conditions prevailing in the gut, like acid pH, proteolytic enzymes and bile salts. They are stable at acid pH (3-5) for 1-3 hours.

The clinical syndromes caused by enteroviruses include neurological disease, cardiac and muscular disease, rash, respiratory disease, ocular disease and neonatal disease. For all members of the group, sub-clinical infection is far more common than clinically manifest disease. Certain serotypes are more frequently associated with epidemics involving a specific syndrome. However, the same serotypes may cause different clinical manifestations or produce no symptoms.

The most famous enteroviruses are the Polioviruses, of which there are 3 distinct types. These viruses cause poliomyelitis, an acute infection of the central nervous system, which can result in flaccid paralysis. The disease can have several forms: abortive poliomyelitis, a minor illness with fever, malaise, drowsiness, nausea, vomiting, constipation and sore throat, non-paralytic poliomyelitis or aseptic meningitis with the additional symptoms of a stiff neck and back, and paralytic poliomyelitis marked by flaccid paralysis. Non-paralytic illness is short-lived and patients recover without permanent damage. Paralytic disease occurs in about 1% of infections and symptoms may persist for months, with residual paralysis lasting years. Mortality among the paralytic cases varies between 4 and 10% depending on the virulence of the virus, the degree of medical assistance and the age of the patient. Recrudescence of paralysis and muscle wasting sometimes appears decades later in some persons who had paralytic poliomyelitis.

Vaccines against polioviruses have been available since the 1950s and efforts are now in progress to completely eradicate poliomyelitis and their causative viruses from the human population by the year 2005. Such global eradication seems achievable because regional eradication has already been achieved in the Western Hemisphere and Western Europe. However, there are no vaccines against the other enteroviruses.

Children are the prime targets of enteroviruses and serve as a vehicle for their spread. It has been calculated that more than 90% of children living under poor sanitary and socio-economic conditions experience infections with a number of the locally prevalent enteroviruses before they reach the age of 5 years. When infection is delayed to older childhood and adult young life, the incidence of paralytic poliomyelitis rises, together with the frequency of the most severe manifestations associated with other enteroviruses.

Almost all enteroviruses can be recovered from the oropharynx and intestine of individuals infected either clinically or sub-clinically. They are generally shed for a month or more in stool of infected individuals. Faecal contamination is the usual source of infections. However, droplets or aerosols from coughing or sneezing also can be a source of direct or indirect contamination for some enteroviruses.

Enteroviruses are found in all parts of the world. Climate is an important factor influencing the circulation and prevalence of these viruses. In tropical and semitropical regions, they are widely distributed throughout the year. In temperate climates, they are rarely present in the winter and are encountered far more commonly during summer and autumn.

Because enteroviruses are shed in faeces and respiratory secretions, and are relatively stable in sewage and water, it is assumed that they are transmitted by faecally contaminated water. Transmission by faecally contaminated water is likely to be one of the main routes for transmission under conditions of poor sanitation and crowding. However, the epidemiological evidence for waterborne transmission is weak despite years of surveillance for these viruses in populations.

2.2.4 Hepatitis A virus

The virus causing hepatitis A (HAV) is a small (27 nm), non-enveloped RNA virus with an icosahedral shape. HAV belongs to the same family of enteroviruses (*Picornaviridae*) and has similar morphological and biological characteristics to these viruses. HAV was previously classified as enterovirus 72, but it is genetically distinct from enteroviruses and is now in a separate genus called Hepatovirus. HAV is extremely resistant to degradation by environmental conditions, as demonstrated by its occurrence in freshwater, seawater, wastewater, soil, marine sediment and oysters. HAV has been found to be more resistant than some other enteric viruses to biosolids and wastewater treatment processes and to persist for as long as a 6 month in sewage-contaminated groundwater. The virus is highly resistant to heat (70°C for 10 min) and acid treatment (pH 1 for 2 h).

Hepatitis A is an acute self-limited disease accounting for approximately 1.4 millions cases in the world per year. The actual burden of disease is probably much higher due to inadequate recognition and reporting. The predominant symptoms are anorexia, jaundice, nausea and vomiting. The symptoms are highly age dependent, with adults and children over 5 years being markedly more susceptible to jaundice compared with children less than 5 years. Duration and seriousness of the disease varies from 1-2 weeks of mild illness to 6-9 months of severely disabling. Mortality rates for hepatitis A are generally less than 1% and death occurs primarily in older people. HAV can be shed before the onset of symptoms and the shedding can continue up to 3 months after resolution of the symptoms.

HAV infections account for 20-25% of clinically apparent hepatitis cases worldwide. The virus is transmitted by the faecal-oral route, either directly by person-to-person or indirectly by ingestion of contaminated food (e.g. shellfish) and water. HAV can occur both sporadically and epidemically. Epidemics are uncommon in developing countries, where children are infected early in life and adults are generally immune. In developed countries

Hepatitis A is still common and often occurs as common source outbreaks due to faecally contaminated food and water. The largest documented outbreak of Hepatitis A resulted in 300,000 cases of illness in Shanghai, China in 1988 and was caused by consumption of faecally contaminated clams.

Although not widely used, an inactivated vaccine against HAV has been available since 1995. Other prevention and control measures based on sanitation and hygiene continue to be the main barriers to transmission.

2.2.5 Hepatitis E virus

The agent causing hepatitis E (HEV) is a non-enveloped RNA virus of 32-34 nm. The virus is classified was previously within the family *Caliciviridae*, but because of genetic and replication differences, it is now unclassified and is likely to be placed in a unique virus family. Compared to HAV, HEV is less stable in harsh environmental conditions like high salt concentration or repeated freeze-thawing. The virus is more susceptible to heat than is HAV.

Hepatitis E has so far been observed almost exclusively in developing countries. Different strains of HEV occur in different parts of the world, with at least 4 main ones: (1) South-East, North and central Asian, Mexico, United States, and Taiwan. Individuals between 15 and 40 years of age are the most frequently affected. The disease closely resembles that described for hepatitis A, although bilirubin levels tend to be higher, and jaundice deeper and more prolonged. The mortality rate is 0.5-3%, but it can be extremely high for pregnant women (10-20%). HEV has been detected in stools 14 days after the onset of jaundice and persists for about 2 weeks. The infection is usually sub-clinical in children. As for hepatitis A, the disease does not progress to chronic hepatitis.

Outbreaks and sporadic cases of HEV have occurred over a large geographic area, most notably in regions with poor sanitation. Outbreaks of hepatitis E are more common in regions with hot climates and are rare in temperate climates. Most HEV outbreaks are due to faecally contaminated drinking water, but food-borne epidemics (raw or uncooked shellfish) have also been reported. Person-to-person transmission appears to be uncommon, perhaps because of the relatively low virus levels in faeces of infected persons.

Epidemic Hepatitis E was first identified in India, and it also occurs in the Middle and Far East, in northern and western Africa, the central Asian Republics of the former Soviet Union, in China and Hong Kong. Both epidemic and sporadic cases of HEV have been reported from southeast and central Asia, the Middle East, northern and western Africa and North America (Mexico). Sporadic cases of Hepatitis E occurring in non-endemic regions have been associated with travel to endemic regions. Recent evidence for the existence of HEV strains in animals (swine, rates, cattle, chickens, etc.) that resemble human HEV strains also raises the possibility of zoonotic transmission as the source of sporadic human cases in non-endemic areas. Experimentally, swine HEV infects primates and human HEV infects swine.

2.2.6 Norwalk virus and other human caliciviruses

The Norwalk virus is the prototype of a group of so-called "small round structured viruses" which are now classified as members of the family *Caliciviridae* on the basis of their nucleic sequence. Members of the family are non-enveloped contain single-stranded RNA surrounded by a capsid with

cup-shaped surface structures. These viruses are generally associated with gastroenteritis in humans.

The human caliciviruses are genetically diverse. They are divided into three major groups, the Norwalk-Like Viruses (NLVs), which are subdivided into two major subgroups, GI and GII, and the Sapporo Viruses. There is genetic diversity within these groups and several different subgroups have been identified. Caliciviruses are endemic in human populations worldwide and there are distinct genetic subgroups that predominate regionally and over time. Norwalk Virus belongs to the GI group of human caliciviruses and these are no longer prevalent in Europe and North America. The GII NLVs are the predominant epidemic caliciviruses in these regions.

The Norwalk viruses are relatively stable. They can survive at pH 2.7 for 3 h, heat at 60°C for 30 minutes and drying on surfaces. The persistence Norwalk Viruses in water, wastewater and soil is similar to that of some other enteric viruses, such as poliovirus and MS2 (a male-specific RNA bacteriophage of *E. coli* or colipage). Information on the survival of human caliciviruses in faeces, sewage and other media are limited because these viruses infect only humans and no laboratory hosts, such as cell cultures or experimental animals are available.

The Norwalk group of viruses tends to infect older children and adults. The illness consists of an explosive episode of nausea, vomiting, diarrhoea and abdominal cramps, some times accompanied by headache, sore muscles and low-grade fever. Symptoms are usually last 12-48 hours for Norwalk virus and for 1-3 days for other human caliciviruses. The shedding of viruses declines after the onset of illness but can persist at declining levels for 1 to 2 weeks.

The Sapporo-like caliciviruses cause illness primarily in children and are endemic worldwide. Most children are infected with at least one calicivirus before leaving primary school. Outbreaks occur mainly in nursery schools and kindergartens, but also in day-care centres, orphanages, maternity hospitals and schools.

The Norwalk-like virus group is one of the major causes of gastroenteritis in adults worldwide. Approximately 40% of outbreaks of gastroenteritis in adults the USA have been attributed to this virus. Common-source outbreaks frequently occur via faecal contamination of water or food (e.g. shellfish and salads). Outbreaks frequently occur in camps, schools, nursing-homes and cruise ships. Because some caliciviruses of cattle and swine are genetically closely related to human caliciviruses, there is now suspicion that zooonotic transmission is possible and deserves consideration in elucidating the natural history of these viruses.

2.2.7 Rotavirus

Rotaviruses are members of the family *Reoviridae*. The family also includes reoviruses, which are commonly found in human stool, but are not associated with gastroenteritis or other illnesses. Rotaviruses are 60-80 nm in diameter, non-enveloped, contain 11 segments of double-stranded RNA viruses surrounded by a protein core and two capsid layers. This three-layered structure of virus proteins causes particles to have the typical appearance of a wheel with spikes. Rotavirus survives at 60° C for 30 minutes. The virus is

stable at acid pH (3.0 - 3.5) and can survive for months outside of the host at temperatures between 4 and 20°C.

Rotaviruses are the major cause of infantile acute diarrhoea in children (95% of children worldwide are infected by age 3 to 5). The disease is a major cause for childhood mortality in Africa, Asia and South America. The infection occurs usually in children between the ages of 6 months and 24 months, with the peak around 12 months. Vomiting generally precedes diarrhoea, which lasts for 4-5 days and can lead to severe dehydration. Asymptomatic infection is the rule in newborns and is quite common in older children and adults, although outbreaks in adult populations have been reported. The virus is shed in stool for as long as 10 days after the onset of symptoms. The levels of rotavirus shedding can be as high as 10^{11}-10^{12} virus particles per gram of stool.

Rotaviruses are major causes of disease worldwide. In temperate regions, infections are most frequent during the winter and early spring months, with high incidence in day-care settings. Rotaviruses are mainly transmitted by the faecal-oral route, although some authors have reported their presence in respiratory tract secretions and other body fluids. Because of their stability in the environment, transmission can occur through ingestion of contaminated water or food or contact with contaminated surfaces.

A rotavirus vaccine was released in the late 1990s, but serious complications of intestinal blockage were reported in enough immunized children that the vaccine was withdrawn from the market. Currently, rotavirus infection and illness must be prevented by adequate sanitation and hygiene and controlled in ill persons by adequate rehydration, supportive care and prevention of spread to other susceptible persons.

2.3 Occurrence in human faeces

Our literature search provided no data on the occurrence of pathogenic viruses in human faeces collected for composting. Only few data exist on the occurrence of pathogenic viruses in pit latrines. A study conducted in poor areas of Texas in 1953 reported the occurrence of both polioviruses (16 out of 220 samples) and coxsackieviruses (10 out of 63 samples) in pit latrines (43). More recently, an investigation on faecal material stored in pit latrines in Botwsana reported concentrations of enteroviruses from 0.3 to 1.5 PFU per gram, and rotaviruses from 90 to 727 virions per gram (44).

The concentration of enteric viruses in sewage may be indicative of their occurrence in human faeces collected for composting. Virus concentrations of 5.000 to 28.000 PFU per litre are commonly found in raw sewage (45). Recent studies have demonstrated that adenoviruses, enteroviruses, HAV and Norwalk virus are common in raw sewage, as they can be detected in samples of 100 ml at frequencies varying between 10 to 100% of samples tested (46,47).

Epidemiological data on the incidence of enteric viruses in the population may be indicative of the occurrence of enteric viruses in human faeces. According to the incidence of human infections (see section 2.2), the distribution of enteric viruses in human faeces should undergo seasonal variations. Adenovirus, astrovirus and rotavirus are more frequent during autumn, winter and early spring, whereas enteroviruses are more common in the summer.

Geographical differences should be also taken into consideration. The HEV virus is likely to occur less frequently in faeces and sewage collected in Europe. However, recent studies have reported HEV detection in sewage in Barcelona, Spain and Washington, DC, which are non-endemic areas. Therefore, the presence of this virus in human wastes is not limited to developing countries. However, the occurrence of HEV and other enteric viruses, such as enteroviruses, HAV and rotaviruses is likely to be higher in developing countries than in industrialized countries due to less coverage of water, sanitation and protection against childhood diarrhoeal diseases in developing countries.

For equal or similar incidences in the human population, viruses faecally excreted at higher numbers and for longer periods are likely to be predominant in faeces collected for composting. HAV is excreted at densities of up to 10^{10} viral particles per gram and the shedding occur for at least 30 days after the onset of the disease and as long as three months (48-51). Also rotaviruses are shed at even higher numbers (10^{10} to 10^{12} viral particles per gram), sometimes for periods up to one month (52-55). The Norwalk-like viruses have been estimated to occur at lower densities in faeces (typically 10^{4}-10^{6} viral particles per gram) (56) and the shedding lasts up to two weeks after infection (57,58). Enteroviruses are generally excreted at densities of 10^{6} infectious units (corresponding to about 10^{8}-10^{10} virus particles) per gram of faeces) and their excretion time is on average 7 weeks (23).

2.4 Occurrence in Denmark

In Denmark, only the incidence of HAV is notifiable. In the year 2000, 81 patients were notified with hepatits A, corresponding to an incidence of 1.5 cases per 100.000 inhabitants (59). According to preliminary data of the WHO Polio Eradication Certification Process, the occurrence of enteroviruses (Coxachie-, Echo, Polio- and Enterovirus 68-71) in faecal specimens from symptomatic patients was 15% in 1998, 13% in 1999 and 23% in 2000 (personal communication from Peter Henrik Andersen, Department of Epidemiology, Statens Serum Institut, Denmark).

The numbers of diagnosed rotavirus infections reported by the major laboratories in the country were 171 out of 2,770 patients tested in 2000 (6%), and 284 out of 10,222 patients tested in 2001 (3%) (personal communication from Francois-Xavier Hanon, Department of Epidemiology Research, Statens Serum Institute, Denmark). Caliciviruses (including the Norwalk virus) account for the vast majority of outbreaks of food-borne viruses and are estimated to be responsible for over 40% of total food-borne outbreaks in the country (60). The contribution of other enteric viruses to food-borne outbreaks can be estimated to be approximately 2-3% (personal communication from Francois-Xavier Hanon, Department of Epidemiology Research, Statens Serum Institute, Denmark).

In another Scandinavian country (Finland), rotavirus appears to be the most common diarrhoea-causing virus in young children. Rotavirus was estimated to be responsible for 54% cases among children less than five years old that were hospitalised for acute diarrhoea in the period between 1985 and 1995 (61). Caliciviruses and astroviruses were detected in 21% and 9% of episodes of acute gastroenteritis in children less than two years of age, respectively (62,63). Also in Sweden, rotavirus is the predominant virus among children affected by gastroenteritis, being recovered from 53% of children attending hospitals with symptoms of gastroenteritis (64).

In Denmark, the concentration of viruses in sewage has been estimated to be 10^3 to 10^5 PFU/100 ml for enteroviruses (including HAV) and 2 to 10^2 PFU/100 ml for rotaviruses (65). Similar or more likely higher concentrations can be expected to occur in human faeces destined to composting, since the concentration of enteric viruses in sewage is reduced as a consequence of dilution.

2.5 Response to physical-chemical factors

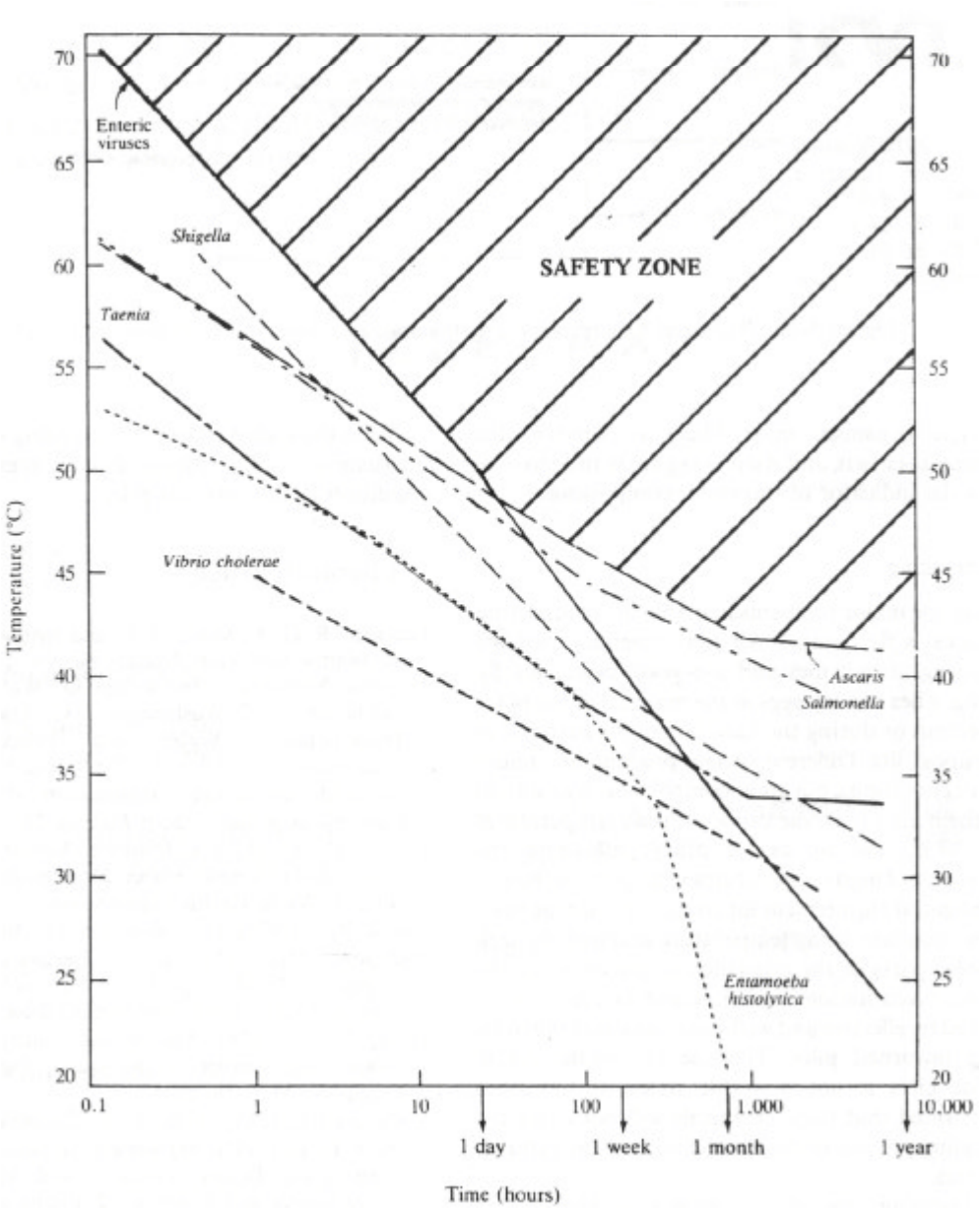
The knowledge of the response of different viruses to physical-chemical factors is essential to evaluate the survival of viruses during storage of faeces and the efficiency of composting in viral inactivation. In particular, the survival of viruses during storage and composting of faeces is closely related to their resistance properties to changes of temperature, pH and moisture occurring during the different phases of the composting process.

2.5.1 Temperature

The principal factor affecting viral survival is temperature. In general, viruses better tolerate low temperatures than high temperatures. Surface proteins are denatured within a short time at temperatures of 55-60°C, with the result that viral particles are no longer able to bind to the host cell (66). Enteroviruses seem to be more resistant than bacteria and parasites when exposed to heat for less than one day, whereas they are more susceptible than other microorganisms for prolonged exposure times (Fig. 2.1).

It is important to recognize that there are marked differences among different viruses in the temperatures and exposure times necessary for viral inactivation. Non-enveloped viruses, like all most important enteric viruses, are generally more heat-resistant than enveloped viruses (66). Among enteric viruses, the most heat-resistant appears to be the HAV, for which temperatures of 60°C for 30 min or 70°C for 10 min are not sufficient for complete inactivation (41,67). However, even HAV is inactivated by heat-treatment at 60°C for 10 hours (68).

Fig. 2.1. Effect of temperature and exposure time on enteroviruses, bacteria (*Salmonella* and *Vibrio choleae*) and parasites (*Taenia* and Ascaris). According to Feachem et al. (23).



The effects of temperature on virus survival have also been extensively studied with respect to the inactivation of viruses in animal slurries (69-77). Table 2.4 reports the data relative to animal viruses belonging to the same families of certain human pathogenic viruses. Also in this case, differences are evident between different types of viruses, with the porcine parvovirus being particularly heat-resistant compared with the other viruses tested.

Table 2.2. Inactivation times for animal viruses in slurry at various temperatures. According to Bøtner (71)

Virus	Family	5°C	20° C	35°C	40° C	45°C	50° C	55°C
Transmissible gastroenteritis virus	Coronaviridae	>8w	2w	24h	>5h	2h 30m	1h	30m
Foot and mouth disease virus	Picornaviridae	>14 w	2w	24h	10h	5 h	1 h	1h
Porcine parvovirus	Parvoviridae	>40 w	> 40w	21w	9w	> 19d	5 d	8d

* w, weeks; d, days; h, hours; m, minutes.

2.5.2 pH

Viruses are generally best preserved at physiologic pH, although some viruses tolerate a wide pH range. While most enveloped viruses are rapidly inactivated at pH 5-6, non-enveloped enteric viruses are able survive gastric acidity (pH=3)(65), or even lower pH levels (e.g. HAV is stable at pH 1 for 2 h). Hence, low pH levels (3 to 5) are not deleterious for enteric virus survival. Most enteric viruses also are relatively stable at moderately high than high pH levels, or at least up to pH 9.5. At pH 10 and higher, the rates of enteric virus inactivation vary among the different viruses. Some viruses are not stable to pH 10 and become inactivated by >99.99% within 1 day. Other enteric viruses are stable at pH 10 for periods of weeks to months. However, most enteric viruses are inactivated by 99.99% at pH 11 or higher within 1 day or less. The low stability of enteric viruses at high pH (pH >11) is used for viral inactivation in sewage sludge by lime treatment (37).

2.5.3 Moisture

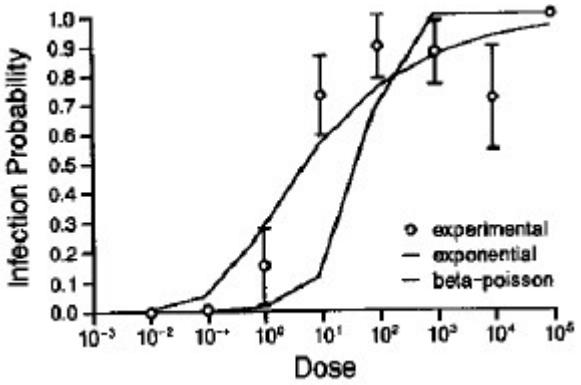
Enteric viruses are susceptible to dry conditions, but only if very low moisture levels are achieved (<5%). Dewatering of raw sludge was shown to effectively inactivate human polioviruses (78). Inactivation was due to disruption of the viral capsid with consequent release of nucleic acids. However, other studies have shown that viruses can persist in relatively dry soils for long time periods. Therefore, other factors in the suspending medium or matrix besides loss of water also may contribute to virus inactivation. Insufficient data are available for comparison of the survival of different types of viruses under dry conditions in different media, as only some virus groups have been studied in certain media. However, some enteric viruses, such as HAV, have been shown to persist for long periods of time under drying or dried conditions.

2.6 Human infectivity and dose-response

The risks associated with survival of pathogenic viruses in human composted faeces are dependent on their human infectivity. Infectivity of enteric viruses and other enteric pathogens can be described quantitatively as a dose-response relationship, which is the relationship between number of virus particles ingested and the probability of resulting infection and disease in humans. As shown in Figure 2.2, the risk or probability of infection increases as the ingested dose of rotavirus to human volunteers increases.

The models that best describe such dose-response relationships are the exponential model and the Beta-Poisson model. The exponential model assumes a random distribution of organisms in the doses to which humans are exposed and that each organism has an independent and identical probability of surviving to initiate infection. The Beta-Poisson assumes that the probability of the microbe surviving to initiate infection is not a constant value but instead varies and is described by a probability distribution, the Beta-Poisson distribution.

Fig. 2.2. Dose-response relationship for infection by a human rotavirus in human volunteers with fitted curves for exponential and Beta-Poisson models.



Most of the available data on the dose-response relationships of enteric virus infectivity for humans have been obtained from human volunteer studies. Only some enteric viruses have been studied because of the difficulties of conducting such human infection studies. However, the available data clearly indicate that most enteric viruses have a high probability of causing infection at relatively low virus doses.

The doses at which viruses have a high probability of causing infection are markedly lower than those for bacteria. For some enteric viruses, such as rotavirus and Norwalk virus, only a few viral particles need to be ingested to cause a high probability of producing disease in humans (Table 2.3). The 50% infective doses of viruses (dose at which the probability of infection is 50%) may vary between 1 and 12,000 viral particles, depending on the virus type and the state of the human exposed to the virus.

Based on dose-response relationships for infection and illness, survival of low numbers of pathogenic viruses (and parasites) in human composted faeces appears to pose a greater hazard in comparison with bacterial pathogens. However, it should be considered that, in contrast to viruses, bacteria can multiply outside of the host and their numbers can therefore increase during storage of faeces and after application of composted faeces to soil.

Table 2.3 Doses of enteric microbes infecting 50% of exposed humans (ID_{50} based on excretion). Adapted from Teunis et al (79,80).

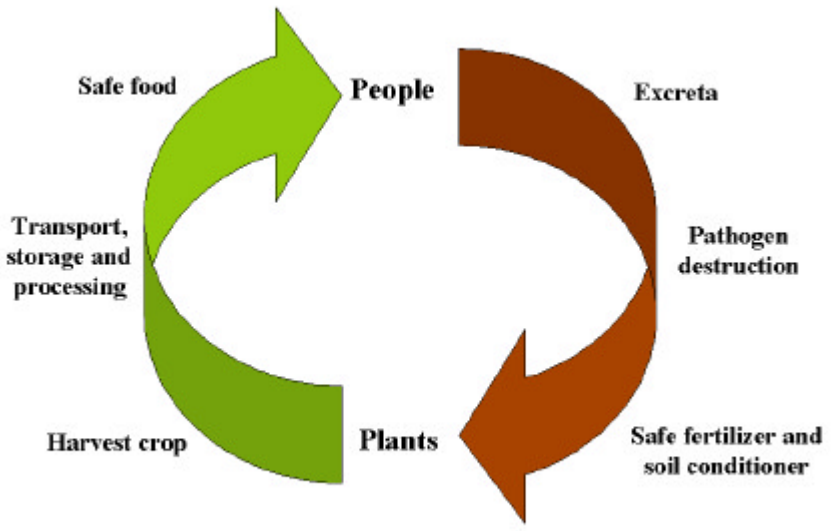
Infectious Agent	Approximated ID_{50}	Comments
Rotavirus	6.1	Ingested in buffered water
Echovirus 12	1000	
Poliovirus 1 sm	1.4	Attenuated vaccine strain
Poliovirus 1 LSc2ab	70,000	"
Poliovirus 1	76	"
Poliovirus 3 Fox	5.5	Attenuated; infants
Poliovirus 3 Fox	5.0	Atenuated; premature infants
Campylobacter jejuni	900	
Salmonella anatum	55,400	
Salmonella typhi	858,000	
Shigella flexneri	35,900	
Shigella dysenteriae	748	
Giardia lamblia	35	
Cryptosporidium parvum	173	

3 Virus survival in composted human faeces

3.1 Introduction

In Denmark, there is an increased interest in using human faeces and urine for agricultural and other purposes. This interest is seen especially when new urban areas and houses are established. Traditional sewerage-based sanitation systems can cause environmental pollution and often do not utilize the nutrients contained in human excreta. An alternative to these systems is the direct recycling of human excreta using treatment systems designed to regard and employ human excreta as a resource to be used rather than as a waste to be disposed. By such systems, the nutrients contained in urine and faeces are returned to soils and plants, thereby contributing to a circular flow of nutrients (Fig. 3.1).

Fig. 3.1. The circular flow of nutrients associated with recycling of human excreta.



An essential prerequisite for safe reuse of human faeces is the removal or destruction of pathogens by composting or other sanitation methods. This chapter describes how and to what extent viruses are inactivated during storage, production and utilization of composted faeces. The mechanisms and the efficiency of viral inactivation by composting are reviewed and evaluated on the basis of the existing literature.

3.2 Virus survival during storage of faeces

Virus survival in composted faeces depends not only on the conditions used for composting, but also on the storage conditions. Storage should therefore be viewed as part of the composting process. Faeces are collected by toilets separating faeces from urine (i.e. urine-diverting toilets) and stored into a

vault beneath the toilet seat. During storage there will normally be no or very little composting effect because of anaerobic conditions and limited temperature increase. Other human waste management systems collect faeces and urine from homes either manually or by automated systems (such as vacuum tankers) and then treat it in bulk by composting or other processes (81).

Viruses decrease in numbers during storage of faeces, due to their incapability to replicate outside of the host and susceptibility to a number of adverse environmental conditions. However, the die-off rate of viruses present in human faeces may show large variations depending on both the type of virus (see section 2.4) and the storage conditions. High pH, low moisture, microbial activity, free ammonia and high temperature are among the most unfavourable conditions for virus survival during storage of faeces.

toilet temperatures = ambient temps

The temperature of faeces stored in latrines generally does not significantly differ from ambient temperature (82). Thus, the role played by temperature in the inactivation of viruses during storage of faeces appears mainly dependent on climate. Studies on sewage applied to soil indicate that viruses can persist for 23 weeks during the winter season in Denmark (83) but for only 2-4 weeks during the summer or fall in Florida (84). Similarly, the survival of animal pathogenic viruses in faeces has been demonstrated to be longer under winter conditions rather than summer conditions (85).

Recent studies have demonstrated that some animal enteric viruses, including enteroviruses, can persist in faeces for a longer time, especially at low ambient temperatures. The inactivation of a bovine enterovirus in liquid cattle manure stored at 20°C was found to be only 2 $\log_{10}$ after 26 weeks (86). The porcine rotavirus was demonstrated to maintain its infectivity after 32 months of storage at 10°C in original stool specimens (87).

viruses survive long periods in low temperature faeces

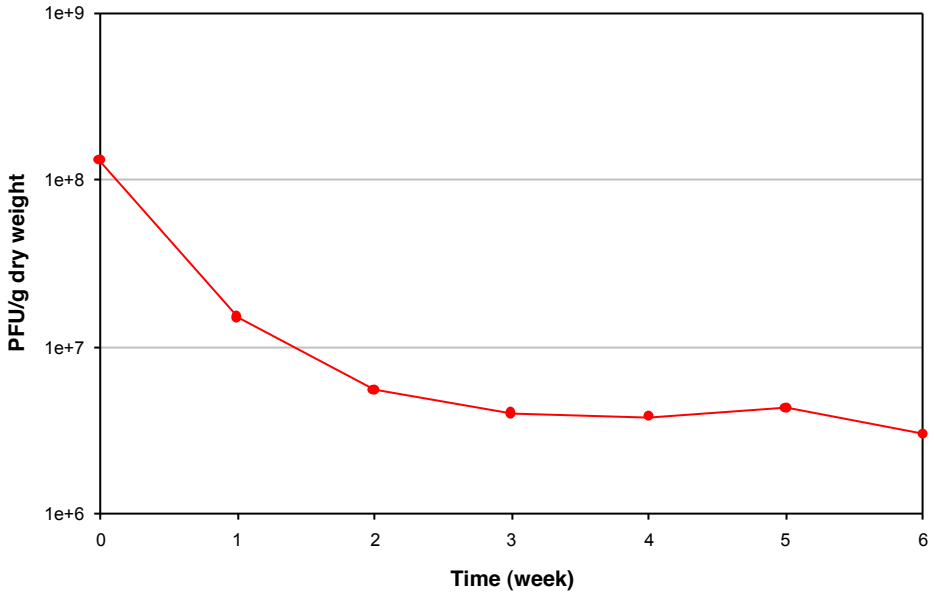
Among human pathogenic viruses occurring in faeces, HAV is a relatively heat-resistant virus and is inactivated more slowly than enteroviruses in a variety of media, including faeces. At lower temperatures, HAV, like other enteric viruses, is very persistent in faeces and other media. For example, HAV is inactivated very slowly in human faeces and manure kept at 5°C, with an observed reduction of only 1-2 log units after 70 days of storage in manure (88) and about 625-1250 days in faeces (89). At 25°C, HAV is relatively persistent in stored faeces (about 139 days for 4 $\log_{10}$ inactivation) when compared to poliovirus and male-specific (F+) coliphages (about 83-84 day). At 40°C, times for 4 $\log_{10}$ inactivation in faeces are about 20-21 days for poliovirus and F+ coliphages, and 29 days for HAV (89).

A number of studies have investigated the survival of viruses in latrines, including urine-diverting vault latrines intended for recovery and potential reuse of both urine and faecal solids. High temperature and high pH levels are the most important physico-chemical factors affecting virus survival (see section 2.5). However, in many latrines, neither high temperature nor high pH levels are achieved. Therefore, extensive enteric microbe reductions are not achieved unless storage times are very long.

The bacteriophage *Salmonella typhimurium* 28B was used as an indicator to study the behaviour of human enteric viruses during storage of faeces collected by urine-diverting toilets (82). The die-off of the phage was slow, with a reduction from 10^8 to 10^6 during 6 weeks of storage at temperatures

between 20 and 35°C (Fig. 3.2). The long survival of the phage in faeces was attributed to the neutral pH conditions (7.8-8.5). Under these pH conditions, the loss of moisture observed during the study (dry weight increased from 85.2 to 96.5%) was not great enough to affect virus survival. On the contrary, it could have reduced viral inactivation by slowing down the biological process of degradation (82).

Fig. 3.2. Mean die-off rate of the bacteriophage *S. typhimurium* 28B during storage of human faeces in urine-diverting toilets. Adapted from Franzen and Scott (82).



A similar study conducted in Vietnam suggested that the combination of high pH and low moisture have a high impact on virus survival during storage of faeces in urine-diverting latrines (90). After 3 weeks of storage, the bacteriophage *S. typhimurium* 28B was reduced 8 log units in faeces with high pH (10.0 to 10.3) and low moisture content (24 to 29%). In contrast, the bacteriophage persisted for 7 weeks in faeces with lower pH (8.5 to 9.4) and higher moisture content (27 to 55%), with a reduction of 2-3 log units only. The high pH of faeces reported in this study was due to the addition of ash originating from wood and leaves (90).

According to a summary report of various studies, Stenstrom (91) stated that enteric microbe reduction in the material of dry latrines was mainly governed by time and high pH. Complete inactivation of indicator viruses was achieved in 6 months or less in association with the use of ash, lime or similar additives for pH elevation. Addition of only moisture-absorbing materials was shown not to ensure efficient microbe reduction.

Austin (92) studied enteric microbe reductions in wood ash-supplemented faecal matter of urine diversion toilets in Eastern Cape province, South Africa. After 10 months of storage in a separate plastic container in the latrine, faeces contained $\log_{10}$ concentrations of 0-3 coliphages per gram. In additional studies, it was shown that microbial reductions were more rapid and extensive in faeces stored on the concrete floor of the latrine vault and turned weekly for aeration than in faeces stored in a closed plastic container. Coliphages were not detected after 2 months of storage at mild to cold conditions (moisture 4-8 % and pH 8.4-8.6) and when latrine faeces were subjected to a temperature

of 50°C for 48 hours, while keeping the moisture content approximately the same (92).

Moe and colleagues (93) studied the performance and stored fecal waste quality of urine-diverting double vault latrines and solar toilets in seven communities in El Salvador. Latrine wastes ranged widely in microbial quality, probably due to high variability in the storage conditions (pH ranged from 5.1 to 12.8 and the percentage of solids ranged from 2 to 98%). Somatic coliphage concentrations varied widely and were as high as 8 $\log_{10}$ per gram. Temperatures of stored latrine material were 20-37.5°C (mean of 27°C), indicating that these toilets are not "true" composting systems because they do not achieve the high internal temperatures (>50°C) typical of aerobic composting. No single physical factor (pH, temperature, moisture content, or storage time) could predict microbial indicator concentration, suggesting that microbial quality is the net result of the effects of a multiple factors (93).

Chien et al. (94) studied the survival *S. typhimurium* phage in various designs of urine-diverting, double-vault latrines in Viet Nam. Phage survival was 23-154 days, with temperatures of 30-40°C, moisture contents of 25.4-58.8% and pH of 8.4-10.3. In general, microbial survival was most influenced by pH. Both pH and moisture content influenced bacteriophage when temperature was below 40°C. To achieve acceptably low levels of microbial risk, 6 months of retention for faecal materials were needed in test toilets (94).

The available data on viral persistence in stored faecal material suggest that in temperate countries the impact of temperature on virus survival is limited to the summer months. At temperatures below 20°C, high pH values (>9.0) in combination with low moisture contents are the most important virucidal factors. Accordingly, wood ash and other substances increasing the pH levels (e.g. lime) can be used to reduce the content of viral pathogens in faeces collected for composting. Soil may be added to faeces for reducing their moisture content, although this practice does not seem to be particularly effective in viral inactivation (82).

Aeration is another factor enhancing viral inactivation during storage of faeces. According to a study on faeces from urine-diverting toilets (91), the die-off of viruses is higher if faeces are collected and stored in heaps and turned weekly, rather than in closed compartments. Similar evidence was provided by a study on animal waste (85), in which a large variety of viruses (i.e. coliphage f2, picorna-, rota-, parvo-, adeno- and herpesvirus) were shown to persist for longer periods under non-aerated conditions. Consequently, the effect of aeration conditions on viral inactivation may have important implications in the design and construction of urine-diverting toilets.

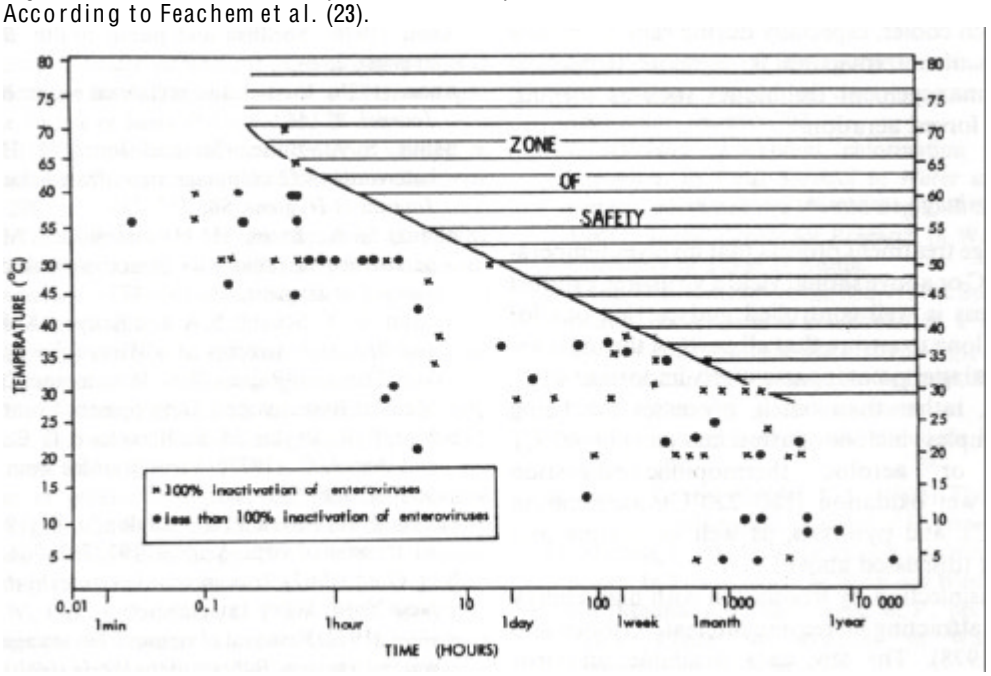
3.3 Factors affecting viral survival during composting

The inactivation of viruses during composting is determined by a combination of chemical, physical and biological factors. The most important cause of viral inactivation is the heat generated during the thermophilic phase of composting. To a lesser extent, viruses are also inactivated by microbial degradation and ammonia. It should be noted that these mechanisms operate at the same time during composting. Thus, viral inactivation should be attributed to a synergistic interaction between them, rather than to each mechanism taken individually.

Environmental factors such as moisture content and pH play a secondary role in viral inactivation during composting. The optimal conditions for composting are given by pH levels of 5.5 to 8.0 and moisture contents of 40 to 60% (8,9). These conditions are not harmful for enteric viruses by themselves (see section 2.5). Accordingly, pH and moisture can affect virus survival only by influencing the metabolic activity of bacteria and fungi and the production of ammonia during composting.

The temperature increase seen during the first phase of an optimal compost process exceeds the temperature levels needed for viral inactivation. Feachem et al. (23) studied the effects of heat and exposure time on enterovirus survival with respect to composting of sewage sludge. According to these authors, exposure at 30°C for 3 months, at 40°C for 2 weeks, at 50°C for 1 day or at 60°C for 2 hours are sufficient to assure complete inactivation of enteroviruses, adenoviruses and reoviruses. The zone of safety described in Fig. 3.3 indicates the possible combinations of temperature and time necessary for complete elimination of enteroviruses in composted sludge.

Fig. 3.3. The influence of temperature and exposure time on survival of enteroviruses. According to Feachem et al. (23).



Due to their remarkable resistance to heat (see section 2.5.1), some enteric viruses (e.g. HAV and parvoviruses) are likely to represent an exception to the safety zone described by Feachem et al. (23). A recent study demonstrated that the bacteriophage *S. typhimurium* 28B is only reduced 1-2 log units after composting at 55°C for 24 hours (95). The inactivation of certain animal enteric viruses (i.e. parvovirus) by composting requires up to 8 days at 55°C (70). However, heat-resistant viruses are appreciably inactivated when compost facilities are operated at times and temperatures specified in accordance with current legislation (Table 3.1).

Problems may occur in the control of temperature during composting. Strauch et al. (96) reported that it is nearly impossible in daily practical operations to constantly maintain the necessary parameters and control them. The authors proposed that composting of sewage sludge should be operated as a two-stage system, with a minimum hydraulic retention time in the system of 5 days and minimal retention time of one day in each reactor. The recommended temperatures and exposure times were either 48 hours at more

than 50°C or 24 hours at 58°C. These temperature and time requirements are largely fulfilled by the current legislation on composting in different countries (Table 3.1).

Among the different systems of composting, enclosed systems ensure the best control of temperature, with horizontal reactors being preferable to vertical reactors (97). Static aerated piles guarantee a better temperature control and therefore also a more efficient pathogen removal compared with windrow systems. The control of temperature is particularly critical in the windrow system because the temperature in the outer part of the windrow is influenced by ambient temperature. This is also true for static compost piles. Thus, the techniques used for turning must ensure that also the outer material is transported into the interior of the windrow and exposed to the temperature and time necessary for inactivation of viruses and other pathogenic organisms.

Table 3.1. Minimum temperature and time requirements for composting in various countries. According to Stentiford (9) and Strauch (28).

Country	Minimum time/temperature requirements
Austria	65°C for 6 days
Belgium	60°C for 4 days
Denmark (in-vessel techniques)[1]	70°C for 1 hour
Denmark (open systems)[2]	55°C for 2 weeks
France	60°C for 6 days
Germany (open windrow)	55°C for 2 weeks or 65°C for at least 1 week
Germany (encased windrow or in-vessel techniques)	60°C for 1 week
Italy	65°C for 2-3 days
Netherlands	55°C for at least 2 days
Switzerland	55°C for at least 3 weeks or > 60°C for at least 1 week
USA (static pile or in-vessel techniques)[3]	55°C for at least 3 days
USA (windrows) [3]	55°C for at least 15 days with at least 5 turnings

[1] Composting of sewage sludge for agricultural use.
[2] Composting of organic waste other than sludge.
[3] Relative to processes to further reduce pathogens (PFRP)(see section 1.7.3).

3.3.1 Microbial degradation and enzymatic activity

Microbial breakdown is likely to play an important role in the destruction of viruses during both storage and composting of faeces. The viral capsid is composed of proteins and therefore susceptible to the action of proteolytic enzymes released by microorganisms during the biodegradation process. Unfortunately, there is a lack of data on susceptibility of viral proteins to the conditions prevailing in compost heaps. However, various studies indicate the importance of bacteria and protozoa in virus removal.

Many bacteria produce proteolytic enzymes inactivating enteric viruses, including certain bacterial species that are prevalent in the mesophilic flora during maturation of compost, for example *B. subtilis* (98). Laboratory experiments have demonstrated that cell-free filtrates of bacterial cultures have an antiviral activity and that such an activity is inhibited by protease inhibitors, indicating that enzymes present in the filtrates are responsible for viral inactivation (98-100). It also appears that viruses may serve as a nutrient source for bacteria, as indicated by the recovery in bacterial cells of labelled viral capsid proteins (98).

In a field experiment, Poliovirus 1 was inactivated more rapidly in mixed waste than in autoclaved mixed waste and bacterium-free filtrate of raw mixed

waste (99). These results suggested a role of microbial activity in viral inactivation. The slower rates of virus inactivation in autoclaved waste could be due to disruption of antiviral bacteria and their products as well as destruction of other heat-labile constituents. The intermediate inactivation of viruses in bacteria-free filtrates of raw waste (compared to raw waste or autoclaved waste) could be due to the absence of antiviral bacteria but the continued presence of heat-labile antiviral chemicals, as well as the destruction by autoclaving of heat-labile chemicals that are protective of viruses.

In a subsequent study on the persistence of HAV in mixtures of septic tank effluent (STE) plus dairy cattle manure slurry, and in mixtures of STE plus swine manure slurry (100), HAV was consistently inactivated more rapidly in the two types of mixed wastes than in STE alone or in a PBS control. Bacterial strains showing antiviral activity on HAV were isolated from manure. In most cases, viral inactivation was associated with bacterial production of proteolytic enzymes. However, the inactivation of HAV by some bacterial strains was not affected by protease inhibitors, suggesting that mechanisms other than enzymatic degradation could be involved in viral inactivation by bacteria. This was in accordance with a previous work documenting the antiviral activity of non proteolytic-substances produced by bacteria (98).

Bacteria are not the only microorganisms able to affect virus survival. Predation by protozoa is a mechanism of viral removal in activated sludge (101). However, protozoa cannot affect the survival of viruses during composting, as these organisms are killed at the high temperatures typically reached during the composting process. In urine-diverting vaulted latrines employing composting or desiccation processes, however, temperatures may not reach those attained during typical bulk composting treatment of other wastes as in static piles, windrows or mixed vessels. Under these lower temperature conditions, other microbes such as parasites and fungi could contribute to virus inactivation and biodegradation.

3.3.2 Ammonia

Destruction or loss of infectivity of viruses during composting is also due to the virucidal activity of ammonia originating from protein degradation. Various authors have demonstrated that molecular ammonia has a virucidal effect on enteric viruses (102-104). This virucidal effect of ammonia is pH-dependent, with the anti-viral activity demonstrated primarily at higher pH levels of 8.5 and above. This is because the activity is mediated by free ammonia and not ammonium ion. The high temperatures reached during the composting process appear to be conducive for viral inactivation by this mechanism, since they enhance both production and virucidal activity of ammonia (105).

3.3.3 Moisture content

Virus inactivation is influenced by moisture content, especially if the moisture content gets very low. Typically, composting is done at moisture content levels at which viruses are stable. It is only when compost or faecal wastes are dried or cured to low moisture levels that virus inactivation is induced. Some virus inactivation is achieved when moisture content declines to about 15%, but generally, rapid and extensive virus inactivation is not likely to be achieved until the moisture content of the material is at or below 10% (106-108).

3.3.4 pH

Virus inactivation is influenced by pH. Most viruses are stable in the pH range of 5 to 9. The extent to which viruses are inactivated above pH 9 and below pH 5 varies with the virus. Most non-enveloped enteric viruses are stable at pH levels as low as 3 and as high as 10 to 10.5. Composting is done at pH levels where viruses are stable, but some latrine wastes are supplemented with lime, wood ash or other alkaline material that may raise the pH to 10 to higher. Under these conditions viruses are likely to be inactivated due to the structural changes associated with exposure to high pH levels (109). However, at moderately alkaline pH levels of 8.5 to 9.5, viruses are also inactivated by the presence of free ammonia (see section 3.3.3). Therefore, there can be synergistic effects of moderately alkaline pH and free ammonia in compost and other faecal wastes.

3.4 Efficiency of composting in viral inactivation

Few field or laboratory studies are available in the literature on the efficiency of composting for viral inactivation in human faeces. The limited data on the inactivation of bacteriophages in latrine wastes have been described in previous sections of this report. The Danish Environmental Protection Agency has given financial support to projects assessing the survival of different pathogens, including bacteriophages, in composted human faeces. The results from these studies should be available in year 2003.

Due to the absence of data on the fate of human enteric viruses during composting of human faeces, the survival of viruses during composting was reviewed on the basis of studies on composting of faecally contaminated sources, such as sewage sludge, liquid organic waste and domestic solid waste containing disposable diapers. Studies on virus survival during composting of manure or animal carcasses are also presented, because the survival of animal viruses during composting can be expected to be similar to that of taxonomically related human viruses.

3.4.1 Composting of sewage sludge

The efficiency of composting of sewage sludge for viral inactivation has been demonstrated by several laboratory studies and field studies at compost facilities. Early studies on virus inactivation by composting determined the effects of the process on male-specific coliphage f2 as a model virus (110-112). In both static pile and windrow composting of municipal wastewater sludge, the titer of coliphage f2 decreased over time. The rate of decrease depended on the type of compost system and the season of the year, with more rapid inactivation in warmer seasons. Reductions of 1 log10 were observed in 4 to 7 days in windrows during dry weather. Rainy weather reduced the inactivation rates by about 50%. Reductions were more rapid in aerated piles than in windrows, probably because higher temperatures were achieved.

Kawata et al. (112) demonstrated that inactivation of seeded coliphage f2 (initial concentration of 10^6 PFU/g) by a windrow composting system required about 50 days for raw sludge and up to 70 days for digested sludge. A similar study by Burge et al. (111) showed that coliphage destruction by aerated static piles was achieved deep in the pile within 21 h, although very small concentrations of the phage (about 0.001%) survived at the edge of the pile. Also in the 1960s, two studies reported that seeded poliovirus type 1 was

eliminated in sludge composted at 38-58°C for 7 days (113) and at 60-76°C for 1 hour (114).

More recent studies have investigated the inactivation of bacteriophages and enteric viruses naturally occurring in raw sludge. Langeland et al. (115) reported that coliphages M2 in raw sludge (approximately 10^3 to 10^4 PFU/g) were completely inactivated after 7 days of composting at 60°C. Carrington et al. (116) documented that enteroviruses in raw sludge (mean concentration of 25.4 PFU/g) were reduced to below the detection limit (0.1 PFU/g) after 28 days of composting at 55°C.

3.4.2 Composting of liquid organic waste

Composting can also be used for sanitation of liquid organic waste. A composting system for different kinds of liquid biodegradable waste was recently tested in a pilot scale (95). The bacteriophage *Salmonella typhimurium* 28B was used as an indicator to evaluate the efficiency of the composting system for viral inactivation in blackwater (faeces, urine, toilet paper and water for flushing) mixed with kitchen organic waste. The results indicated that the die-off was lower at 55°C compared with 60°C. The time required for the inactivation of 90% phages (T_{90}) was calculated to be 14.7 hours at 55°C and 3.5 hours at 60°C.

The authors observed that addition of liquid manure into the waste material reduced the die-off of the bacteriophage (T_{90} was 23.4 hours at 55°C and 13.7 hours at 60°C) (95). The reason of the slower die-off rate in waste containing manure was not investigated. However, the results indicate that the composition of the raw material may influence substantially the die-off of viruses during composting.

3.4.3 Composting of animal faeces

Laboratory studies have shown that composting rapidly inactivate viruses in animal faeces. Monteith and Shannon (117) studied the inactivation of enteric viruses by composting of cattle faeces. Two representatives of the most heat-resistant bovine viruses, i.e. parvovirus and enterovirus, were seeded into the solid fraction of cattle manure at concentrations comparable with the levels found in manure applied in the field (approximately 10^5 PFU/g). The operating temperatures reported in this study were 30°C on day 1, 45°C on day 2 and 60°C for the rest of the experiment. Results showed that neither the parvovirus nor the enterovirus survived composting for 28 days. Thus, composting appeared to be a suitable method for the disinfection of manure for use as a soil conditioner.

In another study, Hirotani et al. (118) used porcine faeces as a model to study the fate of viruses during composting. Porcine faeces containing 10^6 PFU/g of coliphages were kept at 60°C under aerobic conditions. Samples were periodically mixed to maintain aerobic conditions and supplied with water to avoid dryness. No plaques were detected after 5 days, suggesting that more than 99.99% of the coliphages were destroyed under these conditions.

3.4.4 Composting of domestic solid waste

The occurrence of human enteric viruses in domestic solid waste is mainly associated with the presence of faecally soiled diapers, which have been found

to contain average concentrations of 146 PFU of polioviruses type 3 in every gram of faeces (119). Gerba et al. (120) evaluated the effects of composting domestic solid waste containing several faecally contaminated diapers. No viruses were detected in compost treated by in-vessel composting for 100 to 200 days, with temperatures ranging between 57 and 70°C. Furthermore, the lack of enteroviral detection by DNA hybridization suggested that no intact viral DNA was present in the final compost product.

An investigation performed at a Danish composting plant showed that numbers of coliphages were reduced below the detection limits (2 PFU/100 g) during composting of household waste in open-air windrows (121). The raw material was found to contain an average of 5×10^3 PFU of coliphages every 100 g.

3.4.5 Composting of poultry carcasses

The use of composting as an alternative to burial, burning and rendering for disposal of poultry carcasses was recently studied (122). Despite differences in temperature between lower and upper levels of carcasses, two-stage composting (2 cycles of 7 days separated by turning) was found to destroy various avian pathogenic viruses in infected carcasses. The peak temperatures reached during composting were 58.3°C in the upper level and 42.8°C in the lower level (122).

3.5 Virus persistence in soil

Enteric viruses can persist long after application of contaminated sludge to soil, as indicated by their recovery in sludge burials 6 months after the last sludge disposal (123). The persistence of certain viral pathogens in soil represents a potential risk for human health. Viruses may be transported for long distances through soil aquifers, with the consequent possible contamination of groundwater reservoirs (124-126).

High temperature and dry conditions are the two main factors affecting the persistence of viruses in soil (127). While long virus survival times (over 5 months) are possible under cool conditions, at warmer temperatures (<25°C) viruses are likely to be eliminated within 2 weeks (23). Under constant moisture conditions, the die-off of enteric viruses are significantly higher at 27°C than at 15°C (128) and at 23°C than at 10°C (129). Evaporation to less than 5% soil moisture content completely inactivate viruses within 7 days at 15°C and within 3 days at 27°C (128). Soil moisture contents below 2.9% have been shown to have a particularly strong virucidal effect (107)

Other factors affecting virus persistence in soil include soil type. This is because soil type influences the extent of virus adsorption to the soil particles, with clay particles being more adsorptive of viruses than sand, silt or organic particles. Virus survival is enhanced by clay soils compared with sandy and organic muck soils, apparently due to greater adsorption and its protective effect (128,130).Virus type also influences virus survival in soils. The die-off of HAV is lower than for enteroviruses (129,130), and even enteroviruses differ widely in their persistence in soils and sediments. Furthermore, it appears that soil microorganisms can produce antiviral substances increasing the rate of viral inactivation in soil, as suggested by the increased survival of viruses in sterile soils (131,132).

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