# Treatment Technologies for Human Faeces and Urine

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## Abstract

This thesis examines simple, cheap, environmentally friendly and resource efficient technologies for the treatment of source-separated human faeces and urine to enable safe recycling of their plant nutrients for plant production in developing countries.

Composting of faeces-to-food waste (F:FW) in wet weight mix ratios of 1:0, 3:1 and 1:1 was studied in 78 L reactors insulated by 25 mm styrofoam; and of F:FW in wet volume/weight ratios of 1:0, 1:1 and 1:3 in 216 L reactors insulated by 75 mm styrofoam. At both scales, composting without insulation produced temperatures that differed from the ambient by ≤15 °C. A sanitised compost product was produced when the temperature was maintained above sanitising levels (>50 °C) for a sufficiently long time (at least 2 weeks). High moisture levels (>60%) led to low pH (<6), which impeded composting and the attainment of sanitising temperatures.

Incineration of well prepared source-separated faeces with ash as cover material produced high temperatures (800-1000 °C). This process decreased the organic matter, total N and plant-available P by 70->90%. Mass decrease was 15-36% due to high ash content of the incoming material. Incinerating faeces/ash mixtures with DM<90% resulted in a strong smell that lessened when DM was higher. The ash produced by incineration can be used as cover material for faeces during toilet use, which is advantageous in urban areas of developing countries where access to ash is limited.

In urine treatment, a breakpoint concentration of ammonia was found at approximately 40 mM $NH_3$ (*e.g.* 2.1 g $NH_3$-N $L^{-1}$ and pH 8.9 at 24 °C), below which all studied organisms, except *Salmonella* Typhimurium (*S.* Typhimurium), persisted considerably longer irrespective of treatment temperature, showing that urine dilution rate is highly important for pathogen inactivation.

The time to no detection in urine stored in the sun (Uganda; mean temperature±amplitude 24±7.5 °C, $NH_4$-N of 4±1.5 mg $L^{-1}$ and pH 9) for *E. coli*, *Salmonella* and *Ascaris suum* was 11 hours, 14 hours and 40 days respectively. Under similar conditions, *Enterococcus* spp. reached non-detection levels in 50 days, while the phages studied persisted considerably longer. The $t_{90}$ for MS2, Φx 174 and *S.* Typhimurium 28B was 8.2, 37 and 55 days respectively. Fluctuating temperatures in combination with ammonia were shown to inactivate pathogens in urine faster than the same average steady temperature.



*Keywords:* composting, faeces, sanitation, treatment, urine, pathogens, temperature.



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# Dedication

I dedicate this thesis to my father, Mr. Lawrence Buregyeya, for struggling to educate me.

*Det är skönare lyss till en sträng, som brast, än att aldrig spänna en båge.*
Verner von Heidenstam

# Contents

# List of Publications

This thesis is based on the following papers, referred to by Roman numerals in the text:

I Niwagaba, C., Nalubega, M., Vinnerås, B., Sundberg, C. & Jönsson, H. (2009). Bench-scale composting of source-separated human faeces for sanitation. *Waste Management* 29(2), 585-589.

II Niwagaba, C., Nalubega, M., Vinnerås, B., Sundberg, C. & Jönsson, H. (2009). Substrate composition and moisture in composting source-separated human faeces and food waste. *Environmental Technology* 30(5), 487-497.

III Niwagaba, C., Nalubega, M., Vinnerås, B. & Jönsson, H. (2006). Incineration of faecal matter for treatment and sanitation. *Water Practice and Technology* 1(2). doi10.2166/wpt.2006.0042.

IV Vinnerås, B., Nordin, A., Niwagaba, C. & Nyberg, K. (2008). Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. *Water Research* 42(15), 4067-4074.

V Nordin, A., Niwagaba, C., Jönsson, H. & Vinnerås, B. (2009). Inactivation of indicators and pathogens in source-separated human urine at varying temperatures. (Manuscript).

In Paper I Niwagaba and Jönsson planned the study, Niwagaba performed the measurements, analysed the data and did the writing, with revisions by Vinnerås, Nalubega, Sundberg and Jönsson.

In Paper II, Niwagaba, Vinnerås and Jönsson planned the investigation and Niwagaba performed the experiments, data analysis and the writing, with revisions by Vinnerås, Nalubega, Sundberg and Jönsson.

In Paper III, Jönsson and Niwagaba planned the study, Niwagaba performed the measurements, Jönsson and Niwagaba performed the calculations and Niwagaba did the writing, with revisions by Vinnerås, Nalubega and Jönsson.

In Paper IV, Vinnerås and Nordin planned the study, Niwagaba, Nordin and Vinnerås performed the measurements and Vinnerås and Nordin did the writing, with revisions by Niwagaba and Nyberg.

In Paper V, Niwagaba, Nordin, Jönsson and Vinnerås planned the study and Niwagaba and Nordin performed the measurements, data analysis and the writing, with revisions by Jönsson and Vinnerås.

# Abbreviations

| | |
|---|---|
| DM | Dry matter |
| DWD | Directorate of Water Development |
| E. coli | Escherichia coli |
| Ecosan | Ecological Sanitation |
| EcoSanRes | Ecological Sanitation Research Program, Sweden |
| IYS | International Year of Sanitation |
| JSR | Joint Sector Review |
| K | Potassium |
| KCC | Kampala City Council |
| MC | Moisture content |
| MDGs | Millennium Development Goals |
| N | Nitrogen |
| NGOs | Non-Governmental Organisations |
| OM | Organic matter |
| P | Phosphorus |
| PRB | Population Reference Bureau |
| Sida | Swedish International Development Cooperation Agency |
| SWTWS | South Western Towns Water and Sanitation |
| TS | Total solids |
| UBOS | Uganda Bureau of Statistics |
| UDDT | Urine Diversion Dry Toilet |
| UGShs | Uganda Shillings |
| UN | United Nations |
| VS | Volatile solids |
| WHO | World Health Organisation |
| WSSCC | Water Supply and Sanitation Collaborative Council |

# 1 Introduction

The importance of improved sanitation in safeguarding the health and well-being of human kind is well documented (WHO, 2001; Cairncross, 2003; WHO, 2004a; Moe and Rheingans, 2006). The British Medical Journal (2007) reported that according to a survey of 11,000 global respondents, sanitation engineering represented a health breakthrough greater than the discoveries of antibiotics, anaesthesia, vaccines and DNA, and public sanitation was the greatest 'medical breakthrough' since 1840, giving sanitation recognition in saving human lives and reducing poverty. According to Vision 21, sanitation is a basic human right, and one of the major components of poverty eradication (WSSCC, 2000).

Globally, at least 2.6 billion people lack access to basic sanitation (WHO, 2004a) and more than 90% of the sewage in developing countries is discharged untreated (Esrey, 2001; Langergraber and Muellegger, 2005). Millennium Development Goal (MDG) 7 aims at halving the proportion of the world's population without safe drinking water and basic sanitation between 1990 and 2015 (UN, 2002). Recognising the risk that the MDG on sanitation may not be achieved and the fact that sanitation affects other MDGs directly and indirectly, the UN declared 2008 to be the International Year of Sanitation (IYS), in order to raise sanitation awareness amongst UN and other donor agencies, governments and civil society.

The lack of access to improved sanitation potentially contributes to environmental pollution together with its consequences to society. In situations where sanitation is lacking, human excreta may accumulate around homes, in nearby drains and in garbage dumps, leading to environmental pollution (Kulabako *et al.*, 2007). Where conventional sanitation systems are in use, insufficiently treated excreta from latrines and wastewater systems often end up in deep pits and recipient waters. Wastewater effluents contain large amounts of plant nutrients from excreta. Plant nutrients in wastewater

effluents are undesirable because of their potential to cause eutrophication in recipient surface waters, while in deep pits, there is the risk of nutrients, especially nitrogen, leaching to the groundwater. Pathogens from pit latrines directly pollute shallow groundwater, which is often used without any form of treatment for domestic use, including drinking by the great majority of slum dwellers (Barrett *et al.*, 1999; Kulabako *et al.*, 2007).

The national sanitation coverage in Uganda was estimated to be 62.4% in 2008 (MoWE, 2008) and this increased to 67.4% in 2009 (MoWE, 2009). However, in many rural, peri-urban and urban areas of Uganda, sanitation facilities are not equitably distributed. Hence, some areas within regions/districts or even towns can have coverage as low as 10% or less. The households lacking sanitation facilities are often not aware that they statistically spend an unnecessarily large proportion of their limited financial resources nursing the sick, which leads to an increase in unproductive time and exacerbation of the poverty situation.

It has been estimated that, on average, each slum dweller in Kampala spends about Uganda Shillings (UGShs) 25,000/= (US$ 13) per month on water- and sanitation-related diseases (H. Plumm, pers. comm. 2008). The mortality as a result of diarrhoea is estimated to be 440 children per week in Uganda, and evidence suggests that improving sanitation could reduce diarrhoeal diseases by 35-40%, and child mortality by half (WSP, 2000). An important consideration to be aware of is that falling sick as a result of sanitation-related diseases and seeking medical care is no guarantee that the patients will recover, as is evidenced by the mortalities just mentioned.

One way to decrease the environmental pollution in residential areas, as well as in recipient surface waters and groundwater, and thereby decrease the negative impacts on society of untreated excreta, is to safely use the excreta nutrients in plant production (Vinnerås and Jönsson, 2002). The use of excreta-based nutrients closes the nutrient loop, thereby enabling an increase in the sustainability due to recycling of renewable nutrient content (WHO, 2006). The safe use of excreta nutrients can be simplified by collecting the excreta fractions separately, treating them and applying them safely in plant production.

Source-separation of urine and faeces involves the collection of these fractions separately at source. The simplest way to achieve this is by use of a urine diversion toilet (Figures 1 and 2). Urine and faeces can be collected separately using single or double-flushed toilets (Figure 1) or non-flush urine diversion dry toilets (UDDT) (Figure 2). When dry urine diversion toilets are used, the faecal matter and toilet paper (if used) are collected in a container or vault beneath the toilet. The toilet paper can also be collected

in a separate container. The urine is collected in a separate container but if it is not to be used, then it can be diverted into a soakaway pit.

*Figure 1.* Double flush urine diverting toilets of different designs, Sweden. Photos: C. Niwagaba.

*Figure 2.* Dry urine-diverting toilets, Left: Squatting type made of plastic; Right: Pedestal type made of concrete, Uganda. Photos: C. Niwagaba.

In fully functioning source-separation systems, the grey water and solid waste fractions, which together constitute the major fractions of the waste generated in a household (Vinnerås *et al.*, 2006), are also collected separately. The advantage of separately collecting the urine and faeces is that the treatment can be tailored to the specific composition and need for treatment of each fraction, which depends upon the use to which the treated material is to be put, as well as the need to protect the environment from pollution.

Recently, there has been growing interest in using urine as a fertiliser for vegetables, maize, bananas and fruit trees. In fact, the existence of at least

8,000 urine diversion dry toilets (UDDTs) in Uganda (M. Oketch, pers. comm. 2009), the current promotion of UDDTs by various Ugandan NGOs and the existence of a 10 year (2008-2018) National Strategy to promote ecological sanitation gives hope for increased future excreta-derived nutrient use in Uganda, especially as previous studies have not found any serious cultural or social taboos against the use of excreta nutrients in Uganda (Niwagaba and Asiimwe, 2005; Mukama, 2006). Furthermore, the recent large changes in the price of chemical fertilisers on the world market are likely to make the use of excreta fertilisers more interesting.

According to FAOSTAT (2009), the most recent statistics for 2007 reveal that the amount (in tonnes) of fertilisers consumed in Uganda in 2007 was approximately 4000 of total N, 2200 of P (reported as $P_2O_5$) and 1400 of K (reported as $K_2O$). Using results from computations by Jönsson and Vinnerås (2004), and the 2007 mid-year population of Uganda (UBOS, 2007; PRB, 2009), the estimated amount (in tonnes) of nutrients in Ugandan human excreta is approximately 71000 of total N, 11400 of P and 39900 of K. According to FAOSTAT (2009), all of the fertilisers consumed in Uganda were imported. Consequently, if all of the human excreta in Uganda were recycled to agricultural production, there would be no need to import fertilisers and nutrient applications could still be increased by some 10- to 20-fold more than today! In Ethiopia, the results from a material flow model showed that implementing UDDTs in about 33% of the households and recycling their nutrient content would be sufficient to replace the current fertiliser demand in the town of Arba Minch (Meizinger *et al.*, 2009).

An important challenge in the utilisation of excreta-derived nutrients is that they may contain pathogenic microorganisms, which can introduce a disease transmission route that needs to be properly managed. Most of the pathogens of concern are excreted in large concentrations via faeces (WHO, 2006) and only a few of them via urine (Höglund *et al.,* 1998). The major pathogen risk with urine is cross-contamination by faeces, which was measured to 10 mg $L^{-1}$ by Höglund *et al.* (1998). Therefore, both source-separated faeces and urine normally need to be sanitised in order to safely recycle their plant nutrient content to production of food (Schönning and Stenström, 2004; WHO, 2006). This thesis investigates low-cost methods for treatment of human faeces and urine with the aim of promoting safe nutrient recycling.

## 1.1 Objectives

The main objective of this research was to widen our knowledge on treatment technologies for faeces and urine in the search for simple, low-cost, environmentally friendly and resource efficient methods that can facilitate the safe recycling of their plant nutrients for food production.

Specific objectives were to investigate:

1. The effect of different substrate mixtures and of insulation when composting source-separated faeces (Papers I and II),
2. Incineration as a treatment and sanitation method for faeces (Paper III),
3. Indicator/pathogen inactivation in urine, as a function of dilution and temperature (Paper IV),
4. Indicator/pathogen inactivation in urine, when stored in the sun with daily varying temperatures (Paper V).

## 1.2 Structure of the thesis

This thesis is based on Papers I-V. The relationship between these papers is shown in Figure 3.

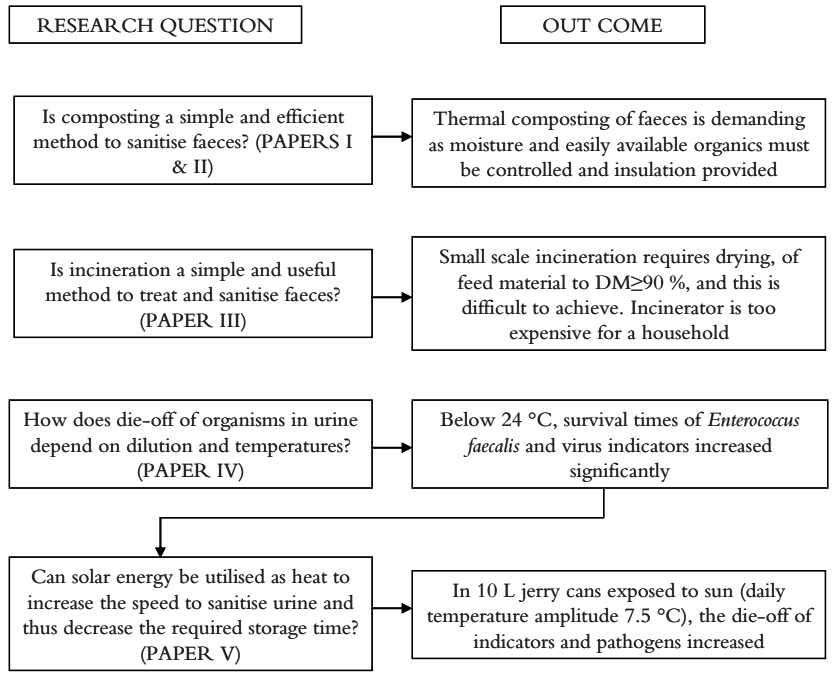


*Figure 3.* Conceptual framework of the studies.

# 2 Literature and Theory

Human excreta consist of faeces and urine, which are the waste products of body metabolism. The appearance, physical and chemical characteristics of urine and faeces depend largely on the health of the person excreting the material, as well as on the amount and type of food and liquid consumed (Lentner *et al.*, 1981; Feachem *et al.*, 1983).

Faeces consist of material that passes through the intestines undigested, mixed with material extracted from the blood stream or shed from glands and the intestines (Guyton, 1992), mucus and bile, which imparts the characteristic brown colour (Featherstone, 1999). Faeces can contain large concentrations of pathogenic viruses, bacteria, cysts of protozoa and eggs of helminths (Faechem *et al.*, 1983; WHO, 2006).

Urine is the excreta fraction that is filtered from the blood by the kidneys (Guyton, 1992). Urine is used by the body as a balancing medium for liquids and salts and the amount of urine excreted by a person therefore varies (Jönsson *et al.*, 2004). Urine largely consists of water, approximately 93-96% (Vinnerås *et al.*, 2006), and large amounts of plant nutrients that are mainly in water-soluble form (Jönsson *et al.*, 2004).

## 2.1 Excreta

### 2.1.1 Generation rate

The amount of faeces produced by a person depends on the composition of the food consumed. Foods low in fibres, such as meat, result in smaller amounts (mass and volume) of faeces than foods high in fibre (Guyton, 1992). The faecal production in the developed countries is approximately 80-140 g/p,d (wet weight) of faeces, corresponding to about 25-40 g/p,d of dry matter (Lentner *et al.*, 1981; Feachem *et al.*, 1983; Jönsson *et al*, 2005;

Vinnerås *et al.*, 2006). Faecal excretion rate in the developing countries is on average 350 g/p,d in rural areas and 250 g/p,d in urban areas (Feachem *et al.*, 1983). In China, Gao *et al.* (2002) measured 315 g/p,d while Pieper (1987) measured 520 g/p,d in Kenya. Schouw *et al.* (2002) measured faecal generation of 15 individuals in three different areas in Southern Thailand and obtained wet faecal generation rates of 120-400 g/p,d. Faecal excretion rate is on average one stool per person per day, but it may vary from one stool per week up to five stools per day (Lentner *et al.*, 1981; Feachem *et al.*, 1983).

The quantity of urine excreted depends on how much a person drinks and sweats, and also on other factors such as diet, physical activity and climate (Lentner *et al.*, 1981; Feachem *et al.*, 1983). Excessive sweating results in concentrated urine, while consumption of large amounts of liquid dilutes the urine. The urine generation rate for most adults is between 1000 and 1300 g/p,d (Feachem *et al.*, 1983). Vinnerås *et al.* (2006) suggested a design value for urine generation to be 1500 g/p,d based on measurements in Sweden, while Schouw *et al.* (2002) found that in Southern Thailand between 600-1200 g/p,d of urine were produced. Based on measurements in Switzerland, Rossi *et al.* (2009) reported a urine generation rate of 637 g/p,d on working days and 922 g/p,d on weekends, which is in agreement with 610-1090 g/p,d reported by Jönsson *et al.* (1999) based on measurements in Sweden.

### 2.1.2 Nutrients in excreta

The nutrient content of faeces originates from the food consumed. It is estimated that the food nutrient content is distributed to the faecal fraction in the proportions: 10-20% nitrogen (N), 20-50% phosphorus (P) and 10-20% potassium (K) (Berger, 1960; Lentner *et al.*, 1981; Guyton, 1992; Vinnerås *et al.*, 2006). About 20% of faecal nitrogen is ammonia, biochemically degraded from proteins, peptides and amino acids, some 17% is found in living bacteria and the remainder is organic nitrogen combined in molecules such as uric acid and enzymes (Lentner *et al.*, 1981). The nutrients contained in faeces in Sweden are on average 550 g N, 183 g and 365 g K per person and year (Jönsson *et al.* 2005; Vinnerås *et al.*, 2006).

Urine contains the largest proportion of plant nutrients found in the household waste and wastewater fractions (Figure 4). The amount of plant nutrients excreted via urine per person and year has been measured at 2.5-4.3 kg N, 0.4-1.0 kg P and 0.9-1.0 kg K (Lentner *et al.*, 1981; Vinnerås *et al.*, 2006).

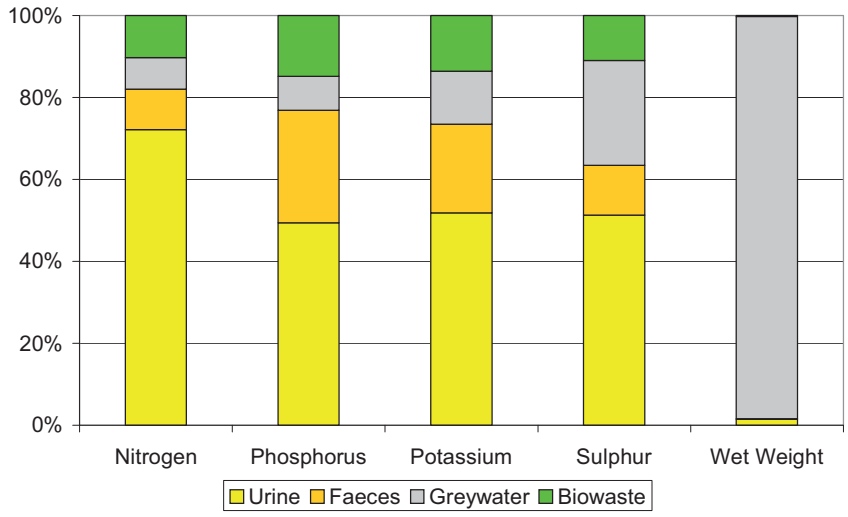


*Figure 4.* Proportions of nutrients found in household wastewater fractions and biowaste in Sweden. Source: Jönsson *et al.*, 2005.

Both Jönsson *et al.* (2005) and Vinnerås *et al.* (2006) analysed measurements on the nutrient content of urine, including previous studies, and found the annual excretion rate per person in Sweden to be about 4000 g N, 330-365 g P and 1000 g K. Together, the nutrients in urine and faeces in Sweden add up to some 4500-4600 g N, 500-550 g P and 1400 g K per person per year (Jönsson *et al.*, 2005; Vinnerås *et al.*, 2006). Based on FAO data on food supply, Jönsson and Vinnerås (2004) estimated the quantity of nutrients in Ugandan excreta to be 2500 g N and 400 g per person per year.

### 2.1.3 Other constituents in excreta

Human faeces and, to a small extent, urine contain trace metals, which if present in excess concentrations could be harmful to humans and to the environment. The amounts of harmful heavy metals in the urine are miniscule (WHO, 2006). This is a result of the biological uptake being small and their excretion being even smaller (Vinnerås, 2002).

Schouw *et al.* (2002) reported the following flows per person per day of heavy metals in human excreta in Southern Thailand: 9-16 mg zinc (Zn), 1.4-1.5 mg copper (Cu), 0.3 mg nickel (Ni), 0.02-0.03 mg Cd, 0.07-0.14 mg lead (Pb), 0.01 mg mercury (Hg) and 0.8-1.1 mg boron (B). The metal content and mass flows in faeces are usually reported to be far higher than in urine. In the study by Schouw *et al.* (2002), the amounts of Zn, Cu, Ni, Cd,

Pb and Hg were larger in faeces than in urine, while amounts of the non-metal elements S and B were larger in urine than in faeces. In Swedish source-separated fractions, Vinnerås *et al.* (2006) reported (per person per day) 11 mg Zn, 1.1 mg Cu, 0.07 mg Ni, 0.02 mg chromium (Cr), 0.02 mg Pb, 0.01 mg Cd and 0.01 mg Hg in faeces, and 0.04 mg Zn, 0.1 mg Cu, 0.01 mg Ni, 0.01 mg Cr, 0.002 mg Pb, 0.001 mg Cd and 0.001 mg Hg in urine. Essentially all the heavy metals in human excreta come from the food ingested and a large proportion of these metals will have been removed from the fields with the crop. Thus, it is possible to recycle excreta fertilisers, provided that they have not been polluted when handled, without threatening the sustainability of the agricultural soil for production of food (Jönsson *et al.*, 2004).

### 2.1.4 Pathogens in excreta

The faeces of a healthy person contain large numbers of bacteria of many non-pathogenic species, referred to as normal intestinal microbiota. Gastrointestinal pathogenic microorganisms do not occur as a natural part of normal intestinal microbiota (Feachem *et al.*, 1983). Their presence in faeces is an indication of infection amongst the population contributing to the faeces analysed. However, on occasion, some of the commensal bacteria otherwise referred to as normal intestinal microbiota may give rise to disease. This situation is likely to happen when the immune system of the human being has been compromised, for example, during sickness or old age, giving rise to opportunistic infections (Madigan and Martinko, 2006).

Most intestinal pathogenic or potentially pathogenic microorganisms enter a new host by ingestion (water, food, dirt on fingers and lips, aerosols caught in the nose and swallowed), through the lungs (after inhalation of aerosol particles) or through the eye (when eyes are rubbed with contaminated fingers) (Feachem *et al.*, 1983), while others may also enter through the skin or wounds. After infecting the host, large numbers of pathogens may be excreted. Depending on the health of the population, several species of pathogenic bacteria, viruses, parasitic protozoa and helminths may be found in the faeces from the population and thus also in its mixed wastewater. From a hygiene point of view, any exposure to fresh/untreated faeces constitutes a risk (Feachem *et al.*, 1983; Schönning and Stenström, 2004; WHO, 2006). Esrey *et al.* (1998) proposed a disease transmission route chart originating from the faeces using seven words all starting with letter F. This is therefore referred to as the F-diagram (Figure 5).

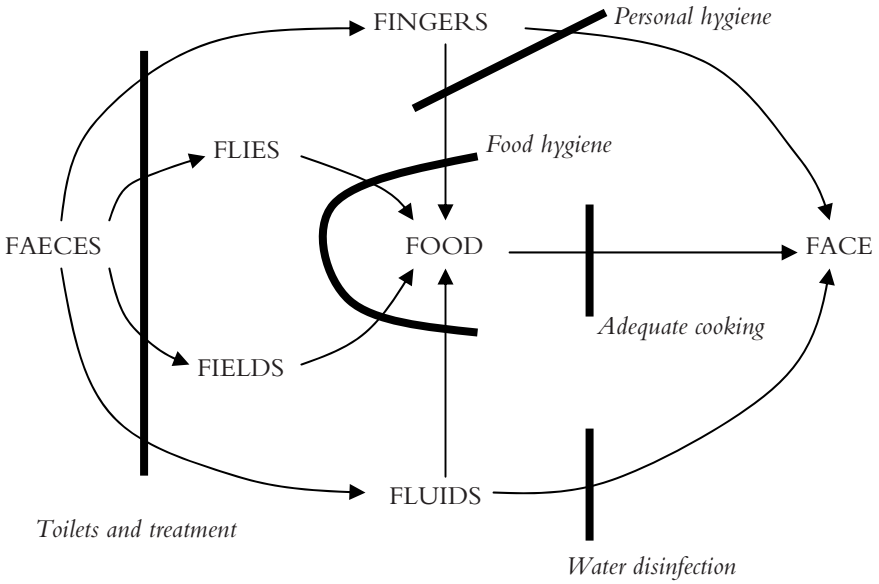


*Figure 5*. The F-diagram, showing the faecal disease transmission routes to a new host and the possible sanitation barriers. Adapted from Esrey *et al.*, 1998.

The pathogens that may be excreted in faeces include bacteria of several species (*e.g. Aeromonas* spp*., Campylobacter jejuni/coli,* pathogenic *E. coli, Pleisiomonas shigelloides, Salmonella typhi/paratyphi, Salmonella* spp*., Shigella* spp*., Vibrio cholerae and Yersinia* spp.), viruses (*e.g.* Enteric adenovirus 40 and 41, Hepatitis A virus, Hepatitis E virus, poliovirus and rotavirus), parasitic protozoa (*e.g. Cryptosporidium parvum*, *Entamoeba histolytica*, *Giardia intestinalis)* and helminths (*e.g. Ascaris lumbricoides* (roundworm), *Taenia solium/saginata* (tapeworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenale/Necator americanus* (hookworm) and *Schistosoma* spp. (blood flukes)) (Schönning and Stenström, 2004; WHO, 2006). The majority of different types of faecal pathogens cause gastrointestinal symptoms such as diarrhoea, vomiting and stomach cramps, while some also cause symptoms involving other organs (Schönning and Stenström, 2004; WHO, 2006).

A few pathogens are excreted via the urine during infection *e.g. Leptospira interrogans*, *Salmonella typhi*, *Salmonella paratyphi*, *Schistosoma haematobium* (Feachem *et al.*, 1983; WHO, 2006), whereas others such as Mycobacterium tuberculosis have occasionally been found in urine during renal TB infection (Daher *et al.*, 2007). Viruses, *e.g.* BK virus and Simian

virus 40, have also been found in children's urine (Vanchiere *et al.*, 2005). *Leptospira interogans* is transmitted by urine from infected animals and its transmission via human urine is low (Feachem *et al.*, 1983). Even though *Salmonella typhi* and *Salmonella paratyphi* can be excreted in urine from persons infected with typhoid and paratyphoid when the bacteria are disseminated through blood, the environmental transmission via urine is low due to the short survival time (just a few hours) of *Salmonella* spp. in urine (WHO, 2006).

In a Swedish study, 22% of the samples of source-diverted urine tested were contaminated with faeces. The contaminated samples were on average contaminated by about 9 mg faeces per litre of urine (Höglund *et al.*, 2002). Safe use of the urine as a fertiliser therefore normally requires that the urine be sanitised.

## 2.2 Treatment of faeces

Fresh faeces should always be considered unsafe due to the potential presence of high concentrations of pathogens (Feachem *et al.*, 1983; WHO, 2006). Thus, faeces should always be sanitised. Treatment and sanitation processes include storage, composting, incineration and chemical treatment.

### 2.2.1 Storage

Ideally, the numbers of microorganisms (including pathogens) in faeces should decrease following excretion, as a result of natural die-off. This is especially true since enteric microorganisms and pathogens (as well as faecal indicators) are acclimatised to body temperature and slightly above, and are thus usually considered to grow well at temperatures in the region of 37-44 °C. However, certain types of bacteria, *e.g. Salmonella* and some indicator organisms, *e.g. E. coli* and *Enterococcus* spp., can increase in numbers when conditions favouring their growth are established in their storage medium/environment (Schönning and Stenström, 2004; WHO, 2006). The extent to which pathogens decrease in numbers during storage depends on factors such as pH, moisture, temperature, nutrient availability, oxygen availability, ammonia concentration and UV exposure (Peasey 2000; Schönning and Stenström, 2004; WHO, 2006; Wichuk and McCartney, 2007; Austin and Cloete, 2008; Winker *et al.*, 2009).

In areas where ambient temperatures reach up to 20 °C, a total storage time of 1.5 to 2 years (including the time of storage during primary treatment, *i.e.* storage under the toilet vault) will eliminate most bacterial pathogens, provided the faecal material is kept dry, and will substantially

reduce viruses, protozoa and parasites (Schönning and Stenström, 2004; WHO, 2006). In areas with higher ambient temperatures (up to 35 °C), a total storage period of one year will achieve the same result, as pathogen die-off is faster at higher temperatures (Schönning and Stenström, 2004; WHO, 2006). This agrees well with Strauss and Blumenthal (1990), who suggested that one year was sufficient for inactivation of different types of faecal pathogens under tropical conditions (28-30 °C), whereas 18 months would be needed at lower temperatures (17-20 °C). However, longer survival times of 2-3 years have been reported for *Ascaris* at 22-37 °C (Moe and Izurieta, 2004). Vinnerås *et al.* (2007) reported that 50 days of storage of faecal matter at 20 °C did not reduce *Enterococcus* spp. and at 4 °C *Salmonella* spp. was not reduced either. Thus, at low temperatures prolonged storage times are needed to achieve sufficient sanitation of faeces.

### 2.2.2 Composting

Composting is the microbiological degradation of organic material to a humus-like stable product under aerobic, moist and self-heating conditions. Composting is often performed in order to convert potentially degradable waste into a beneficial product, to disinfect material that might be contaminated with pathogens and also for bioremediation of hazardous waste (Haug, 1993). The product from a well functioning and managed thermophilic compost process is usually free of pathogens and plant seeds and can be beneficially applied to land, supplying nutrients for plant growth, humus and organic matter for soil improvement (Epstein, 1997; Arvanitoyannis *et al.*, 2006; Arvanitoyannis and Kassaveti, 2007).

When a well conditioned substrate (sufficient energy, nutrients, moisture, structure *etc.*) is composted, aerobic degradation of its organics occurs. The process is exothermic, *i.e.* heat is generated, resulting in increased temperature. The heat produced either remains in the compost mass or escapes by conduction, convection and radiation, or is lost with the outgoing gas. To keep the material undergoing composting hot enough for sanitation, sufficient amounts of the heat generated should remain in the compost matrix. This requires, at least on the small and medium scale, that the compost be well insulated. The outer parts of large compost piles act as insulators to the inner parts of the pile, resulting in a temperature gradient within the pile, with the highest temperature in the interior (Finger *et al.*, 1976).

The degradation of the organics is a biochemical process performed by microorganisms. The factors that influence microbial growth affect the composting process. These factors relate to substrate composition, including

*e.g.* moisture, oxygen, pH, temperature and C/N ratio (Miller, 1993; Haug, 1993).

**Moisture:** The moisture of compost substrates provides a medium for the transport of dissolved nutrients required for the metabolic and physiological activities of microorganisms (McCartney and Tingley, 1998). The maximum rate of transfer of nutrients and waste products takes place in a liquid environment at 100% MC (Finger *et al.*, 1976), but in these conditions operating aerobic composting systems becomes hard to achieve. Optimum moisture for successful composting of various types of waste is reported to be in the range 25-80% (Ahn *et al.*, 2008) and 50-60% (Suler and Finstein, 1977; Bishop and Godfrey, 1983; McKinley and Vestal, 1984; Tiquia *et al.*, 1998). The wide range of optimum moisture content reported for the various substrates undergoing composting reflects their different structural properties. Highly structured substrates, *e.g.* wood chips, can compost at high moisture contents, whereas poorly structured substrates, *e.g.* food waste, require lower moisture contents to compost. At low initial moisture (*e.g.* <25%), early dehydration of the compost mass easily occurs (Liang *et al.*, 2003), which arrests the biological process, thus giving physically stable but biologically unstable composts (de Bertoldi *et al.*, 1983). For most substrates, moisture content exceeding 60% may produce anaerobic conditions through water-logging, which inhibits aerobic compost degradation (Schulze, 1962; Tiquia *et al.*, 1996) and can also result in problems with acid production and smell.

**Oxygen:** To properly function, the microorganisms involved in aerobic composting of substrate organic matter should be supplied with oxygen. Oxygen can be supplied to composts by mechanical aeration, convective air flow (passive aeration), diffusion and physical turning of the compost mass (Epstein, 1997). Whatever the method used for oxygen supply, the diffusion of oxygen in the aqueous layer around the substrates and into the pores of the substrate is important during composting. The resistance to oxygen diffusion through the compost matrix causes an oxygen gradient across the pile, with the lowest oxygen concentrations in the interior (Finger, 1976).

**pH:** In the initial phases of the composting process, pH usually declines due to the formation of organic acids, which are produced through fermentation of easily degradable organic matter (Beck-Friis *et al.*, 2003). Organic acids interfere with microbial cellular functions by entering the cells when the acids are in their undissociated form, *i.e.* when the pH is low (Sundberg, 2005). The organic acids tend to decrease the pH during the initial phase of composting but the carbonic and ammonia systems balance the pH reduction with varying success, depending on the substrate

(Sundberg, 2003). Later in the process the pH rises because the acids are consumed and ammonium is produced (Beck-Friis *et al.*, 2003), and the pH usually stabilises around 7.5-8.5 (Jeris and Regan, 1973; Beck-Friis *et al.*, 2003). Most bacteria cannot survive at pH 3 and below, and they begin to succumb at pH>10.5 too, with significant kills occurring at pH>11.5 (Haug, 1993).

**Temperature:** From the start of the composting process, the temperature increases due to microbial metabolism. Temperature increase within composting materials is a function of initial temperature, metabolic heat evolution and heat conservation (Miller, 1993). Based on temperature development, the composting process can be divided into three main phases, namely the mesophilic, thermophilic and curing phases. The mesophilic phase is characterised by increasing temperatures up to about 40 °C. The transition from mesophilic to thermophilic takes place at about 40-45 °C. The thermophilic phase is defined by temperatures from 45 °C to 70 °C and sometimes even more (Miller, 1996) and the curing phase is characterised by temperatures sinking below 40-45 °C again (Chiumenti *et al.*, 2005). Thermal sanitation mainly occurs in the thermophilic phase.

Temperature affects the rate of decomposition, which can be defined as the rate of carbon dioxide evolution. Temperatures below 20 °C have been demonstrated to slow or impede the composting process (Mosher and Anderson, 1977). The temperature at which maximum decomposition occurs probably depends on the type of substrate composted and is reported to be in the range 50-67 °C (Suler and Finstein, 1977; Haug, 1993; Miller, 1993; Richard and Walker, 1999; Eklind *et al.*, 2007). When temperatures above 50 °C are maintained for at least one week, pathogen inactivation is achieved according to Schönning and Stenström (2004) and WHO (2006). The higher the temperature margin in excess of 50 °C and the longer this temperature is maintained, the better the sanitation effect. High temperatures cause protein denaturation, leading to the destruction of cells (Madigan and Martinko, 2006).

The temperature in a composting heap is usually hotter at the centre than at the edges (Haug, 1993). To attain and keep high temperatures in the entire pile, insulation should be provided to prevent heat loss, especially for small compost heaps (Karlsson and Larsson, 2000; Björklund, 2002; Vinnerås *et al.*, 2003a). Insulation is also required when composting in small heaps under tropical conditions (Karlsson and Larsson, 2000). Even when insulation is provided, there will still be parts of the compost with low temperatures, for example near the air inlet. To expose the material in such

low temperature zones to high temperatures, the compost should be mixed sufficiently and repeatedly (Epstein, 1997; Vinnerås *et al.*, 2003a).

**C:N ratio:** The C:N ratio is considered among the factors affecting the compost process and compost quality (de Bertoldi *et al.*, 1983; Michel *et al.*, 1996). In growing, microorganisms require digestible carbon for energy and nitrogen for cell synthesis (Epstein, 1997). During aerobic metabolism, microbes use about 15 to 30 parts C for each part N (Haug, 1993). At C/N ratios of 15 to 30, nitrogen is present in sufficient amounts for cell synthesis and no rate limitation should be imposed (Haug, 1993). If the initial C/N ratio is greater than 35, the microorganisms must go through many lifecycles, oxidising off the excess carbon until a suitable C/N ratio for their metabolism is reached (de Bertoldi *et al.*, 1983). When composting substrates with low C/N ratios, excess nitrogen is lost via ammonia volatilisation, and this is higher at high pH and temperature (de Bertoldi *et al.*, 1983; Eklind *et al.*, 2007). The loss of N decreases the value of the compost as a fertiliser and when lost as ammonia or nitrous oxide, also pollutes the air.

**Substrate conditioning for moisture and energy:** Feed conditioning is an important aspect that determines how successful the compost can be in attaining sanitising temperatures. Since a faeces/ash mixture may not contain enough organics to ensure that sanitising temperatures (>50 °C) are maintained for a sufficiently long duration (at least one week) to attain sanitation, there may be a need to co-compost with food waste or other organic waste. Haug (1993) has developed rules of thumb that can be used to roughly estimate whether the energy in the material is sufficient for thermal composting. Since the largest energy usage in composting is that for the evaporation of water, it is possible to use the ratio of water to the mass of biological volatile matter (W) to roughly estimate whether there is sufficient energy to heat and evaporate the water. The ratio W is calculated according to Equation 1 according to Haug (1993).

$$W = \frac{(X_s - X_s S_s)}{k_s V_s S_s X_s} \qquad \text{Eqn. (1)}$$

where $k_s$ is the fraction of the substrate volatile solids degradable under composting; $V_s$ is the volatile solids content of the dry solids; $S_s$ is the fractional solids (dry matter) content of the substrates; and $X_s$ is the wet weight of the feed substrate.

The calculated W is then compared with the recommended literature value, W<8, to check whether the compost substrate has sufficient energy

for temperature elevation and water evaporation. If W>10, normally insufficient energy is available to achieve temperature elevation and sufficient water evaporation. Another important parameter, energy ratio (E) is calculated according to Equation 2 according to Haug (1993).

$$E = \frac{k_s V_s S_s X_s H_s}{(X_s - S_s X_s)} \qquad \text{Eqn. (2)}$$

where $H_s$ is the heat released per gram of biodegraded volatile solids. An estimate of $H_s$ = 23.24 MJ $g^{-1}$ degraded $V_s$ (5550 cal $g^{-1}$ degraded $V_s$) is given by Haug (1993). As a rough rule of thumb, composts with E>700 theoretically posses sufficient energy for composting and drying, while composts with E<600 lack sufficient energy (Haug, 1993).

### 2.2.3 Incineration

Incineration of faeces offers a treatment method that not only destroys pathogens, but is also a compact and rapid process, *i.e.* inactivation can be achieved quickly. In addition, the amount of material remaining decreases, as only ashes remain. The possibility to re-use the ashes as cover material during the collection phase in UDD toilets means there is little additional need for disposal. Thus the frequently encountered problem of providing cover material for the collection phase can be solved.

Incineration increases the temperature to high levels such that short exposure should be enough to inactivate any pathogens present. Low-cost small-scale incinerators made of steel sheets have been promoted by international agencies, mainly for disposal of healthcare waste. In most of these applications, incinerators are used to prevent health risks arising out of possible scavenging of sharps, with the associated possibility of transmission of diseases such as hepatitis and HIV/AIDS (WHO, 2004b).

Incineration of materials with inorganic or organic chlorides produces more dioxins than materials without chlorides (Shibamoto *et al.*, 2007). Dioxin formation occurs at temperatures above 450 °C and is reduced significantly at temperatures above 850 °C (Shibamoto *et al.*, 2007). This agrees with EU directives (89/429/EEC discussed in Nasserzadeh *et al.* (1995)), which state that air pollution is minimised when the combustion chamber temperature reaches at least 850 °C and the gases are exposed to this temperature for two seconds or more in the presence of at least 6% oxygen.

Literature on the burning of different types and sources of straw contains reports of 90-100% losses of N, S and C (Partridge and Hodgkinson, 1977;

Heard *et al.*, 2000) and 24% losses of P, 35% of K and 75% of S (Heard *et al.*, 2000). According to Jönsson *et al.* (2004), ash from incineration of faeces contains large proportions of the P and K, which, like plant ash, can fertilise the soil for agricultural purposes.

### 2.2.4 Chemical treatment

Chemicals that can be used to treat faeces for pathogen reduction include acids (*e.g.* phosphoric acid), bases (*e.g.* ammonia and lime) and oxidising agents (*e.g.* chlorine). Some chemicals for disinfection contain substances of agronomic value, *e.g.* $Ca(OH)_2$, $NH_3$, KOH and $PO_4^{3-}$. The use of these disinfecting chemicals is preferable for substrates that are to be recycled as fertilisers, as the nutrient content of the disinfectant increases the fertiliser value of the product (Winker *et al.*, 2009; Vinnerås *et al.*, 2009).

Urea has been investigated for sanitation of faecal matter (Vinnerås *et al.*, 2003b; Nordin *et al.*, 2009a,b; Vinnerås *et al.*, 2009) and manure (Ottoson *et al.*, 2008). The treatment by urea functions via enzymatic degradation, which produces uncharged ammonia, and thus ammonia is the disinfecting agent in urea treatment systems. When urea degrades, the pH increases and when pH>9 is achieved, the majority of the ammonium/ammonia is uncharged ammonia and thus the disinfection of bacterial cells increases even more as a result of ammonia toxicity (Warren, 1962; Pecson *et al.*, 2007). Addition of urea at a dosage of 30 g ammonia nitrogen per kilogram of faeces (3% ammonia nitrogen) is sufficient for the material to be considered generally safe after 2 months at 20 °C (Vinnerås *et al.*, 2003b). In the study by Vinnerås *et al.* (2003b), addition of urea to faecal matter resulted in a pH increase to approximately 9.3. This produced an efficient disinfection of *E. coli*, *Enterococcus* spp. and *Salmonella* spp. within 3 weeks (>6$\log_{10}$ reduction) and a reduction of the chemical resistant phage *S.* Typhimurium 28B, corresponding to a decimal reduction within 7.5 days, or 45 days for 6$\log_{10}$ reduction.

Ammonia at high pH inactivates bacteria (Warren, 1962; Nordin *et al.*, 2009b), viruses (Cramer *et al.*, 1983; Pesaro *et al.*, 1995; Nordin *et al.*, 2009b), *Cryptosporidium* oocysts (Jenkins *et al.*, 1998) and *Ascaris* eggs (Ghiglietti *et al.*, 1997; Pecson *et al.*, 2007; Nordin *et al.*, 2009a). Nordin *et al.* (2009a) studied the inactivation of *Ascaris suum* eggs in faeces by ammonia at storage temperatures corresponding to different ambient temperatures (4, 14, 24 and 24 °C) and found that uncharged ammonia in concentrations of ≥60 mM was efficient in sanitising faeces. A 6$\log_{10}$ reduction in *Ascaris suum* eggs was achieved within 1 month of storage at 34 °C and within 6 months at 24 °C. However, at 14 °C or lower, the inactivation of *Ascaris suum* eggs

was low, with viable eggs after 6 months of storage at $NH_3$ concentrations of ≥60 mM.

Treatment of faeces from source-separating dry toilets with 1% urea achieves *Salmonella* reduction levels that meet the requirements for safe reuse of faeces as fertiliser (*i.e.* $6\log_{10}$ reduction) within 2 months at 14 °C or within 1 week at 24 °C and 34 °C (Nordin *et al.*, 2009b). With respect to *S.* Typhimurium phage 28B, the addition of 2% urea at 24 °C and 34 °C produces a safe fertiliser for unrestricted use within 8 months and 1 month respectively (Nordin *et al.*, 2009b).

## 2.3 Temperature-time relationships to sanitise faeces

In composting, several processes kill pathogens. These include competition between indigenous microorganisms and pathogens, antagonistic relationships between organisms, the action of antibiotics produced by certain fungi and actinomycetes, natural die-off in the compost environment (which is non-ideal for enteric pathogens), production of toxic by-products such as gaseous ammonia, nutrient depletion, and thermal destruction (Pereira-Neto *et al.*, 1987; Haug, 1993; Epstein, 1997; Wichuk and McCartney, 2007). Because of the ease with which temperature can be measured by facility operators, the use of elevated temperatures is considered to be the most reliable method for inferring compost sanitation (Feachem *et al.*, 1983; Vinnerås *et al.*, 2003a). Consequently, most regulatory agencies use temperature as a means of inferring the achievement of pathogen destruction (Haug, 1993).

All pathogens have threshold temperatures beyond which their viability ceases (Madigan and Martinko, 2006). The mechanism of temperature inactivation differs for different types of pathogens. Elevated temperatures irreversibly inactivate enzymes of bacteria, protozoa and helminths, thereby resulting in cellular inactivation (Madigan and Martinko, 2006; Wichuk and McCartney, 2007). For viruses, thermal inactivation occurs as a result of damage to the viral structure through denaturation of viral surface proteins (Wichuk and McCartney, 2007).

The degree of thermal inactivation of pathogens is a function of both the temperature and time of exposure (Feachem *et al.*, 1983; de Bertoldi, 1998; Wichuk and McCartney, 2007). Several authors have studied the temperature-time relationships that result in a safely sanitised compost. Beauford and Westerberg (1969) reported that *Salmonella newport*, poliovirus type 1, *Ascaris lumbricoides* ova and *Candida albicans* were effectively killed in aerobic composting of sewage sludge at 60-70 °C within 3 days. According

to Feachem *et al.* (1983), the time-temperature combinations lethal to all pathogens excreted in faeces, including the most resistant *Ascaris* (with the possible exception of hepatitis A virus at short retention times), are: 1 hour at ≥62 °C, 1 day at ≥50 °C, and 1 week at ≥46 °C. *Salmonella* spp. and *E. coli* are undetectable within 24 hours of composting at temperatures higher than 50 °C (Lung *et al.*, 2001; Hess *et al.*, 2004). Grewal *et al.* (2006) found that composting dairy manure at 55 °C killed off *E. coli*, *Listeria* and *Salmonella* spp. within 3 days.

Most literature on the sanitation of composts report temperatures in excess of 50-55 °C as sanitising. The higher the temperature beyond the region of 50-55 °C, the shorter the time of inactivation and *vice versa*. It must be noted that the sanitising temperatures (>50-55 °C) should be attained by all of the particles in the compost matrix in order for the material to be safely sanitised. Schönning and Stenström (2004) and WHO (2006) recommend thermophilic composting of source-separated faeces at temperatures >50 °C for at least one week to ensure safe sanitation. In the United States of America, the compost is regarded as hygienically safe if a minimum of 55 °C is maintained for 3 consecutive days during composting in aerated static pile or in-vessel reactors, while for windrows, temperatures greater than 55 °C should be maintained for at least 15 days with a minimum of 5 turnings during the high temperature period (USEPA, 1999). In Sweden, the voluntary rules for certification of compost from source-separated household waste distinguish between open and closed windrow composting as follows: In open windrow composting, the rule calls for 55 °C during three periods of 7 days' duration or 65 °C during three periods of 3 days' duration with turning between each period and in addition the compost should cure for at least 6 months before being used. In closed systems, the rules require 55 °C during 7 days or 65 °C during three days (SP, 2009).

## 2.4 Treatment of urine

### 2.4.1 Storage

The factors responsible for the persistence (and/or die-off) of microorganisms during storage of source-separated human urine are temperature, pH and ammonia, which is affected by the dilution of the urine (Höglund, 2001; Nordin *et al.*, 2009a; Chandran *et al.*, 2009). Treatment of urine by storage has been investigated at 4-5 °C and at 20 °C (Höglund, 2001) and at 4, 14, 24 and 34 °C (Nordin *et al.*, 2009a). Studies

on the die-off of various types of microorganisms in stored urine, combined with a risk assessment, resulted in the recommendation that when urine is stored for 6 months at 20 °C, it should be safe to use as a fertiliser for any crop (Schönning and Stenström, 2004; WHO, 2006).

The majority of nitrogen in fresh urine is urea (Jönsson *et al.*, 1997). Urea dissociates to ammonium in a reaction catalysed by urease (Equation 3).

$$CO(NH_2)_2 + 3H_2O \xrightarrow{urease} 2NH_4^+ + HCO_3^- + OH^- \qquad \text{Eqn. (3)}$$

Ammonium is in equilibrium with dissolved ammonia (Equation 4); and dissolved ammonia is in equilibrium with gaseous ammonia (Equation 5).

$$NH_4^+ + OH^- \leftrightarrow NH_3(aq) + H_2O \qquad \text{Eqn. (4)}$$

$$NH_3(aq) \leftrightarrow NH_3(g) \qquad \text{Eqn. (5)}$$

Urea dissociation normally increases the pH of the urine to 8.8-9.2 (Jönsson and Vinnerås, 2007). Studies of urine in which the dissociation of urea into ammonia was prevented from occurring showed that decreasing the pH to 3 also had an inactivating effect on the pathogens (Hellström *et al.*, 1999), which agrees with Haug (1993) for pathogens in compost.

In aqueous solutions, total ammonia is present in two forms, the ammonium ion ($NH_4^+$) and uncharged ammonia ($NH_3$) (Emerson *et al.*, 1975; Erickson, 1985; Arthur *et al.*, 1987; Ip *et al.*, 2001). The relative concentration of each of these forms is pH- and temperature-dependent as described by Equation (6) according to Erickson (1985) and Equation (7) according to Emerson *et al.* (1975) for an aqueous solution with just ammonium and ammonia:

$$K_a = \frac{[NH_3].[H^+]}{[NH_4^+]} \qquad \text{Eqn. (6)}$$

$$pK_a = (0.09018 + 2729.92)/(273.2 + T) \qquad \text{Eqn. (7)}$$

where T is temperature in °C.

The proportion of the total ammonia concentration that is ionised at any given pH is governed by the dissociation constant (pK) of the molecule, which varies with the temperature and the type of solution (Emerson *et al.*, 1975; Körner *et al.*, 2001). The ratio of un-ionised ammonia to ammonium ion increases by 5-10-fold for each unit increase in pH up to the pKa value

and by about 2-fold for each 10 °C rise in temperature over the 0-30 °C range (Erickson, 1985). For example, at pKa 9.0, the respective proportion of un-ionised ammonia at pH 6, 7, 8 and 9 is approximately 0.1, 1, 10 and 50% (Warren, 1962).

The toxicity of ammonia to organisms, animals and plants has been studied by various authors (Warren, 1962; Arthur *et al.*, 1987; Ip *et al.*, 2001; Körner *et al.*, 2001). Ammonia at low pH is toxic only in overwhelming quantities whereas at high pH, much smaller amounts of ammonia can be lethal (Warren, 1962). According to EPA (1998; *cit.* Ip *et al.*, 2001), the threshold concentration of total ammonia ($NH_3$+$NH_4^+$) resulting in unacceptable biological effects in freshwater is 3.48 mg N $L^{-1}$ at pH 6.5 and 0.25 mg N $L^{-1}$ at pH 9. Ammonia is lethal to microorganisms when its un-ionised form enters the cell by penetrating the cell wall (Warren, 1962).

### 2.4.2 Treatment by temperature

The available literature presents the treatment of urine as a function of time, temperature and ammonia concentration or pH (Höglund, 2001). The data show that pathogen inactivation is faster at high temperatures (*e.g.* 20, 24 or 30-34 °C) than at 4-5 °C or 14-15 °C (Höglund, 2001; Chandran *et al.*, 2009; Nordin *et al.*, 2009a).

In the above studies, pathogens were studied at a constant or near constant temperature. However, the temperature often varies between day and night, and temperature amplitude (peak-to-peak) between day and night can be of the order of magnitude of 10 °C or more. Such cyclic changes in temperature may stress the pathogens, killing them faster in accordance with the tyndalisation process (Stanbury *et al.*, 1995, *cit.* Vinnerås, 2002).

# 3 Summary of Papers

## 3.1 Composting (Papers I and II)

The aim of these studies was to investigate whether composting is a simple and efficient method to sanitise source-separated faeces collected with ash as cover material and, in particular, how to achieve sanitising temperatures and maintain them sufficiently long to achieve sanitation.

To increase the organic fraction of the source-separated faeces and thereby improve its potential to compost, the faeces/ash mixture was sieved prior to composting on a quarter-inch (6.35 mm) sieve. The sieved faeces were mixed with food waste to give compost substrates containing faeces: food waste (F:FW) by wet weight mix ratios of F:FW = 1:0, 3:1 and 1:1 (Paper I). The composting experiments were then scaled up to 216 L reactors using sieved faeces/ash and food waste in wet mix ratios of 1:0, 1:1 and 1:3, first according to volume and then according to weight (Paper II).

To prevent excessive drying of the material during composting, the moisture was monitored visually and moisture lost was replaced by sprinkling water followed by mixing. Temperature was measured two to four times daily (at 9.00 and 12.00 and occasionally also at 15.00 and 18.00 during the intensive part of composting, or within 20 days from the start of composting) using a portable digital thermometer (Model 307, Taichung, Taiwan) at the four corners and in the middle (Papers I and II).

### 3.1.1 Insulation

The temperature in non-insulated reactors in Paper I did not increase beyond 45 °C. The highest temperature above the surrounding ambient in non-insulated reactors was only about 14 °C, while it was 20-35 °C in insulated reactors (Paper I).

On a few occasions single point measurements reached 50 °C in non-insulated reactors, but this was not maintained for even a day (Paper II). In non-insulated reactors, the temperature at the centre was on average 2.5-2.6 °C higher than that in the corners and 7-10 °C higher than the ambient (Table 1; Paper II). In insulated reactors in which other factors, *e.g.* high moisture/low pH, did not inhibit the process, the temperature in the centre was on average 0.3-2 °C higher than that in the corners and 20-34 °C higher than the ambient (Table 1; Paper II).

Thermal insulation of the reactor walls is important as this decreases the loss of internally generated heat by conduction, convection and radiation. In large-scale composting, insulation of inner material is provided by the outer part of the heap (Finger *et al.*, 1976), but small composting reactors need to be insulated to retain enough of the heat to raise their temperature to sanitising levels (Epstein, 1997). However, insulation is of great value for large composts too, as it decreases the material in low temperature zones, which is especially important when sanitation is the objective.

Composting experiments at the 78 L and 216 L scale showed that insulation is important for reaching thermophilic temperatures and maintaining them for sustained periods, even in the tropics (Table 1; Papers I and II). The results, which showed that the non-insulated composts did not reach sanitising temperatures, agree with the findings by Karlsson and Larsson (2000) and Björklund (2002). Heat loss from the non-insulated composts is one probable reason for the low increase in temperature in their experiments. With the exception of those substrates in Paper II that were too wet, composting in large reactors (216 L) with thick insulation (75 mm) produced higher temperatures than composting in smaller reactors (78 L) with thinner insulation of 25 mm (Paper I). Both the scale and insulation probably contributed to this result.

The compost reactors used in the experiments were insulated with styrofoam all around the reactor. Covering the top of the reactor with an insulated lid is especially important, as most of the energy is lost through the upper surface. Amongst the low income earners in Uganda, insulation could be provided using styrofoam. Styrofoam packaging material is considered waste material after goods have been unpacked and it is common to find pieces of styrofoam of varying thickness and shape in the normal streams of waste, especially in urban areas. Therefore, where a number of pieces can be found, they can be put together and tied onto compost reactors, using sisal strings, or even banana fibre. Styrofoam can also be used for filling the gap in a double wall structure that can be built around the compost reactor to provide insulation.

Table 1. *Summary of temperature data in compost reactors in Paper II*

| Exp. | Run[a] | [b]$T_{max,av}$ °C | [c]$(T_c - T_{com,Av})$ °C | [d]$T_c - T_a$ °C | [e]Time >50 °C Days |
|---|---|---|---|---|---|
| 1 | V1:0 | 60.9 | 0.8±1.8 | 18.6±6.9 | 4 |
| | V1:1 | 67.9 | 0.3±1.6 | 31.1±5.0 | >18[f] |
| | V1:3 | 74.1 | 0.5±1.6 | 34.0±6.4 | >18[f] |
| 2 | W1:0aN | 41.6 | 2.5±3.2 | 6.8±5.9 | 0 |
| | W1:0a | 60.8 | 1.9±2.4 | 18.9±8.3 | 6 |
| | W1:1a | 71.6 | 1.8±2.4 | 31.6±10.2 | 25 |
| | W1:3a | 41.0 | 0.8±1.2 | 2.8±5.8 | 0 |
| | W1:3aF | 42.5 | 0.6±1.2 | 2.7±6.1 | 0 |
| 3 | W1:0bN | 49.3 | 2.6±2.8 | 9.5±7.3 | 0 |
| | W1:0b | 66.3 | 1.5±2.2 | 20±10.1 | 12 |
| | W1:1b | 38.1 | 0.2±1.1 | 4.0±3.2 | 0 |
| | W1:3b | 36.7 | 0.2±1.1 | 1.2±3.5 | 0 |
| | W1:3bF | 39.0 | 0.3±0.9 | 1.9±3.6 | 0 |

[a] *The runs are denoted by their faeces:food waste mix ratios by volume (V) or weight (W). F denotes runs with fresh food waste (collected and used on the same day), while food waste 1-14 days old was used in the other runs. N denotes runs in reactors without insulation, while a and b distinguish between runs in experiment 2 and 3.*

[b] $T_{max,av}$ *(°C) = Maximum temperature recorded during each measurement, where the maximum temperature is the mean for five measurement points (centre and four corners).*

[c] $(T_c - T_{com,Av})$ *= mean difference (over the measurement period), between centre temperature and mean corner temperature on each measurement occasion,*

[d] $(T_c - T_a)$ *= mean differences between centre temperature and ambient temperature over the measurement period,*

[e] *Time in days is the time during which the temperature (mean for all five points) exceeded 50 °C,*

[f] *Temperature measurements in Experiment 1 were performed over a period of 18 days only. Temperatures were measured over 40 days in Experiments 2 and 3, Mean ambient temperature was 29.7, 24.9 and 25.6 °C in Experiments 1, 2 and 3 respectively.*

Large compost heaps can be insulated using materials such as tarpaulin or heavy duty polyethylene, applied as a cover to the heap. Tarpaulins or heavy duty polyethylene materials are flexible, *i.e.* they adapt to the outer shape of the compost heap, thereby providing good cover for the compost and decreasing both heat and water losses. Another way could be to apply a layer of soil or old compost to act as an insulator to reduce heat and water losses. However, the effectiveness of these suggestions to provide insulation should be investigated before being implemented on full scale.

### 3.1.2 Easily degradable substrates

Composting sieved faeces/ash mixture alone resulted in a temperature increase (Papers I and II). The temperature increased to sanitising levels in insulated compost reactors containing only faeces/ash mixture but the temperature was not sustained beyond 3 days in a row (Paper I). Composting faeces/ash mixtures containing food waste at the 78 L scale

maintained temperatures at/above 50 °C for 4-8 days in a row, even though the temperature was often sustained at ≥50 °C up to day 12 of composting (Paper I). The duration of temperature at/above 50 °C when composting faeces/ash mixture alone at the 216 L scale was somewhat longer, 4-12 days (Paper II). When the faeces/ash were mixed with food waste at the 216 L scale, sanitising temperatures (>50 °C) were sustained for >2 weeks, provided that high moisture/low pH did not inhibit the composting process (Table 1, Paper II).

Faeces contain little easily degradable material, as most of this has been taken up by the human intestine (Lentner *et al.*, 1981). The content of VS in collected source-separated faeces decreases even further when ash is used as cover material during the collection phase. The lack of maintenance of high temperatures and their quick decrease in composting of the faeces/ash mixtures without food waste was linked to their low fraction of readily degradable organics, which led to their exhaustion and the temperature decreasing to below 50 °C in less than two weeks (Table 1; Papers I and II). This happened even though most of the ash had been sieved out of the faeces/ash mixture. If the faeces/ash mixture had not been sieved, the compost would probably not have reached 50 °C at all. Karlsson and Larsson (2000) did not attain sanitising temperatures when they composted faeces/ash that was not conditioned to increase the easily degradable organics. The lack of easily degradable organics in their substrates could have partly contributed to the low temperatures reached.

### 3.1.3 Moisture and pH

The moisture content of the starting substrates and of the samples taken at any one time during the composting was 40-60% in Paper I, while it was 43-73% in Paper II. Substrates with initial moisture content in the range 37-57% rapidly self-heated to sanitising temperatures (Figure 2 in Paper I; Figures 2, 3 and 4 in Paper II), attaining >65 °C in just two days, while those with moisture content above 63% either self-heated slowly or did not self-heat at all (Paper II). This shows that it is better to start the compost process somewhat drier, and then add water when needed, than to start with a high MC that may inhibit the composting process. Moisture content in the range 40-60% is considered optimal for composting operations (Golueke, 1977, *cit.* Epstein, 1997; Haug, 1993; Chiumenti *et al.*, 2005). For the faeces/ash and food waste substrates, there seems to be a breakpoint in moisture content around 60-65% beyond which composting is impeded, limiting the temperature increase (Paper II).

The MC of substrates with initial MC>63% remained high, causing noticeable water-logging during the composting. The mixtures in fact became wet pastes. Spreading the mixtures on a polyethylene sheet and drying them in the open air to about 50-60% MC did not make them heat up, probably because they had lost their structure. According to Golueke (1977, *cit.* Epstein, 1997), MC >60% in composts affects particle aggregation and air-filled porosity, which limits transport of the oxygen necessary for the composting process.

Moisture influences the pH. In one experimental run in Paper II with an initial moisture content of 63% and low pH, self-heating occurred very slowly, reaching above 65 °C after 37 days (Figure 6; Figure 4 in Paper II). As can be seen in Figure 6, there appears to be a relationship between moisture and pH, with materials having high initial moisture content (>65%) tending to exhibit low pH during composting. This agrees well with other authors and is probably a result of organic acid formation and accumulation under anaerobic conditions (Sundberg *et al.*, 2004; Nakasaki *et al.*, 2009). Organic acids are suppressive to microbial activity and growth at low pH (Sundberg *et al.*, 2004). This could have inhibited the microbial activity in the experimental runs with high MC, inhibiting temperature rise even after sun-drying (Paper II). The formation of the cakes and loss of structure could also have inhibited the composting process.

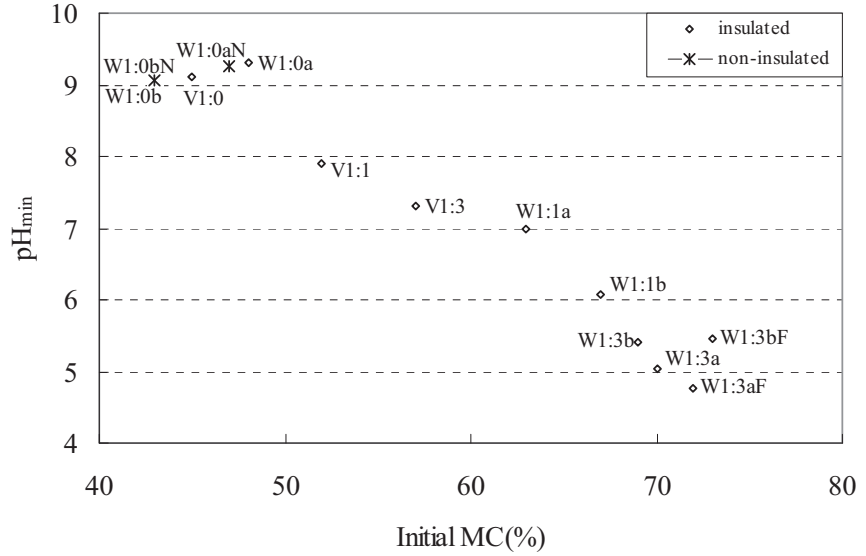


*Figure 6.* Minimum pH during composting plotted against the initial moisture content of the substrates (Paper II).

The higher the proportion of faeces/ash mixture, the higher the initial pH of the substrates. Materials that had minimum pH equal to or higher than 6.9 (and up to 9.3), either from the start or at a later time, composted well, attaining thermophilic and sanitising temperatures (Papers I and II). Materials with pH of about 6 and lower did not attain thermophilic temperatures. There appears to be a pH breakpoint between 6 and 7, below which the thermophilic composting of substrates/materials is impeded and above which the progress of the composting process proceeds well, attaining thermophilic and sanitising temperatures (Figure 6; Paper II). This agrees with Haug (1993), Epstein (1997) and Sundberg (2005). The rapid increase in temperatures in substrates with pH up to about 9.8 shows that up to this pH the composting process can proceed (Papers I and II).

### 3.1.4 Sanitation of composts

In runs where sanitising temperatures (>50 °C) were maintained only for short durations (<4 days), *E. coli* was detected in all samples in concentrations $>10^3$ cfu $g^{-1}$ (Paper I). In some runs, where a temperature of >50 °C was maintained for more than 4 days, *E. coli* decreased to below the detection limit but then sometimes reappeared later in the experiment (Figure 7a; Paper I). In the experiments where >50 °C was maintained for 4-8 days, a reduction of $>3\log_{10}$ for *E. coli* was achieved (Paper I).

In runs which did not heat to sanitising temperatures, *Enterococcus* spp. counts were almost the same in all the samples taken at the end of the experiment as in the starting samples. Where >50 °C was maintained for less than 4 days, *Enterococcus* spp. remained detectable (detection limit $10^2$ cfu $g^{-1}$) in all samples (Paper I). When >50 °C was maintained for >4 days, a reduction of $>4\log_{10}$ for *Enterococcus* spp. was achieved. The F:FW=1:0 compost, which maintained ≥50 °C for just 2-3 days, was not sanitised, as the counts of *Enterococcus* spp. increased between days 11 and 15 (Figure 7b) (Paper I). In the F:FW = 3:1 and F:FW = 1:1 composts, which maintained ≥50 °C for 4-8 days, the *Enterococcus* spp. decreased with time until there was no detection (Figure 7b; Paper I).

In the 216 L reactor, the faeces/ash mixture alone exceeded 50 °C for ≤12 days in insulated reactors, but did not reach or maintain 50 °C in non-insulated reactors (Paper II). In both experiments in Paper II, where >50 °C was maintained for at least six days, *E. coli* and total coliform concentrations (initially $10^3$-$10^5$ cfu $g^{-1}$) decreased to below detection, as did the *Enterococcus* spp. in runs with >50 °C for about 2 weeks (Table 2; Paper II).

(a)

(b)

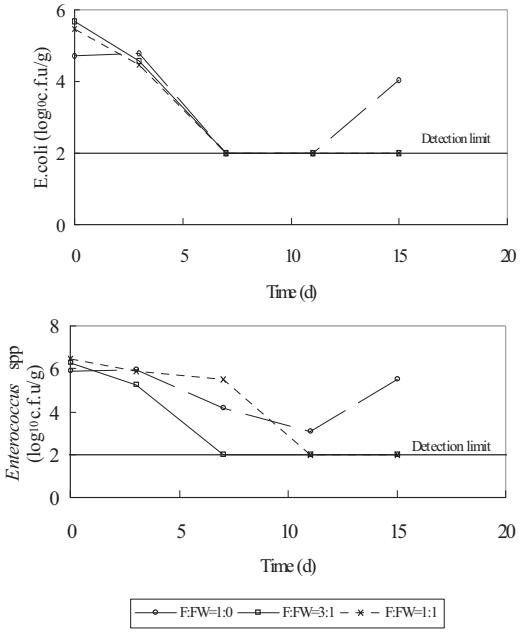


*Figure 7.* Concentrations ($log_{10}$cfu $g^{-1}$) of (a) *E. coli* and (b) *Enterococcus* spp. in runs in the 78 L reactor reaching sanitising temperatures (Paper I).

Table 2. *Counts of total coliforms and Enterococcus spp. (cfu $g^{-1}$) in Paper II*

| Organism | Run | Day 0 | Day 6 | Day 12 | Day 18 | Day 43 |
|---|---|---|---|---|---|---|
| Total coliforms | V1:0 | $4.2\times10^5$ | $5.0\times10^4$ | $3.2\times10^3$ | n.d. | n.d. |
| | V1:1 | $2.4\times10^5$ | $9.0\times10^3$ | n.d. | n.d. | n.d. |
| | V1:3 | $1.2\times10^5$ | $2.1\times10^4$ | n.d. | n.d. | n.d. |
| *Enterococcus* spp. | V1:0 | $3.7\times10^6$ | $2.2\times10^4$ | $1.1\times10^4$ | $1.0\times10^3$ | $1\times10^3$ |
| | V1:1 | $1.5\times10^6$ | $2.7\times10^4$ | $2.3\times10^3$ | n.d. | n.d. |
| | V1:3 | $2.1\times10^6$ | n.d. | n.d. | n.d. | - |

*n.d. Not detected. Detection Limit $10^2$ cfu $g^{-1}$.*

No *E. coli* or total coliforms were detected in material that exceeded 50 °C for at least six days. *Enterococcus* spp. decreased below detection in material that exceeded 50 °C for at least 2 weeks, but remained detectable after 1.5 months in material that exceeded 50 °C for less than two weeks, suggesting that at least 2 weeks above 50 °C combined with mixing is needed to achieve sanitation.

The composts that were sanitised were turned 2-3 times a week during the high active phase of composting, when the temperatures were rising and also as the temperatures were sanitising (above 50 °C). Turning and mixing

of materials/substrates by hand using a spade was easier to do well at the bench-scale (78 L) (Paper II), but harder at the larger, 216 L scale (Paper II). This might be one explanation why *Enterococcus* spp. decreased to below detection after 11 days of composting in 78 L reactors, which maintained sanitising temperatures for only 8 days (Paper I), but not in the 216 L reactors, even when sanitising temperatures were maintained for 12 days (Paper II). This was in spite of turning all the composts 2-3 times during the high active phase at each scale.

The results of composting studies (Papers I and II) suggest that sanitation is attained for composts maintaining ≥50 °C for 2 weeks during which the compost is turned at least 4 times.

## 3.2 Incineration of faeces (Paper III)

This study was carried out to determine whether incineration is a simple and useful method to sanitise source-separated faeces, also considering its possibility to decrease the amount of faecal material to be disposed of by urban dwellers, who may not have gardens on which to apply the material.

The studies were performed on faeces (including toilet paper and ash added as cover material in the toilet) collected from UDDTs used by high school girls. The faeces were collected using woven papyrus baskets placed directly under the toilet vault and stored under a drying shed for 2-6 months. The faeces for the experiments were collected in separate 50 kg bags without mixing the different batches of two- and six-month old faeces.

The faeces were incinerated in a locally manufactured incinerator made out of steel sheets. It contained a metallic grating on which a perforated steel sheet was mounted for supporting the faeces (Figure 8).

Loose ash was decreased by sieving the collected faeces/ash mixture on a quarter-inch (6.35 mm) aperture sieve (Paper III). The sieved faeces, toilet paper and faecal-bound ash were sun-dried on a polyethylene sheet in the open at an average ambient air temperature of about 28 °C for 2-3 days to decrease their moisture content before incineration.

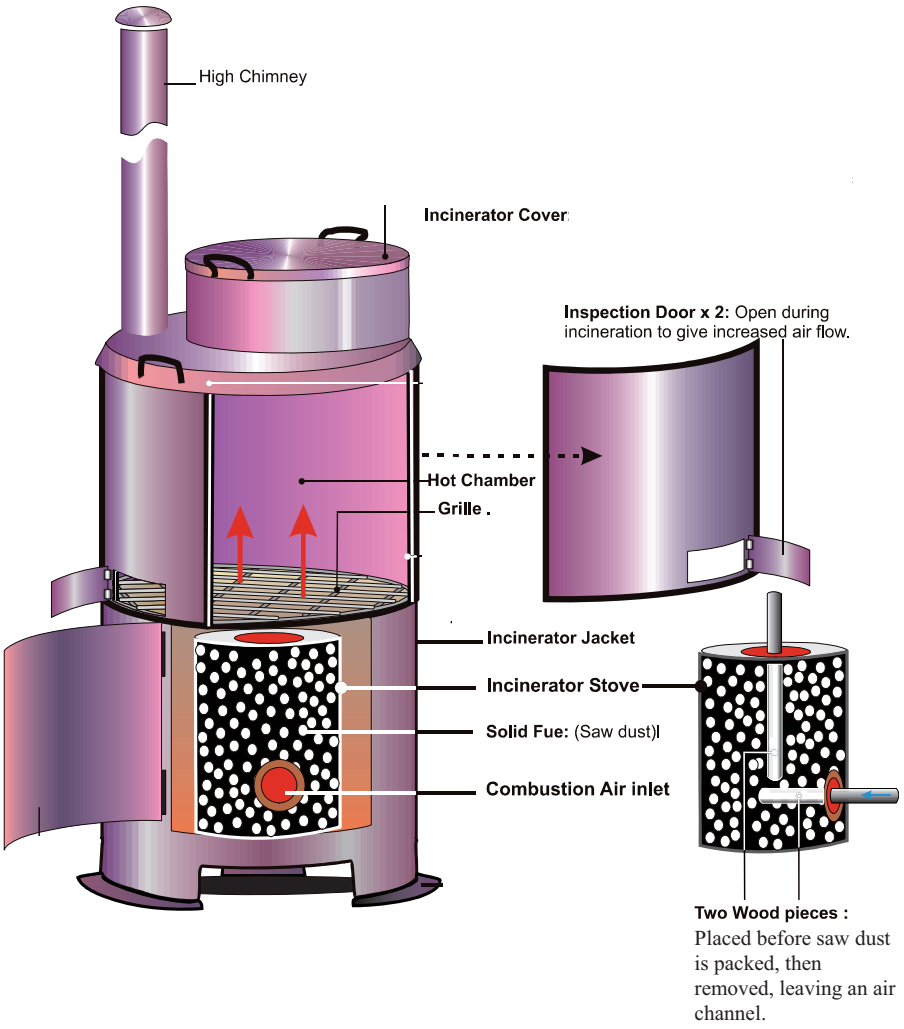


*Figure 8.* Incinerator used in the experiments (Paper III). Source: Supplier product sheets, M.K. Musaazi, 2006.

The fire was started and driven by a sawdust-fuelled stove underneath the metallic grate (Figure 8). The experiments were monitored and the highest temperatures attained during each experiment recorded. Within 45 minutes to 1 hour, the sieved faeces on the metal sheet grate ignited at temperatures of 800 °C and beyond.

Samples were collected before and after incineration and analysed for moisture and organic matter content (Table 3), plant nutrients (N, P, and K), and bacteria. Analysis was performed on crushed samples, *i.e.* faeces and toilet paper were crushed to obtain a homogeneous sample.

Table 3. *Moisture (MC) and organic matter (OM) content in the different trials on incinerating crushed faecal matter (Paper III)*

| Expt. No | Time in drying shade | $MC_{in}$ (%) | $OM_{in}$ (%) | $MC_{out}$ (%) | $OM_{out}$ (%) |
|---|---|---|---|---|---|
| 1 | 6 months | 12.4 | 8.8 | 4.3 | 1.9 |
| 2 | 2 months | 5.3 | 14.6 | 4.2 | 0.1 |
| 3 | 6 months | 22.8 | 18.7 | 1.4 | 0.4 |

Using the moisture and organic matter content of the material before and after incineration, the mass loss was calculated (Table 4).

Table 4. *Mass balance for sun-dried faeces over the incineration (Paper III)*

| Expt. No. | Time in drying shed | Initial mass (kg) | Final mass (calculated, kg) | Mass loss (kg) | Mass loss (%) |
|---|---|---|---|---|---|
| 1 | 6 months | 14.7 | 12.5 | 2.2 | 15 |
| 2 | 2 months | 16.0 | 13.5 | 2.5 | 15 |
| 3 | 6 months | 17.5 | 11.2 | 6.3 | 36 |

Total losses of incinerated material, as shown in Table 4, were low due to its high initial ash content. However, for the organics the mass loss was >90%.

By comparing the plant nutrients before and after the incineration, the loss of nutrients was calculated. Calculated nutrient losses of total N and plant-available P are shown in Table 5, where total N and available P losses are in the range 90-94% and 70-94%, respectively.

After sieving and sun-drying, only low numbers of microorganisms were found in the material before incineration, and thus no measurements of these were performed on the outgoing material. During the incineration, the small particles that passed though the holes on the metallic plate and grill fell into the bottom chamber where the stove was. This whole chamber maintained a high temperature.

Table 5. *Losses of total N and plant-available P in the incineration experiments (Paper III)*

| Expt. No. | Total N | | | Available P | | |
|---|---|---|---|---|---|---|
| | Before incineration (g $kg^{-1}$ ash) | After incineration (g $kg^{-1}$ ash) | Loss (%) | Before incineration (g $kg^{-1}$ ash) | After incineration (g $kg^{-1}$ ash) | Loss (%) |
| 1 | 1.56 | 0.14 | 91 | 0.21 | 0.06 | 70 |
| 2 | 1.69 | 0.10 | 94 | 0.33 | 0.08 | 75 |
| 3 | 2.34 | 0.23 | 90 | 0.49 | 0.03 | 94 |

The advantage of incinerating source-separated faeces from UDDTs is that the ash can be recycled as cover material in toilets, thereby solving the often encountered problem of lack of ash in densely populated areas using electricity for cooking. The ash, especially that with a high pH (>9) is useful in affecting chamber conditions and helps kill pathogens in primary and secondary treatment sanitation systems (Moe and Izurieta, 2004; Austin and Cloete, 2008; Niwagaba *et al.*, 2009).

The wet mass of faeces and toilet paper is about 60 kg per person and year, while the mass of ash in faeces is only about 7 grams per person and day (2.5 kg per person and year) (Vinnerås *et al.*, 2006). The mass loss in incineration is thus 90-96% and only a small mass of ash has to be disposed of or recycled as cover material after incineration.

The disadvantages of incineration in small-scale incinerators include the handling of initially unsanitised faeces/ash mixtures and the need for preparation by drying them to low moisture content (about 10% and preferably less), as the smoke smells very bad if material with a MC above 10% is burnt (Paper III). There is also a risk of air pollution by furans and dioxins, but this decreases when the combustion temperatures reach at least 850 °C and the gases are exposed to this for two seconds or more in the presence of at least 6% oxygen (Nasserzadeh *et al.*, 1995). In large-scale continuous processes, this risk should be low as the start-up time is minimal in relation to total operation time.

The challenge of incinerating faeces with large amounts of loose ash remains to be solved. One way is to use cover materials that can burn, *e.g.* sawdust or a mixture of ash and sawdust. When sawdust is applied as cover material during the collection phase, the smell and fly problems are decreased in the same way as with ash (Kinobe, 2009).

The N losses (90-94%) observed when incinerating (Table 5) are in line with reports by Partridge and Hodgkinson (1977) of estimated total loss of N and S and no loss of P and K during the burning of straw; and with reports by Heard *et al.* (2000) of N losses of more than 90% from the

burning of different types and sources of straw. For other nutrient fractions, Heard *et al.* (2000) obtained average losses of 24% of P, 35% of K and 75% of S in the smoke and particulate matter that drifted away. The incineration of faeces/ash mixtures resulted available P losses in the range 70-90% (Paper III; Table 5), which is considerably higher than that reported by Heard *et al.* (2000) even though the values reported by Heard *et al.* (2000) were measured as fertiliser equivalents ($P_2 0_5$). Depending on the temperature of incineration (450-900 °C), total P solubility in the ash differs. Therefore, the rather high loss of available P could be due to poor solubility in the solution (*i.e.* Bray 1) used to dissolve the ash (E. Otabbong, pers. comm. 2009) in the analysis for available P.

Handling the initially unsanitised source-separated faeces/ash mixture during sun-drying may expose workers to health risks. This can be decreased by the use of protective wear, *e.g.* gloves and face masks. These will probably also assist in breaking possible cultural beliefs against such handling of faeces.

The large losses of N when source-separated faeces are incinerated suggest that this method of treatment is not good when aiming at plant nutrient recycling. However, incineration can be of interest in situations with dense populations, where space for agricultural reuse of treated faeces is limiting.

The MC of ≤10% for the faeces not to smell badly during incineration is hard to achieve. This has the potential to not only pollute the air, but it also generates a negative attitude among users.

In order to sanitise the material, incinerator design and operation should be optimised to ensure that all outgoing material is exposed to high temperatures.

## 3.3 Urine storage and treatment (Papers IV & V)

These studies examined the dependence of organism inactivation on dilution (thus ammonia concentration) and temperature (Paper IV) and how solar exposure affects the speed of organism inactivation and the storage time needed to sanitise urine (Paper V).

In Paper IV, three urine dilutions and four storage temperatures were investigated in triplicate for 7 months under laboratory conditions. The dilutions (urine:water) ranged from undiluted urine (6.0 g $N_{NH3/NH4}$ $L^{-1}$) through 1:1 to 1:3 (1.5 g $N_{NH3/NH4}$ $L^{-1}$), representing urine dilutions that can be found in practice in sanitation systems, and the temperature was studied at 4, 14, 24 or 34 °C to cover a range of ambient storage temperatures that

can be found in various parts of the world. The die-off of *Salmonella* Typhimurium (*S.* Typhimurium), *Enterococcus faecalis* (*E. faecalis*), *S.* Typhimurium phage 28B, enterobacteriophage MS2 and coliphage Φx 174 was studied at the above dilutions and temperatures.

In Paper V, the treatment of urine was studied under field conditions in Kampala, Uganda, during storage for 3 months in 10 L plastic jerry cans kept at three ambient exposures; completely out in the sun (S), partly in the sun adjacent to a wall (W) and inside a ventilated room (R). The experiments were supported by complementary studies on urine in 50 mL centrifuge tubes in a laboratory incubator programmed to mimic a cyclic temperature pattern similar to that obtained from the exposure in the sun in the field study.

The motivation for the studies in Paper V is that most of the available literature has largely examined the die-off of various pathogens in stored urine at constant temperature. However, in real-life small treatment systems, *e.g.* storage in jerry cans, the urine experiences diurnal temperature variations. Direct sunlight to exposed containers has the potential to increase the temperature of their contents. The number of hours of bright sunshine is important to increase the temperature of urine in exposed containers. The estimated monthly average daily relative sunshine duration in Kampala ranges between 0.31-0.62, corresponding to 3.7-7.4 hours (Mubiru and Banda, 2007; Mubiru *et al.*, 2007), which agrees with the estimated 2-3 hours of bright sunshine per day during the studies in Paper V.

In the field study, *E. coli* O157:H7, *Enterococcus* spp., *S.* Typhimurium and eggs of *Ascaris suum* were studied (Paper V). In the laboratory *E. faecalis* was studied instead of *Enterococcus* spp. and the phages studied in Paper IV were included.

### 3.3.1 Dilution and temperature

Dilution of urine affects its ammonia concentration (Paper IV). The calculated concentrations of uncharged ammonia ($NH_3$) in urine:water dilutions of 1:0, 1:1 and 1:3 at 24 °C were 156 ± 11, 60 ± 4 and 24 ± 6 mM respectively (Paper IV). Below un-ionised ammonia concentration of approximately 40 mM, organism inactivation was slow and showed little dependence on ammonia concentration (Figure 9).

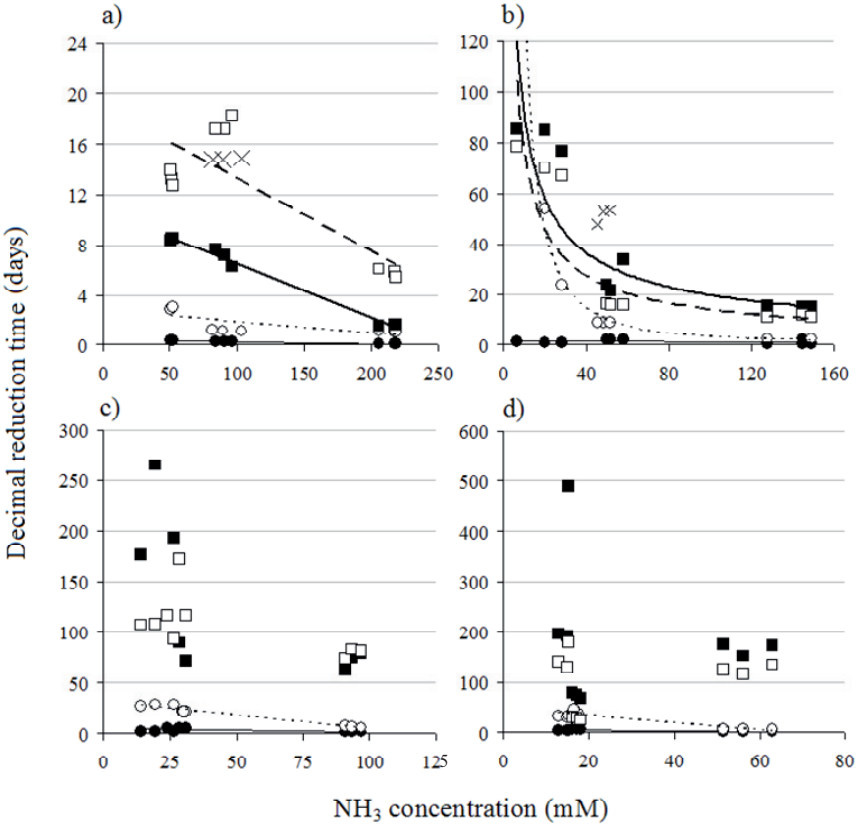


*Figure 9*. Time (days) for one decimal reduction ($t_{90}$) plotted as a function of uncharged ammonia for *S.* Typhimurium (●), *Enterococcus faecalis* (○), MS2 (■) and Φx 174 (□) and *S.* Typhimurium phage 28 B (×) in urine stored at temperatures a) 34, b) 24, c) 14 and d) 4 °C. The correlation trend is indicated with thin lines for the bacteria (*Enterococcus faecalis* broken line) and with bold lines for the phages (Φx 174 broken) (Paper IV).

The pH was only slightly affected by the dilution rates studied, making it difficult to isolate single inactivation effects due to pH alone (Paper IV). The pH values in Paper IV compare well with those obtained in Paper V on urine collected from Uganda. The average pH of the collected urine was 9 and this decreased slightly to 8.8 after spiking with the organisms (Paper V). The less diluted the urine, the smaller the differences in pH between the temperatures (Paper IV). The pH of concentrated urine (1:0) was 9.1 at 4

and 14 °C; and 9.0 at 24 and 34 °C. The pH of urine diluted 1:1 was 8.8 at 4 and 14 °C; 8.7-8.8 at 24 °C; and 8.8 at 34 °C. The pH of urine diluted 1:3 was 9.1 at 4 °C; 8.9 at 14 °C; and 8.7 for both 24 and 34 °C (Paper IV).

At 4 and 14 °C, the reduction in *E. faecalis* and phages (MS2, Φx and 28B) was slow, as shown by large $t_{90}$ values (Table 6), even when free ammonia was 50 mM and the inactivation rate was less correlated with free ammonia concentration (Paper IV). Thus, for storage at 4-14 °C, long retention times up to 6 months are needed to sanitise the urine (Schönning and Stenström, 2004; WHO, 2006).

Table 6. *Decimal reduction ($t_{90}$) in days ±S.D(%) for the bacteria S. Typhimurium, E. faecalis, MS2, Φx 174 and phage 28B at different temperatures and urine concentrations (adapted from Paper IV)*

| Temperature (°C) | Urine:water mix | *Salmonella* Typhimurium[a] | *E. faecalis* | MS2 | Φx 174 | 28B |
|---|---|---|---|---|---|---|
| 34 | 1:0 | <0.1 | <1.1±16 | <1.6±19 | <5.7±36 | 1.5±3[b] |
| 34 | 1:1 | 0.2±3 | <1.2±3 | 6.9±10 | <5.1±5[b] | <15±2 |
| 34 | 1:3 | <0.3±11 | <3.0±38 | 8.4±18 | 13±34 | 14±49[b] |
| 37 | 0:1 | 24[b] | 16[b] | - | 10[b] | 65[b] |
| 24 | 1:0 | 0.6±18 | 2.3±13 | 15±3 | 12±10 | 12±19 |
| 24 | 1:1 | 2.1±5 | 9.1±5 | 25±22 | 16±3 | 51±6 |
| 24 | 1:3 | 1.0±53 | 47±93 | 82±9 | 71±8 | 59±37 |
| 24 | 0:1 | 31 | NR | - | 22 | 80 |
| 14 | 1:0 | <1.2±5 | 6.4±100 | 71±13 | 79±6 | 18±7 |
| 14 | 1:1 | 5.3±7 | 21±2 | 89±24 | 130±21 | NR |
| 14 | 1:3 | 2.3±6 | 28±4 | 200±20 | 100±9 | 169±58 |
| 14 | 0:1 | NR | NR | - | NR | NR |
| 4 | 1:0 | 2.1±9 | 6.3±20 | 160±8 | 120±7 | NR |
| 4 | 1:1 | 6.5±9 | 42±9 | 73±7 | 28±9 | NR |
| 4 | 1:3 | 5.0±7 | 33±61 | 240±44 | 150±15 | NR |
| 4 | 0:1 | NR | NR | _ | NR | NR |

*NR = no reduction detected during 182 days, except for the temperature control (0:1) which was studied during 40 days, [a]For Salmonella, an initial concentration from the batch-wise contaminated urine was included in the function fitting as reduction took place rapidly; [b]performed in 37 °C.*

At 24 °C, the die-off of *S.* Typhimurium was fast irrespective of the dilution according to the $t_{90}$ values in Table 6. The $t_{90}$ values for *E. faecalis* increased greatly (about 20 times) from undiluted urine via 1:1 to 1:3 dilution (Table 6). The $t_{90}$ values for MS2, Φx and 28B differed slightly only in undiluted urine and they all increased to $t_{90}$ values about 5 to 6 times as long for 1:3 dilution (Table 6; Figure 9; Paper IV).

At 34 °C, the reduction rates of all organisms investigated showed a linear correlation with $NH_3$. At this temperature, *S.* Typhimurium and *E. faecalis* were reduced to below detection limit in less than a day and 3 days respectively, resulting in very short $t_{90}$ values (Table 6). This result, as well as the reduction in the otherwise stable phages, with $t_{90}$ of 2 days to 2 weeks (Table 6), indicates that storage of urine for more than 2 months at 34 °C is not necessary, even when it is used to fertilise lettuce. At 34 °C and for urine diluted 1:0, 1:1 and 1:3, no viable eggs of *Ascaris suum* were found from days 7, 8, and 10 respectively and for each day 440 to 1,446 eggs observed (Nordin *et al.*, 2009a). Based on the analyses of *E. coli*, *S.* Typhimurium and MS2, Chandran *et al.* (2009) suggested that undiluted urine stored for just about 1 week at 30 °C should be safe for use as a crop fertiliser.

Generally, a doubling in biological activity is expected for every 10 °C increase in temperature (Madigan and Martinko, 2006). Temperature affects the rate of permeability of ammonia into a cell, with low permeability at low temperatures (Jenkins *et al.*, 1998).

Based on Figure 9 and the short $t_{90}$ values (Table 6), low or no numbers of organisms remain after just short storage times at ≥24 °C when the concentration of free ammonia in urine is above 40 mM, *e.g.* 2.8 g $N_{NH3/NH4}$ $L^{-1}$ and pH 8.8 (Paper IV). At higher pH or temperature, a somewhat lower ammonia concentration would result in the same concentration of un-ionised ammonia ($NH_3$). The sanitising concentration of approximately 40 mM of free ammonia below which almost all organisms showed a much long survival time at 24 °C according to $t_{90}$ values (Figure 9; Paper IV) was exceeded by all dilutions at 34 °C and by 1:0 and 1:1 at 24 °C, whereas at 4 and 14 °C, only the undiluted urine (1:0) reached this dilution (Paper IV). Thus, temperature and free ammonia work synergistically to sanitise urine.

### 3.3.2 Microbial inactivation

The $t_{90}$ values for *E. faecalis* were higher than those for *S.* Typhimurium, especially when the urine was diluted (Paper IV, Table 6). The reduction in *S.* Typhimurium was not strongly related to different urine dilutions studied, as $t_{90}$ was not affected by the 1:1 and 1:3 dilutions and was at most 6.5 days (Table 6).

The reduction in *E. faecalis* at 34 °C was fast regardless of urine concentration, whereas at lower temperatures the undiluted urine resulted in significantly shorter $t_{90}$ than the diluted urine (Table 6, Paper IV). For undiluted urine, the $t_{90}$ was below 7 days at all temperatures, while in the 1:3 dilution it was 33 days at 4 °C. In the temperature controls at 34 °C only a

small reduction was detected during 40 days of study and at 24 °C and below the reduction was slight during that time (Table 6, Paper IV).

In Paper IV, *E. coli* O157:H7, which was studied only in urine dilution (urine:water) of 1:1 at 4 and 34 °C, was found to be more sensitive to ammonia than *S.* Typhimurium. This agrees with the results from ammonia treatment of *S.* Typhimurium and *E. coli* 0157:H7 according to Mendez *et al.* (2004). No breakpoint in the inactivation of *S.* Typhimurium by $NH_3$ was found, with the lowest studied $NH_3$ concentration being 15 mM, which is in agreement with Park and Diez-Gonzalez (2003), who found 5 mM$NH_3$ to be the lowest inhibitory concentration for *S.* Typhimurium and *E. coli* in broth solution. Thus, *E. coli* and *S.* Typhimurium seem very sensitive to uncharged ammonia and will always be killed-off rapidly, even at lower concentrations of uncharged ammonia than are normally found in source-separating sewage systems. *E. coli* and *S.* Typhimurium should thus not be of concern in urine collected and stored for short durations (days to a few weeks) from these systems, especially at temperatures above 20 °C.

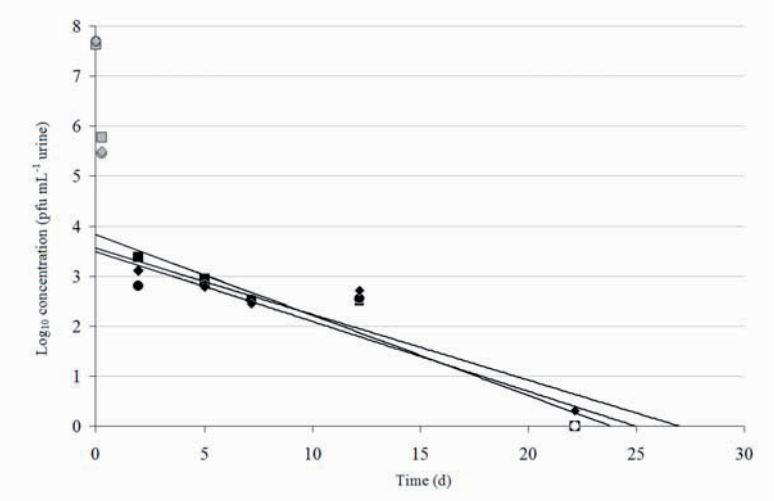


*Figure 10.* Log concentration (pfu $mL^{-1}$) of MS2, studied in triplicate, as a function of time (days) in urine diluted 1:1 at 34 °C showing the breakpoint between two sets of inactivation rates especially given that the grey points are far above the line; and the regression of an exponential function (shown as a straight line as the y-axis is logged) based on the latter rate. Samples excluded from the regression are marked with grey and ND values indicated with unfilled symbols (Paper IV).

Reduction kinetics for MS2 and Φx 174 deviated from first order kinetics, with initial fast reduction followed by a breakpoint, after which a slower reduction took place. This is illustrated by the reduction in MS2 phage in urine diluted 1:1 and stored at 34 °C (Figure 10). The supposedly initial fast reduction phase was excluded from the fitting as it would have overestimated the sanitation efficiency and was explained by artefacts such as adhesion to particles in the urine (Paper IV).

Chandran *et al.* (2009) observed a fast reduction (within 1 week) of MS2 in urine stored at 30 °C and at 15 °C, which differs very much from the model shown in Figure 10 and in Paper IV. Their urine was not diluted; and it was stored for 6 months in closed containers prior to their studies, resulting in a high concentration of ammonia/ammonium of 8.57 g $L^{-1}$. The ammonium concentration in the urine studied by Chandran *et al.* (2009) was about twice that in Paper IV and holding it at 30 °C led to high levels of aqueous ammonia, which is the probable explanation for the rapid sanitation of the urine.

At 4 °C and 14 °C, the $t_{90}$ for Φx 174 and MS2 ranged from 28 to 240 days, with little correlation to urine dilution, and no difference in sensitivity was observed between the two phages (Paper IV). There was little or no reduction in *S.* Typhimurium phage 28B at 4 and 14 °C. This agrees with Höglund *et al.* (2002), who obtained very little reduction in *S.* Typhimurium phage 28B at 5 °C within 200 days.

Höglund *et al.* (2002) reported a considerably shorter inactivation time for rhesus rotavirus ($T_{90}$ = 35 days) compared with that achieved for *S.* Typhimurium phage ($T_{90}$ = 71 days). Studies of the ammonia sensitivity of coated viruses, *e.g.* Avian influenza, also indicate a much faster reduction for coated viruses compared with the phages used in Papers IV and V (Emmoth *et al.*, 2007). Thus, pathogenic viruses seem to be more sensitive to ammonia treatments than the phages studied, thereby giving a safety margin when the urine is treated, as suggested in Papers IV and V.

## 3.4 Urine storage at varying temperatures (Paper V)

### 3.4.1 Organism inactivation

In the field study in Uganda, the fresh urine contained neither *Salmonella* spp. nor *E. coli*, whereas *Enterococcus* spp. were found in high concentrations ($10^6$ cfu $mL^{-1}$ urine). Upon spiking, the initial concentration of all organisms studied was $10^5$-$10^8$ cfu $mL^{-1}$ urine. *S.* Typhimurium and *E. coli* were inactivated rapidly and were not detectable after 24 hours of exposure of

urine at any of the three exposure locations (sun, wall and room). In the complementary laboratory study, *E. coli* and *S.* Typhimurium were reduced from 6$\log_{10}$ to non detectable concentrations (10 cfu $mL^{-1}$ urine) in just 11 and 14 hours, respectively.

*Enterococcus* spp. was much more resistant than the other bacteria studied and in the field study it was detected until the last sampling at day 37 but at concentrations close to the detection limit (10 cfu $mL^{-1}$), with almost similar $t_{90}$ values for all locations (S, W and R; Table 7). In the complementary study, *Enterococcus* spp. reached non-detectable concentration at day 6 ($t_{90}$ = 1.8 days). The inactivation rate for *Enterococcus* spp. was more than 4 times higher in the complementary studies than in the field study (Table 7).

Of the three phages studied, MS2 was most sensitive and on day 22, it was close to the detection limit (1 cfu $mL^{-1}$ urine). The ΦX and *S.* Typhimurium phage 28B, which were sampled for 42 and 48 days, respectively, showed slower inactivation, with $t_{90}$ values of 37 and 55 days, respectively (Table 7).

Table 7. *Reduction coefficients with standard error, k±SE ($log_{10}$ cfu/pfu $d^{-1}$) for the linear regression of exponential inactivation of the organisms studied. Time for 1$log_{10}$ reduction ($t_{90}$) is given as mean/upper 95% confidence interval in days*

| Organism | Study, mean temperature ± daily variation | N | k±SE (-log cfu/pfu $d^{-1}$) | $T_{90}$ (days) |
|---|---|---|---|---|
| *S.* Typhimurium | 24 ±7.5 °C | 8 | 8.19±0.39 | 0.1/0.1 |
| *E. coli* O157:H7 | 24 ±7.5 °C | 10 | 8.40±0.52 | 0.1/0.1 |
| *Enterococcus* spp. | 24 ±7.5 °C | 8 | 0.57±0.05 | 1.8/2.1 |
| | 24 ±6.2 °C | 18 | 0.13±0.01 | 7.7/9.4 |
| | 24 ±6.2 °C | 18 | 0.13±0.01 | 7.6/9.4 |
| | 24 ±6.2 °C | 19 | 0.13±0.01 | 7.7/9.1 |
| MS2 | 24 ±7.5 °C | 9 | 0.12±0.03 | 8.2/16 |
| Φx | 24 ±7.5 °C | 10 | 0.03±0.00 | 37.0/43.0 |
| 28B | 24 ±7.5 °C | 11 | 0.02±0.01 | 55.0/64.0 |

In the field study the viability of the *Ascaris suum* eggs in the sun location was 1% at day 42, while the viability at the wall and room locations was 4±1% and 40±5%, respectively (Figure 11) The NaCl end-controls from day 40-42 showed no significant reduction compared with the initial viability.

In the complementary study, a lag phase with no inactivation or even higher viability than the initial was observed until day 15. From day 24, when the viability had decreased to 12%, a decreased inactivation rate was observed (Figure 11). On day 40 no viable eggs were found (Figure 11). Lag phase models for shouldered survival curves of *Ascaris suum* were fitted with

the best fit for the sun location for an equation giving the die-off for a lag time of 18.5 days and a $T_{90}$ of 27.6 days (Table 8).

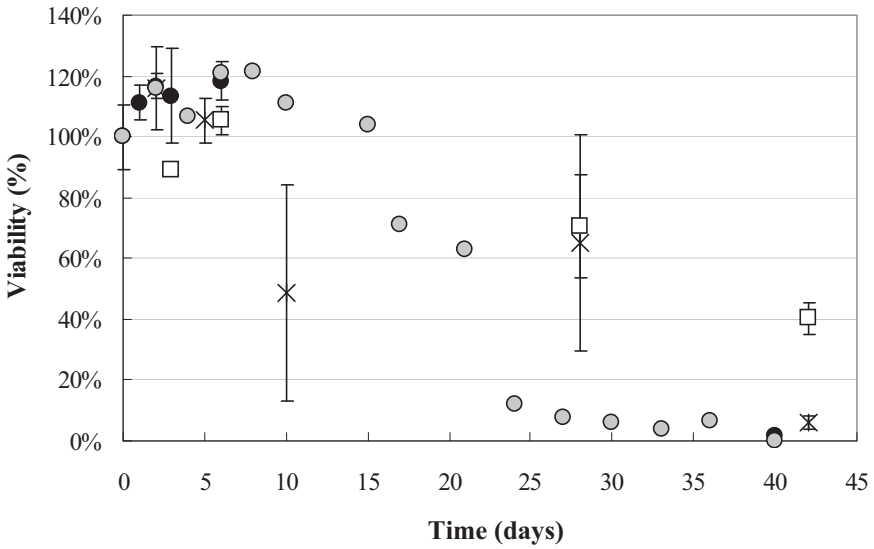


*Figure 11.* Viability of *Ascaris suum* eggs (% of initial viability) incubated in urine in 10 L jerry cans exposed to sun (●), wall (×) and room (□) location. The shaded circles (○) are inactivation data retrieved from the incubator setup with temperature adjusted to mimic the daily temperature changes as in the sun location (Paper V).

Table 8. *Reduction models for the inactivation of Ascaris suum eggs. Times of lag and for 1log$_{10}$ reduction ($t_{90}$) are given as mean/upper 95% confidence interval in days*

| Location | Decay model | Lag (days) | N | p≤ | $T_{90}$ (days) |
|---|---|---|---|---|---|
| Sun (complementary) | $N_t = N_0(1-(1-e^{-0.249t})^{99})$ | 18.5(35) | 14 | 0.01 | 27.6/50 |
| Wall | $N_t = N_0e^{-0.0652t}$ | 0 | 14 | 0.01 | 35.6/54.7 |
| Room | $N_t = N_0e^{-0.0227t}$ | 0 | 9 | 0.01 | 83.3/113 |

For the wall and room locations, the lag phase was less pronounced and a plain exponential inactivation equation was used (Table 8) although due to the few data points and large variation between replicates, linear regressions fitted almost equally well.

### 3.4.2 Container size and colour

In the main study, the variation in temperature in the urine at all exposures as well as the ambient temperatures followed a distorted sinusoidal curve. The lowest temperatures were recorded in early morning, between 04.00 and 08.00 hours. The highest temperatures were experienced between 10 00 and 16 00 hours. The jerry cans in the sun location showed higher

temperatures, especially during the day, than the jerry cans at the wall and room locations. The daily mid-point and bottom temperature amplitudes in the 10 L jerry cans in the sun location were 7.5 °C and 6.2 °C respectively (Paper V). The difference between sun and wall locations was that the sun hit all four sides of the jerry cans in the sun location, while at the wall location, the wall prevented the suns rays from hitting one side of the jerry can directly, *e.g.* three sides were exposed. The daily mid-point and bottom temperature amplitude in the 10 L jerry can at the wall location was between 4.7 °C and 4.3 °C, while that in a similar size jerry can at the room location was much less, just 1.2 °C and 1.1 °C at mid-point and bottom respectively. The minimum daily mid-point temperature in the 10 L jerry can at the room location was 20.9 °C, while it was 21.3 °C and 20.6 °C in the jerry cans at the wall and sun locations respectively (Table 9). Maximum temperature was 22.2, 26 and 28.1 °C for the room, wall and sun locations respectively (Table 9).

Table 9. *Mean day temperatures: average (av), maximum (max), minimum (min) at the sun, wall and room locations for the different colour and size jerry cans at the depths shown for the 10 L and 20 L jerry cans. Ambient temperatures were measured by thermocouples placed at the sun, wall and room 2 locations. Average temperature is given for the two thermocouples in the room location.*

| Container (colour/position) | Sun | | | Wall | | | Room | | |
|---|---|---|---|---|---|---|---|---|---|
| | Av | Max | Min | Av | Max | Min | Av | Max | Min |
| 10L-Yellow middle | 24.1 | 28.1 | 20.6 | 23.6 | 26.0 | 21.3 | 21.6 | 22.2 | 20.9 |
| 10L-Yellow bottom | 23.5 | 26.8 | 20.6 | 23.1 | 25.3 | 21.0 | 21.5 | 22.0 | 20.9 |
| 20L-Yellow middle | 23.9 | 27.2 | 20.8 | | | | 21.6 | 22.0 | 21.0 |
| 20L-White middle | 23.8 | 26.8 | 20.9 | | | | 21.6 | 22.0 | 21.0 |
| 20L-Green middle | 24.1 | 27.6 | 20.9 | | | | 21.7 | 22.1 | 21.1 |
| Ambient | 23.1 | 30.7 | 19.3 | 22.7 | 28.2 | 19.3 | 21.5 | 23.2 | 19.7 |

The 20 L jerry can at the room exposure showed the same lowest temperature of 21 °C. There was no significant difference ($p<0.005$) in temperatures in the 20 L jerry cans of different colours. The amplitude at mid-point in the 20 L yellow jerry can was 1.2 °C lower than that in the 10 L yellow jerry can.

A high temperature and high ammonia content are a good combination to sanitise urine. When combining the results from the present studies (Papers IV and V) with those reported by Nordin *et al.* (2009a), it appears that storage of urine from large systems with a free ammonia concentration of 50 mM at about 24 °C produces a well-sanitised fertiliser within 2 months of storage. At temperatures exceeding 24 °C, a low ammonia concentration can be expected to give the same results, while at

combinations of high temperature and high ammonia concentrations, a much shorter storage time is sufficient, *e.g.* days to a few weeks (Nordin *et al.*, 2009a; Chandran *et al.*, 2009). When urine is stored with daily varying temperatures, the die-off of most pathogens is faster than for storage at fixed temperatures (Paper V). Fluctuating temperatures in combination with ammonia inactivate bacteria and *Ascaris suum* eggs faster than the same average steady temperature. At a mean temperature of 24 °C and daily temperature amplitude of 7.5 °C, safely sanitised urine can be expected after storage for 1.5 months (Paper V).

# 4 General Discussion

## 4.1 Treatment systems for faeces

### 4.1.1 Composting (Papers I and II)

Insulation is important for compost processes, especially on small scale, to reach and maintain high temperatures long enough for the material to be sanitised even under tropical conditions (Paper I and Paper II). Compost reactors lacking insulation did not heat to sanitising temperatures (Paper I) and their average temperatures remained lower than sanitising temperatures and were often just slightly higher or almost equal to the ambient after 2 weeks (Figure 12).

The maximum temperature above the ambient achieved in composting source-separated faeces/ash in non-insulated 216 L reactors was 22 °C, while 42 °C was achieved in insulated reactors (Figure 12; Paper II). A maximum of 34 °C, which was about 15 °C higher than the ambient, was achieved by Björklund (2002) when composting source-separated faeces mixed with soil/lime, garden soil, food waste and garden waste in poorly insulated compost piles in Mexico. He used straw for insulation, which inevitably allowed heat loss through its voids via convection and radiation. When Karlsson and Larsson (2000) composted source-separated faeces mixed with ash in Ethiopia, only about 10 °C above the ambient was achieved in four non-insulated compost piles. Vinnerås *et al.* (2003), on the other hand, insulated their composts by using 200 mm cell plastic on all sides of the compost. This was probably essential in achieving sanitising temperatures (>50 °C), which were maintained sufficiently long (>1 week) in spite of the low ambient temperature of approximately 10 °C.

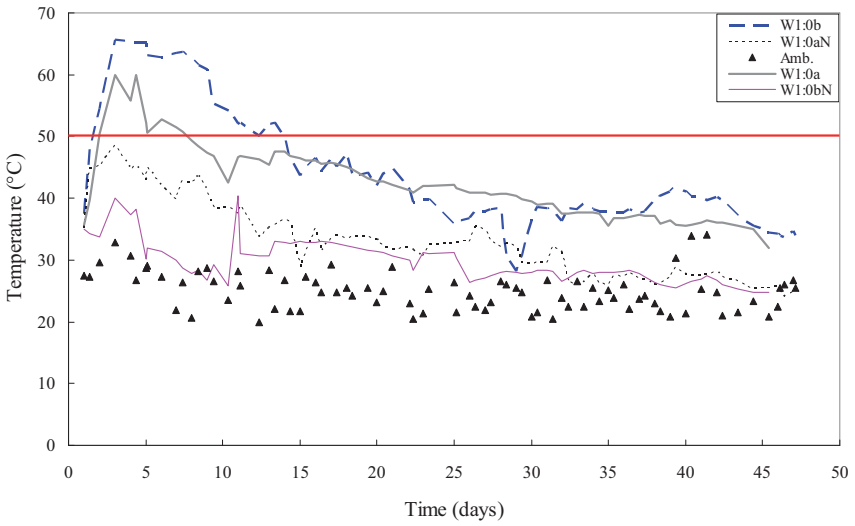


*Figure 12.* Temperature versus time in reactors with similar substrates and with/without insulation in Paper II. The thick lines (dotted/not dotted) represent temperature in compost reactors that were insulated; thin lines represent temperature in non-insulated reactors. ▲is the ambient temperature.

The temperature profile of composts decreases from the centre of the pile to the outside. In order to preserve the heat and thereby expose the majority of the compost material to high temperatures, insulation is needed (Haug, 1993; Epstein, 1997; Vinnerås *et al.*, 2003a). Compost materials are poor heat conductors (Finger *et al.*, 1976) and thus the outer part of the pile acts as an insulator to the inner part of the pile, decreasing the heat loss and thus keeping high temperatures in a large proportion of the pile (Epstein, 1997). However, in composts without external insulation, a larger proportion of the outer parts does not reach high temperatures and these become cold zones where proper sanitation is not achieved (Haug, 1993). Considering that the volume of material in the cold zones of non-insulated composts may be 20-50% of the entire compost pile (Haug, 1993), a significant part of the compost may not be sanitised. Such composts should be mixed by turning, and each time the pile is turned, the redistribution of material should be such that all compost particles are exposed to sanitising temperatures for a sufficient time to be sanitised (Haug, 1993). When sufficient mixing to ensure that all compost particles reach sanitising temperatures cannot be ensured, one way to increase the proportion of the compost in the sanitising temperature zone during composting, and thereby sanitise it, is by providing insulation.

Papers I and II, as well as data in the literature, show that for sanitation by thermal composting, external insulation is a great advantage, and for small composts it is essential. In Papers I and II, insulation was provided using styrofoam packaging material. This is part of the waste stream and is readily available in Uganda, at least in cities.

The energy source of the compost is the fraction of organics in the substrate and the availability of this affects the heat production in the compost. The heat production together with the loss regulates the temperature increase during composting. The relatively low temperature change in insulated reactors containing mixtures with only faeces/ash in Paper II was attributed to low organic matter content, VS (23 and 32%) in these mixtures. This resulted in these substrates not maintaining sanitising temperatures for a sufficient duration necessary for sanitation to occur. The above VS content of the faeces/ash substrate was achieved after sieving the source-separated faeces on a quarter inch (6.35 mm) sieve. Thus, if the source-separated faeces/ash mixture had not been sieved, the VS content would have been lower than the 23 and 32% achieved in Paper II. In the studies by Björklund (2002) the VS of dried source-separated faeces with soil/lime mixture was 27.3%, and after conditioning with garden soil, old compost and food waste, it decreased to only 9.9% (Björklund, 2002). None of these mixtures reached sanitising temperatures, which was partly attributed to low organic matter content.

To increase the organic fraction of the compost substrates, food waste were added (Papers I and II) to the faeces/ash mixtures. This increased the VS and, in all cases where the moisture did not become excessively high (>60%), it also increased the temperature reached and/or the time for which sanitising temperatures were maintained. This is in line with Vinnerås et *al.* (2003), who conditioned source-separated faeces and toilet paper with artificial food waste (dog food) and amendment (straw) and attained a starting organic matter content of 84%. Their compost quickly heated to >50 °C and maintained temperatures >50 °C for about 10 days.

In Paper II, at the 216 L insulated reactor scale, temperatures above 50 °C were reached for substrate mixtures with a VS content down to 23 and 32%. These sanitising temperatures were only maintained for 6 to 12 days, although this was sufficient to reduce *Enterococcus* spp. below the detection limit in one of these cases. Substrates with VS >65% containing food waste maintained temperatures above 50 °C for more than 18 days, sufficient to reduce *Enterococcus* spp. Thus it seems that a VS content of

between 30 and 65% is needed to maintain sanitising temperatures sufficiently long for complete sanitation.

When composting source-separated faeces separately, a limited degree of degradation of organics (30-40%) was achieved (Paper II), whereas when food waste was included and at MC ≤60%, the degradation exceeded 60%. The well functioning composts maintained sanitising temperatures much longer, >2 weeks, when food waste was included in the mixture, than without it (Paper II). High degradation is an indication of high compost activity, resulting in more heat being produced, which increases the temperature. To increase the VS content, the faeces/ash mixture used in Papers I and II was sieved to decrease its ash content. This practice is unhygienic and poses a health risk to the workers and should therefore be replaced by better ways of increasing the proportion of organics of source-separated faeces. From Paper II, it is also clear that adding large fractions of food waste might lead to the MC becoming too high. Thus, alternative ways of increasing the VS are of interest. One such method is to replace the ash used as cover material in the toilet by sawdust (or any other dry organic matter, rice husks, *etc.*) or by a mixture of ash and sawdust. Using a mixture of 50% sawdust (TS) and 50% wood ash (TS) as cover material will achieve >25% VS in the collected faecal fraction, which is in the range of that achieved after sieving in the present studies. However these combinations of ash and sawdust still remain to be tested for their function as amendments during collection, as well as during composting.

Haug (1993) gives a rule of thumb for the amount of water that a compost can contain and still reach thermophilic temperatures. An energy ratio, E (Calculated according to Eqn. 1), below 600 cal $g^{-1}$ and a water ratio, W (Calculated according to Eq. 2), above 10 mean that the thermodynamic requirements for temperature elevation and water evaporation during thermophilic composting are not met. Figure 13, in which the calculated E and W values for all the composts in Paper II are plotted, shows that the rule of thumb for E and W agree well with the results. All well functioning composts, except one, had E>600 cal $g^{-1}$ and W<10. The exception is the W1:0 compost in Experiment 2 (Paper II), which had E=500 cal $g^{-1}$, close to 600 cal $g^{-1}$, while its W was 11, *i.e.* just over 10 (Figure 13). This shows the strength of the simple rule of thumb that E and W are valuable for predicting compost performance.

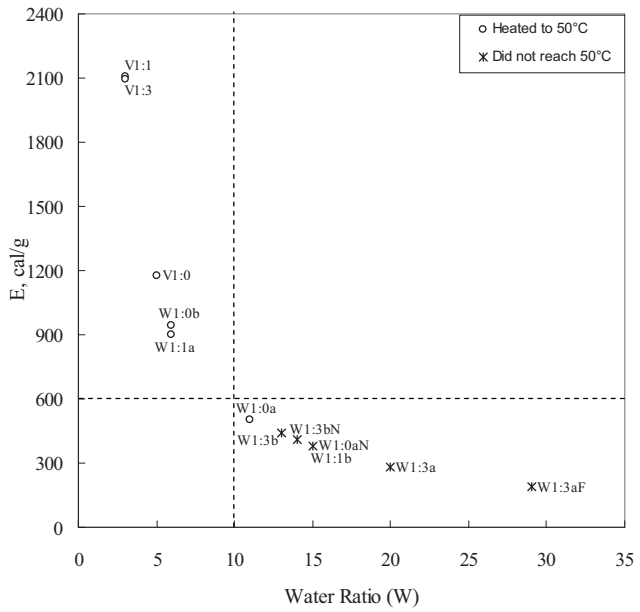


*Figure 13*. Theoretical energy ratio, E (cal $g^{-1}$) versus water ratio, W. Dotted lines are limit values for non-functional composts (Haug, 1993). Descriptions of the compost mixtures are given in Table 1 (Paper II).

The food waste used to increase the organic matter fraction of the source-separated faeces in Paper II presented difficulties in determining the right amount of moisture at the start of the process. This is due to the fact that the fist test (Llewelyn, 2005) or visual inspection to estimate the MC of fresh food waste mixture is unreliable, since a lot of water contained in the initially intact cells of the food waste is not squeezable or visible. This leads to large risks of underestimating the starting moisture of the compost mixture. When the cells of the food waste disintegrate after a few days in the compost, this results in loss of structure and increased amounts of free water (Paper II). Composting substrates at moisture contents exceeding 65% proceeded with decreasing pH, indicating organic acid formation and accumulation under anaerobic conditions (Paper II Figure 6; Sundberg *et al.*, 2004; Nakasaki *et al.*, 2009). Therefore, it is better to start the composting process with a somewhat dry mixture and then add water when needed, rather than risk having too wet a substrate. The effect of the high moisture content of food waste can be dealt with by decreasing the amount added to source-separated faeces or by addition of drier substrates. This was done by Vinnerås *et al.* (2003) when they composted source-separated faeces mixed with artificial pelleted food waste (dog food) that had a dry matter content

of 26.9% and when mixed with faeces and amendment produced a mixture with dry matter content of 28.9%. In addition, Vinnerås *et al.* (2003) added more amendment to the compost after four days in order to increase the dry matter content.

Starting substrates with high pH (9-10) seemed to overcome the initial pH-induced lag phase (Papers I and II) otherwise encountered in composting processes (Sundberg, 2003; Sundberg *et al.*, 2004; Sundberg and Jönsson, 2005). High pH can be achieved by addition of ash. The need for ash, *e.g.* in composting of highly acidic food waste or sewage sludge, is an important management route for the product of incineration processes (Paper III). The application of ash to composts not only helps to increase their pH, thereby avoiding acidic conditions as was experienced in Paper II, but it also acts as a bulking agent and increases dry matter content (Fang *et al.*, 1999; Gaind and Gaur, 2003). In the composts in Paper II that contained high moisture levels (>65%) the pH dropped below 6, hampering the process and the temperature increase (Figure 6). So even with ash amendment to the compost, too high a water content resulted in lower pH level and an inadequately functioning compost.

In the preparation of compost substrates, it is important to carefully estimate the MC of all fractions, especially of any food waste included. The E and W should be estimated in order to ensure there is sufficient energy and organic substrate to manage the ingoing water content. It is better to start with the compost mixture a little on the dry side and then add some water after a few days or if the compost becomes too dry.

The compost produced can reach non-detection levels of pathogenic organisms when the process is managed in such a way that all compost particles attain high temperatures (>50 °C) and maintain them sufficiently long (>1-2 weeks) (Paper I; Paper II; WHO, 2006). Achieving these conditions is complicated, especially for non-insulated composts where the temperature profile decreases from the centre to the edges such that about 20-50% of the material is at sub-lethal temperatures. In such cases, pathogenic bacteria that survive in compost zones experiencing low temperatures may grow and increase in numbers, thereby rendering the compost unsafe. Furthermore, recontamination of the compost pile from external sources and (or) re-growth from undetectable levels is a potential occurrence that can explain bacteria presence beyond the high temperature phase of composting (Papers I and II; Haug, 1993). Re-growth is a problem only for certain types of bacteria indicators and pathogens such as *E. coli* and *Salmonella*. Re-growth is less likely to occur in high temperature areas of a

compost heap than in cool spots (Wichuk and McCartney, 2007). Sidhu *et al.* (2001) suggested that re-growth of bacteria is low in material that contains an active flora, such as that which has been well composted.

Long-term survival (up to 6 weeks) of *Salmonella* spp. in mature, composted sewage sludge at 36 °C has been reported (Hussong *et al.*, 1985). Re-growth of *Salmonella* spp. was not supported in mature non-sterilised compost but occurred in irradiation-sterilised compost at the rate of 0.65 doublings per hour for 24 hours at 36 °C (Hussong *et al.*, 1985). Even longer-term survival (up to 3 months) of *Salmonella enterica* serotype Enteritidis at 25 °C was reported in experimental biowaste composts by Lemunier *et al.* (2005), who also studied *E. coli* and *L. monocytogenes* and found them to survive in 4-week-old compost but not in compost older than that. Studies by Elving (2009) show that re-growth can be associated with the maturity level of the compost. Re-growth was less likely in cold zones of mature compost compared with high levels of growth, over $2\log_{10}$ during one week, of *e.g. Salmonella* spp. in cold zones of fresh compost.

To prevent the re-growth of pathogens in low temperature zones from affecting the safety of the entire compost, the compost should be mixed such that all the particles of the compost experience sanitising temperatures for a sufficient duration to be sanitised, *i.e.* during mixing as much material as possible material should be moved from cold zones to hot zones. Naturally, it helps to insulate the compost to minimise the incidence of zones experiencing low temperatures (less than sanitising, 50 °C). However, even insulated composts need to be mixed well, as the bottom and the zones around the air inlet are normally cool. In Papers I and II, mixing was carried out 2-3 times per week for the first 1-3 weeks when temperatures were >50 °C and composts maintaining >50 °C for more than two weeks were sanitised. Thus, at the scales investigated and in insulated reactors, it seems sanitation is ensured if the temperature is maintained at >50 °C for more than two weeks and during this time the compost is mixed 3-6 times.

Composting at two scales, using 78 L reactors insulated with 25 mm thick styrofoam and 216 L reactors insulated with 75 mm thick styrofoam insulation (Papers I and II) produced noticeable differences in the time at which the high temperatures were maintained. Composting faeces/ash and no food waste (V1:0, W1:0) produced higher temperatures (≥50 °C) that were maintained longer (4-12 days) at the 216 L scale (Paper II) compared with the temperatures (≥50 °C) and time (0-3 days) achieved at the 78 L scale (Paper I). A similar observation was found when composting faeces/ash and food waste in the ratio of 1:1 in Paper I (8 days ≥50 °C) and Paper II

(>18-25 days at ≥50 °C) for functioning composts that were not inhibited by high moisture and low pH. Thus, Papers I and II clearly show that the larger scale is an advantage from a temperature point of view, and this can be explained by the relatively smaller heat losses from the larger reactor, compared with the smaller one. However, two disadvantages were also noted for the larger scale: It was more difficult to thoroughly mix all the material in the larger reactors, which is probably why a longer time at sanitising temperatures (>50 °C) was needed in the larger reactors than in the smaller ones; and quite a lot of food waste was required when filling the large reactors, which makes them difficult to manage on a household scale.

Composting is an intricate process, requiring a high level of skill and expertise to make it function properly, *i.e.* heating the pile and ensuring that all particles of the compost pile hold sanitising temperatures (>50 °C) for a sufficiently long duration (>1-2 weeks) to be sanitised (Papers I and II; Feachem *et al.*, 1983; Haug, 1993; Vinnerås *et al.*, 2003a; WHO, 2006; Wichuk and McCartney, 2007). Based on this as well as the challenges described above, composting does not seem to be a suitable process for sanitising source-separated faeces collected with inorganic cover material in the developing countries because: i) It is a major challenge to achieve a well-functioning thermophilic composting process with the unskilled personnel available to work in such plants in the developing countries, even if they are given much training; ii) the nature of involvement and level of expertise needed for the process to sanitise material limits the possibility for household level applications; and iii) the amounts of organic waste needed for co-composting the faeces/ash mixture are fairly large and will not always be available. In a study in one peri-urban and one rural settlement just outside of Durban in South Africa, the average amount of faecal fraction collected per person and day was 234 grams (just faeces) and that of kitchen waste was 77 grams (H. Jönsson, pers. comm. 2009). One reason for the small amounts of kitchen waste was that a very large proportion of the diet was based on maize porridge of different varieties and the maize meal for this was bought. The families had very little vegetables and meat, and food leftovers were essentially non-existent. Thus, whether there is sufficient kitchen waste depends very much on the local circumstances.

It is important to control the moisture level well and for this the material needs to be protected from rain water. This was achieved by composting under an iron roof shade in Papers I and II. When composting in the open, the effects of rainwater can be decreased by covering the compost, *e.g.* using a polyethylene sheet, which also doubles as an insulator. Insulation has to be

fitted so that there is provision for escape of exhaust gases from the top of the compost.

To perform composting as the treatment process for source-separated faeces, trained personnel are needed. If the required expertise for proper composting is not met, the risk is large that the compost will not be sufficiently sanitised and thus can pose risks to users and consumers. One way to increase the involvement of trained personnel in composting is to engage the private sector to run composting as a business. In situations where no competent skilled personnel are available to perform and oversee composting operations, composting by unskilled personnel is unlikely to meet the necessary temperature and time for sanitation. Thus additional barriers are needed in order to counter the diseases from composts that are either not sanitised or in which re-growth has occurred.

One way to introduce composting of faeces in society might be to include the faeces as an additional substrate at composting plants already having a well performing compost process. In this, the faecal material can be introduced into the hot zones of the already functioning and hot composts, which would minimise the handling of non-sanitised faecal material. To introduce faecal co-composting at such plants, subsidies by the local authority, *e.g.* municipality, may be needed for support until the composting business becomes profitable. To protect the potential market, the local authority should put in place legislation and quality standards that marketable compost should meet.

### 4.1.2 Incineration (Paper III)

Incineration is a compact process that does not require a lot of space. Therefore it is suitable for application in dense urban settlements where there is limited space for the use of treated faeces in agriculture, other treatment processes and/or final disposal methods. The final product of incineration, the ash, is an inert small proportion that can easily be handled. Besides, the ash from the incineration process can be used as cover material in the toilet and when larger amounts are generated from incineration of faeces than can be used as cover materials, the excess ash can be co-composted with highly acidic and wet organic solid waste fractions (*e.g.* kitchen food waste) as described above.

The difficult part of incineration is to prepare the faeces to a moisture content ≤10% in order for the material to burn at high temperatures with acceptable smell, *i.e.* a smell comparable to that of burning cow dung (Paper III). This is compounded by the need to sieve out the loose ashes, which otherwise extinguished the stove used for ignition during the incineration

trials (Paper III). If sawdust could be used instead of ash, perhaps there would be no need for sieving, given that the source of the fire to start up incineration was in fact sawdust (Paper III). Another alternative would be a modified incinerator, where the flames from the ignition stove are diverted horizontally to below the grate, as with this design ash falling through the grate would not affect the function of the stove.

Small-scale incineration can be an alternative treatment method for source-separated faeces in hot climates where it is possible to reach the required MC≤10% and where the recycling of N and P is not a priority. This can be in areas with natural soil fertility, but in other areas the necessary N and P can be supplied by using the urine. The preparation of the faeces for incineration, *i.e.* drying, has to be well managed so as not to present risks of disease transmission to the personnel. In large facilities, it should be possible to use the heat from the incinerator to dry the faeces and to decrease the smell by using the air from drying for the incinerator, thus thermally oxidising the odorous compounds. As in the case with composting, site workers should use protective gear and washing facilities should be provided at the site of incineration.

At high temperatures (800-1000 °C) similar to those achieved in Paper III, it is possible to safely sanitise the faeces by incineration. The design and construction of the incinerator, as well as its firing, should ensure that all outgoing material is burnt at high temperature, *e.g.* with the design described above, so that there is no risk of pathogens in the ashes, especially if they are to be used as cover material in toilets. This is also necessary for achieving low emissions of hazardous gas emissions. In addition, it is important to use high chimneys to dissipate and dilute the smoke from the incinerator high up in the air in order to protect the workers and neighbouring people and to use highly efficient incinerators to decrease environmental pollution (Nasserzadeh *et al.*, 1995).

### 4.1.3 Storage

Storage of faeces is a simple, cheap way to treat human excreta that does not require a high level of technical expertise by the users. However, in storage treatment sanitation systems where other factors, *e.g.* ammonia and temperature, are not sufficient to influence the die-off, whether a cover material is used or not (*e.g.* ash, wood shavings/sawdust, dry soil, sodium hydroxide or straw), storage alone does not sanitise faeces (Austin and Cloete, 2008; Sherpa *et al.*, 2009), unless a long retention time is allowed (WHO, 2006). The storage time is suggested to be at least 1 year at ambient temperatures >20-35 °C and 1.5-2 years at 2-20 °C (WHO, 2006).

Ensuring that the storage time in reality is this long requires good procedures and bookkeeping.

The current design and construction of Ecosan toilets in Uganda comprises two faecal vaults with a filling time of 6-8 months (DWD, 2003) which are used one at a time, so that upon emptying the second vault, the faeces from the first vault are applied in the garden. This system is inadequate considering that faeces will not be sufficiently sanitised by storage during this period and the faeces held in the vault for 6-8 months should not be applied in the garden without secondary treatment to decrease their pathogen content. Attaining hygiene safety using source-separated faeces without secondary treatment would require changes to the design and the construction of two faecal vaults, each with a filling time of 1-1.5 years, since the average temperature is greater than 20 °C. With this design, the faeces would be ready for use in the garden when the second toilet vault was full. However, this would mean that the existing vaults would have to be increased by about 100%, which of course has implications for the cost of the toilet, and would be even more impractical for communal or institutional Ecosan toilets. The alternative is to construct a separate storage space, as has been done at Kalungu Girls Boarding Secondary School. Where storage space is not available, secondary treatment of faeces by prolonged storage becomes limiting. In that case, other secondary treatment processes, including incineration and composting, should be considered. A compact and rapid treatment method that can be applied when lack of space prohibits prolonged storage as the treatment option, and also for offsite treatment, is chemical treatment, *e.g.* using urea. Another way to minimise the risk of disease transmission is by burying the faeces, preferably at shallow depth and in such a way that their plant nutrients can be well utilised.

### 4.1.4 Burying faeces

Burying faeces decreases the spread of pathogens in the environment. A procedure of burying faeces and applying a topsoil cover of 25 cm has been adopted in eThekwini Municipality in South Africa as a secondary treatment measure for faeces from urine diversion dry toilets (Guness *et al.*, 2005). With such systems, it is expected that additional sanitation takes place in the fields due to natural die-off and out-competition by the more resistant soil organisms, while at the same time the spread of pathogens in the environment is minimised. Corrales *et al.* (2006) found higher prevalence of intestinal helminth infections among people using latrine biosolids in agriculture than those burying biosolids. Burying faeces is therefore a simple and inexpensive treatment method for faeces that minimises the handling

and that can also decrease faecal-related diseases amongst the people re-using the faeces. However, faeces should not be buried in areas with shallow groundwater (distance from pit bottom to groundwater should be >1.5 m) or within close proximity (<30 m) to water sources. There are several advantages of burying faeces in such a way that trees/bushes and other plants can utilise their plant nutrients. Plants need large amounts of water, and thus if the faeces are buried close to trees or bushes, the risk of groundwater contamination is minimised as the plants use most, if not all, the water and thus minimise the infiltration to groundwater. Furthermore, trees and bushes protect the ground, and thus the faeces, from being dug up again before they are sanitised.

### 4.1.5 Use of urea

To decrease the storage time for source-separated faeces from urine diversion dry toilets (UDDTs), urea treatment has been suggested (Vinnerås *et al.*, 2003; Vinnerås, 2007; Vinnerås *et al.*, 2009; Nordin *et al.*, 2009a&b) as well as ammonia treatment for urine (Paper IV; V; Nordin *et al.*, 2009a).

Urea is stable and harmless in its undegraded state (Vinnerås *et al.*, 2009) and can be applied easily without posing a risk to the operator. It can therefore be easily applied in household level treatment of source-separated faeces. In closed containers, the ammonia from the degradation of urea can dissipate and migrate through the faecal material and treat it (Vinnerås *et al.*, 2009), thus eliminating the need for unhygienic handling of untreated matter *e.g.* hand-mixing the urea with source-separated faeces. Treatment of faeces by urea may be interesting to farmers who perhaps would have used urea as a fertiliser on their fields. By adding it to the excreta the urea serves as the treatment agent, while the agronomic value of the sanitised faeces is the sum of the value of the urea and of the excreta. For poor people living in urban settlements who cannot afford urea and perhaps with less motivation to perform agriculture due to lack of space, urea treatment may not be an option for treating their excreta. However, an interesting innovation is the single-use, self-sanitising biodegradable toilet (Peepoo bag) which is basically a 25 μm thick 15×40 $cm^2$ bag containing a thin layer of urea. It is priced to be affordable to poor people (Vinnerås *et al.*, 2009). During defecation the Peepoo bag can be held by hand or placed in a small cut bottle, *e.g.* 1.5 L polyethylene terephthalate (PET) bottle, after which the bag is sealed with a simple knot. The management of the excreta can then be handled by the private sector, possibly engaged in agriculture or handling and/or processing the Peepoo bags further and selling the products as fertiliser to farmers.

## 4.2 General discussion/recommendations on faecal management

The current recommendations for the treatment of source-separated faeces for safe plant nutrient recycling according to WHO (2006) are: 1) Storage at ambient temperature (2-20 °C) for 1.5-2 years; 2) storage at >20-35 °C for >1 year; 3) alkaline treatment at pH>9 followed by storage for >6 months at temperature >35 °C and moisture content <25%; 4) and composting at >50 °C for >1 week as a minimum requirement, suggesting that longer times should be aimed at, especially when the temperature of >50 °C cannot be ensured for all particles in the compost. In addition, WHO (2006) suggests that the above recommendations be supported by a multi-barrier approach as presented in section 4.5. Depending on the scale of composting and when mixing during the high active phase is done manually and is thus more inefficient at large-scale than small-scale, composting at >50 °C may reach sanitation after 1-2 weeks (Papers I and II). Furthermore, composting of just source-separated faeces/ash mixtures separately often does not reach 50 °C and even when it reaches this temperature it does not maintain it for long enough for the material to be sanitised. Thus, source-separated faeces should be mixed with substrates containing a high fraction of easily degradable organics in order to be sanitised by composting.

The studies in Paper III suggest that incineration can be performed as a treatment and sanitation method of source-separated faeces provided that it is possible to dry them to low moisture content (≤10%). Incineration should be the aim in densely populated areas, as the possibility to reuse ash in toilets solves the lack of space for their disposal and also the lack of ash.

While sanitation of composts can be achieved during one week of composting at 50 °C, the volume of material, which affects the possibility to mix it well, especially when mixing is manual, affects the possibility to sanitise the material during this period. Thus, compost management should aim at keeping temperatures greater than 50 °C for 2 weeks, supported by mixing 4-6 times during this time.

According to Nordin *et al.* (2009a), treatment of faeces with 2% urea sanitises it in 2 months at 20-30 °C and in 2 weeks above 30 °C with respect to *Ascaris suum*; while with 1% urea, *S.* Typhimurium reduction levels that meet the requirements for safe reuse of faeces as fertiliser (*i.e.* 6 $\log_{10}$ reduction) are reached in 2 months at 14 °C or within 1 week at 24 °C and 34 °C (Nordin *et al.*, 2009b).

## 4.3 Treatment systems for urine (Papers IV and V)

The urine from urine diversion dry toilets (no mix and low flush systems) is sometimes diluted (urine:water) to about 1:0-1:3 (Jönsson *et al.*, 1997; Jönsson *et al.*, 1999). In small systems, *e.g.* household level, urine whether diluted or undiluted can be used immediately upon excretion (Jönsson *et al.*, 2004; WHO, 2006). In large systems, there should be restrictions on the use of urine as fertiliser on food crops if it has been stored at temperatures <20 °C, due to the high risk of survival of viruses as shown by the persistence of Φx and MS2 in Paper IV. This agrees with results of studies of parasites in sewage sludge, using indigenous *Ascaris* (Pecson *et al.*, 2007) and *Ascaris suum* (Pecson and Nelson, 2005). The inactivation of *C. parvum* in ammonia solution infected with oocysts from calf faeces (Jenkins *et al.*, 1998) and in urine (Höglund and Stenström, 1999) as well as *S.* Typhimurium phage 28B (Höglund *et al.*, 2002) was slow at low temperatures in combination with low ammonia content (Jenkins *et al.*, 1998; Nordin *et al.*, 2009a). The results from Paper IV show that the urine with uncharged ammonia concentration in excess of 40 mM at temperatures above 20 °C can be used for unrestricted use after storage for only 2 months in large systems, based on the criteria of: $6\log_{10}$ reduction in *S.* Typhimurium, which had a $t_{90}$ value of less than one week (Table 2; Paper IV) and a $4\log_{10}$ reduction in *C. parvum*, which had a $t_{90}$ value of less than two weeks (Jenkins *et al.* 1998). These two are the major zoonotic microorganisms, as some *Salmonella* can be excreted in the urine (Faechem *et al.*, 1983) and thereby result in high concentrations in the urine, while *C. parvum* is found in the faeces and is thereby expected to be found in lower concentrations in the collected urine. *E. coli*, *S.* Typhimurium, *E. faecalis* and MS2 all had low $t_{90}$ values, both at 24 and 34 °C, and they agree well with data in Chandran *et al.* (2009), confirming that urine diluted/undiluted (urine:water of 1:0/1:1) is sanitised rapidly during storage at high temperatures. The inactivation does not seem to be connected to one single factor but the combination of temperature and ammonia concentration seems to regulate the speed of inactivation.

Several studies indicate that there can be an effect from varying the temperature on the speed of inactivation. At the temperatures investigated in Paper V (average 24 °C), the inactivation of *S.* Typhimurium and *E. coli* was so fast, $t_{90}$= 4 and 5 h respectively, that it was not possible to determine any effects of temperature fluctuation. The inactivation of *E. faecalis* and MS2 at average temperature±amplitude 24±7.5 °C (Paper V) was faster than inactivation of these organisms at a constant average temperature of 24 °C (Paper IV). The inactivation of phage S. Typhimurium 28B ($t_{90}$=55 days) at

24±7.5 °C (Paper V) was comparable to the inactivation at a stable temperature of 24 °C ($t_{90}$=51 days) (Paper IV). For Φx the inactivation was slower at the variable temperature ($t_{90}$=37 days) (Paper V) compared with the static temperature ($t_{90}$=16 days) (Paper IV). Therefore, the effect of the change in temperature on viral inactivation is unclear and requires further studies.

*Ascaris suum* egg inactivation in Paper V exhibited a lag phase, also observed by Ghiglietti *et al.* (1997) and Nordin *et al.* (2009a). The inactivation of *Ascaris suum* eggs at fluctuating temperature (average 24 ºC and amplitude 7.5 ºC) reached the no viability level at day 40 in the laboratory study (Paper V). At constant temperatures of 34 ºC and 24 ºC for the undiluted urine (pH, 9-9.1), the time to reach the no viability level for *Ascaris suum* was 7 and 73 days respectively (Nordin *et al.* 2009a). The cans with wall exposure in the field study had the same average temperature (24 ºC) as those with sun exposure but had smaller amplitude, about 4.7 ºC instead of 7.5 ºC, and the viability at the end of the study was 4% at the wall (3 repetitions) compared with 1% in the sun (1 observation). In the cans stored in the room, which had both lower average temperature of 22 ºC and lower amplitude (1.2 ºC), the 40% viability at day 42 was considerably higher than for the sun-exposed cans.

The temperature-dependent inactivation by ammonia and the effect of increased amplitude, which is shown by the differences in inactivation for the different locations and by comparing with Nordin *et al.* (2009a), may be explained by increased permeability of the shell lipid layer with increased temperature (Wharton, 1980). This temperature-ammonia synergy was also confirmed by Nordin *et al.* (2009) when increasing temperature from 24 to 34 ºC, which reduced *Ascaris suum* egg inactivation time by 80% at equal $NH_3$ concentration and pH.

The faster organism inactivation at varying temperature seems to mainly be connected with organisms that are active outside the host, *i.e.* bacteria and parasites, and not viruses (Paper V). The higher inactivation of bacteria and parasites for a temperature amplitude of similar average to a stable temperature agrees with Semenov *et al.* (2007), who studied *E. coli* 0157:H7 and *S.* Typhimurium in small microcosms at 7, 16, 23 and 33 °C at daily temperature oscillations with three amplitudes (0, ±4, ±7 °C) and found their survival to significantly decrease with increasing mean temperature and increasing amplitude. Scherm and van Bruggen (1994) suggested that the linearity of the temperature response implies a relatively greater sensitivity to temperature temporarily higher than the mean compared with temperatures temporarily lower than the mean, resulting in low organism survival at

fluctuating temperatures. In addition, the inactivation at similar ammonia concentrations with different temperatures shows a faster inactivation at higher temperatures (Paper IV; Nordin *et al.*, 2009a).

Based on results from Höglund and Stenström (1999) of *C. parvum* reaching non detection levels (1/300) after about 2 months, an approximate reduction of $6\log_{10}$, and the measurements of *Salmonella* and phages in Paper IV, it can be suggested that two months of storage of urine at 24 °C are sufficient for the urine to be used to fertilise crops not intended for human consumption. Taking into account the eggs of *Ascaris suum*, about 1.5 months of storage at fluctuating temperatures with average 24 °C and an amplitude of about 7.5 °C for all the urine seems to be sufficient not to cause a risk of re-use in relation to helminths either, and thus this urine could be used in larger systems for food crops, were it not for the risk for viruses. Viral agents, as measured by coliphage Φx 174 and bacteriophage *S.* Typhimurium phage 28B seem to persist longer than two months. However, most human and animal viruses seem to have higher sensitivity than these phages (Grabow, 2001), suggesting that the risk may be lower for human and animals viruses than for the bacteriophages used as indicators for virus survival in these studies (Papers IV and V).

## 4.4 General discussion/recommendations on urine management

The current recommendations for treatment of urine for safe plant nutrient recycling according to WHO (2006) are: 1) Storage at 4 °C for ≥1 month for urine to be used on food and fodder crops that are processed before human and/animal consumption; 2) storage at 4 °C for ≥6 months for urine to be used on food crops that are processed before consumption and on all fodder crops except grassland for fodder; 3) storage at 20 °C for ≥1 month for urine to be used on food crops that are processed; and 4) storage at 20 °C for ≥6 months for urine for use on all crops.

Later studies suggest two months at >20 °C when the ammonia content is approximately 50 mM $NH_3$ and less than 10 days at 34 °C at 50 mM $NH_3$, considering *Ascaris suum*, bacteria and viruses (Paper IV; Nordin *et al.*, 2009a), which agrees well with, although is a little more conservative than, Chandran *et al.* (2009). In the above studies, the criteria of sanitation being achieved were reaching $6\log_{10}$ reduction for bacteria and viruses and no viability for *Ascaris* eggs with more than 1000 eggs being counted ($>3\log_{10}$). The time of treatment for urine in small systems can be considerably shortened by storage of the urine containers in solar radiation (Paper IV). The higher the peak temperatures and the larger the amplitudes reached, the

more efficient the inactivation (Paper V; Semenov *et al.*, 2007). By using solar heat it is possible to significantly decrease the storage time for safe reuse of collected human urine.

## 4.5 Multi-barrier approach

Papers I and II suggest that composting should be performed such that >50 °C is maintained for at least two weeks for the compost to be sanitised; and that this should be supported by sufficient mixing during the high active phase. According to Elving (2009), there are higher risks of re-growth of bacteria in fresh compost than in mature compost. Therefore, for increased safety against microbial contamination, the compost should be kept until it is mature.

Furthermore, the storage of urine at mean constant temperatures in Paper IV and at varying temperatures in Paper V, combined with literature values, suggest that storage times of a few weeks at 30-34 °C to 2 months of storage at 20 °C are sufficient to allow for unrestricted use of urine undiluted/diluted to urine:water of 1:0/1:1. At temperatures below 20 °C the reduction rate of parasites, *Ascaris suum*, viruses and phages slows down significantly with $t_{90}$ over 2 months. According to WHO (2006), urine stored at low temperatures should not be used for human consumption of non-processed crops. This agrees well with the studies performed in Papers IV and V. However, the zoonoses investigated show reduction rates so that the storage time needed could probably be shortened in combination with restriction in usage of the fertiliser.

For both faeces and urine, there is also the initial risk of handling initially unsanitised material *e.g.* in collection, transportation and preparation/start-up of treatment processes. In addition to the safety handling procedures proposed earlier, it is important to include additional safety barriers to protect the workers and consumers.

For faeces and urine, safety barriers which should be adopted include allowing for one month waiting time between the time of application of fertiliser and harvest, while faeces should be incorporated into the soil before crop establishment (WHO, 2006). Restriction of crops, for example fertilising non-edible vegetation like trees and flowers or use on crops not to be eaten raw, is another measure that has been suggested (WHO, 2006).

Other safety barriers for the urine include a close-to-ground application technique, which minimises the formation of aerosols, and incorporation into the soil after spreading, thereby not only minimising the exposure but also decreasing ammonia loss through evaporation (Höglund *et al.*, 2002).

Hygiene education and promotion including hand washing with soap after working with excreta should be emphasised for the workers involved in handling and treating faeces and urine.

## 4.6 Practical aspects

### 4.6.1 Management of source-separated faeces

Composting in reactors similar to those investigated in this thesis requires local technical expertise, *e.g.* a carpenter to make the compost reactors and a skilled person to prepare the substrates and to operate and monitor the process. Alternatively, composting can be performed in heaped windrows or in pits, in which case no carpenter is needed. There is a need for mixing space, for example a concrete surface or a polythene sheet, and spades for mixing. However, where available or affordable, a mixer similar to the concrete mixer could be used.

The reactors used in these studies, *e.g.* 78 L (Paper I) for household level use, cost UGShs. 30,000-60,000 (USD 15-30) per unit and the 216 L (Paper II) for use by institutions or small communities of about 300 persons (*e.g.* schools, restaurants and blocks of houses) cost UGShs. 100,000-250,000 (USD 50-125) per unit. The cost of the compost reactors reported here includes materials and labour, and the range is given because of different costs depending on the materials used. Cheaper reactors can probably also be built using locally available materials, *e.g.* waste timber and insulation by old cloths or soil.

Low cost incinerators, like that used in Paper III, can be made in local workshops where the materials required, *i.e.* steel sheets, can be obtained. The incinerators cost about UGShs. 450,000/= (USD 225). An incinerator costing this amount can serve a community estimated to comprise about 500 people, with the requirement for burning being performed occasionally when the faecal mixture has been dried. As such an incinerator does not have to be used daily, it can also be utilised to incinerate community solid wastes that have to be handled by incineration. Therefore, it can be multipurpose and the cost of the incinerator can be shared amongst the community using it.

The drying of faeces is cumbersome and can be difficult to achieve during periods with few or no sunny hot days, *e.g.* during rain periods. However, during extended periods of hot sunny days, the drying process contributes to pathogen reduction in subsequent handling of the faecal matter. Where the space requirement for drying faeces is limited, collection

and management outside dense settlements, perhaps by the private sector, should be explored. This should be possible because most urban areas are surrounded by peri-urban and rural settlements, where space for storage is possible to find; and the distance ranges in most developing cities from just a few kilometres (*e.g.* 1-5 km) to intermediate distances (*e.g.* 6-10 km) or longer (>10 km) depending on the size of the city. Moreover, it is mostly in these areas close to the city that intense agriculture and horticulture are practised and hence nutrient recycling is possible. If collection systems are available, *e.g.* by the private sector, which can also engage in treatment, branding and/certification of treated material, it should be possible to treat faeces by prolonged storage in areas outside the city. The same private sector can perform other treatments *e.g.* composting, urea treatment and incineration in order to obtain sanitised products.

Workers handling initially unsanitised material before treatment should decrease their health risks *e.g.* by using protective gear such as overalls, gumboots, gloves and, when needed, nose and mouth masks. Hygiene education, including hand washing with soap after working with excreta, is probably the most important hygiene measure for workers and households involved in the collection, transportation, treatment and reuse of excreta.

Another relatively simple and inexpensive secondary treatment method is to bury and apply a thin layer of topsoil (25 cm) through which the root system of plants growing in the topsoil can access the nutrients in the excreta, thereby reusing the nutrients and improving agricultural productivity.

For verification of the composting and incineration processes, analyses of important process parameters are needed. However, such analyses, *e.g.* for physical and chemical parameters, require good laboratories and equipment such as pH meters, dry matter ovens and organic matter furnaces. Pathogen die-off studies require good microbiology laboratories, as well as trained laboratory staff for performing the analyses. Once the process has been verified and the personnel trained, the visual process control supplemented with temperature measurements should suffice.

Capacity building for the community and private sector, *e.g.* education/ training and awareness creation are needed in order to increase the technical expertise to manage the treatment processes for faeces and to increase acceptance for reuse of treated faeces.

### 4.6.2 Management of source-separated urine

In Uganda, the present practice in urine management is to collect it in 5-20 L plastic jerry cans (Mukwano Industries Uganda Ltd), and then use it

immediately on small scale or store it for periods ranging from one week to 2 months. In a few cases, *e.g.* at institutional level, polyethylene tanks (Crestanks Uganda Ltd) of varying sizes ranging from 100-1000 L capacity are used for urine collection and storage. Underground tanks consisting of a pit lined with heavy duty liner have been proposed for larger storage volumes (Vinnerås and Jönsson, 2007).

The 20 L jerry cans used in the study of inactivation of urine during storage (Paper V) are used for packaging cooking oil. Therefore, these containers are available at a low price on the local market and are already being used to collect and store urine in Uganda. Thinner containers (and perhaps also smaller capacity), as often used in solar water disinfection (SODIS) technology for drinking water, can be used in order to increase the surface area exposed to solar radiation and increase contact, thereby increasing the temperatures and amplitudes to disinfect the urine. A typical type is the PET bottles of 1-3 L capacity that can be exposed for 5-6 hours in the sun in a similar way as is done for SODIS (Wegelin *et al.*, 2001). Considering that about 1.5 months seems to be sufficient to achieve safety for most pathogens in urine stored at average temperature±amplitude of 24±7.5 °C (Paper V), a shorter storage time can be used when the average temperature and amplitudes exceed these. Simple techniques for increasing the temperature of the urine tanks can also be used, *e.g.* solar reflectors, thereby giving a high amplitude and thus a faster reduction in pathogens.

In underground tanks and large surface tanks, the temperature is likely to be fairly stable, with little or no amplitude. Thus, under tropical conditions where ambient temperatures of ≥20 °C are achieved, storage, *e.g.* for about 2 months may be performed to disinfect the urine. Under such conditions, it should also be ensured that the containers in which the urine is stored are kept closed as tightly as possible in order to avoid ammonia loss, and this is more important at high temperatures.

### 4.6.3 Transportation and handling

The transport and handling of initially unsanitised material to be composted is a management challenge that presents a health risk to the workers and to some extent also to the public, *e.g.* along the route where the material is transported. For the workers, this problem can be solved by use of protective gear, *e.g.* nose and mouth masks, gumboots and overalls, combined with washing facilities for cleaning up after work. For the general public, it is important to transport the unsanitised faecal material in sealed containers in order to avoid spills. In the case of accidental spills, the

accident site should immediately be well cleaned up. Equipment for this should be carried by the transport vehicle.

Furthermore, the transport and handling of urine and faeces may be costly and thus low-cost transportation methods need to be investigated.

# 5 Conclusions and Recommendations

- To successfully thermally compost dry source-separated faeces/ash in small to medium-sized reactors and keep sanitising temperatures (>50 °C) for sufficiently long (at least 2 weeks) to disinfect the material, source-separated faeces should be mixed with material containing easily degradable organics (*e.g.* food waste) to an organic matter content of at least 30-70% and the reactor should be well insulated.
- For successful composting of the above-mentioned materials, the moisture content should not exceed 60%. The compost process turns anaerobic when composting substrates at moisture content exceeding 65% and thermal sanitising temperatures are not reached.
- Thorough mixing of the material (2-3 times per week for at least 2 weeks during the high active phase of composting) is needed to ensure that all material is exposed to high temperatures and thereby sanitised.
- The difficulty in achieving the required operating conditions to sanitise by thermal composting of source-separated faeces collected with inorganic cover material hampers the application of this process.
- The water content for successful small-scale incineration of faeces, without excessive and unbearable odour, should be ≤10%. The incinerator should be designed so that loose ash falling through the grate is safely sanitised and does not extinguish the fire.
- The expected mass loss and the possibility to use ash from the incineration of source-separated faeces/ash in the toilet as cover material, thereby simultaneously solving the commonly encountered problems of lack of ash and the cost of excreta disposal, could make

incineration an interesting faecal treatment option in high density peri-urban areas.

- The large losses of N (90-94%) during incineration suggest that this method is not good when the aim is plant nutrient recycling.
- The dilution of urine either intentionally or by water from the low flush sanitation system affects the ammonia concentration and thus organism inactivation, especially at temperatures <20 °C.
- For safe, unrestricted use, when urine has 40 mM of uncharged ammonia ($NH_3$) at temperatures >20 °C, the storage time of 6 months needed according to WHO guidelines could probably be shortened to about 2 months. However, to be safe in respect of viruses, more than 2 months of storage are needed.
- At 34 °C, where the concentration of $NH_3$ was above 40 mM even when urine was diluted 1:3, fast inactivation of all organisms was observed and the storage time for unrestricted use could probably be shortened to about 10 days.
- Fluctuating storage temperatures that can be achieved under natural ambient conditions due to temperature differences between day and night have an additional tyndalisation effect that stresses the pathogens, thereby increasing their die-off.
- *Escherichia coli* and *Salmonella* spp. should be of no concern in urine of 4±1.5 mg $L^{-1}$ $NH_4$-N and pH 9 when stored at temperature/amplitude of 24±7.5 °C for more than one day, as their die-off is high, reaching no detection within a few hours.
- It is recommended that storage and urea treatment be further developed as simple treatment methods for source-separated faeces, and that composting and incineration only be used when a high level of technical expertise is available to operate the systems.
- It is recommended that urine be treated by storage in completely closed containers exposed to the sun in order to maximise the synergistic effect of ammonia and increased temperature and amplitude.

# 6 Remaining Issues and Research Needs

One way to increase the organics of collected source-separated faeces for composting and/or incineration is to replace wood ash by sawdust or a mixture of wood ash and sawdust as cover material(s). Therefore, there is a need to investigate the composting/incineration processes when sawdust or a mixture of wood ash/sawdust are used during the collection phase.

Immediately introducing the unsanitised material to the inner hot parts of the pile and then, after its sanitation, moving it for maturation in the outer part of the pile would be interesting. In this way the material would be sanitised already when it is mixed for the first time, thus decreasing the health risk to the workers.

Material handling in and around the compost and incinerator needs to be studied in order to devise good and practical handing instructions to minimise the risk of pathogen spread.

Further studies need to be undertaken to find appropriate means of reducing smells and hazardous emissions from local incinerators and associated material handling. The incinerator design needs to be optimised for safe treatment of faeces.

Studies on how to increase temperatures to sanitising levels and how to achieve large daily temperature amplitudes to stress pathogens to death could be valuable to decrease the storage time needed for sanitising urine and faeces.

There is a need to investigate cheap and resource-efficient collection systems and to stimulate private sector involvement in these practices, including treatment, branding/certification and sale of treated excreta.

Finally, studies are needed to determine the social and cultural acceptability, as well as impediments regarding the use and adoption of the different treatment methods for the faeces. Such studies are also needed for the re-use of the end products, compost, ash and stored faeces, as fertiliser.

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