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76

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Development of a Continuous Single Chamber Vermicomposting Toilet with Urine Diversion for On-site Application

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Zusammenfassung

Menschliche Ausscheidungen stellen in Entwicklungsländern noch immer ein ernstzunehmendes gesundheitliches Risiko dar. In den meisten Ländern ist das Fehlen angepasster technischer Lösungen die Haupteinschränkung für die Implementierung von funktionierenden Entsorgungssystemen.

In dieser Arbeit wurde ein Durchfluss-Modellreaktor für die Behandlung von Fäkalien durch Wurmkompostierung untersucht. Der Komposter wurde für Bedingungen konzipiert, wie sie in Systemen mit urinseparierenden Trockentoiletten zu finden sind, und basierte auf vertikaler Beschickung und kontinuierlicher Behandlung mit Regenwürmern der Art *Eisenia Foetida*.

In dem Testsystem bewegte sich das fäkale Material, das zur Bestimmung der Retentionszeit mit unterschiedlich gefärbten, biologisch nicht abbaubaren Glasmarkern versetzt und kontinuierlich in dünnen Schichten auf den oberen Teil des Reaktors aufgebracht wurde, nach unten und kam anschließend in Kontakt mit den nach oben wandernden Regenwürmern. Untersuchungen zum Einfluss des Feuchtigkeitsgehalts sowie zur Hygienisierung wurden allerdings im Chargenbetrieb durchgeführt.

Das Ziel dieser Studie war es, die Vermikompostierung als Behandlungsmethode für getrennt von Urin erfasste Fäkalien in einem kontinuierlichen Einkammer-Trockensystem zu untersuchen. Dazu wurden 1) die Hauptdimensionierungskriterien, 2) die optimalen Umgebungsbedingungen (im Chargenbetrieb) und 3) die chemische und die mikrobielle Kinetik des Prozesses ermittelt. Die Studie zielte darauf ab, die folgende Frage zu beantworten: Wie beeinflussen Dimensionierung und Umgebungseinflüsse den Abbau der organischen Verbindungen und die Dynamik der Nährstoffe? Zwei wichtige Dimensionierungsparameter, nämlich die oberflächenbezogene Beschickung des Komposters und die anfängliche Besatzdichte mit Würmern wurden in den kontinuierlichen Kompostern mit drei unterschiedlichen Beschickungsraten und zwei Besatzdichten untersucht. Der Einfluss des Wassergehalts der Fäkalien wurde im Bereich von 60 bis 80 % im Chargenbetrieb ermittelt. Die Leistung der Vermikompostierung wurde bewertet durch den Abbaugrad des organischen Materials (als Information zur Stabilisierung des Komposts), den Gehalt an löslichen Nährstoffen (organisch gebundener Kohlenstoff, Ammonium, Nitrat und Phosphor), und an Gesamtnährstoffen einschließlich des organischen Gesamtkohlenstoffs, der organischen Feststoffe und des Gesamt-Kjeldahlstickstoffs.

Zur Aufklärung der Hygienisierung wurde die Inaktivierung von sechs Indikatorbakterien (*Enterococcus spp.*, *E. coli*, *Salmonella spp.*, Fäkalcoliforme, *Enterobacter spp*. und *Shigella spp.*), die üblicherweise zur Beurteilung der mikrobiologischen Qualität von Abwasser verwendet werden, in einem Batchversuch mit 60-tägiger Kompostierung des Materials in An- und Abwesenheit von Würmern quantifiziert.

Unter Berücksichtigung der verschiedenen untersuchten Parameter ergab sich eine optimale Leistung (40 % Reduzierung des organischen Feststoffgehalts bei einer mittleren Aufenthaltszeit von 120 Tagen) für eine Beschickungsrate von 1,2 kg Material pro kg Würmer und Tag bei einer anfänglichen Wurmdichte von 2,2 kg Würmern pro m^2.

Ein Wassergehalt der Fäkalien von 70 % erwies sich in Batch-Experimenten als optimal und führte zu einer fast 80%igen Reduzierung des organischen Kohlenstoffgehalts innerhalb von 96 Tagen. Auch Wassergehalte von 65 und 75 % erwiesen sich als geeignet, wobei in der gleichen Zeit 74 bzw. 54 % des organischen Kohlenstoffgehalts entfernt wurden.

Die Reaktionskinetik des Prozesses wurde durch deutliche Veränderungen der Ammonium- und Nitratgehalte sowie des pH Wertes und der elektrischen Leitfähigkeit charakterisiert. Sie wurde von den verschiedenen Auslegungsparametern und Wassergehalten des Testreaktors beeinflusst. In den Kompostern ohne Wurmbesatz konnten keine vergleichbaren Kinetiken beobachtet werden. Als Folge der Kohlenstoffverluste durch die Mineralisierung während des Abbaus wurde in den Kompostern mit Würmern eine Zunahme der Schwermetallgehalte während des Prozesses erwartet. Die Schwermetallkonzentrationen (Ni, Cd, Pb, Zn und Cu) nahmen jedoch durchweg ab. In 36 Wochen wurde eine Reduktion der Schwermetalle zwischen 45 und 90 % registriert.

Die Abnahme der Indikatorbakterien zeigte, dass eine Hygienisierung während der Stabilisierung des Fäkalmaterials sowohl in Gegenwart als auch - meistens - in Abwesenheit von Regenwürmern zu verzeichnen war. Die Keimzahl-Reduzierung war jedoch stärker, wenn Regenwürmer beteiligt waren: *Escherichia coli* (3.74 log Abnahme mit Würmern gegenüber 0.26 log Abnahme ohne Würmer), Faekalcoliforme (5.0 gegenüber 0.29 log Abnahme), *Enterococcus faecalis* (5.22 log Abnahme gegenüber 0.10 log Zunahme), *Salmonella spp* (5.58 gegenüber 0.59 log Abnahme), *Shigella spp* (2.50 gegenüber 1.54 log Abnahme) und *Enterobacter spp.* (3.77 gegenüber 0.36 log Abnahme). Die Indikatorbakterien lagen auf einem Niveau, das zu einer Klassifizierung des Endproduktes in die Gütestufe B nach den US-EPA-Richtlinien für behandelten Klärschlamm führen würde.

Aus den Ergebnissen lässt sich schlussfolgern, dass das Modellsystem bei ähnlichen Umgebungsbedingungen wie im Labormaßstab (Wassergehalt und Temperatur) erfolgreich in den Pilotmaßstab überführt werden kann. Für eine gut funktionierende Wurmkompostierung sollte der Wassergehalt der Fäkalien mindestens 65 % und maximal 80 % betragen. Die Temperatur sollte zwischen 20°C und 25°C liegen. Unter diesen Bedingungen kann bei dreimonatiger Aufenthaltszeit der Fäkalien im Wurmkomposter ein Kompost gewonnen werden, der von der Stabilisierung her einem behandelten Klärschlamm der US-EPA-Güteklasse B entspricht. Für einen ausgereiften Wurmkompost bedarf es jedoch einer längeren Aufenthaltszeit (> 13 Wochen).

Entgegen der bestehenden Auffassung, dass eine Wurmkompostierung keine Option für trockene Sanitärsysteme sei, bestätigt diese Arbeit die Realisierbarkeit sogar bei kontinuierlicher Betriebsweise. Die Einkammer-Wurmkompostierungstoilette ist für dezentrale Systeme anwendbar und sollte daher auch im Pilotmaßstab untersucht werden.

Abstract

Human excreta continue to be a serious health burden in the developing world. Lack of context-relevant technical options is the main restricting factor in implementing viable sanitation schemes in most countries.

In this thesis, a laboratory scale flow-through model reactor for the treatment of faecal matter by vermicomposting is presented. The system was setup to investigate conditions likely to be experienced in urine diverting dry (UDD) sanitation systems based on vertical loading, continuous-flow vermicomposting. In the test system, that was designed to utilize the feeding habits and reproductive cycles of the earthworm *Eisenia foetida*, faecal matter tainted with differently coloured non-biodegradable glass markers and fed continuously in thin layers to the upper part of the reactor flows down and subsequently comes in contact with upward-migrating earthworms.

The aim of the study was to investigate the feasibility of the vermicomposting technology as a treatment method for faecal matter in a continuous single chamber dry sanitation system. The aim was achieved through the objectives of investigating the major design criteria, establishing the optimum environmental requirements and understanding the chemical and microbial kinetics of the process. Thus, the study was designed to provide answers to the questions: How do design parameters and environmental factors affect carbon loss and nutrient dynamics? Two important design criteria: loading rate and initial worm inoculation density, and a process control parameter – moisture content – were investigated. Three loading rates, three stocking densities and five moisture regimes (60, 65, 70, 75 and 80%) were independently studied. The performance of the system was assessed by the rate of organic matter stabilisation and contents of leachable nutrients such as water soluble organic carbon, ammonium, nitrate and phosphorus, and total nutrients including total organic carbon, volatile solids, heavy metals and total Kjeldahl nitrogen.

In addition, the system was assessed with respect to pathogen inactivation by monitoring six sanitation indicator bacteria (SIB) during a 60 day period in order to obtain information about the hygienisation effect of the vermicomposting process. The reactors for this experiment were operated in batch mode.

Considering the various parameters evaluated a feeding rate of 1.2 kg-feed /kg-worm /day with 40% volatile solids reduction after 120 days solids retention time and an initial inoculation density of 2.20 kg worms/m^2, optimized performance.

The 70% moisture content was found to be most favorable for faecal matter vermicomposting resulting in nearly 80 % organic carbon (OC) reduction within a 96 day treatment period in batch reactors. The 65 and 75% moisture contents were found to be adequate with respectively 74 and 53% OC reduction.

The process was characterized by a clear pattern in the change of ammonium and nitrate contents, as well as changes in pH and electrical conductivity at the different design parameters and moisture regimes for the test reactors. For most of the controls, no specific patterns were observed. As a consequence of carbon losses by mineralization during decomposition, increases in the total amounts of heavy metals during the process were

expected. However, the concentrations of heavy metals (Ni, Cd, Pb, Zn, and Cu) consistently decreased by 45 to 90 % in thirty-six weeks, obviously ingested by earthworms which were separated prior to analyses.

The dynamic change in SIB populations showed that the hygienisation effect during stabilisation of faecal matter with and without earthworms both result in pathogen reduction in most cases. However, the reduction was greater when earthworms participated in the decomposition process: *Escherichia coli* (3.74 log reduction with worms vs. 0.26 log reduction without worms), Faecal coliforms (5.0 vs. 0.29 log reduction), *Enterococcus faecalis* (5.22 log reduction vs. 0.10 log increase), *Salmonella spp* (5.58 vs. 0.59 log reduction), *Shigella spp* (2.50 vs. 1.54 log reduction), and *Enterobacter spp.* (3.77 vs. 0.36 log reduction). SIB were detected at levels that would grade the end-product as hygienisation Class B, according to US-EPA guidelines for use of biosolids.

It can be concluded from the results that the model system on the laboratory scale can successfully be applied to a pilot-scale reactor when environmental conditions (moisture content and temperature) are similar to those found to be optimum in this study. For efficient vermicomposting, moisture content should be at least 65% but below 80%. Temperature should be maintained between 20 and 25°C.

Overall, this thesis says that contrary to existing perception that vermicomposting-based treatment is not an option in dry sanitation systems; that it is feasible, even on a continuous-flow basis. Thus the continuous single chamber vermicomposting toilet is a feasible alternative for on-site application, however, depending on further validation at field-level pilot trials.

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1 Introduction

The sanitation situation all over the world has always been problematic. But today, in developing world's urban and peri-urban areas the excreta management situation is dire. Three scenarios can be identified: When sewerage systems are the means of sanitation, treatment facilities are mostly non-functional. Consequently, generated wastewater is disposed untreated. When septic tanks are the main sanitation systems, the faecal sludge generated is amenable only to a few suitable pre-treatment options. When unsewered ('dry') toilets are the predominant sanitation installations, high-strength faecal sludges are generated with yet no treatment options. Consequently, they are disposed of untreated. All three scenarios represent a one-way traffic of resources with consequences on food security, the environment and human health. Innovative sanitation approaches that close material cycles, and protection human and environmental health are needed.

1.1 General Perspectives

Solution of the problems faced by society today, namely; fresh water scarcity, water pollution and decline of soil fertility depends upon how society deals with its wastes, notably how it deals with human excreta (Esrey *et al.*, 1998). The range of strategies used to solve problems raised by excreta, solid and industrial wastes and runoff water is what is referred to as sanitation.

1.1.1 Current global sanitation situation

In 2010, approximately 60 % of the world population had access to any type of improved sanitation facilities (Figure 1.2). Among the 2.6 billion people in the world who do not use improved sanitation facilities, by far the greatest number (72%) is in Asia, but there are also large numbers in Sub-Saharan Africa. This includes 1.2 billion people who have access to no facilities at all (WHO/UNICEF, 2010). They are obliged to defecate in the open or use unsanitary facilities, with a serious risk of exposure to sanitation related diseases. While sanitation coverage has increased from 49 % in 1990, a huge effort needs to be made to expand coverage to the MDG target level of 75 % by 2015. As Figure 1.1 shows, the sanitation coverage varies significantly around the world.

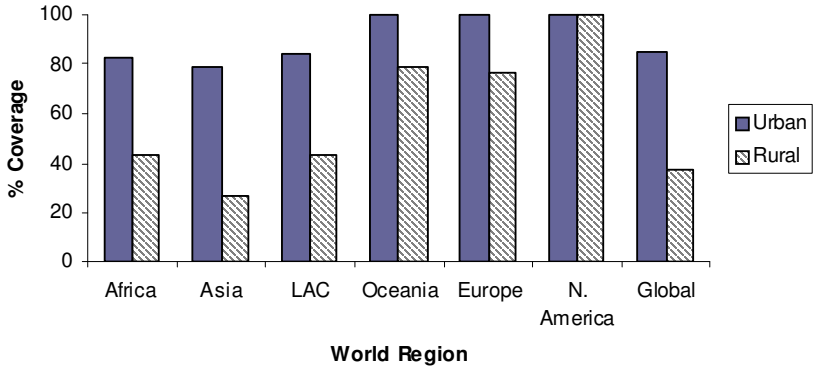


Figure 1.1: Global coverage of sanitation by region (Adapted from WHO/UNICEF 2000, WHO/UNICEF, 2010)

The figure shows that the situation is particularly severe in rural areas where coverage lags behind that reported for urban areas.

While Figure 1.1 shows the differences between regions, it does not bring out the fact that the percentage coverage is barely increasing over time. Figure 1.2, attempts to illustrate this point. Furthermore, the global statistics illustrated in Figure 1.1 hide a critical situation in some of the developing regions, notably in sub-Saharan Africa where coverage is standing still or even decreasing. According to WHO/UNICEF (2010) the sanitation coverage in the sub-Saharan Africa region is a mere 31%.

Considering the shortfall of 40 % in coverage, it is clear that efforts need to be redoubled if the Millennium Development Goals on sanitation must be achieved.

Figure 1.2 shows the world's population is expected to increase from 6.5 billion in 2010 to 7.2 billion in 2015. In 2010, 40 % of people (2.6 billion) had inadequate sanitation. Considering the population trend, meeting the MDG target means an additional 2 billion people will need to be served with improved sanitation. However, 1.4 billion people will remain without improved sanitation. Winpenny (2003) reports that progress towards meeting the MDG sanitation target is the slowest of all MDGs, with an enormous gap existing between the intended coverage and actual reality.

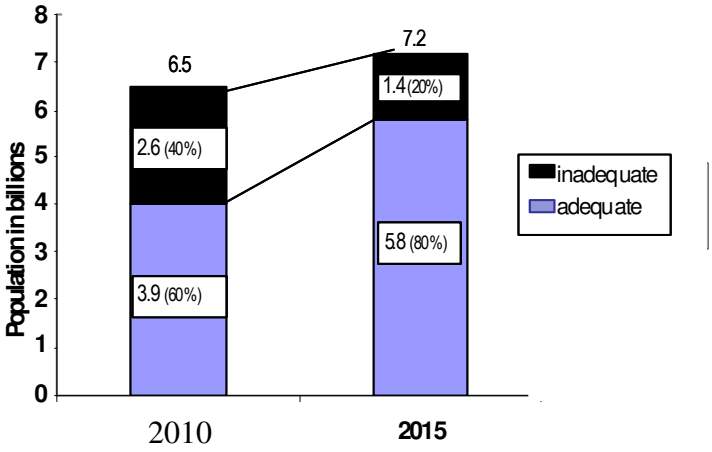


Figure 1.2: Sanitation coverage backlog (Adapted from WHO and UNICEF 2008)

For many reasons, conventional approaches cannot solve the problem. Sewerage systems with centralised wastewater treatment plants are not appropriate for developing countries as they lack the necessary resources such as finance, water, technical and institutional capacity. On-site disposal systems, such as septic tanks or pit latrines that are widely used in developing countries, do not offer a sustainable solution since often they lead directly to groundwater pollution.

1.2 Approaches in wastewater management and sanitation

Existing conventional approaches for managing human excreta fall into two categories: waterborne systems and dry systems. Tayler (2003 *et al.*) describes them as off-site and on-site systems, respectively. Off-site systems (also referred to as 'wet' or 'flush and discharge' systems) remove both faeces and wastewater from the site for disposal or after treatment to elsewhere. On-site systems retain both liquid and solid excretions on or near the site where they are produced. These two systems were developed on the premise that excreta are waste which is suitable only for disposal (Esrey *et al*., 2001).

This section presents a focused review of these two sanitation approaches, with emphasis on their merits and limitations with respect to material recycling, public health and environmental protection.

1.2.1 Off-site sanitation

Off-Site sanitation consists of a sewer network and a centralised wastewater treatment plant (WWTP). In this system, sanitation waste (household wastewater, storm water, municipal and industrial water) is collected and piped to a central WWTP where it is treated mechanically, combined with biological and/or chemical treatment.

1.2.1.1 Historical overview

In the 19th century it was realised that decentralised sewage discharges and handling of human excreta were the main causes of major outbreaks of infectious diseases such as cholera (Reijinders, 2001).

Therefore, in major European cities, sewers were established to conduct the wastewater away from dwellings to nearby water courses, and subsequently into the sea (Cooper, 2001). This approach has been developed and operated for more than one hundred years. In industrialised nations, owing to advanced technological methods, it has been possible to achieve high standards in wastewater management. The developing nations present a different picture, where sewerage systems are the means of sanitation, treatment facilities are either lacking or in poor performance. While sewerage systems decreased the incidence of diseases linked to latrines, they have been implicated in a number of problem areas.

Otterpohl *et al*., (1997) argue that there are several disadvantages that become exceedingly important with today's world-wide promotion of centralisation sanitation concepts when considered on a larger time scale.

1.2.1.2 Merits and limitations of off-site sanitation

Proponents of centralised wastewater treatment concepts contend that the systems are efficient in solving acute pollution problems and require relatively small capacities per inhabitant. Indeed, this sanitation approach offers three main advantages.

It is convenient for the users as they are not responsible for the operation and maintenance of the facilities.
The systems achieve a high removal of organic matter, nutrients and pathogenic bacteria.

It is well adapted to urban areas. The space required for WWTP 0.5 - 2 m2/person.

While the off-site approach offers these advantages it also presents some major problems. These are discussed in the following paragraphs:

The investment and operation costs are high. The construction of a sewer network is expensive. About 65% of the total investments are required for the sewers. Operation and maintaining the system also represents additional costs, especially relating to energy costs.

Water-borne sanitation systems mix the food and water cycle. This wastes valuable nutrient and water resources. Important plant nutrients that could be recycled into food production are flushed away (Figure 1.3) only to be removed again from the wastewater at high cost.

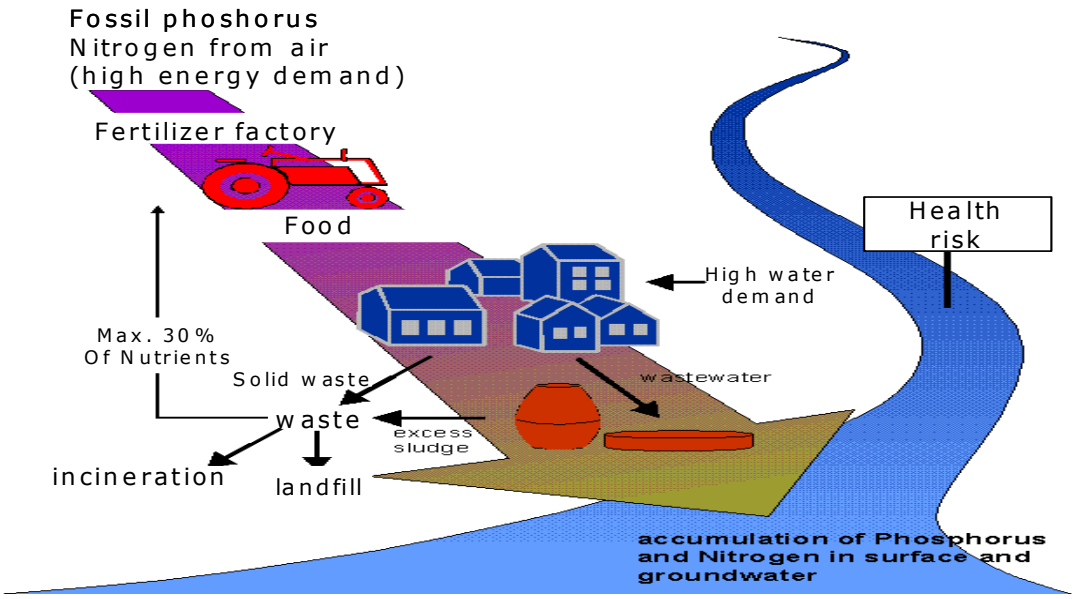


Figure 1.3: The linear flow system of the centralized flushing sewer system (Otterpohl *et al*. 1997)

In most cases, nutrients are not recovered as they are washed away with the purified wastewater and emitted to rivers and the sea where they give rise to another problem, eutrophication. Consequently, more nutrients will need to be produced for agriculture at high energy cost, and causing depletion of fossil resources. These factors impact food security in a world where malnutrition and famines are abound (Ilesanmi, 2006).

The water demand of the systems is high. About 25-40 litres of water are needed for one person daily only to flush the waste to the sewage system (Strauss *et al*., 2000). Annually, an estimated 15,000 litres of water are required to flush away about 400-500 litres of urine and about 50 kg of faeces per capita (Esrey *et al*., 2000). These figures show that flush and discharge requires a huge quantity of water to transport away relatively small amounts of waste and therefore generating a larger volume of polluted wastewater (Esrey *et al*., 2004).

They bring about dilution of nutrients. Sewerage systems not only consume high amounts of freshwater but also dilute nutrients (nitrogen, phosphorus) and organic substances to such an extent that only a small part can be reclaimed for use in agriculture. The mixing of wastewater

streams, (yellow, brown, grey) results in very large, highly diluted amounts of wastewater (Figure 1.3) to be processed, which in turn requires large areas, expensive treatment plants, complex processes and high energy input to achieve desired levels of treatment (Ilesanmi, 2006).

Wastewater discharge impact water bodies and cause environmental problems. Ecological balance is disturbed when nutrients are released into water bodies and a mixing of the nutrient and water cycle occurs. Raloff (1998) reports risks posed by pharmaceutical residues or metabolites entering into water bodies. Groundwater pollution is also a problem associated with this system. Experts concede that it is virtually impossible to operate a sewer system without incidences of leaks. Infiltration of groundwater into the sewer, and the much more serious problem of ex-filtration of wastewater into groundwater, are potential problems in this system, thus the "backbone" of the conventional system – the sewer, is a potential source of contamination of groundwater.

Handling of sewage sludge presents challenges. Sludge is the residual semi-solid material left from wastewater treatment processes. It contains unwanted substances such as heavy metals, pharmaceuticals, pesticides and detergent residues that make its agricultural application problematic in spite of its high content of available plant nutrients. It is therefore another waste to dispose of appropriately often at significant cost.

1.2.2 On-site sanitation

On-site sanitation refers to the range of actions related to the treatment and reuse/disposal of domestic waste water that cannot be transported by off-site systems. The boundary between the two systems (on- site and off-site) is not always as clear and well-identified (Chocat, 1997). In this context, and according to CREPA (2004), one talks about:

There is direct and immediate benefit as long planning and building of a sewer system is unnecessary.
As the produced wastewater flows are small, their treatment is cost-effective and efficient in contrast to the expensive treatment at centralised wastewater treatment plants.
The required calculable investments yield a direct benefit as expensive building and maintenance costs do not incur.
Fertiliser, compost and biogas can be produced instead of disposing of sewage with an undefined mix of pollutants.

On-site systems, such as pit latrines, can be simple and relatively low cost but have many drawbacks as discussed in the following paragraph. Most existing on-site sanitation systems lack faecal sludge treatment. Due to the large volume of the underground pit needed, it is not suitable for densely populated urban areas. Digging a new pit when the old one is full often leads to the question where to build the new one. If the groundwater table is high, or the ground is very hard it is not possible to construct the toilets. In areas where they are used there is the risk that the surrounding groundwater will be contaminated from seepage or from water flowing in during floods. Due to the high humidity levels in the pits, disease vectors breed rapidly, producing diseases such as filariasis, yellow fever and cholera. Furthermore, issues such as bad odour, fly/mosquito breeding, pit collapse or the distance from the house make the case that this cannot be a suitable alternative. Another issue of importance concerns the

agricultural sector. Latrines offer little or no possibility for recycling of nutrients in human excreta.

The main selection criteria for on-site or off-site sanitation are population density and water volume. High population density and high water volume will lead to off-site systems. Low population density leads to on-site sanitation.

1.3 New trends in Sustainable Sanitation

The new trend in sanitation, referred to as sustainable sanitation, focuses on finding locally adapted solutions to sanitation problems. The main objective of a sanitation system is to protect human health by providing a clean environment and breaking the cycle of disease. In order to be sustainable, however, a sanitation system should also be economically viable, socially acceptable, technically and institutionally appropriate, and protect the environment and natural resources (SuSanA 2008-short statement). This statement tells us that because of different regional or local environmental, economic and socio-cultural conditions, sustainable sanitation systems can only be realized in a context-specific way. Otherwise stated; in order for sanitation to be successful, systems that are tailored to fulfil their function in the specific local environment need to be implemented.

Sustainable sanitation encompasses, in general, the following criteria:
Disease prevention. The sanitation system must be capable of destroying or isolating faecal pathogens.
Environmental protection. It must prevent pollution and conserve valuable water resources.
Affordability. The sanitation system must be accessible to the poor.

Acceptability. It must be aesthetically inoffensive and consistent with cultural and social values.
Simplicity. The sanitation system must be robust enough to be easily maintained (Rockström, 2005).

Any sanitation system that meets these requirements is sustainable, be it a centralised or decentralised system. Centralized sewerage systems should not be considered as the only possible solution for sanitation. Both low-tech and high-tech systems with source control can avoid many problems of the conventional on-site and end-of-pipe technologies by respecting different qualities of wastewater and waste and by treating them for reuse. A locally adapted and optimised system can be planned and designed for any community, taking into account local conditions, the water and soil conditions, the agricultural and environmental situation, technical capacity and available financial resources. Otterpohl *et al.* (1997) points out that the wide range of existing technical solutions makes this concept adaptable to various local conditions, ranging from sanitation concepts for urban and rural areas to disaster relief measures and the rebuilding of infrastructure after natural disasters and conflicts.
To sum up, sustainable sanitation emphasises that sanitation should be dealt with as a multi-step process (and not a single point) where waste products are accounted for from the point of generation to the point of ultimate destination. Ideally, resources contained in “wastes” would be used beneficially, which could be referred to as ‘closing the loop’ (Tilley *et al.*, 2008).

1.4 Ecological sanitation

When sanitation practices fail to convert the wastes back into resources, they don't meet the important criteria of sustainable sanitation (Esrey *et al.*, 2000). Ecological sanitation (EcoSan) is an emerging sanitation concept based on an ecosystem approach. This new approach is rooted in the idea that 'waste' and wastewater are not waste. Rather, they are resources that can be recovered and reused.

1.4.1 Underlying principles of EcoSan

The separation of wastewater streams of different qualities and their respective appropriate treatment for reuse is common in industry and is fundamental for this new concept (Otterpohl *et al*., 1999). Therefore, the underlying principle of EcoSan is the separate collection of the different flow streams in wastewater and subsequent targeted treatment of the wastes. Unlike most conventional sanitation methods, ecological sanitation processes human waste to recover materials that would otherwise be discarded. Esrey *et al*. (2003) thus defined an EcoSan system as one that:

Recovers and recycles nutrients and organic matter.
Prevents disease and promotes health.
Protects the environment and conserves water.

These three principles underpin the ecological sanitation system approach.

EcoSan represents a shift in the way people think about and act upon human excreta. Because the various waste flows, notably urine and faeces, are separated at source, the EcoSan approach is sometimes referred to as "Source control sanitation". Figure 1.4 illustrates the cyclical (close loop) flow of materials in EcoSan. The concept is not based on any specific sanitation technology, but is rather a new philosophy in handling substances that have so far been seen simply as wastewater and water-carried waste for disposal. Therefore, any system that offers economically and ecologically sustainable and culturally acceptable sanitation solution that aim to close the natural nutrient and water cycles is an EcoSan system.

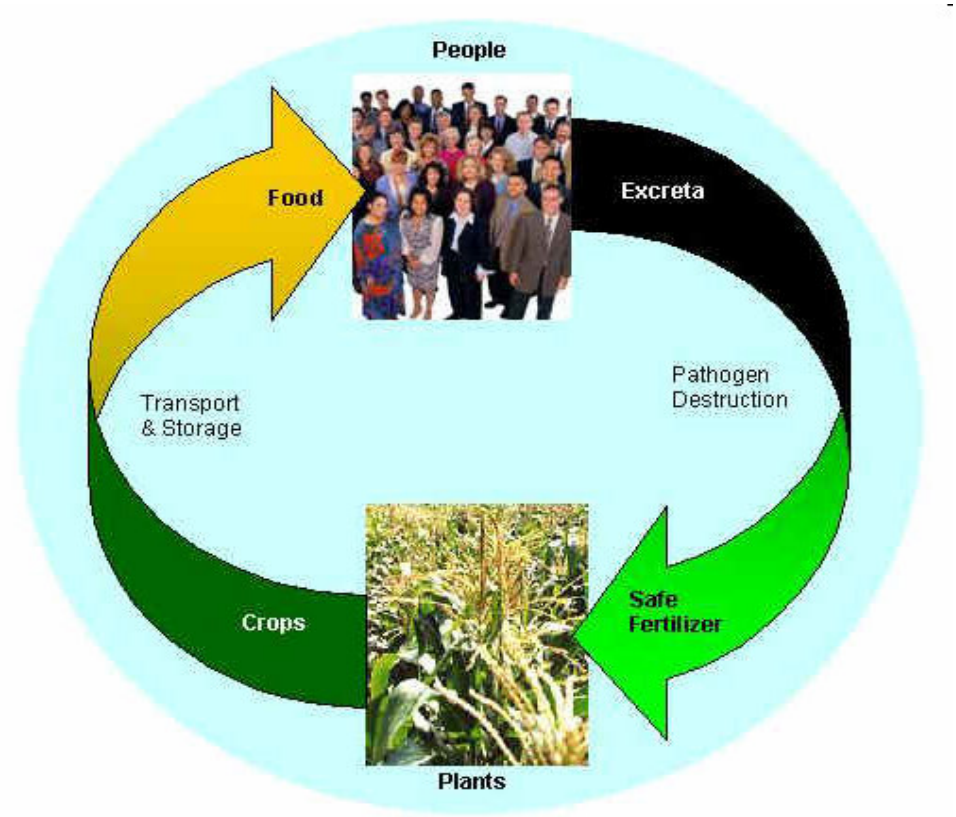


Figure 1.4: System approach to ecological sanitation closes loop on local material flows (Earle 2001)

1.4.2 The necessity of ecological sanitation

Water is a scarce resource as only 1% of global water is drinkable. Often, water of drinking quality is used in flush toilets. The mixing of faeces and urine with flush water and greywater inhibits economic reuse of human excreta. The hygienically unsafe, small quantity of faeces contaminates large amounts of water (Figure 1.5) via conventional flush toilets and sewerage (Otterpohl *et al*., 2004). Very little, if any, recovery of useful products such as fertilizers (nitrogen, phosphorus and potassium) from wastewater is achieved.

As Figure 1.5 shows, there are enormous differences in the individual domestic wastewater flows. The figure leads to three important conclusions:

Very high quantity of grey water that is virtually unpolluted (except for the presence of chemicals used in modern households) is produced domestically. Its treatment usually requires only relatively simple methods.
Organics (COD: 47%) and pathogens (statistics not shown) are mostly concentrated in the faeces. The health risk of wastewater is virtually caused by faeces alone. Separation, with little or no dilution, would allow for proper treatment and production of soil fertiliser.
Although urine is only approximately 1% of the total wastewater amount, it contains most of the soluble nutrients. Separate collection and targeted treatment of urine would make the urine suitable for agricultural reuse. By properly managing urine, treatment costs as well as fertilizer costs can be reduced.

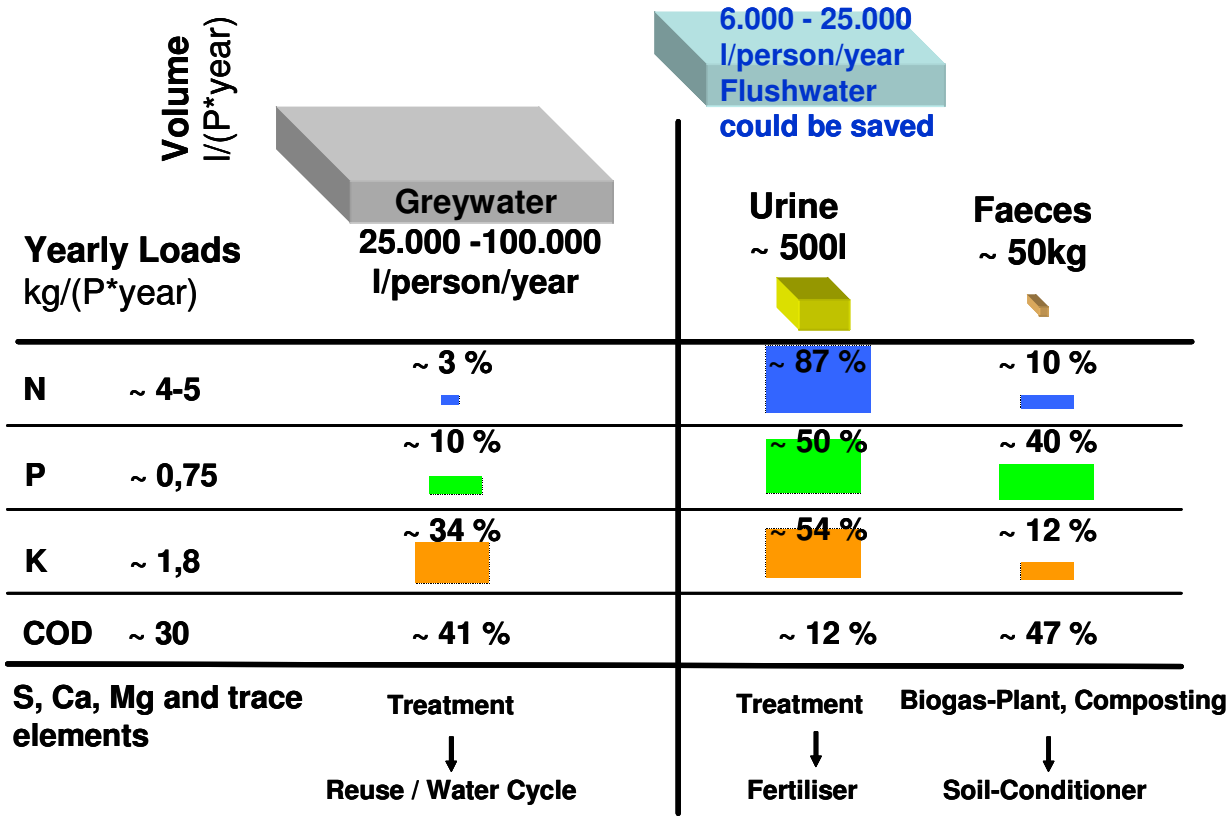


Figure 1.5: Characteristics of the main components of household wastewater and their potential reuse options (Otterpohl *et al.* 1997)

Conventional sanitation systems misplace these nutrients, dispose them off and turn the cycle into a linear flow (Winblad and Simpson-Hébert, 2004). In ecological sanitation, urine and faeces are separated at source and are not mixed with water. This not only saves water but also avoids the contamination of large volumes of water with pathogens. Furthermore, the separation of urine and faeces makes it easier to recover and recycle nutrients such as phosphorous and nitrogen. If separated, urine can be used as a fertiliser after diluting with water. After faeces have been dried, they are free from pathogens, diseases and odour. They can then serve as soil conditioner for agriculture, returning a significant part of the nutrients and trace elements to the soil. The greywater may be used for irrigation after treatment and also for recharging the local aquifer. This closes local cycle, helping to improve food security and to conserve soil fertility.

Ecological sanitation reduces the need for pipelines - the most expensive part of a traditional sewer network and can provide both the poor and the wealthy with sustainable sanitary systems at an affordable cost.
The approach is flexible, and centralised can be combined with decentralised, waterborne with dry sanitation, high-tech with low-tech, etc. By considering a much larger range of options, optimal and economic solutions can be developed for each particular situation.
It eliminates large quantities of blackwater, which is the main fraction carrying disease causing organisms, and pollute water supplies specifically for the poor in developing

countries. Ecological toilet systems for the poor enhance their dignity, quality of life and health.
It saves at least 20-40% of the domestic water consumption (Gardner, 1997). Adding water saving devices or recycling greywater makes it possible to save even more water. This is of key importance since water is a major limiting factor for development in many countries. After filtration, greywater can be used for irrigation, groundwater recharge or even for production of potable water.
The approach enables 80-90% of the nitrogen, phosphorus and potassium in excreta and wastewater to be recycled for agricultural use (Vinneras, 2002). This provides inexpensive local fertilisers that help long-term poverty alleviation through enhanced food production and a series of local business opportunities.
It facilitates energy production from organic waste resources and creates local business opportunities for construction, operation and maintenance of sanitary facilities and sale of fertiliser products.

1.4.3 Advances in Ecological sanitation

In recent years many successful EcoSan programmes have been implemented in various parts of the world, in rural and sparsely settled urban areas. Quite a lot of experience has been gathered in these areas and a variety of solutions are available that can be recommended for large-scale, widespread implementation in accordance with existing local technical, physical, cultural and socio-economic conditions. Although initial experiences with EcoSan systems are available from densely populated urban areas, Huber *et al*. (2004) point out that, there is need for further research and development to gain necessary experience in these more complex areas that would allow EcoSan systems to be implemented on a large scale, to demonstrate the technical feasibility and benefits of this new approach. Consequently, a few challenges still have to be overcome, including:

Increasing awareness of the alternatives offered by EcoSan. The water closet and centralised sewers are perceived as the ultimate solution. Knowledge about the concept of ecological sanitation must be communicated to engineers, decision makers and stakeholders.
Integrating resource reuse needs to be included into sanitation planning processes from the very beginning. Cultural taboos and attitudes can hinder the use of excreta-based fertilisers. Information and participatory planning is important to change this.
Lobbying for the revision of legal frameworks and technical standards. Existing legislation in many countries favours conventional centralised sanitary systems and need to be revised to encompass ecological sanitation.

These challenges can be overcome and ecological sanitation can be introduced as the new, promising, holistic and sustainable approach to providing safe sanitation, reduce poverty, contribute to food security, preserve the environment and maintain the natural basis of life in industrialised, developing and emerging countries (Huber *et al.* (2004).

2 Review of research issues

Having demonstrated that conventional 'wet' or 'dry' sanitation may not be sustainable; this section sets out to provide the background with regards to human excreta management in developing countries. It identifies and discusses the key issues related to source control sanitation. And outlines how some of the major characteristics and thinking relating to excreta management need to change in order to address concerns of public health, environmental degradation and food security. It goes on to present an overview of existing solutions for excreta management, their limitations, and the need for innovative sanitation solutions that are context relevant, protect human health and promote efficient use of resources. Furthermore, it introduces the vermicomposting concept as an innovative excreta treatment option, and identifies the recent efforts made to integrate this technology in source control sanitation. It points out the way vermicomposting uptake occurs, underlining the importance of understanding the process as prerequisite for designing systems for integration into source control sanitation. Then finally, it introduces the continuous flow vermicomposting approach as an innovative alternative to batch systems.

2.1 Background - current practice and problems in human excreta management

Inadequate excreta and wastewater management continue to be responsible for a significant proportion of the world's infectious disease burden. The WHO estimates that worldwide there are 4 billion cases of diarrhoea (resulting in 2.2 million deaths) per year, 200 million people with schistosomiasis, and as much as 10% (about 400 million people) of the population of the developing world are severely infected with intestinal worms (WHO/UNICEF, 2010). Particularly critical are the food insecurity and living conditions in the developing world, notably Africa and Asia where the availability of nutrients for agricultural needs is in sharp decline.

For maximum health protection, it is important to treat human excreta as close to the source as possible before it gets introduced into the environment. This calls for the development of innovative sanitation systems that allow human excreta to be managed at the household or community level.

Compared to wastewater management, little effort has been directed to the development of technologies to treat human excreta adapted to conditions prevailing in developing countries. Consequently, excreta management is still an unresolved issue in several developing countries. Here people live and raise their families in health and life-threatening homes and neighbourhoods, often lacking access to basic sanitation. In rural areas families seek to dispose of their excreta using the cheapest means possible; and where the sanitation systems can not be afforded open defecation in fields and on surface waters is practiced. In peri-urban areas where mostly poor people live in overcrowded, cramped housing conditions, without roads, safe water and drainage facilities; little attention is given to sanitation. Unable to afford the 'expensive' sanitary systems; human excreta, from home bucket toilets, are typically dumped untreated into streams and open drainage ditches. In urban centres, everyday thousands of tons of excreta from on-site sanitation (OSS) installations are discharged untreated into the urban and peri-urban environment – either spread in agricultural fields or discharged indiscriminately into drainage ditches, lanes, onto open spaces and into inland waters, estuaries and the sea (Ingallinella *et al*. 2002), often with severe environmental

consequences. Diseases resulting from the improper management of human excreta are among the main constraints to the economic development of many developing countries. In urban centres of developing countries where OSS systems (septic tanks, unsewered toilets and aqua privies) are the predominant form of excreta disposal, numerous obstacles are encountered in the collection, transportation and treatment phases. Among the challenges are; the inadequacy of excreta collection and haulage systems; difficulty to access OSS installations for emptying trucks, the non-affordability of mechanised pit emptying by urban populace; excessive haulage distances to designated treatment or disposal sites in cities; and the lack of satellite sites and low-cost treatment options. Using the figure of 1 L FS/cap/day (Koné & Strauss, 2004) as an average excreta generation rate in urban areas; in the order of 1,000 m^3 of excreta or faecal sludge (FS) should be collected in a city of 1 million inhabitants. However, Koné and Strauss (2004) report that daily collection rates for cities much larger than this (such as Accra and Bangkok) rarely exceed 300-500 m^3. According to these workers, a major fraction of the excreta are disposed of unrecorded and clandestinely within the urban settlement area. Few practical options are available for management of the excreta. The state of research in adapting low-cost technologies for treating excreta in developing countries shows two trends, (Koné *et al*. 2004):

Where septic tanks are the predominant type of on-site sanitation installations and, hence, septage is the only or predominant type of faecal sludge generated, treatment schemes might be devised which provide primary treatment of the septage and its by-products (biosolids and liquids). Such schemes might comprise a pre-treatment stage such as constructed wetlands with solids loading rates (SLR) $\leq$ 250 kg TS/m^2/year, settling tanks/ponds or unplanted drying beds (SLR $\leq$ 200 kg TS/m^2/year) followed by a combination of polishing units such as ponds, wetlands, or floating macrophytes-based systems (Koné *et al*., 2004).

Where the predominant types of OSS installations are unsewered toilets, no suitable treatment schemes are available to date. Koné and Strauss (2004) point out that the highly concentrated, fresh, and biochemically unstable faecal sludges collected from 'dry' (unsewered) toilets constitute a great challenge for treatment. The ammonia content of this type of sludge is often high (400-4000 mg NH_4^+-N/L) since urine and faeces are collected together. This concentration will inhibit algal growth in pond systems and impair plant growth in constructed wetlands. Appropriate low-cost treatment options for this kind of human wastes have not yet been developed. The workers, Koné and Strauss, argue that pond systems, although cheap, are likely to fail in such localities where high-strength sludges exhibiting such excessive ammonia contents are generated. Co-composting (combined composting) appears to be a reliable option, but field data from engineered plants validating them do not exist. Sanitation systems with sewer network and a central wastewater treatment system are not a suitable solution for developing countries. Heavy investment and operating costs, high water consumption and elimination of nutrients in the treatment processes are only some arguments against a wide implementation of the systems. Koné and Strauss (2004) therefore, recommend that solutions should be sought in different domains: 1), in developing adequate treatment options, and 2), in seeking alternative on-site sanitation systems.

Indeed, unless alternative sanitation systems that are context-relevant, safe, cheap and sustainable are developed soon, the above situation is likely to last for several decades, since for most developing countries, city-wide sewered sanitation is neither affordable nor feasible.

2.2 Source separation of human waste

Presently, three types of sanitation systems namely (i) open defecation, (ii) drop and store and (iii) flush and forget are in use. As explained in the previous sections, these systems do not provide neither acceptable nor sustainable solutions as either they are considered non-hygienic or they convert the point problem into diffused problem (Yadav, 2008), or involve high initial investment as well as high operation and maintenance costs. Source separation of human excreta is the first line of defence against disease infections.

2.2.1 Physical, chemical and biological characteristics of faecal matter

The composition of faeces (Table 2.1 and 2.2) depends upon food intake and health status, and varies from region to region. In industrialised countries, because of high consumption of protein based diets (fish and meat), the average daily production is 100-200g (wet weight) per person, whereas in developing countries the production varies between 130 and 520g/person/day (wet weight), due to a more vegetable/fibre diet. The most important organic substance groups in faecal matter are carbohydrates, proteins and lipids (ATV, 1996).

Of the nutrients from the food consumed, 10-20% of the nitrogen, 20-50% of the phosphorus and 10-20% of potassium will be found in the faecal fraction, while the rest is found in urine (Berger, 1960; Lentner *et al*., 1981; Guyton, 1992; Frausto da Silva & Williams, 1997). The distribution depends upon the composition of the food consumed, especially in the case of phosphorus where calcium regulates the amount of phosphorus available for uptake in the digestive system (Frausto da Silva & Williams, 1997). Of the faecal nitrogen, about 17% is contained in bacteria fraction and about 10% is found as ammonia from degradation of urea, peptides and amino acids. The remaining proportion of the nitrogen is found in different organic compounds such as uric acid and different enzymes or peptides (Vinneras, 2001). The nitrogen in faeces is about 50% water-soluble (Trémolières *et al*., 1961). The degradation of proteins and other nitrogen-based compounds produces indol, skatol and thiols, which are responsible for the unpleasant odour of faeces. Anaerobic degradation of sulphur produces H_2S which also contributes to odour problems. Phosphorus is found mainly as granular calcium phosphate in faeces (Frausto da Silva & Williams, 1997), but some phosphorus is found in organic compounds and a very small amount as soluble phosphorus ions (Lentner *et al.*, 1981). Potassium is mainly found in its water-soluble ionic form (Berger, 1960). A high proportion of heavy metals consumed pass through the intestine undigested, while many of the heavy metals inhaled are taken up by the body (WHO, 1995).

The biological properties of human faeces are influenced by sanitary conditions and environmental factors, notably climate. A large number of non-pathogenic microorganisms occurs in the faeces of a healthy person as a normal part of the natural intestinal micro biota. The gastrointestinal tract harbours between 100 and 400 different types of this natural flora. Some of these commensal bacteria, as they are commonly called, are often used as indicators of faecal contamination and may occasionally give rise to diseases. Faeces contain two main groups of pathogens – bacteria and viruses, and two groups of parasites – protozoa and helminths. Table 2.2 below presents the common pathogens occurring in human faeces and their environmental survivability.

Table 2.1: Human Faeces Composition (Expanded from Faechem *et al*., 1983)

Parameter	**Content**
Quantity Wet, g/p/d	100-400
Quantity Dry, g/p/d	30-60
Moisture content, %	70-85
Organic Matter, (% of dry weight)	88-97
Carbon, (% of dry weight)	44-55
Nitrogen, (% of dry weight)	5.0-7.0
Phosphorus, (P_2O_5), (% of dry weight)	3.0-5.4
Potassium (K_2O), (% of dry weight)	1.0-2.
C/N ration	8.2
BOD (g)	11.1
COD (g)	33

Table 2.2: Faecal pathogen and indicator survival in different environmental media (Adapted from Rose and Slifko 1999; Schwartzbrod 2000,)

Pathogen survival (time in days unless otherwise indicated)				
Organism	Freshwater	Saltwater	Soil	Crops
Viruses	11-304	11-871	6-180	0.4-25
E. coli	ND	ND	215	ND
Salmonellae	<10	<10	15-100	5-50
Cholera	30	+285	<20	<5
Fecal coliforms	<10	<6	<100	<50
Protozoan cysts	176	1 yr	+75	ND
Ascaris eggs	1.5 yr	2*	1-2 yr	<60
Tapeworm eggs	63*	168*	7 months	<60
Trematodes	30-180	<2	<1*	130**

ND No data; *Not considered an important transmission pathway; **Aquatic macrophytes

2.2.2 The need for source-separation of human excreta

Studies have shown that human excreta are a good source of plant nutrients and organic matter for soil amendment. Presently worldwide, there is a growing interest in sanitation systems that use negligible amounts of water and permit recycling of materials to generate fertilizers for supporting agricultural needs. For example, of the total nutrients in domestic waste, urine contains approximately 80 % of the nitrogen (N), about 50% of the phosphorus (P) and roughly 60 % of the potassium (K), whereas faeces contain about 10 % of the N, 25 % of the P and 20 % of the K (Vinneras *et al*., 2006; Niwagaba *et al*., 2009).

Mixing these two components - urine and faeces - not only results in the formation of malodorous compounds but also complicates the recovery of nutrients from each fraction. The slurry that results contains a high number of enteric microorganisms (Heinnoen-Tanski and Savolainen, 2003). Diverted urine contains very small amounts of pathogens that mostly originate from faecal contamination (Johnsson, *et al*., 1997). Most of the salts (sodium chloride) consumed by humans are excreted in urine. Salts are toxic to living organisms above their minimum requirement (Yadav, 2008), and can inhibit composting organisms. The nitrogen in urine is readily converted to ammonia, which is toxic to composting microorganisms and contributes to the odour problem.

If all of these fractions are collected separately and recycled, 92 % of the nitrogen, 85 % of the phosphorus and 63 % of the potassium out of the total flow of nutrients in the biodegradable fractions (urine, faeces, greywater and biodegradable solids) would be recycled (Vinneras, 2001). Faeces may be considered as clean fertilizer (Schonning *et al*., 2002; Niwagaba *et al.,* 2009) since it contains lower concentrations of heavy metals in comparison to farmyard manure and artificial phosphorus fertilizers. Jönsson *et al*. (1999) and Kärrman (2000) have demonstrated, by analysing sewage treatment alternatives using Life cycle analysis and Systems analysis, that urine diversion was a good alternative with respect to water-recipient preservation, energy usage and nutrient recycling.

The objectives of source separation sanitation systems are to minimise the discharge of eutrophying substances into water bodies and to reduce the reliant on fossil resources in agriculture. Urine and faeces, the two most nutrient-containing fractions, can be collected separately, treated and recycled. By recycling urine and faecal nutrients, much energy can be saved as the load on wastewater treatment plants decreases (Vinneras, 2002). Consequently, adopting urine-diverting systems combined with faecal matter separation and treatment may be a reliable approach for minimising resource depletion, safeguard water bodies and subsequently protect human health and promote socio-economic well-being.

2.2.3 Source-separating systems

In the discussion about new types of waste management systems that source-separate wastewater fractions, interest and emphasis are laid on the composition of the various fractions. Various systems for separately collecting urine and faeces have been developed and implemented. A commonly used system is the flush urine-diverting (UD) toilet, characterised by its two separate bowls (Figure 2.1). Urine is collected in a smaller front bowl whereas a larger rear bowl serves for faecal matter collection. Studies by Jönsson *et al*. (2000) and Lindgren (1999) have shown that between 50% and 80% of the urine is collected. Another emerging model is the urine diverting dry (UDD) (Figure 2.2) toilet that does not require the use of water for flushing thereby allowing for collection of undiluted faecal solids.

Figure 2.1: Urine diverting (UD) toilet

Figure 2.2: Urine diverting dry (UDU) toilet

Several commercial separating systems are available. The two main ones are filtration and Aquatron, or a combination of both in some cases. The Aquatron separates by a combination of a whirlpool effect, gravitation and surface tension (Vinneras & Jönsson, 2002); while the filtration systems are based on filters with a filter cake that complements the filter with a retention time of several months (Vinneras, 2001).

2.2.4 The need for linking sanitation and agriculture

Worldwide, many agricultural lands have been moderately or severely degraded, greatly undermining the ability of farmers to sustain production at current levels or improve productivity through more intensive farming. With limited availability of additional land for crop production, coupled with a steady decline in yield for several major crops, there are concerns about the ability of agriculture to feed a growing world population that is projected o exceed 7.5 billion by the year 2020. Chemical fertilisers, though effective to a certain extent, cannot be relied on. The lifetime of global economic phosphorus reserves is estimated at between 60-130 years (Steen, 1998). Besides, the mining and refining of raw materials for phosphate production generate considerable amounts of hazardous substances that cause environmental problems.

Production of nitrogen-based fertilisers by reduction of nitrogen to ammonia is a costly process, relying heavily on non-renewable resources, oil and gas, (Greenwood & Earnshaw, 1998), which are estimated to reach their global peaks in 10 and 20 years respectively (Bentley, 2002). This suggests that chemical fertilisers are neither economical nor sustainable. Therefore, strategies for increasing agricultural productivity will have to focus on restoring degraded land by returning organic matter to soils and using available nutrient resources more effectively and sustainably. One option is to return to cropland the organic matter and nutrients in faecal matter.

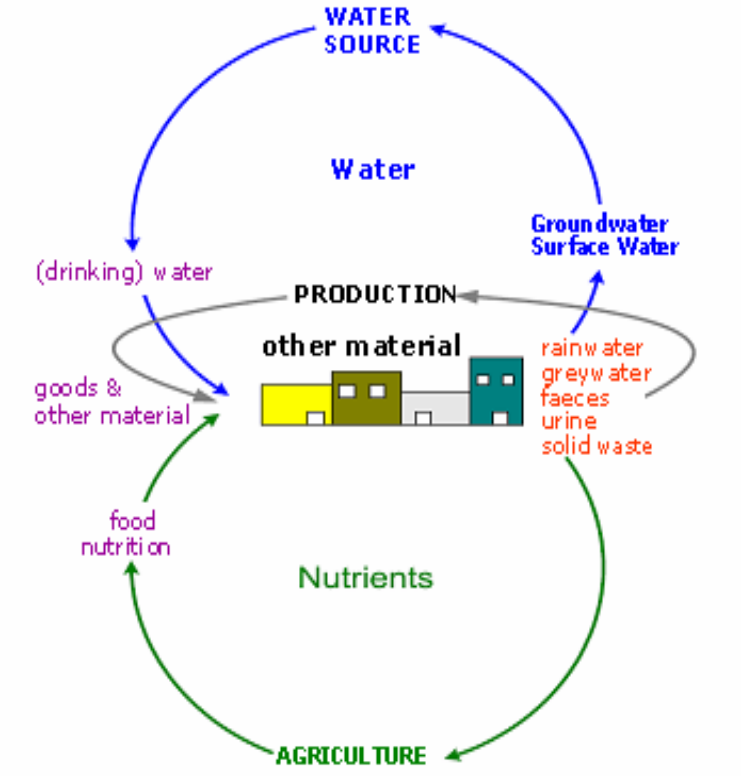


Figure 2.3: Material recycling in sanitation (Langergraber and Muellegger, 2005)

Presently, the flow of nutrients from farmland to society is largely a one-way process based on 'linear' sanitation solutions – 'flush and discharge' and 'drop and store'. A large

proportion of the plant nutrients consumed are excreted; normally via the urine and faeces (Guyton, 1992). If not recovered, the nutrients are permanently removed from the food chain. Figure 2.3 illustrates a natural cycle in which materials are moved from cropland to home and back to agriculture.

The current global food production scenario lends credence to the argument that 'closing the loop on nutrient cycles' is a necessity for sustainable agricultural productivity. Food insecurity, characterised by deficient nutritional status, is a major contributor to the global burden of disease (Lopez *et al*., 2007). Together with diarrhoeal diseases, this situation contributed 18.6% of the 2001 burden of disease measured in Disability Adjusted Life Years (DALYs). It is therefore imperative to seek cost-effective alternative methods of managing human waste.

Vermicomposting has been shown to be an alternative option for managing human excreta and recycling nutrients in sanitation systems. Vermicomposting is the use of earthworms to decompose organic waste at ambient temperatures, optimally between 20 and 25ºC (Edwards *et al*., 1984; Aston, 1988). It is a widely used low-input, un-mechanized means of utilizing both agricultural and municipal wastes in less industrialized countries (Ashok Kumar, 1994; Werner and Cuevas, 1996). Amending soils with vermicompost increases soil organic matter content, cation exchange capacity (CEC), and total nutrient levels, while lowering soil bulk density, improving infiltration, and reducing surface runoff (Bulluck *et al*., 2002a; Gonzalez and Cooperband, 2002). Research has shown that nitrate leaching to groundwater can be reduced when soil is amended with compost compared to inorganic fertilizers (Maynard, 1993) or with semi-composted farmyard waste compared to pig manure slurry (Beckwith *et al*., 1998).

2.2.5 Trends in faecal matter recycling

With human excreta gaining more recognition as a source of nutrients and organic material for soil improvement, the search for simple, cost-effective, reliable and scale independent sanitation methods for recycling of faecal matter has intensified. This section examines the advances in this domain. In order to provide a full understanding of how new trends have evolved, existing conventional treatment methods are first of all briefly presented.

2.2.5.1 Existing conventional methods

Current conventional methods for the treatment of faecal matter include composting, thermal treatment, storage/dehydration, and chemical treatment. The efficiency of hygienisation by each treatment method depends on various factors as discussed in the sections that follow.

2.2.5.1.1 Composting

Composting technology has been widely used for the processing of source-separated human faeces (WHO, 2006). Thermophilic or microbial composting, as it is sometimes called, is a natural process by which a consortium of microorganisms, notably bacteria, decomposes organic material to simple end products such as carbon dioxide (CO_2), ammonia (NH_3), water and heat. In the absence or limited supply of oxygen, anaerobic microorganisms dominate and produce compounds such as methane (CH_4), hydrogen sulphide (H_2S), organic acids, and a

variety of substances. To differentiate this from aerobic composting, it is called anaerobic digestion.

Several factors work in concert in the breakdown of organic matter during the composting process. These include moisture, microbial populations, oxygen (O_2), and a balance of carbon (C) and nitrogen (N) (Mcclintock, 2004). C to N (C/N) ratio of 25:1 or 30:1, moisture content of 50-60%, oxygen concentration > 5%, pH of 6.5 to 8.0, temperature of 55-60 °C, time in the range of 10 to 14 weeks, etc are considered to be optimum for the composting process (Rynk, 1992). Microorganisms in the organic matter (OM) consume the readily available C. As it is metabolized, temperatures increase in the compost pile and carbon dioxide (CO_2) is released. As a result, the pile is newly populated with thermophilic, or heat-loving, bacteria that consume the rest of the degradable C. As microbial activity slows, temperature decreases, allowing for colonization by fungi that slowly consume much of the remaining recalcitrant forms of C-lignin and cellulose. This is followed by the maturation phase, where the compost slowly cools down to ambient temperature. Therefore, the composting process itself can be divided into four sequencing stages as shown in Figure 2.4.

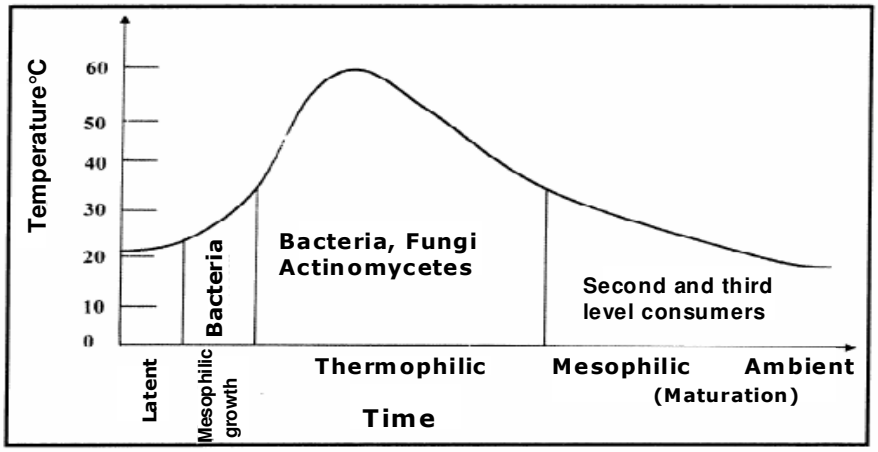


Figure 2.4: Pattern of temperature and microbial growth in compost piles (adapted from Polprasert, 1996)

Vinneras *et al*. (2003) report that the time needed to reach and maintain the temperature levels required for the proper composting of separated faeces is approximately 10 – 15 days.

2.2.5.1.2 Thermal treatment

The purpose of thermal treatment is to inactivate pathogens. Microorganisms have different sensitivity to heat treatment. While some microbes can withstand high temperatures for longer periods of time, others are capable of withstanding lower temperatures for longer time periods. This phenomenon is exemplified in the case of enteroviruses and ascaris. The former are more resistant to higher temperatures than the latter, but the Ascaris are more resistant at lower temperatures Vinneras *et al*. (2002) [Figure 2.5]. Vinneras *et al.* (2003) investigated a method (Table 2.3) to mathematically evaluate and estimate the safety margins of pathogen inactivation during thermal composting of faecal matter.

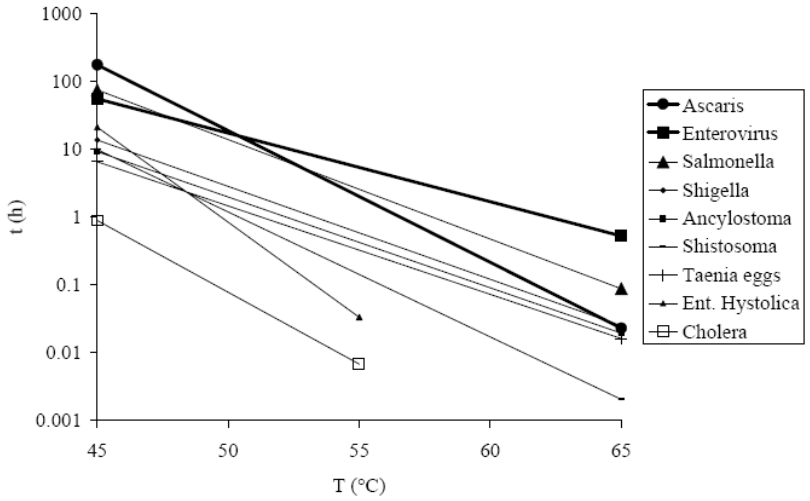


Figure 2.5: Time for total inactivation of nine pathogens at temperatures between 45°C and 65°C. The equations for the inactivation times are taken from Vinneras *et al*. (2002), and according to Feachem *et al*. (1983)

In Table 2.3, the inactivation time of the organisms is presented as a function of temperature. For instance, for Ascaris, inactivation can only be guaranteed above 45°C.

Table 2.3: Equations derived from Feachem *et al*. (1983) for the time in hours (t) required to attain no viable organisms of different pathogens at different temperatures (T) in Celsius

Organism	Type	Equation (Ed)
Enteroviruses	Virus	$t = 55.9 \times 10^{-0.101(T-45)}$
Cholera	Bacteria	$t = 0.89 \times 10^{-0.2117(T-45)}$
Salmonella	Bacteria	$t = 75.4 \times 10^{-0.1466(T-45)}$
Shigella	Bacteria	$t = 13.8 \times 10^{-0.1369(T-45)}$
Enteric hystolica	Protozoa	$t = 21.3 \times 10^{-0.2806(T-45)}$
Ancyclostoma	Helminth	$t = 9.31 \times 10^{-0.1340(T-45)}$
Ascaris	Helminth	$t = 177 \times 10^{-0.1944(T-45)}$
Schistosoma	Helminth	$t = 10.0 \times 10^{-0.1844(T-45)}$
Taenia	Helminth	$t = 6.6 \times 10^{-0.1306(T-45)}$

The inactivation of the pathogens is logarithmic and even if the time interval is shorter than that needed to achieve no viable organisms, a partial inactivation is obtained, which can be seen as a number of logarithms of the total inactivation. The heat for thermal treatment can be derived in two ways; either an external source (such as electricity or oil) or the generated during biodegradation of organic matter.

2.2.5.1.3 Storage

Storage is a commonly used faeces treatment method, but is a low impact alternative for attaining improved hygienisation of faecal matter. As a function of time, some inactivation of pathogens occurs during storage (Brock *et al*., 1994). Nevertheless, storage is not a reliable method; although Faechem *et al*. (1983) as well as Mitshcerlich & Marth (1984) report that the efficiency of storage on pathogen control increases with increasing temperature. According to Vinneras (2002), the mechanisms dominating the reduction of pathogens during storage of wastewater products are mainly competition from other organisms and starvation. Due to this non-specific effect that differs according to local circumstances, the effects of storage are difficult to predict and to specify.

Tests on the survival of different Salmonella sub-species during storage of faeces and sewage sludge have shown that the materials still contain viable organisms after 100 days of storage (Mitscherlich & Marth, 1984). When storing sludge with high water contents, fermentation of the sludge occurs and produces fatty acids that lower the pH (Haug, 1993). This drop in pH can result in a higher inactivation rate of pathogens (Mitscherlich & Marth, 1984; Brock *et al*., 1994).

2.2.5.1.4 Chemical treatment

The addition of ash to latrines is an old practice, which is still on-going. Presently, in many developing countries, wood ash is added to toilets and the increased pH may lead to sanitisation of faecal material (Franceys *et al*. 1992; Austin 2001; Moe *et al*. 2001). Recently, various researchers have investigated the feasibility of different chemicals, including acids (phosphoric acid), bases (ammonia) and oxidising agents (chlorine).

For treatment with bases, the main chemicals so far investigated are: ammonia (NH_3) and potassium hydroxide (KOH). Allevi *et al*. (1994), after application of similar concentrations of these chemicals to faecal matter report a much higher inactivation of bacteria by the ammonia-based compounds. Deactivation using NH_3 has been investigated at different pH and temperatures. According to Allevi *et al*. (1994), a pH value over 10 at temperatures above 10°C is sufficient for inactivation of bacteria. Temperatures above 10°C are sufficient for having good effects and at temperatures below 5°C no effect from the ammonia will be detected. One main effect from increasing the pH is the amount of free non-ionised ammonia produced. To get free ammonia the pH has to exceed 8 (Turner & Burton, 1997). The amount of free ammonia depends on the temperature, the pH and the ammonium concentration (Svensson, 1993).

The inactivation mechanisms of viruses by ammonia have been investigated (Burge *et al*., 1983; Turner & Burton, 1997), and for single strand RNA viruses such as the poliovirus, the inactivation is caused by RNA rupture for temperatures below 50°C (Vinneras, 2002). However, further work needs to be carried out to validate these results.

For inactivation of Ascaris eggs in sewage sludge, a treatment of 2 months with an initial pH of 12.5 is required (Gaspard *et al*., 1995). Gantzer *et al*. (2001) investigated liming of sewage sludge reaching a pH of 12.4 for approximately 24h, without achieving a significant reduction in nematode eggs. According to Vinneras (2002), the application of lime to the sludge was a

probable reason for the poor performance. This highlights the limitations associated with the use of chemicals for treatment of faecal matter and the fact that care must be taken to ensure that the chemical reaches all portions of the material through proper mixing. Vinneras *et al*. (2006) suggest that a hygienically safe product can be obtained by addition of urea to separated faeces. The urea is degraded to ammonia, and they found out that the pH was raised from 8.0 to 9.0 within an hour of urea addition. Nordin (2007), investigated ammonia-based sanitation with separated faeces and concluded that urea treatment of faeces collected with ash is possible, however, if the pH is greater than 10 in the material, urea will not be degraded (into NH_3) and thus will not contribute to inactivation.

Various researchers (Ward, 1978; Cramer *et al*., 1983 and Burge *et al*., 1983) have pointed out that the main reason for the disinfection effect by ammonia is that at high pH and temperature, ammonia is found in the uncharged state, (i.e. NH_3). As stated above, the amount of free ammonia is regulated by the equilibrium between uncharged free NH_3 and the charged ammonium ion (NH_4^+). Increasing the temperature or pH, forces the equilibrium towards formation of free ammonia. Ammonia is in equilibrium between the uncharged state and the +I state (Equation 1).

$$\left(NH_4^+\right)_{aq} \leftrightarrow \left(NH_3\right)_{aq} + \left(H^+\right) \quad \text{…….. Equation 1.}$$

2.2.5.1.5 Limitations of existing faecal matter treatment methods

Studies have shown that temperatures high enough to achieve adequate hygienisation are normally not reached during faecal matter storage in single household compost toilets (Møller *et al*., 2003). Storage of organic wastes, on the other hand, can produce other problems, especially towards the surrounding environment through emissions, both of eutrophying, acidifying and greenhouse gases (Flodman, 2002).

During the composting of faecal matter, temperatures have been observed to increase only by 10-15°C above ambient temperature (Bjorklund, 2002b). In general, the main limitations linked with composting are the energy intensiveness of the process, the need for frequent turning of the material, the long duration (usually above 6 months) of the process, the loss of nutrients during the prolonged treatment, and the heterogeneous nature of the product (Ndegwa and Thompson, 2001). Anaerobic digestion, on the other hand, leaves pathogens intact as it is a low-temperature process. Often, the anaerobic process results in partially oxidised products which are only slightly stabilised. Such products can cause problems, especially phytotoxicity or odours (Strauss e*t al.*, 2000).

Thermal treatment also presents major challenges since using an external heat source consumes energy and adds to O & M cost. Before incineration the raw untreated matter has to be transported to a treatment plant and to avoid negative environmental effects from the incineration, the gases produced have to be cleaned. Some valuable nutrients, namely nitrogen, are lost during the process.

Although the literature suggests that chemical treatment provides promising results, this method has a major drawback arising from the fact that apart from economic concerns the

continuous utilisation of chemicals will cause environmental problems. Consequently, this technique cannot be recommended for large scale implementation.

As a result, there is need to investigate alternative treatment methods that circumvent the above limitations and at the same time providing safe end-products.

2.3 Vermicomposting as a new trend in faecal matter management

Innovative methods in faecal matter recycling in recent years have focused mostly on biological techniques. Interest in this approach has increased with the realisation that ecological sanitation (EcoSan) could be an option for closing the loop on material cycles.
Indeed, during recent years the methodology of solid waste management has shifted from conventional disposal strategies such as incineration, landfill etc. to conversion of sludge into value added products (Liang *et al*., 2003). As mentioned in the previous chapter, this approach underpins the principle of EcoSan. EcoSan concepts are gaining popularity the world over, with the rate of adoption of systems that allow nutrient recycling rising rapidly in several parts of the world, notably where water and soil fertility problems are most evident. Dry sanitation systems (with urine diversion) are one of such EcoSan concepts which are gaining rapid acceptance and recognition in the 'loop-closing' domain. However, the triple-pronged problem of storage, treatment and transportation of faecal matter generated from the dry sanitation systems is becoming increasingly acute in communities where the technologies have been adopted on a large scale.

Vermicomposting, which is alternatively called earthworm vermistabilisation, worm composting, or annelic consumption (Wang *et al.,* 2006), is an old approach in the field of soil sciences but a novel concept in biosolids and solid waste management that holds considerable promise in the field of ecological sanitation. The process involves complex mechanical, chemical and biological transformations. It is not only a rapid, easily controllable, cost-effective, energy saving and zero waste process, but also accomplishes most efficient recycling organics and nutrients (Eastman *et al.,* 2001). Hence, the definition of Hand *et al.* (1988) of vermicomposting as a low-cost technology system for processing of organic solid wastes. As pointed out by Edwards *et al.* (1988), earthworms differ considerably in their growth rates, reproductive rates, size, and ability to withstand handling and in their environmental requirements. The question then arises which of the earthworm species are the most suitable for large scale vermiculture (Reinecke, *et al.,* 1992), be it for the treatment of organic wastes or for production of worm protein as a supplement for animal feed. For vermistabilisation of sewage sludges, Neuhauser *et al.* (1980) have contributed towards answering this question, whereas for the stabilisation of faecal sludge, Shalabi (2006) has provided valuable clues.

2.3.1 Vermicomposting technology development

Human beings have long-recognized the ability of the earthworms to transform organic wastes into humus-rich material with soil fertilizing capabilities. Indeed, Aristotle had referred to earthworms as the "intestines of the earth" (Kale, 1993). Bouché (1987) traces the birth of vermiculture, the production of earthworms, to the fishing bait industry in the US in the early part of the 20th century whose rise was a function of a growing urban population. Mcclintock (2004) points out that by the late 1940s, worm growers were promoting earthworms as an effective means for farmers to improve the fertility of their soils, often

making claims that lacked scientific basis. However, their claims were later given backing by several workers including Teotia *et al. (*1950) who suggested the use of earthworms for accelerating the mineralization of organic matter in fields. In the 1970s, as environmental concerns moved into the public consciousness, researchers in various parts of the world began exploring the earthworm's potential to mitigate waste disposal and soil reclamation (Mcclintock, 2004), notably the US, Britain, France, and Japan. On the strength of the results pouring in from these sources, research programmes were initiated in the early 1980s aimed at using earthworms to break down organic wastes. One of such works carried out at Rothamsted in the UK (Edwards *et al.*, 1985) has largely contributed to our present day knowledge in this field.

Application of earthworms for the conversion of sludges or biosolids into compost was first attempted by Mitchell (1997). Later, these investigators studied the potential role of the earthworm (*Eisenia foetida*) on the stabilisation of sewage sludge in drying beds, with promising results. Since then, several researchers (Benitez *et al.,* 1999; Bansal and Kapoor, 2000; Kaushik and Garg, 2004; Loh *et al.,* 2005; Garg *et al.,* 2006; Monroy *et al.,* 2006; Shalabi, 2006; Suthar and Singh, 2008; Khwairakpam and Bhargava, 2009; Warman and AngLopez, 2010) have amassed scientific data supporting the viability of vermicomposting as a source of fertility and as a means of waste management (Eastman *et al.,* 2001; Edwards, 1998; Ndegwa and Thompson 2001; Wong and Griffiths, 1991), disease suppression (Szczech *et al.,* 1993), and bio-remediation (Ma *et al.,* 2002). A comprehensive review of the literature on the evolution of the vermitechnology, with emphasis on substrate types, stabilization efficiency, inactivation and ultimate disposal and utilization is provided by Wang *et al.* (2009).

2.3.2 Need of vermicomposting for the management of human excreta

Wastes generated from agricultural activities including animal excreta and crop residues often require little treatment before disposal or reuse. Some can be directly added to the soil without any pre-treatment as such wastes may possess limited or no toxic pollutants (Lerch *et al*., 1992). But for wastes such as sludge from sewage plants and faecal matter from dry toilets much attention is required since they can produce toxicity and may exhibit depressive effects on metabolism of microorganisms (Ayuso *et al.*, 1996). Furthermore, a wide variety of pathogenic microorganisms have been reported to be present in sludge generated from the treatment of municipal wastewater (Hassen *et al*., 2001). Most scientific works on excreta management suggests that these solid wastes should be biocomposted to achieve biological transformation of the organic matter and to avoid potential risks of pathogens before applying to soil (Beloso *et al.,* 1993; Gliotti *et al.,* 1997; Masciandaro *et al.,* 2000). Composting of solid wastes brings about stabilization of the organic matter and effectively reduces pathogen concentrations in sludge to very low levels (Burge *et al.,* 1987; Millner *et al.,* 1987). However, absolute removal of pathogens becomes difficult to achieve and many survive the composting process (Russ and Yanko, 1981; Sidhu *et al.,* 2001). Incorporation of earthworm in the composting process has been considered to be an appropriate technology for biowaste management for producing nutrient enriched compost (Kumar *et al*., 2004).

The development of alternative worm-based treatment systems that could be successfully applied in medium to high density peri-urban settlements may be a major step towards addressing the excreta management problem in most developing countries. While home and

municipal-scale vermicomposting has rapidly increased in popularity in the industrialized world since the early 1990s, there is limited information documenting the application of the technology for the management of human faeces. In particular, there is limited scientific data to date reporting its use in the treatment of source-separated human faeces. Basja *et al.* (2002) and Gajurel (2003) reported the first attempts at applying vermicomposting for the treatment of human faeces. Most recent studies by Shalabi (2006) involving vermicomposting of faecal matter in batch systems have demonstrated the feasibility of this technique, producing end-products (vermicast) with suitable characteristics for land application.

2.3.3 Vermicomposting versus Thermophilic Composting

While composting and vermicomposting both involve the decomposition of organic matter by microorganisms, there are key differences in the biochemical and physical factors controlling the processes. Composting is a decomposition process that passes through a thermophilic stage (45 to 65°C) where microorganisms (mainly bacteria, fungi and actinomycetes) liberate heat, carbon dioxide and water. Heterogeneous organic waste is transformed into a homogeneous and stabilized humus-like product through aeration or turning. Vermicomposting is an accelerated decomposition process of organic material that, in contrast to composting, involves the combined action of earthworms and microorganisms and does not involve a thermophilic stage. The earthworms are the agents of turning. A major criterion in setting up a composting system is to stack waste in sufficiently large quantities to ensure that the heat generated in the breakdown of organic matter is retained in the waste pile. This condition stimulates the proliferation of thermophilic microbes, which then carryout the decomposition activity. For vermicomposting, a key factor is to maintain temperatures below 35°C throughout the process, beyond which earthworms will die. Therefore, a critical design criterion for vermicomposting systems is the feeding rate. The common approach is to apply waste frequently in thin layers, a few centimetres thick, to beds or boxes containing earthworms in order to prevent overheating and help keep the waste aerobic (Fredrickson and Ross-Smith, 2004). Shalabi (2006) suggests that earthworms accelerate waste decomposition rather than being the direct agent. Loehr *et al*. (1988) posit that the rapid degradation of organic matter may be due to the increased aeration and other factors brought by earthworms. Fredrickson and Ross-Smith (2004) however, points out that the processing rates will crucially depend on many factors such as the system being used, the processing temperature, and nature of the waste and the ratio of the earthworms to waste.

Based on an analysis of the work published by various authors, it can be concluded that composting and vermicomposting are essentially different types of processes involving different process control parameters, biological agents, reactor operation techniques and process conditions.

2.3.4 Species selection for Vermicomposting

While the literature repeatedly mentions *Eisenia foetida* as a suitable species for vermicomposting, there is indeed a wide range of species proving effective as decomposers. The range of species actually depends on the type of substrate. Edwards *et al*. (1984) evaluated the suitability of six different species for decomposing agricultural waste and sludge: *E. fetida, Dendrobaena veneta, D. subrubicunda, Lumbricus rubellus, Eudrilus eugeniae*, and *Perionyx excavatus*. They found that while *D. veneta* showed rapid growth and attained a greater weight at maturity than *E. fetida*, its cocoons produced fewer hatchlings. For

practical applications, the choice of species depends largely on temperature. Ashok Kumar (1994), showed that *E. fetida, E. eugeniae, P. excavatus* and *P. sansibaricus* are well suited to southern regions of India where summer temperatures are lower than in the north. In tropical regions, *E. eugeniae*, the African night crawler, is commonplace as a composting worm and as a source of protein meal (Ashok Kumar, 1994; Kale, 1998). Aston (1988) report lethal temperatures from 25 to 33°C for earthworms common to temperate regions and 34 to 38°C for tropical and sub-tropical species, yet this range is higher than those reported by Edwards (1988): 30°C for *P. excavatus* and *E. eugeniae*. Neuhauser *et al*. (1988) measured growth and reproduction of five species (*D. veneta, E. fetida, E. eugeniae, P. escavatus*, and *Pheretima hawayan*a) in aerobically digested sewage sludge. They observed little variation in growth between 15 and 25°C, whereas 30°C was associated with reduced growth rates, and 35°C with mortality.

2.3.5 Scientific relationships and engineering aspects of vermicomposting

The goal of any composting process is to achieve maximum reduction of volatile solids in order to stabilisation of the organic matter. Therefore, if earthworms are to be useful in the breakdown of faeces they must increase the rate of volatile solids reduction, thereby increasing the stabilization rate. To achieve this objective, and ensure the success of the vermicomposting process, from a technical and economic consideration, certain basic factors need to be evaluated to determine the requirements for optimum development, growth and productivity of earthworms in organic wastes. This section, therefore, examines the scientific relationships and technical aspects of vermicomposting.

2.3.5.1 Scientific relationships

The life cycle or earthworms is affected by a number of factors, which, consequently, determine the rate of waste processing, vermicompost production and the number of earthworms produced. These include temperature, moisture, aeration, earthworm density and waste characteristics (pH, salt content, carbon to nitrogen ratio and ammonia concentration). Recently, it has been shown that earthworms have well-defined limits of tolerance to these factors, notably temperature, moisture levels, ammonia and salt concentrations, and that wastes are processed much more efficiently under a relatively narrow range of favourable chemical conditions. To accelerate the rate of volatile solids reduction during the vermicomposting process, these factors and the relationships between them need to be understood. If their limits are greatly diverged from, earthworms may move to more suitable zones in the waste, leave the waste or die, so that the wastes are processed only slowly (Dominguez & Edwards, 2004). If these parameters are optimised, both earthworm activity and the rates of organic waste processing increase dramatically.

In the following sections the state of the research with regards to the relationship between earthworm growth, processing activity and the factors stated above is presented.

2.3.5.1.1 Temperature

Scientific evidence to date suggests that worms exhibit complex responses to changes in temperature. Neuhauser *et al*. (1988) investigated the potential of various earthworm species to grow in sludge and concluded that the different species have optimum growth temperature ranging between 15 and 25°C. In this study, the researchers report that cocoon production was

restricted more by temperature than by growth and the species studied produced most of the cocoons at 25°C. Further knowledge on this topic was provided by C. Edwards (1988) who monitored the life cycles and optimal conditions for survival and growth of four earthworm species; *E. foetida, D. veneta, E. eugeniae, and P. excavatus*. Each of them differed considerably in terms of their responses and tolerance to different temperatures. The optimum temperature for *E. foetida* was 25°C (Figure 2.6), and its temperature tolerance was between 0 and 35°C. *D. veneta* had a rather low temperature optimum and rather less tolerance to extreme temperatures. The optimum for *E. eugeniae* and *P. excavatus* was around 25°C, but they died at temperatures below 9°C and above 30°C.

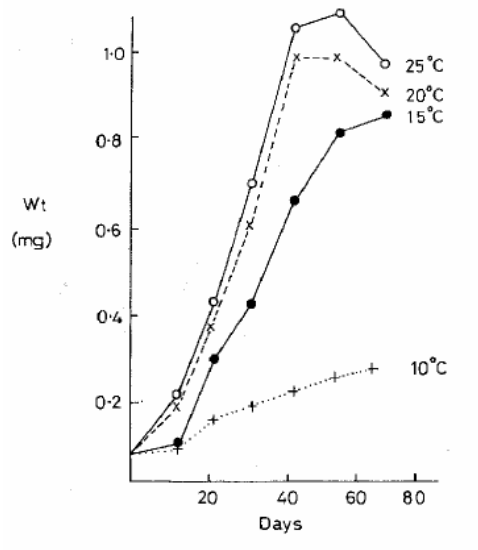


Figure 2.6: Growth of *Eisenia foetida* at different temperatures (Edwards 1988).

Shalabi (2006) investigated the optimum temperatures of *E. foetida* and *D. veneta* by monitoring their processing activities at four temperatures (10, 15, 20 and 25 °C) in human faeces and concluded that while they both perform well in the range 20-25 °C, they appear to be at their optimum at 25 °C (Figures 2.7 and 2.8). This finding agrees with the results of Neuhauser *et al*. (1988) and Edwards (1988) who showed that maximum cocoon production occurs at 25 °C suggesting optimum worm activity.

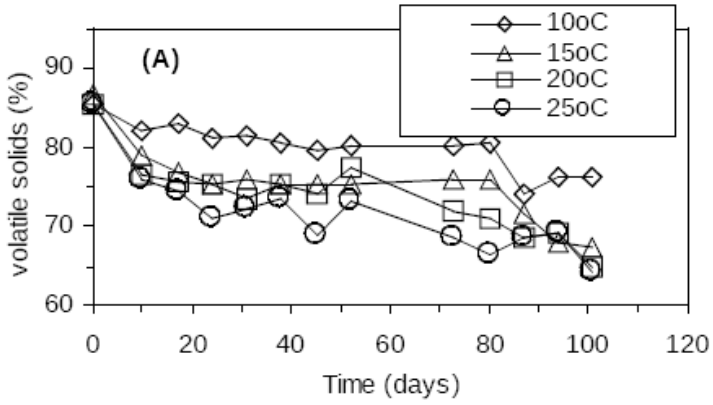


Figure 2.7: : Influence of temperature on volatile solids reduction in human faeces by *E. foetida* (Shalabi 2006)

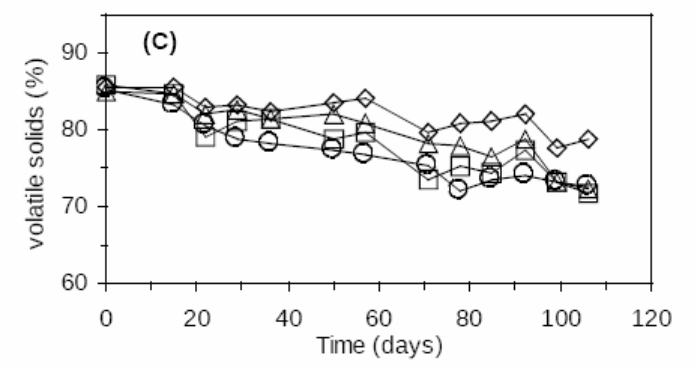


Figure 2.8: Influence of temperature on volatile solids reduction in human faeces by *D. veneta* (Shalabi 2006)

2.3.5.1.2 Moisture

Several workers have demonstrated that there is a relationship between moisture content of waste and the growth and productivity of earthworms (Figure 2.9). In vermicomposting systems, the optimum range of moisture contents has been reported to be between 50 to 90 % (Dominguez and Edwards, 2004).

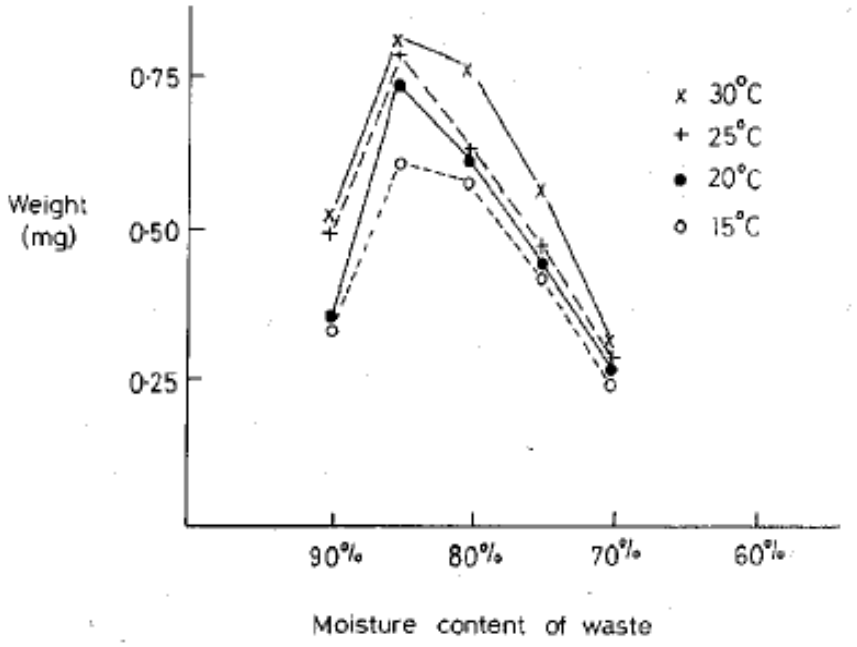


Figure 2.9: Growth of *Eisenia foetida* at different moisture contents (Edwards 1988).

According to a review by Dominguez and Edwards (2004), it seems likely that a lowering of the growth rate due to low moisture conditions can also retard sexual development so that earthworms of the same age could develop clitella at different times under different moisture conditions.

2.3.5.1.3 Ammonia and salt content

Earthworms cannot survive in organic wastes (e.g. fresh human faeces) with large amounts of inorganic salts. They prefer salt contents less than 0.5% (5 mg /g) in the substrate. Worms are also very sensitive to ammonia and die in wastes containing high levels of this chemical. Both ammonia and inorganic salts have very sharp cut-off points between toxic and non-toxic, that is, <1 mg/g of ammonia (Figure 2.10) and < 0.5% salts (Dominguez and Edwards, 2004).

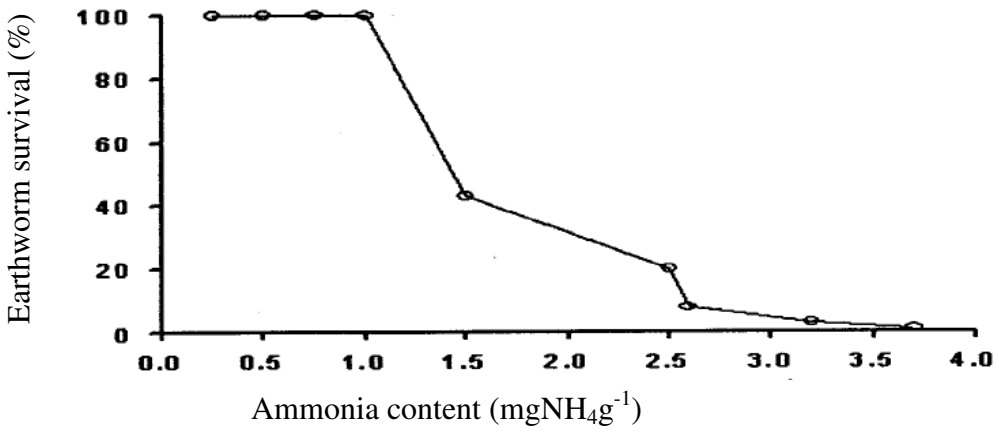


Figure 2.10: Survival of *E. foetida* at different ammonia contents (Dominguez and Edwards, 2004)

2.3.5.1.4 Carbon to nitrogen ratio

Carbon and nitrogen are the two main elements in organic wastes and their proportion (C: N) is of importance to the decomposition process. The bulk of the organic matter should be carbon (C), with just enough nitrogen (N) to support the decomposition process. Several workers have suggested that a ratio of 30 parts C to 1 part N is optimum. According to Bertoldi *et al*. (1983), if the C/N ratio is greater than 35, microorganisms must go through many life cycles, oxidizing off the excess carbon until a more convenient C/N ratio for their metabolism is reached. The C/N ratio of microbial cell is roughly 10 and this theoretically would be the ideal value for their metabolism. However, this is not the case in practice, as the low C/N value will result in N loss through ammonia volatilization at high pH and temperature values. In general, for organic waste treatment, a C/N ratio in the range of 25-30:1 is considered suitable. Higher values will slow the rate of decomposition, and lower ones will result in N loss.

2.3.5.1.5 pH

Earthworms that are suitable for vermicomposting have been shown to be generally tolerant to pH. However, when subjected to a pH gradient, they move towards the more acidic material, with a preference of pH 5.0 (Figure 2.11) (Dominguez and Edwards, 2004). Edwards and Bohlen (1996), report that worms will avoid acidic soil environments of pH less than 4.5, and extended exposure to such environments could produce lethal effects on worms.

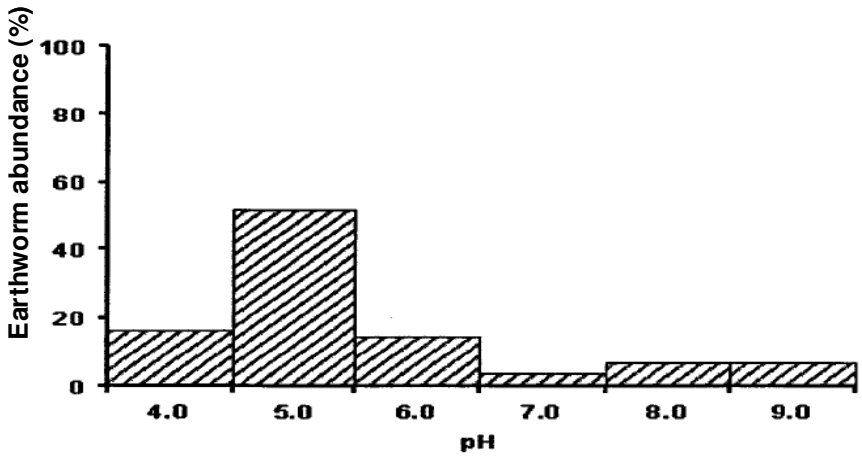


Figure 2.11: pH preference of *E. foetida* (Dominguez and Edwards, 2004)

2.3.5.1.6 Engineering aspects of vermicomposting

The engineering of earthworm-based systems to efficiently and economically process large volumes of wastes is a subject of much concern, as rising volumes of wastes are generated with increasing populations worldwide. Vermicomposting is the only bioprocess, outside the ones involving one or other type of animal farming, in which a multicellular animal is used in reactor systems to generate a product other than the animal offspring (Abbasi *et al*, 2008). These authors maintain that all other engineered bioprocesses, except a few which are based on plants, revolve around the use of enzymes, bacteria, micro fungi, or microalgae in mobile or immobilized modes. Such processes have been intensively studied, modelled, designed, and engineered (Shuler and Kargi, 2007). Abbasi *et al.* (2008) point out that no design or operation criteria based on bioprocess engineering principles has been developed for vermicomposting. Most scientific literature and data to date, relating to the technical aspects of vermicomposting systems have been derived from bench-scale research projects, conducted under controlled conditions. Considerable work has been going on on the science of vermicomposting, especially with respect to biotic and abiotic factors which influence vermicast production, earthworm growth and fecundity (Edwards, 2003). Studies are also being increasingly reported on the 'vermicompostability' of newer substrates and newer earthworm species (Gajalakshmi and Abbasi, 2008). But the aspects of vermicomposting process design, control, operation, and optimization are by-and-large still unaddressed (Abbasi *et al*., 2008).

Existing vermicomposting systems are reported to present a number of practical problems. Firstly, applying waste to beds and periodic earthworm harvesting by hand are considered labour intensive and costly. Most existing practical vermicomposting facilities are based on the model of open-air, bed systems containing a bedding material and an inoculum of earthworms. Waste is then applied to the surface of the beds and this is subsequently decomposed by the worms. The design and construction of typical beds often leads to several technical problems, including difficulties in applying waste to beds and inadequate drainage.
Alternative systems that minimise labour, operational problems and increase waste processing rates need to be investigated. The technical aspects of such systems such as basic design criteria (e.g. starter stocking density, feed application rates, system temperature and moisture

specifications) and performance levels such as waste processing rates and worm productivity need to be established.

2.3.6 Practical systems of vermicomposting

Unlike thermophilic composting that depends on temperature of the organic material, vermicomposting can be successfully managed on large or small scales, and is perhaps most widely practiced in kitchens in homemade bins on a small scale (Mcclintock, 2004). On an industrial or commercial level, however, there are generally three main vermicomposting systems: windrows, single-batch reactors, and continuous flow systems (Edwards, 1998; Sherman-Huntoon, 2000). These are briefly reviewed in the following sections.

2.3.6.1 Windrows

Windrows are extensively used both in the open and under shade, but require either a lot of land or large buildings. Windrows range from 1 to 2.5 m wide and can be as long as 0.5 km. According to Mcclintock (2004) feedstock should be repeatedly placed on top of the windrow at a thickness of 3 to 10 cm. Haimi and Huhta (1986) claim that worms can work a maximum thickness of 5 cm of sewage sludge. Mitchell (1997) reported two-fold greater reductions of organic matter in windrows 20 cm deep than in windrows 30 cm deep. Different variations of windrows exist. Two common types are described below.

2.3.6.1.1 Static Pile windrows (batch)

Static pile windrows are simply piles of bedding with feed layered on top that are inoculated with worms and allowed to stand until the processing is complete (Figure 2.12). This method of vermicomposting is implemented at the Periar Maniammai College of Technology for Women (PMCTW), TamilNadu, India for decomposing the organic wastes produced in the campus. Wastes from various sources such as hostel, kitchen, canteen, college and agricultural fields of the campus are collected and segregated manually. Each bed consists of about one .

Figure 2.12: Static pile windrows (Courtesy: J. Walker)

2.3.6.1.2 Top-fed windrows-continuous flow

The continuous flow top-fed windrows are similar to the windrows described above, except that they are not mixed and placed as a batch, but are set up as a continuous flow operation. This means that the beddings is placed first, then inoculated with worms, and then covered repeatedly with thin (less than 10 cm) layers of food. The worms tend to consume the food at the food/bedding interface, and then drop their casting near the bottom of the windrow. By this, a layered windrow is created over time, with the finished produce on the bottom, partially consumed material in the middle and the fresher food on the top. The main disadvantage of this system is that the covering layer must be removed and replaced every time the worms are fed, creating extra work for the operator. The advantage is mainly the greater control the operator has over the worms' environment. Parameters like feeding rate, moisture levels, etc can be modified at each feeding. This tends to result in a higher efficiency system with greater worm production. The wedge system is a modified version of the windrow method in which new feedstock is added at a 45-degree angle against one face of the mature windrow (Sherman-Huntoon, 2000).

2.3.6.2 Single-batch reactors

Batch reactors range in size from small boxes or bins to large, walled beds, or troughs. While the most feasible for experimentation and home-scale vermicomposting, Edwards (1998) points out that batch systems are too labour-intensive for effective production since vermicompost must be removed before new waste can be added. However, the total removal of material following vermicomposting allows for cleaning and is a means of avoiding mite infestation (Beetz, 1999). Without labour constraints; this method has proven effective in Cuba, where large concrete feeding troughs serve as batch reactors (Werner and Cuevas, 1996).

2.3.6.3 Continuous flow systems

Several workers (Edwards, 1998; Beetz, 1999; Sherman-Huntoon, 2000) have described the continuous flow system (CFS) as a raised-bed system that allows air flow and harvesting through a mesh grate below, with feedstock evenly distributed along the top via a mechanical tracked gantry. Mcclintock (2004), reports that although continuous flow reactors have the highest initial capital investment of all vermicomposting systems, they have the greatest potential for processing substantial quantities of organic waste. One 36.5 × 2.4 m bed made of dairy industry machinery in Washington is capable of processing 2.7 Mg of waste daily (Sherman-Huntoon, 2002). According to Chambers (2002), a farmer who produced high-quality vermicompost in eight to twelve months in a windrow system was able to produce the same quantity in 40 to 60 days after switching over to a continuous flow reactor. Two models of the CFS exist: continuous vertical flow systems and continuous horizontal flow systems.

Continuous vertical flow systems. In this model the material is fed from the top, flows through the reactor (and the worms' gut) and comes out at the bottom. Continual processing of the waste is achieved by adding waste, on a scheduled basis, in thin layers to the top of the bed and collecting the processed material through the base. The idea is that the worms will finish composting the bottom tray and then migrate to the one above. When a sufficient number of worms have migrated the bottom tray can be collected (Figure 2.13) and should be relatively free of worms. The CFS provides an easier method of harvesting.

Continuous Horizontal Flow systems. A continuous horizontal flow bin is another type of bin that relies on the earthworms habits of migrating towards a food source in order to ease the process of harvesting. The bin is usually constructed to be similar to a non-continuous bin but twice as long (horizontally). The bin is divided in half, usually by a large gauge screen of chicken wire. Only one side is used initially. When that half becomes full, the other half is filled with bedding and organic matter. In time, the worms will migrate to the side with the food and the compost can be collected.

2.3.7 Physical, chemical and biological characteristics of vermicompost

The relatively rapid decomposition of organic matter in vermicomposting is brought about by the grinding muscular gizzard of the earthworm, aided by enzymatic secretions such as cellulase, protease, amylase, lipase and chitinase. The process transforms nutrients into plant available forms (Edwards, 1998). Buchanan, (1988) suggest that mineralization may be affected by organic carbon present in the substrate. However, phosphorus (P), molybdenum (Mo), iron (Fe), and zinc (Zn) content may also be indirectly related to mineralization by their effect on soil microbial activity. Some researchers have observed that although nutrient content in a finished vermicompost varies according to the initial feedstock, the nutrient content of the digested waste is often lower than that of the raw material (Mcclintock, 2004). Other workers have reported higher content of nutrients in the vermicompost compared to the parent material (Table 2.3).

Table 2.4: Effect of worm activity on nutrients in organic waste (Edwards *et al., 1984*)

Organic waste	*Nitrate-N (ppm)*	*Readily soluble P (% d.m.)*	*Exchangeable K (% d.m.)*	*Exchangeable Ca (% dm)*	**Exchangeable Mg (% dm)**
Cattle waste (unprocessed)	8.8	0.11	0.19	0.35	0.05
Cattle waste (vermicomposted)	259.4	0.18	0.41	0.59	0.08
Pig waste (unprocessed)	31.6	1.05	1.49	1.56	0.45
Pig waste (vermicomposted)	110.3	1.64	1.76	2.27	0.72
Potato waste (unprocessed)	74.6	0.19	1.94	0.91	0.24
Potato waste (vermicomposted)	1428.0	0.22	3.09	1.37	0.34

Buchanan (1988) report that nitrogen loss is balanced by a reduction in volume, nevertheless the overall N content of a vermicomposted waste may be lower than the feedstock due to volatilization, leaching, or denitrification. Soluble salts and electrical conductivity (EC) generally increase over the course of the vermicomposting (Kale, 1998; Masciandaro *et al*., 1997), but Warman and AngLopez (2002) report an eventual decrease in electrical conductivity, supporting the earlier findings of Elvira *et al*. (1998).

2.4 Conclusion

Combining the results of the many studies and reviews conducted, it is clear that the vermicomposting technology may provide a practical option for the management of human waste. Studies at Hamburg University of Technology (TUHH) have shown that vermicomposting can be integrated into source control sanitation systems. The research work at TUHH with very promising results – although not yet fully published in the scientific literature – showed that earthworms, notably *E. foetida*, will grow and reproduce well in human faeces. The work, which involved the use of Rottebehaelter (rotting sacks) to collect faecal solids from flush toilets showed the great potential of the vermitechnology as a low cost component of ecological sanitation. Prior to the work at TUHH, many generalizations regarding the use of vermicomposting as a treatment option for faecal matter or faecal sludge have been put forward, but very limited scientific evidence had been published. Indeed, almost all of the research reviewed on vermicomposting is based on a variety of other organic wastes (agricultural wastes and sewage sludge), whereas basic scientific questions relating to faecal matter have not yet been answered. This fact is made known by the work of Shalabi (2006), which has answered some key questions especially with regards to temperature requirements and also brought to light a number of fundamental questions that need to be investigated to ensure the technical and economic success of faecal matter treatment by vermicomposting. The results of the research conducted by Shalabi (2006), although not yet published in the scientific literature, are promising and merit continued research. For example, he forwarded many generalisations regarding the possible utilisation of *E. foetida* as candidate for human excreta degradation; however, more scientific evidence is needed to validate this species under UDD toilet conditions since the work was based on flush system-derived faecal matter solids with high carbon content and low conductivity. Furthermore, the many works presented in this review attest to the multiple benefits linked with the vemicomposting technology, notably its usefulness to material recycling between sanitation systems and agriculture.

However, a note of caution needs to be sounded to guard against the general optimism and "acceptance" of vermicompost as 'ideal' material for soil structure and fertility improvement. Indeed, most of the works reviewed also emphasize the risks of vermicompost handling and use in agriculture with respect to health issues. Further research is needed to more fully understand the impact of vermicomposting on pathogen survival. The application of the technology to excreta management is still in its infancy, and there is need to undertake a more in-depth investigation into design criteria of a faecal matter treatment system and the scientific relationships among major parameters that allow for optimisation of the system before engaging in widespread promotion and adoption of the technology.

2.5 Objective of the study

This study is part of an ongoing effort at the Hamburg University of Technology (TUHH) to investigate the feasibility of the vermitechnology as a faecal matter management option. More specifically, the study evaluates the potential of continuous flow vermicomposting for the stabilization of faecal matter in urine diverting dry (UDD) toilets, focusing on the fate of different parameters during organic matter transformations. No research of note has been conducted into the operating characteristics of continuous flow vermicomposting systems with faecal matter as substrate.

Scientific information on vermicomposting of sewage sludge and livestock excreta including pig waste, horse waste and cattle dung are available. But for verimcomposting of human excreta, the scientific literature is still scanty or completely empty.

However, recent work on vermicomposting of faecal matter undertaken in TUHH at the Institute of Wastewater Management and Water Protection has contributed to our knowledge on this subject, although research work has been executed with faecal matter solids filtered from the toilet discharges and not from urine diverting dry toilets. Faecal matter solids derived from flush toilets are expected to be less saline. Therefore, research with raw faecal matter is necessary as salt content of the substrate might influence activity (performance) of earthworms. Researchers are still in the dark on many basic questions relating to the environmental requirements of *E. foetida* in faecal matter. For the implementation of a continuous flow vermicomposting system, information on basic design criteria is still needed since methods of inoculating worms, loading rates, optimum stocking rates and reactor geometry are referred to but not investigated for any of the worm species studied in the previous work by Shalabi (2006). Therefore, this study is aimed at advancing the state of the research by providing data that may be essential for design of efficient vermicomposting systems for faecal matter resulting from urine diverting dry toilets.

3 Materials and Methods

3.1 Material collection and pre-processing

Two main types of material were used in this study; earthworms and human faeces. These are briefly described in the following sections. Others such as chemicals, growth media and glassware were standard materials.

3.1.1 Substrate origin and preparation

Faecal material was obtained from composting toilets (CTs) in an ecological settlement, Neu-Allermöhe, Hamburg. The settlement is made up of apartments consisting of 114 inhabitants and each apartment uses a composting chamber (3.5-5 m³) connected by a down-pipe with a toilet-seat directly on top. To recover faecal solids from the facility, the CTs' user interface were converted to urine diversion mode by retro-fitting with urine diverting inlets, and the faecal material collected in plastic boxes installed in the interior of the CTs. Collected material was transported to the laboratory of the Institute of Wastewater Management and Water Protection where it was thoroughly homogenised and analysed for physicochemical parameters prior to loading the reactors.

3.1.2 Earthworms

Earthworms, *Eisenia foetida*, were obtained from Regenwurmfarm Tacke, Borken-Burlo, Germany and cultured in the laboratory of the Institute of Wastewater Management and Water Protection.

3.2 Continuous-flow vermicomposting units, experimental set-up and monitoring

3.2.1 Design of continuous flow reactors

Twelve reactors made from polyvinyl chloride (PVC) were used in the experiments (except where otherwise mentioned). The reactors were 19.6 cm in diameter and 60 cm deep (300 cm^2 exposed top surface, 18 l volume). Each reactor sat on a supporting frame, overlained by a screened (metal) bottom with nominal 1 cm x 1 cm openings. Thus the base of each reactor was open to the air and free to flow-out of decomposed substrate. Substrate added to the top moves downward for 4-6 months while being processed by the earthworms and microorganisms. The earthworm, *E. foetida*, is a top feeder and moves upwards towards the new source of food. This feeding habit was used as the basis of design, so that the material removed from the bottom was nearly worm free, and the reactors' total earthworm population was maintained. Furthermore, the reactors depth (60 cm) was chosen to ensure that the residence time for the substrate as it descended was long enough to allow for earthworm reproduction. The residence time must be longer than the worms' egg/cocoon incubation period (average 23 days) – worms hatch and move upwards replacing mortalities and maintaining or increasing the reactors' population as needed (Herlihy, 2006). Thus earthworm population was self-regulating by natural selection.

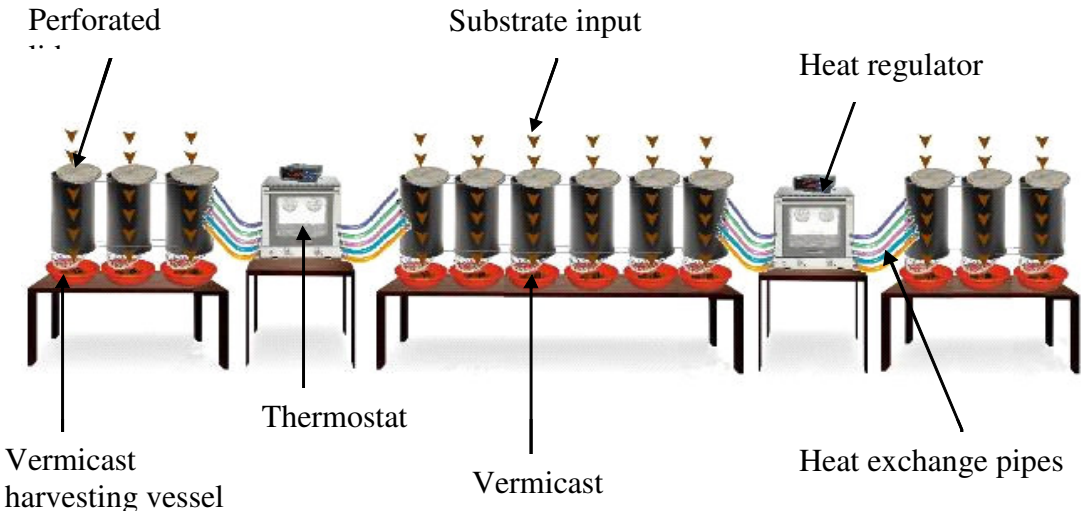


Figure 3.1: Schematic diagram of the continuous flow reactors (Housing of the reactors not shown. See Figure 3.2)

Four sets of reactors, all run in triplicate were made. Each set was housed in a separate rectangular tank (108 x 40 x 50 cm^3) filled with water (Figs 3.1 and 3.2). Water temperatures were maintained within the range 24 – 25.5 °C and controlled by thermostats connected to the tanks. Most of the time, the water temperature was nearly constant at 25 °C, varying only for short periods, thereby staying within the zone for optimum earthworm metabolism and activity according to Hartensein (1982) and Shalabi (2006). The air humidity inside the reactors was measured using a Hygrothermometer (Thies-clima) that was placed inside the reactors. All twelve reactors were covered with plastic lids with small perforation in the centre. The perforation was covered with a fairly dark fibre material that allowed limited amount of light into the reactor while permitting free-flow of air. Earthworm-processed material; which was a mixture of decomposed (vermicompost), partially decomposed material and worm castings (vermicast); was collected in plastic vessels at the base of each reactor. The system is sketched in Figure 3.1 and photographed in Figure 3.2.

Figure 3.2: Photograph of the continuous flow reactors housed in tanks

3.2.2 Adaptation process and operation of continuous flow reactors

Attempts to grow *E. foetida* directly on fresh faecal matter were entirely unsuccessful and resulted in the dead of worms. It was observed that earthworms became inactive within 25 minutes upon inoculation in the substrate and died within two hours. This was probably caused by high electrical conductivity, (approx. 2.8 mS/cm) brought about by excess ammonia (>1 mg/g) and soluble salts. However, worms fared better and subsequently acclimated to grow well when placed into a mixture of fresh substrate and original worm medium.

Therefore, to condition worms with the substrate's environment, an adaptation process was carried out. Bedding consisting of a mixture of three parts of original worm medium to two parts of shredded straw (by weight) was prepared and placed on the floor of the reactors. Each reactor was then initially loaded with 500g (wet weight) substrate, applied evenly across the reactor surface, giving two layers: bedding-substrate layers. The reactors were started by introducing desired number of *E. foetida* adults, which, in turn were randomly picked from a crop-residue-fed culture of over 4000 worms. The average moisture content of the entire bedding was maintained at 75 ± 5 %. Initially, all worms migrated to the bottom layer made mostly of vermicasts (worm medium). After about 3 days, a large proportion of the worm population entered into the intermediate zone located just beneath the surface of the substrate, and then into the actual substrate and distributed all over the core. Evidence, therefore, suggested that the worms were adapted to the substrate. That is, a smooth transition into the new medium had been attained and the worms considered adapted. Experiments were started immediately when worms had converted almost all the substrate into soil-like material indicating active feeding behaviour. As worms processed the substrate, their castings sank down, 'flowed' through the screen and were collected.

Two main design criteria; loading rate and initial worm inoculation density were evaluated. In addition, moisture requirements and the fate of pathogens during the vermicomposting process were investigated. The study was conducted in five parts. Part one was concerned with establishing an optimum loading rate for the continuous flow system. Loading rate is crucial for smooth functioning of the system, as excess loading can raise temperatures through heating or development of anaerobic conditions. The second part was a study of the initial worm stocking density. This was important as the amount of reactor space would affect earthworm behaviour and influence survival, reproduction and performance of individuals. The third part investigated the optimum moisture requirement of *E. foetida* in faecal matter. Moisture and temperature are the major environmental factors influencing performance. Part four was concerned with monitoring microbial populations during composting and vermicomposting by means of sanitation indicator bacteria (SIB), and assaying the final product for presence of SIB that survived the process. The investigation during this experimental phase concentrated on the process effectiveness in eliminating pathogenic organisms present in the raw material. This elimination is necessary to provide a hygienically safe, end-product. The last part dealt with earthworm salinity preference or avoidance response, and focused on establishing salt tolerance boundaries for the earthworm, *E. foetia.*

Although the experimental setup was fundamentally the same for all parts (except where stated), the methodological approach was different for each of them. A brief description of each part is provided below.

3.3 Experiments

3.3.1 Moisture requirement experiments

As with the previous experiments, the material was thoroughly homogenised and the initial moisture content determined by oven drying at 105°C for 24 hours, subsequently, appropriate quantities of deionised water were added to five separate 500 g sub-samples to obtain the following moisture levels: 60%, 65%, 70%, 75% and 80%. Known weights (50 ± 2g) of juvenile worms were introduced into each of the reactors to provide a density of 2.30 kg worm/m^2. Three replicates for each moisture level were established. The same setup without earthworms, served as controls. The reactors, which were 10.8 cm in diameter and 24 cm deep, were operated in batch mode. To maintain the moisture content of the material in the reactors, replicates were covered with moistened foam material, which were opened briefly (approx. 3 minutes) daily to allow exchange of gases. The moisture content of the material was determined weekly by weighing and drying 3 - 5 g homogeneous sub-samples at 105°C for 48 hours. Distilled water was then added to adjust the substrate moisture contents to the desired level. Substrate parameters were examined before, during (weekly basis) and at the end of the experiment. All determinations were carried out in triplicate. The experiments were performed in batch mode and at ambient conditions, room temperature 21 ± 1.5 °C.

3.3.2 Loading rate experiment

This section investigated the influence of feed loading rate on worm processing capacity. The aim was to determine the optimum rate at which faecal matter can be added to the continuous flow reactors, expressed as the amount of material added per square meter of reactor surface per day (kg feed m^{-2} day^{-1}). The objectives were to (1) better understand the effects that substrate loading rates have on substrate residence time, (2) determine how control of loading rates affects the stabilisation process and development of suitable physical-chemical conditions, and 3) identify a loading rate that allows for optimisation of the continuous flow vermicomposting system. Vermireactors were setup as detailed earlier (section 3.2.1) to investigate three loading rates at 6.7, 10 and 13.3 kg feed/m^2/day. The three loading rates were designated as light, moderate and heavy. To achieve these loading rates, the reactors were fed with 200g, 300g and 400g substrate respectively. These treatments corresponded to an application depth of 3mm, 6.5mm and 9mm respectively. Each reactor was inoculated with 50 mature earthworms (approx. 90g). This provided feeding rates of respectively 1.63, 1.22 and 0.82 kg-feed/kg-worm/day for the light, medium and heavy loadings. Two replicates for each feeding rate were established. For each loading rate, one reactor without earthworms was setup to serve as control. Initially, all reactors were fed daily for 10 days, followed by scheduled feeding twice a week until the end of the experiments (120 days). Markers (spherical glass pebbles) were added to the material during pre-processing to date the different layers of substrate. A different colour marker was introduced after every week. All reactors were maintained at 25 ± 0.5 °C throughout the experiment, and the average moisture content of the reactors was maintained at roughly 70 % periodic monitoring and appropriate replenishment as detailed earlier. The raw substrate was analysed at every loading, harvested vermicasts was also analysed, and at the end of the experiment samples from the different layers were analysed in duplicate and averaged.

3.3.3 Initial worm inoculation density experiment

In this part of the study, the influence of initial worm stocking density on worm processing efficiency was investigated. Each reactor was inoculated with 50g, 70g and 80g adult specimens of *E. foetida* (wet weight) to provide the stocking densities equal to1.67, 2.33 and 2.67 kg-worms m^{-2} respectively. Three replicates for each stocking density were established. A set of three reactors without earthworms served as control and consisted of the same amount of substrate. During the first 15 days the reactors were continuously fed (daily basis) 200g substrate until 3 kg was reached and thereafter feeding was scheduled at 250g twice per week.

3.3.4 Earthworm avoidance-response tests

In order to optimize decomposition conditions, it is desirable to understand the optimal salinity for growth and activity of earthworms. Therefore, the aim of this part was to determine the response of *E. foetida* to different levels of salinity. The specific objective was to determine the upper levels of salinity that can be tolerated by the worm *E. foetida* and the influence on its processing activity.

E. foetida were exposed to five salt concentrations in five independent trials. 2 kg of faecal material was rendered salt-free by eluting with deionised water at 1:10 (w/v). The raw faecal material and eluted (salt-free) material were combined in various proportions and homogenised to obtain desired electrical conductivities (EC). Five EC levels were set up at 1.2, 1.6, 2.4 and 2.7 mS/cm in deionised water extracts of the samples. These corresponded to salt concentrations of 3.4, 6.9, 14.0 and 27.5 g salt/kg DS respectively. Worms were placed in a five-compartment plastic chamber and were allowed unrestricted movement between the different compartments. After 24-48 hours, each compartment was assessed for presence of worms. That is, avoidance response.

3.3.5 Influence of vermicomposting on microbial populations.

This experiment monitored the evolution of six bacteria populations during vermicomposting within a 60 day period. Two groups of reactors, each consisting of three 18 L PVC units, were setup as previously and operated in batch mode. One group was designated as test reactors and the other group as controls. Thus, three replicate reactors were maintained per group. Each reactor was loaded with 4 kg substrate adjusted to the same initial moisture content of 70 % based on earlier findings. The test reactors were then seeded with *E. foetida* (on top of the substrate). The sampling regime consisted of collecting three representative samples, with respect to different depths, from each reactor on various days using a steel pipe auger (approx. 0.5 cm inside diameter). Augers were autoclaved, for sterilization, at 120 °C for 45 minutes after each sampling event. All reactors were maintained at 25 ± 0.5 °C throughout the experiment, and moisture levels kept fairly constant by periodic (every two days) sprinkling of equal amounts of distilled water.

3.4 Chemical and microbiological analysis

3.4.1 Chemical analysis

Analyses were performed immediately after samples were collected and when this was not possible, samples were preserved by refrigerating at 4 °C to shut down microbial activities (decomposition process) until analyses could be done. Vermicompost in this study refers to mixture of worm castings and decomposed and partially decomposed substrate. All samples were analysed in triplicate and the results were averaged. All chemical analyses were conducted using standard methods as described in the "Methods book for compost analyses" of the FCQAO (BGK, 1994). The following paragraphs summarise the parameters analysed and the methods used.

Water content and dry substance: determined gravimetrically by weighing the mass loss after drying sample at 105° C up to a constant weight (48 h).

Volatile solids: DIN 38414, S 3 (burning at 550°C; determination as loss on ignition)

Salt content: determined in an extract of deionised water (ratio: 1: 10; wet weight of solids to volume of deionised water) after 2 h of stirring and filtration.

pH: determined electrochemically in a suspension of sample in 0.01 molar $CaCl_2$ solutions (ratio: 1:10; wet weight of solids to volume of deionised water). The pH in the suspension was determined with a calibrated pH meter.

Soluble nutrients and carbon: determined by extraction of samples by liquid (deionised water, $CaCl_2$ solution or CAL solution) for two hours. The suspension was separated by centrifugation followed by filtration with fine pored filter paper. The liquid phase was further analysed for the following constituents:

- Ammonia (NH_4^+-N) : DIN 38306 (1983)
- Nitrite: DIN EN 26777 1993)
- nitrate DIN 38405 (1979) and
- Kjedahl-nitrogen DIN EN 25663 H11 (1994). The mineral nitrogen compounds were analysed with a double ray photometer from Jasco type V550.
- Phosphorus and Potassium: Phosphorus was determined in an extract of sample with CAL solution using Technicon Autoanalyser. The analysis of potassium was done in the Central Analytical Laboratory of the TUHH by flame photometry.
- Water-soluble carbon (WSC): WSC was extracted with distilled water (solid to liquid of 1:5)

Total nutrients and carbon: Samples were milled to <0.25mm and given to TUHH central laboratory for analysis:
Kjeldahl nitrogen (TKN): DIN EN 25663 H11 (1994).
Total nitrogen: DIN 19684

Heavy metals: disintegration with aqua regia (DIN 38414, S7); determination according to ISO 11885 (Pb, Cr, Cu, Ni, Zn), ISO 5961 (Cd).

3.4.2 Microbiological analysis

Pathogen indicators and culture media: three sets of bacterial analyses each with a different growth medium (Chromocult® Coliform, Slanetz-Bartley, and m-FC Agars) and plating technique (Spreading, Membrane filtering) were carried out independently. With Chromocult® Coliform agar, the number of *Escherichia coli* and fecal coliforms (CFU/g) was determined for every analyzed sample. *Enterococcus faecalis* was identified through Slanetz-Bartley agar, while m-FC agar was used to monitor the populations of *Shigella spp.*, *Salmonella spp.*, and *Enterobacter spp.* The three culture media were prepared using standardized protocols and reagents, always working under sterile conditions. It is important to note that although m-FC agar is commonly used to identify faecal coliforms, in this experiment it was successfully used to differentiate between other bacteria. A example of the growth media is photographed in Figure 3.4.

Figure 3.3: Photograph of one of the pathogen cultures used in the experiments plates.

Sample preparation for microbial analysis: 1 g (wet weight) of sample was diluted with 9 ml of saline peptone solution in a screw cap tube and was homogenized by rotation (Vortex mixer) at 250 rpm for 40 min at 22°C [the final samples (worm product) needed around 60 min]. The resulting slurries were serially 1:10 diluted [0.1 ml of homogenate/slurry (1st dilution) with 0.9 ml of saline peptone solution] in nine steps. Five aliquots (0.1 ml) of each dilution were spread in the Petri dishes containing the required growth media. Therefore, five replicates were used per dilution in each of the bacterial analysis. The moisture content of each fecal sample was determined by drying 1 g at 105°C for 24 h. All microbial counts (CFU) were then expressed per gram of dry weight.

Bacterial incubation: Samples spread in the Chromocult® Coliform Agar were incubated for 24 hours at 35-37°C, while the dishes containing the Slanetz-Bartley Agar were left in the oven for 48 hours at 47°C. The m-FC agar samples were incubated for 24 hours at 44.5°C. This process was carried out in an electric oven, working aerobically.

Isolation and quantification of isolates: Dishes with visible colonies after incubation (30-300) [15-150 in the Slanetz-Bartley agar] were counted. The average of CFU in the five replicates of each dilution was used as starting point for further calculations. The average number of colonies was then calculated according to 1 g of original sample (number of colonies multiplied with the dilution factor, taking into account the dilution of the aliquots as well).

4 Results and Discussion

4.1 Moisture requirements

In this part of the study investigations were carried out in order to answer the following questions:

- *Which is the optimum level of moisture for faecal matter vermicomposting?*
- *How are the dynamics of faecal matter degradation and chemical properties of the composted material affected with earthworms at different moisture contents?*

It is known from recent research (Shalabi, 2006) that carbon loss will be greater and occur more rapidly with earthworm inoculation, and that in spite of rapid carbon declines, C:N ratios would increase or remain relatively stable. Therefore, a short maturation period and a greater concentration of nutrients in inoculated reactors are expected.

Earthworms have 75% to 90% water of their body weight (Grant, 1955). Enzymatic reactions require minimum moisture content of living organisms. On the other hand, moisture contents exceeding a certain level may inhibit access of the worms to oxygen thus impairing their activities in organic matter degradation. Gray *et al.* (1971) have pointed out that optimal level of moisture is required for vermicomposting as well as for microbial flora present in the process. Under adverse dry conditions of substrate earthworms release large amount of water from their bodies for their survival, and their activity get reduced (Edward and Lofty, 1977). The available moisture in a substrate is expressed as a pF value, which is the logarithm of the negative pressure or tension in the substrate, and indicates the water holding capacity of the substrate (Abrahamsen, 1971; Reinecke and Venter, 1985). Determination of the pF value of organic substrates is complicated and was not attempted in this study. Moisture content in this study is expressed as a percentage.

In a pre-trial study to establish the minimum moisture requirement of *E. foetia*, it was observed that at moisture levels of 40%, 45%, 50% and 85% all the worms had died by day 3. The lowest moisture level at which some of the earthworms (70%) survived the pre-trial period (20 days) was 55%. This finding was the basis of the choice of the five moisture levels investigated. In the following sections chemical parameters, analysed for the purpose of monitoring progress of the decomposition process, are discussed. The average composition of the collected raw material is given in Table 3.1. The chemical content profiles are presented in Figure 4.1 to Figure 4.12.

Table 4.1: Mean chemical characteristics (dry wet basis) of faecal matter from UDD toilets in Neu-Allermöhe used in the experiments (mg/g)

pH	EC*	DM (%)	C	N	P	C:N	K	Ca
7.14	2.8	83	4.59	nd	19.63	7.3	37.71	28.91
Mg	Zn**	Cu**	Pb**	Ni**	Cr**	Cd**		
9.27	276	87.3	10.23	241	513	0.26		

DM: dry matter, nd: not detected, EC: electrical conductivity, *mS/cm, ** mg/k

4.1.1 pH

The pH of wastes is one of the most important factors controlling the rate of decomposition and maturity of the product. It influences the activities of microorganisms and thus the rate of stabilization of organic matter. However, Rynk *et al*. (1992) have pointed out that when waste is comprised of a mixture of organic materials the composting process is relatively insensitive to pH, largely because of the broad spectrum of microorganisms involved. pH does become important in mono stream substrates such as faecal matter which often have a high percentage of nitrogen. High pH, (above 8.4), enhances the conversion of the ammonium ion, NH_4^+, to ammonia gas, resulting not only in nitrogen loss from the material but also causing inhibition of nitrifying microorganisms. Loss of nitrogen, in the form of ammonia, to the atmosphere not only causes nuisance odors but also reduces the nutrient value of the product.

The pH profiles of the substrate during a three month processing period with and without worms at different moisture contents are presented in Figures 4.1a and 4.1b.

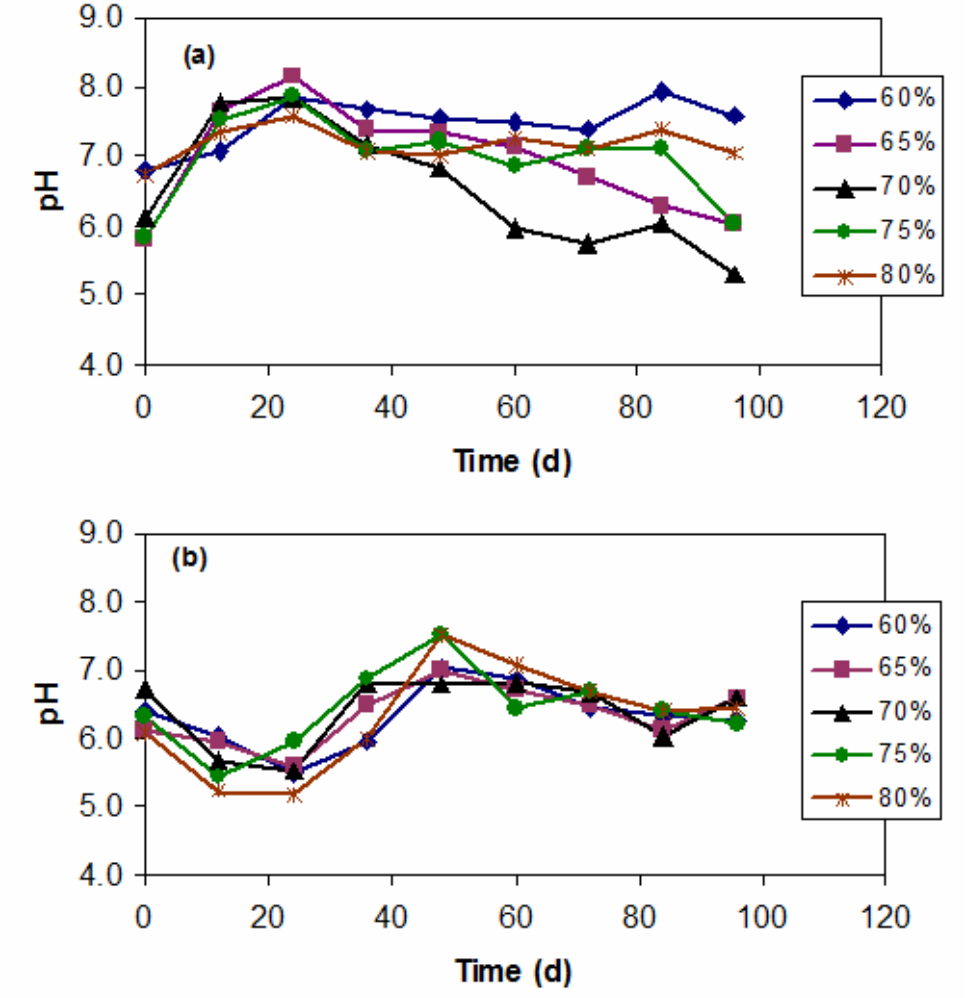


Figure 4.1: Moisture content and earthworm effects on pH. (a): Test (with worms), (b): Blank (without worms)

For the results shown in Figure 4.1a, the initial pH ranged from 5.8 to 6.8, indicating the substrate was slightly acidic to neutral. During vermicomposting, the pH values at all five moisture levels increased rapidly up to day 24, afterward a rapid decrease was recorded up to day 48, followed by a gradual decrease up to day 72, except for the reactors at 70% moisture content where the decline was most pronounced after day 36. The initial low pH of the substrate could be attributed to the formation of volatile organic acids (VOA) under temporary anaerobic conditions. VOA formation was expected because of the high moisture content (>80%) of the fresh faecal matter. The rise in pH observed during the first 24 days after inoculating with earthworms was likely due to VOA breakdown facilitated by aerobic conditions brought about by earthworm movements. As the pH was below 8.2 throughout the experiment it was unlikely that high amounts of NH_3 could have been present. The least variation in pH was observed with the reactors maintained at 60 % moisture content. This was probably due to reduced chemical and microbial activities resulting from low moisture content.

Overall, the final pH ranged from 5.3 to 7.6, with the lowest recorded in the reactor at 70% moisture level. The pH decline was not clearly evident in the reactors at 60 and 80 % moisture, where the pH remained fairly constant after day 36 in the range 7.1 ± 0.25 and dropped markedly after the day 84 (Fig. 4.1a), registering relatively small increases compared to the rest of the treatments. This correlated with the lower levels of nitrates recorded at these moisture levels (Fig. 4.9). Obviously, high and low moisture contents led to low nitrification rates.

Nitrification is linked to generation of protons. Therefore, the low pH recorded in the test reactors at the end of the experiments might have been due to nitrification, and probably also to the production of CO_2 and the formation of humic and fulvic acids. This effect was clearly evident at the 65 % and 70 % moisture levels, with the effect more pronounced at 70 % moisture.

For the blanks (without worms) shown in Figure 4.1b, in contrast to the test reactors (with worms), an initial decline in pH was recorded up to day 12, reaching values as low as 5.17 in the reactors at 80 % moisture. This could be attributed to anoxic conditions in the control which favored accumulation of organic acids. This was not the case in the test reactors presumably due to the aerating effects of worms that allowed the breakdown of VOAs. pH in the controls increased drastically after day 24 up to day 48, afterwards a gradual decrease was detected over the rest of the experimental period. Unlike the test reactors, the pH trend was similar at all moisture levels except for the blanks.

Other researchers have reported similar results (Gunadi and Edwards, 2003; Garg *et al*., 2006) to those observed in this study. Short *et al.* (1999) reported an initial increase in the pH and subsequent decrease towards the end of vermicomposting. Benitez *et al.* (1999) have reported a decrease in final pH values of sewage sludge from 8.0 to 6.7 following 8 weeks of vermicomposting. Atiyah *et al*. (2000) have observed a decrease in pH of cow manure towards the end of curing after 4 weeks of vermicomposting. Albanell *et al.* (1988) have reported pH decreases from 9.1 to 7.2 during vermicomposting of sheep manure mixed with industrial cotton wastes.

Ndegwa e*t al*. (1999) have attributed the shift in pH from the initial near neutral towards acidic conditions to the bioconversion of organic material into various intermediate species of organic acids. Ndegwa *et al.* (2000) explained these observations by suggesting that the pH shift towards acidic conditions occurs because of the higher mineralization of organic nitrogen compounds and subsequent transformation of ammonia to nitrites or nitrates. Haimi and Huhta (1986) and Elvira *et al.* (1998) had come to the same conclusion. Elvira and colleagues concluded that 20 to 43 % of total organic carbon was lost to CO_2 production and extractable carbon increased, as did the humic fraction.

However, other researchers have reported a pH trend during vermicomposting of organic material that was different to that observed in the present study. Datar *et al*. (1997) and Nogales *et al.* (1998) have reported an increase in pH with time throughout the curing period. Komilis and Ham (2006) reported that decomposition of organic matter leads to formation of ammonium ions and humic acids and could be the possible reason for increment in pH. Buchanan, 1988)) have attributed the increase to $CaCO_3$ secretions in the worm gut that have an alkalizing effect on the substrate.

As explained in the introduction, the pH requirement of earthworms has been investigated extensively and they have been shown to be generally tolerant to pH within certain limits. Various authors have suggested different limits: Rivero-Hernandez (1991) suggested 7 to 8 as ideal limits for *E. foetida*. Kristiana *et al.* (2005) recommended 6.5 and 7 as the optimum pH range for earthworm growth. Earlier work by Edwards (1988) suggested that 5 is the ideal pH for *E. foetida.* Edwards and Bohlen (1996) have reported that worms will avoid acid soil environments of pH less than 4.5, and extended exposure to such environments could produce lethal effects on worms. For the end-product (vermicompost), a neutral to slightly acidic (6.0-7.5) pH has been recommended. Steps should be taken to control the pH if it exceeds 8.4, where it becomes potentially harmful to plants, and is associated with odour and ammonia loss.

In the present study, the final pH (5.3 to 7.6) falls within the recommended range for agricultural application.

4.1.2 Salt content

The salt levels are presented in Figures 4.2a and 4.2b, with the initial concentrations ranging between 3 and 8 g/kg DS (equivalent to conductivities between 1.85 and 2.75 mS/cm). Salt concentrations increased markedly in both the blanks (Fig. 4.2b) and test series (Fig. 4.2a) during the first 48 days of curing. The change in salt concentration was well correlated ($r = -0.64$) to volatile solids (VS) reduction rate (Fig. 4.3). Similar to the changes in TOC and VS, changes in salt concentrations were clearly observed at 65 and 70% moisture. The salt profile at 75% moisture did not differ to the profiles of the 60 and 80% moisture levels.

A similar trend was observed in the blanks (Fig. 4.2b) except that the increase in concentration reduced markedly after day 24 with the highest salinity recorded at 75% moisture. The increase in salt concentrations was due to loss of organic matter, and additionally to release of different inorganic ions (such as phosphate, ammonium and potassium).

Similar observations to those found in this study were reported by Masciandaro *et al.* (1997) and Kale (1998) who noted that soluble salts and electrical conductivity initially increase during the vermicomposting process. However, Elvira *et al.* (1998) and Warman and AngLopez (2002) have reported that the initial increases are followed by decreases over time as the waste stabilizes. Tajbakhsh *et al*., (2008) have observed a 40 % reduction in electrical conductivity of vermicompost by the end of week 12 in comparison to the initial value of week 0. Elvira *et al*. (1998) and Warman and AngLopez (2002) have reported an eventual decrease in EC during the vermicomposting process. They attributed the decrease to elevated pH and ammonia loss.

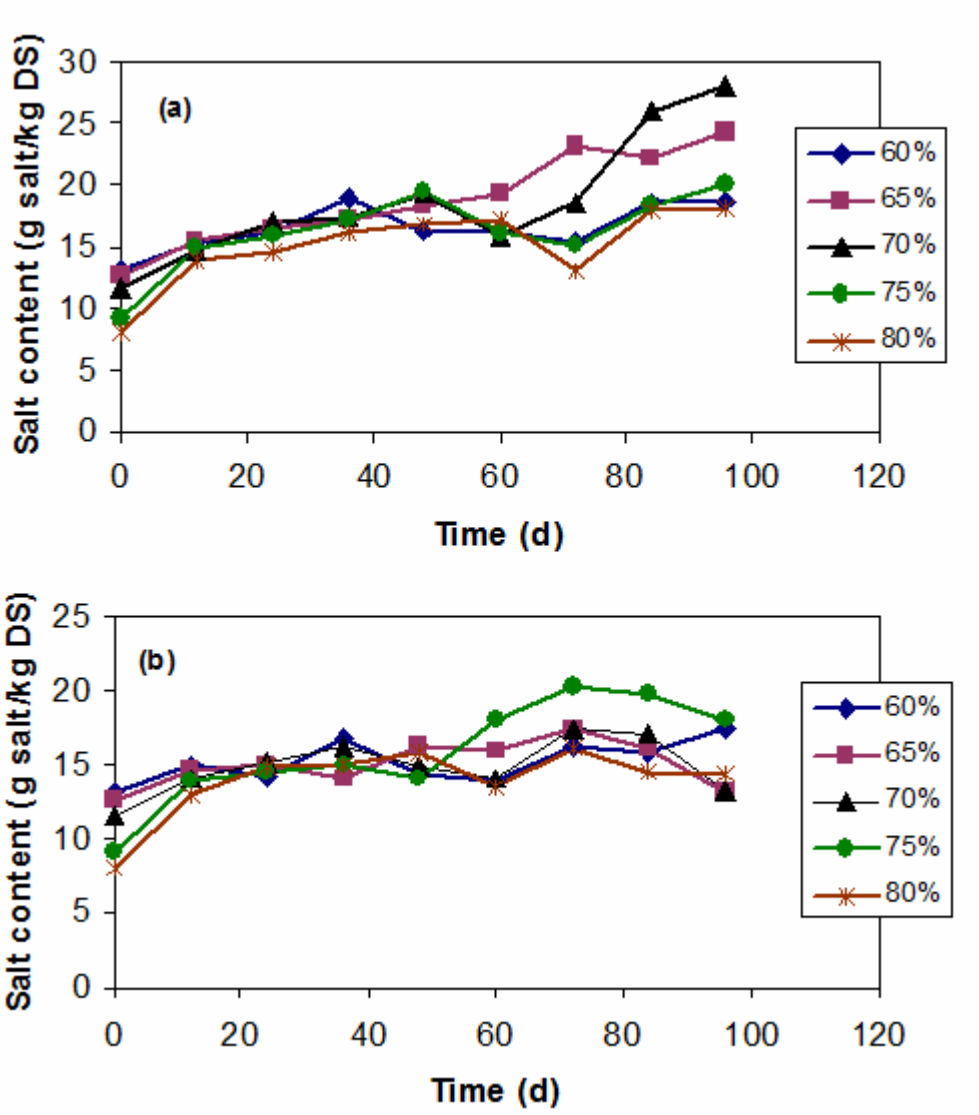


Figure 4.2: Moisture level and earthworm effects on salt content. (a): Test (with worms), (b): Blank (without worms)

The final salt concentrations (13 to 24 g/kg DS; approx. 3.2 to 5.4 mS/cm) found in this study are higher than those reported by Khwairakpam and Bhargava (2007) who observed a rise in conductivity from 2.44 mS/cm to 3.75 mS/cm during composting of cattle waste using *E. foetida*, *E. eugeniae*, *P. excavatus* and their combinations over a 45 day period. Also, Gomez-

Brandon *et al.* (2008) have reported smaller increases in electrical conductivity (EC) during composting of cattle manure in which EC values rose from about 1.3 mS/cm to 3.1 mS/cm during 180 days of curing.

However, similar concentrations to those observed in this study have been reported by other authors (Wong *et al*., 2001). Gomez-Brandon *et al*. (2008) have pointed out that the EC affects the quality of composts in a large way because it reflects their salinity and suitability for crop growth.

Electrical conductivities (EC) observed in the vermicomposts of this work ranged from 3.2 – 5.4 mS/cm. Salt tolerance limits of the most tolerant plants are 3 mS/cm. A further confirmation of this threshold value has been given by Soumaré *et al*. (2002) who have recommended 3 mS/cm as maximum concentration for agricultural application.

Results of the present study suggest that, from salinity consideration, vermicompost from faecal matter may not be suitable for land application

Figure 4.3 below shows the relationship between the total volatile solids and the salt content of the substrate during the experimental period. Although the coefficient of determination was low (R^2 = 0.404), the correlation between the two variables was highly significant ($p<0.01$). Decreasing levels of volatile solids were associated with increasing salt content. Predicted salt content = -0.31VS (%) + 45.8.

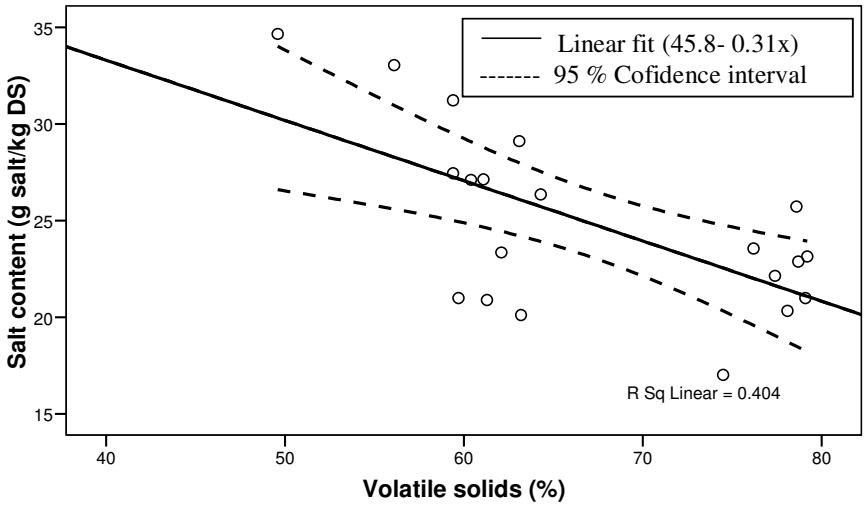


Figure 4.3: Correlation between volatile solids and salt content during the experimental period. (Scatter plot showing fit with confidence interval).

4.1.3 Volatile solids

Faecal matter contains a high proportion (approx. 65 to 85 % of the total solids) of organic matter. The most important substance groups are carbohydrates, proteins and lipids (ATV, 1996).

This work reports organic matter as the total weight loss on ignition. Although this method is common and gives adequate results, it is not 100% accurate. This is because chemically bound water and other volatile inorganic substances such as ammonia-compounds and some carbonates were also recorded. Pabsch (2004) suggested that a more accurate expression is "volatile solids" (VS). This is not the same as organic matter, but will be used throughout in this text.

The organic matter content of wastes is of importance for two main reasons. First of all, it is, among others, one indicator of the grade of stability (Pabsch, 2004) of the treated material. Stability, according to the US-EPA, is defined as the point at which readily degradable substrate is diminished so that its decomposition rate does not control the overall rate of decomposition. Decomposition rate expresses the effectiveness of the composting process and is an important parameter to assess the process. Because only the volatile solids can be decomposed during the composting process, the decomposition rate is usually expressed as the reduction in volatile solids (Qiao and Ho, 1997). As stability is related to vector attraction, the US-EPA recommends a 38 % reduction of volatile solids (in the case of sludge treatment) as one alternative for vector attraction reduction. Any degradable material that remains characteristically degrades so slowly that vectors are not drawn to it (US-EPA, 1999). Secondly, the reduction of organic matter will diminish the mass of the faecal charge considerably. Pabsch (2004) has pointed out that such a reduction will significantly reduce the expenses for handling and recycling or disposing of faecal matter.

In the VS profiles shown in Figures 4.4a and 4.4b, both the test reactors (with worms) and the controls (without worms) initially had similar VS levels, roughly 80%. From the VS profiles, it can be seen that moisture content of the substrate had an effect on organic matter reduction rate. For test and blank reactors, the VS levels decreased over the course of curing, with a markedly greater rate and extent for the test reactors than for the controls (blanks). The decreasing pattern suggests that earthworms participated in the decomposition process and accelerated the reduction of VS, and that moisture level influenced the degradation rate. The rate of decomposition was very rapid during the first 48 days at the 65, 70, 75% moisture levels resulting in up to 24% VS reduction (70% moisture level) (Fig. 4.4a), with an unexpected slight increase at the 70% moisture after day 36. Subsequent decomposition was slow at the above three moisture levels, resulting in a further loss of 2.7% and a maximum VS reduction of 34.7%, occurring at 70%.

The fact that there was a marked difference in final VS values at the different moisture contents suggests that moisture may be critical for organic matter decomposition. Reinecke and Venter (1987) have shown that a moisture difference of as little as 5% in the substrate has significant influence on earthworm growth and activity.

The VS reductions recorded during the process seemed to be due for the most part to the mineralization of organic matter with production of CO_2 and NH_3. Shalabi (2006) reported a similar trend during the vermicomposting of faecal solids filtered from blackwater. While monitoring the performance of two worm species, *E. foetida* and *Dendrobaena veneta,* at four temperatures (10, 15, 20 and 25°C); he observed a rapid decrease in VS in the first 10 days at all temperatures.

In the present study, very low VS reduction (3.5% and 2.9%) were recorded at 60 and 80% moisture levels (Fig. 4.4a) respectively, indicating that low and high substrate moisture were less favourable for earthworms.

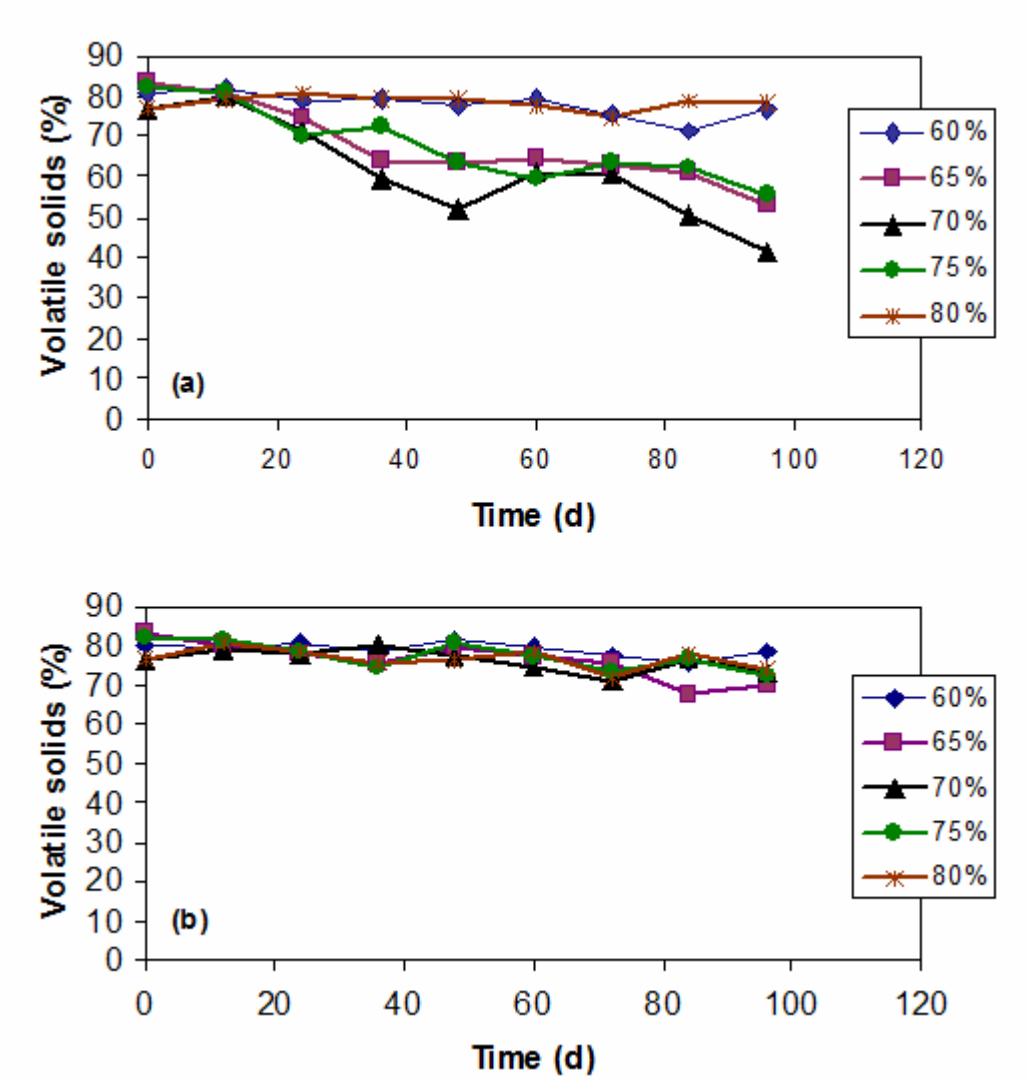


Figure 4.4: Moisture level and earthworm effects on volatile solids. (a): Test (with worms), (b): Blank (without worms)

For all the blanks shown in Figure 4.4b, reductions of VS were clearly lower than the test series (with worms). During the 96 days of curing, the VS content showed negligible changes, attaining a maximum reduction of 14.7% (at 65% moisture) which compares poorly with the value (34.7%) obtained from the test series. VS reductions for the controls, at all moisture levels except 65%, were comparable to the test units at unfavourable moisture contents (60 and 80%).

VS reduction rates of the present study were generally higher than those reported by some authors. Ramos *et al*. (2005) found 13 to 22% reductions with various biosolids mixtures and cow manure. Sampedro *et al*. (1996) observed 12.7% reduction after 90 days using

Eisenia andrei on solid pulp and paper mill sludge and Neuhauser *et al*. (1988) who obtained a 28% reduction after 4 weeks with activated sludge, also using *E. foetida.*

Other authors have reported VS reductions higher than those observed in this study. Fredrickson *et al*. (1997) have reported 37% VS reductions after two months vermicomposting of municipal solid waste compared to a 30% reduction within four months composting without worms. Datar *et al*. (1997) observed VS reductions of 50 to 67% in vermicomposting of solid waste using the earthworm *Eudrilus eugeniae.*

The reduction rates found in this study were similar to those reported by Shalabi (2006) who observed a reduction in the mean VS content by approximately 34% after 130 days vermicomposting 40 kg faecal solids using 2000 *D. veneta* worms and *E. foetida*. Similar observations to those of this study have also been reported by Singh *et al*. (2004), Atiyeh *et al*. (2000), Kavian and Ghatnekar (1991); and Hartenstein and Hartenstein (1981).

In spite of the high difference in VS reduction between the control and test series found in this study, the vermicomposting results still fall way short of the target (38%) set by the US-EPA, as cutoff value for reducing vector attraction. This suggests that the vermicompost obtained in this study was not stable and require further extended curing time.

4.1.4 Total organic carbon

Figures 4.5a and 4.5b show the total organic carbon (TOC) concentrations; which ranged between 164 g/kg and 316 g/kg at the end of the experiment from an initial value of 390 g/kg. TOC content at all five moisture levels declined over the entire experimental period, with the decrease more pronounced at 65, 70 and 75% substrate moisture (Fig. 4.5a), indicating that moisture levels influenced TOC reduction as has been discussed for VS reduction. It can be suggested that these water levels favoured earthworm activity. In comparison, decreases in the remaining series (60 and 80%) were minor indicating that decomposition conditions were relatively unsuitable. TOC correlated highly ($r = 0.93$) with the reduction of volatile solids with the exception of the reactors at 60% and 80% moisture level where a weak correlation was found. Lower TOC reduction rates than in the other series were obviously due to reduced worm activity caused by unsuitable moisture conditions.

At the end of the experiment, the highest mean percentage reduction of TOC (57.8%), compared to its initial level was recorded for 70% moisture content, followed by 75% moisture content (47.2% TOC reduction), and 65% moisture content (37% TOC reduction). This was consistent with the conclusions drawn from the changes in volatile solids reduction rate, which was an indication that the two parameters were strongly correlated.

For the blanks shown in Figure 4.5b, the TOC decrease was virtually nil at all moisture levels, except at 65% and 70% moisture where some reductions were observed after day 60. The highest percentage reduction (16.4%) attained at 65% moisture was much lower than the lowest (18.9%) recorded in the test reactors involving worms.

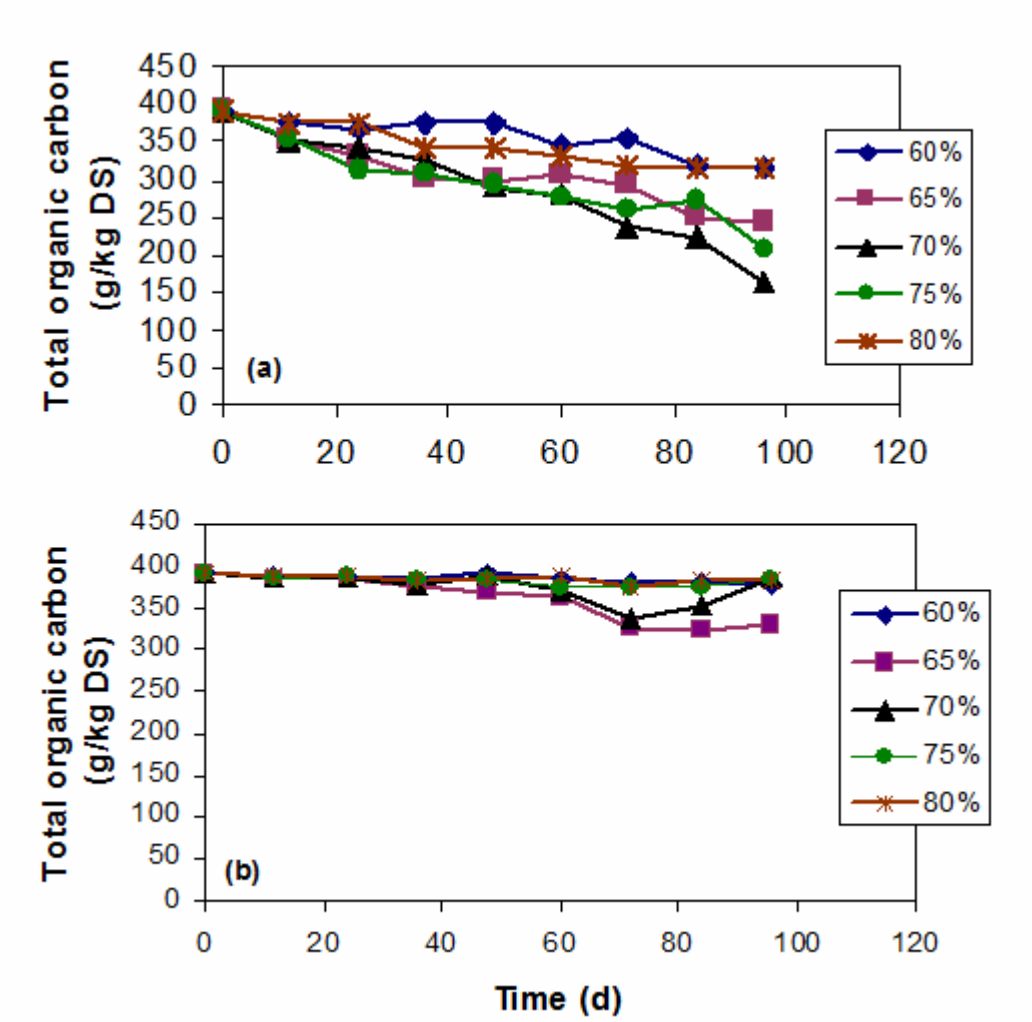


Figure 4.5: Moisture level and earthworm effects total organic carbon.(a): Test (with worms), (b): Blank (without worms)

Several workers have observed a similar trend in TOC change during composting and vermicomposting of various wastes. A large proportion, between 27% and 52%, of the TOC is lost as CO_2. The reduction rates observed in this study are higher than those reported by: Sampedro *et al.* (1996) who have observed 8.9% reduction in TOC after 90 days of vermicomposting wastewater sludge from paper-pulp industry; Benitez *et al*. (1999) who have recorded lower TOC reduction of 40.3% in a mixture of anaerobically digested paper mill sludge and sewage sludge after 18 weeks of vermicomposting; Nogales *et al*. (1999) who found TOC reduction of 39-53% after 63 days of vermicomposting biosolids alone and with wood shavings material; Elvira *et al.* (1998) who achieved between 20 and 43% TOC reduction after vermicomposting a mixture of paper mill sludge, dairy sludge and cattle manure for six months; Kaushik and Garg (2004) who have observed 26.5-52.5% TOC reduction in different mixtures of cow dung, solid textile mill sludge and agriculture residue after 11 weeks vermicomposting under laboratory conditions; and Garg *et al*. (2006) who have reported 35.9% TOC reduction during the vermicomposting of a mixture of textile mill sludge and biogas slurry using *E. foetida*.

However, Garg and Kaushik (2005) have reported TOC reduction rates higher than those found in this study. The authors observed reduction rates ranging between 33 and 66% after vermicomposting different organic wastes for a period of 100 days using *E. foetida*.

The processing activities of earthworms are directly related to their growth and reproduction. High survival rate and rapid weight gain imply higher reduction of TOC. The growth of *E. foetida* in organic matter substrates with different moisture contents has been extensively studied. The optimal moisture contents found in this study are slightly lower than those reported by Kaplan *et al*. (1980) who observed best survival and maximum weight gained at moisture contents between 75% and 85% in horse manure and activated sludge, whereas in this study, it was observed that TOC reduction rate declined remarkably at moisture levels beyond 75%. Gunadi *et al*. (2002) reported even higher optimal values (of 85% and 95%), for growth of *E. foetida* in activated sludge, than those found in this work. Satchell (1983) noted that 85% of the worms placed in a moisture gradient selected moisture conditions of 81% - 85%, whereas Palsania *et al*. (2008) observed that the moisture content of 75 ± 5% is the optimal range at which the vermicomposting process is fastest. The latter authors made the conclusion after investigating the effect of moisture content variation over kinetic reaction rate during vermicomposting of sugar cane bagasse using *E. eugeniae*.

Also, Muyima *et al.* (1994) have reported moisture levels near to those found in this study: the moisture preference for clitellate worms ranged between 67.4 and 84.3%, whereas the optimum moisture content for growth and maturation of juvenile worms was 75%.

The variation in these findings suggests that the optimum moisture content for earthworm activity varies according to the type of substrate and according to the species of earthworm.

4.1.5 Water extractable organic carbon

The water extractable organic carbon (water extractable OC) represents the most easily biodegradable carbon fraction during the composting process because it consists of sugars, organic acids, amino acids and phenols, apart from the soluble but recalcitrant fraction of fulvic acids (Garcia *et al.,* 1991). Therefore, it can be used as a good indicator of stability. However, caution is needed when using water extractable organic carbon as a maturity index because, as Gulyas (2003) has pointed out during biodegradation of organics water soluble organics like hetero polysaccharides and humic substances are also formed as transformation products which are recalcitrant towards biodedgradation.

In the present study, the initial water extractable OC values ranged between 4.0 and 4.5g C/kg dry solids. The concentration of water extractable OC declined with time for both the test and control series (Figure 4.6a and 4.6b), which is consistent with the literature (Chefetz *et al*., 1998; Inbar *et al*., 1993; Garcia *et al*., 1991). For the test series (with worms), the greatest decline occurred during the first 24 days, except in the reactors at 80% where an unexpected sharp rise was observed after day 12, followed by a slow decrease during the rest of the period. For the remaining reactors, water extractable OC remained fairly constant or decreased only slightly until after day 60 and day 84 when marked decreases where observed in the reactors at 65 and 70% moisture content. Overall, the decrease rate was highest at 65, 70 and 75% moisture levels compared to moisture contents of 60 or 80%.

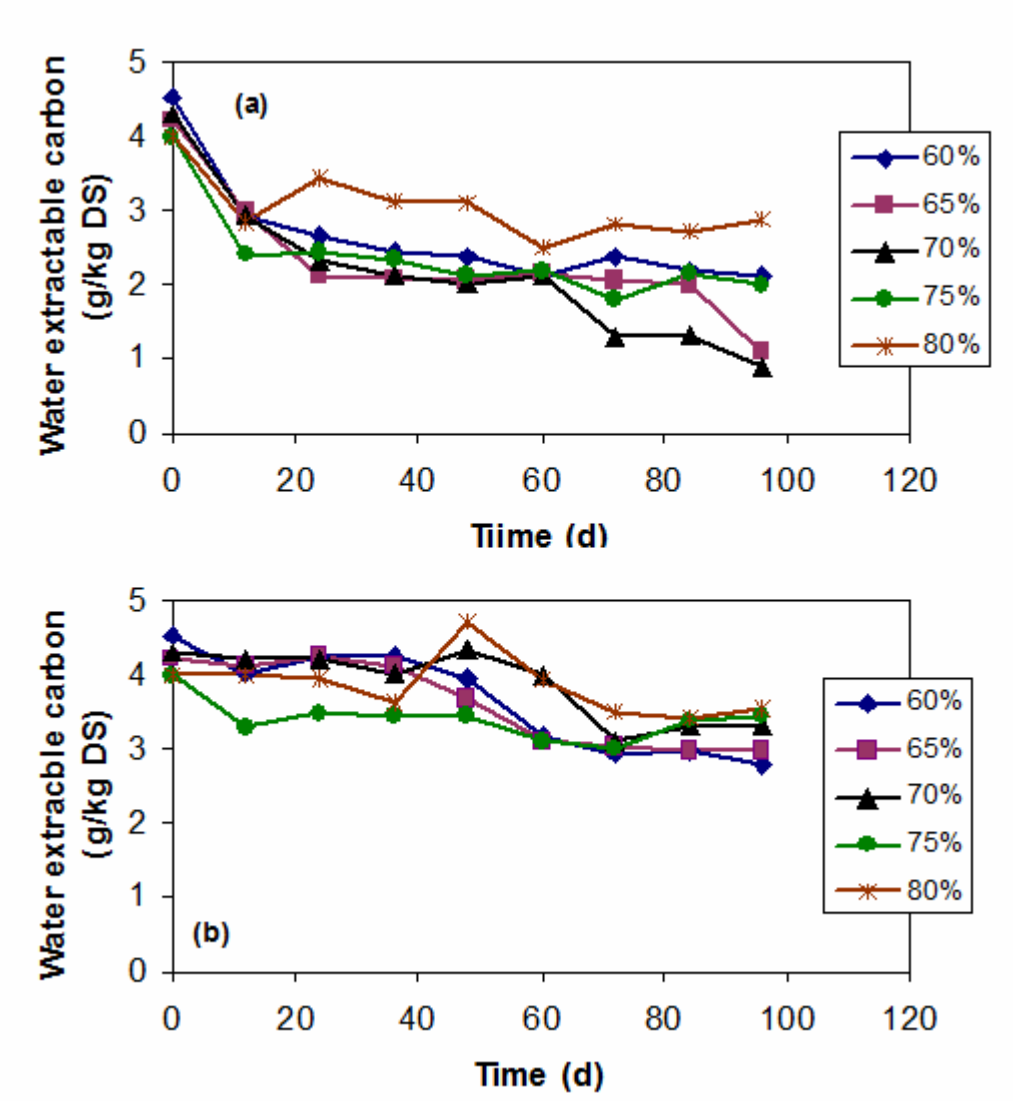


Figure 4.6: Moisture level and earthworm effects on Water extractable organic carbon. (a): Test (with worms), (b): Blank (without worms)

For the blanks (Fig. 4.6b), water extractable OC reduction was apparent during the first 48 days, decreasing fairly faster between day 48 and day 72, and gradually reached a plateau thereafter up to the end of the experiments. However, a slightly different trend was observed in the reactors at 75% moisture, where the decrease was relatively faster, and at 80% moisture content where an unexpected increase was observed after day 36. Gomez-Brandon *et al.* (2008) have explained this surprising observation by suggesting that the degradation of solid polymeric material in the composting substrate may lead to the formation of soluble organic matter which increases the water extractable OC concentration.

As discussed earlier, total water extractable OC (with worms and without worms) decreased with curing, but the reduction was clearly more pronounced when worms participated in the decomposition process (Fig. 4.6a). The decrease of water extractable OC with treatment time has been reported previously (Bernal *et al.,* 1998; Chefetz *et al*., 1998; Hue and Liu, 1995). Similar trends to those observed in this study were reported by Gomez-Brand *et al.* (2008)

during composting of cattle manure. They found a decline in the water extractable OC from 7000 to 2200 mg/kg (dry weight) during 270 days of treatment. Similar observations were also made by Elvira *et al*. (1998) who obtained a final product with water extractable OC < 1.5%, and Ramos *et al*. (2005) who reported final values < 0.5% after vermicomposting 9 different mixtures of textile and household wastes with cow manure and oat straw for 60 days. Shalabi (2006) has reported decreases from 20000 and 23000 mg/kg to 1200 and 1600 mg/kg dry solids after 130 days of curing. Shalabi (2006) also observed low reductions in water extractable OC for the controls, recording up to 9200 mg/kg dry solids at the end of the investigations.

Said-Pullicino and Gigliotti (2007) have attributed the decrease in water extractable OC to the continuous mineralization of soluble organic compounds, and the repolymerization and condensation pathways that lead to the formation of complex organic substrates with low solubility in water which tend to flocculate out the solution. Several researchers agree that water extractable OC is the most active part of carbon and indicative of compost stability. Thus it is commonly used as an indicator for monitoring the decomposition process. Various reference (cutoff values) values are cited in the literature.

Hue and Liu (1995) have suggested using 10g of water extractable OC per kg dry matter as the threshold value for stable compost, based on 17 composts. Other authors have proposed different threshold values: Eggen and Vethe (2001) and Garcia *et al*. (1991) have proposed 0.5% as the cutoff point. Bernal *et al*. (1998) have proposed water extractable OC values < 1.5% as the index for stable compost based on 7 composts, whereas Zmora-Nahum *et al*. (2005) have recommended 4 mg DOC/g dry weight as the reference value indicating compost maturity.

In the present study, the water extractable OC of the raw faecal matter was low, less than 5g/kg dry matter. This suggests that while water extractable OC reduction can generally be correlated with other stability indices, such as volatile solids and C:N ratio, a decision cannot be made on the maturity and stability of the end product based on water extractable organic carbon. The low initial value for fresh faecal matter and the wide range of threshold values recommended in the literature are indications that this indicator should be weighed against observed decomposition traits and other stability/maturity indicators.

4.1.6 Water soluble ammonium

The ammonium-nitrogen (NH_4^+-N) concentrations decreased in both the test reactors (with worms) and the controls (without worms) throughout the period of experimentation (Figs. 4.7a and 4.7b), dropping from initial levels of 1830 mg/kg to about 30 mg/kg and 100 mg/kg dry solids during the first 36 days, both with and without earthworms respectively. The presence of earthworms has caused a more rapid loss of ammonium-nitrogen (3 folds over control) after day 12. In the test reactors, ammonium concentrations reached about 20 mg/kg after day 96 compared to about 70 mg/kg without earthworms.

A similar trend to that found in this study has been reported by Short *et al*. (1999) who observed a decrease in NH_4^+-N from an initial value of 2148 to 416 mg NH_4^+-N mg/kg after 4 weeks of vermicomposting waste paper sludge.

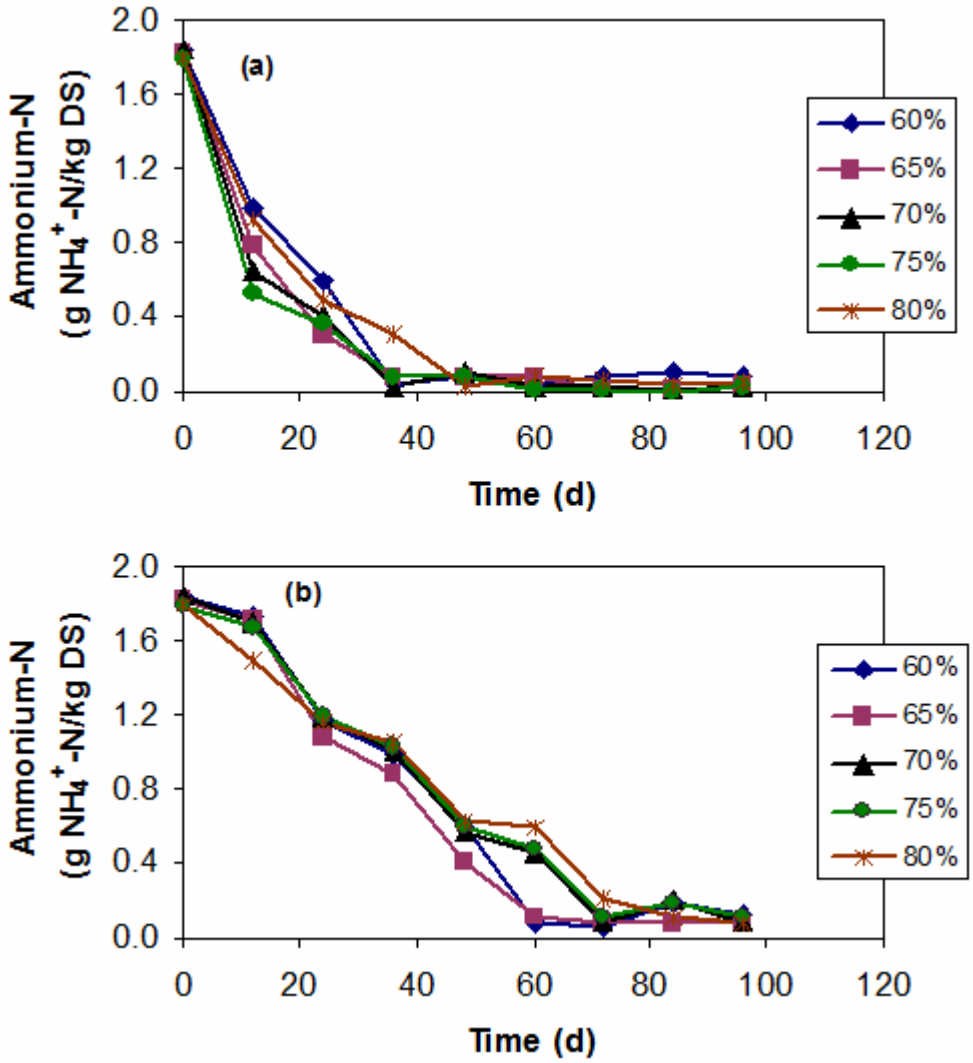


Figure 4.7: Moisture level and earthworm effects on water soluble ammonium. (a): Test (with worms), (b): Blank (without worms)

Several other researchers have demonstrated that NH_4^+-N concentrations generally decrease during decomposition, indicating stabilisation of the organic matter. Hand *et al.* (1988) reported that a large proportion of the initial NH_4^+-N was lost within two weeks. However, they also reported some conversion of the NH_4^+-N to NO_3^- -N. Atiyeh *et al* (2000) observed a rapid decrease in NH_4^+-N during the first four weeks of treatment with and without earthworms. Maboeta and Van Rensburg (2003) have reported more than 92% decrease in NH_4^+-N within four weeks and more than 100% increase in NO_3^- -N. Shalabi (2006) reported a rapid decrease of ammonia during the first 10 days, followed by a plateau for the rest of the experimental period, reaching values ranging between 20 and 47 mg/kg NH_4^+-N, which was similar to the range of values observed in the present study.

The concentration of NH_4^+-N decreased due to its conversion to nitrate-nitrogen (NO_3^--N). Many researchers (Gomez-Brandon *et al.,* 2008; Benito *et al*., 2003; Hand *et al.* 1988) have

attributed the decreasing NH_4^+-N concentrations to both NO_3^--N formation and volatilization as ammonia (NH_3) at high pH and temperature. Ammonium (NH_4^+) and ammonia (NH_3) are in chemical equilibrium in the aqueous phase via ammonium hydroxide (NH_4OH). Under conditions with high pH and high temperatures, the equilibrium is shifted towards ammonia that can be released into the atmosphere (Gebel, 1991). In the present study, ammonia volatilization was likely to take place as pH approached 8 (See Fig. 4.1).

Benito *et al*. (2003) have pointed out, after composting pruning wastes and grass clippings for 190 days that decreasing amounts of NH_4^+-N combined with increases in NO_3^--N concentrations towards the end of composting suggest that intensive biological decomposition has been slowed down and maturity has been reached. Based on this assumption, some researchers have suggested threshold values of ammonium concentration as indicators of compost maturity and stability. Zucconi and De Bertoldi (1987) have recommended a concentration of 400 mg NH_4^+-N/kg as cutoff value, whereas Bernal *et al*. (1998) have used the ratio between NH_4^+-N and NO_3^--N and recommended 0.16 as threshold value. In this study, the NH_4^+-N concentration (20 mg NH_4^+-N /kg) obtained at the end of the experiments was well below the recommended threshold value.

For the profiles shown in Figure 4.7a, the NH_4^+-N has exhibited a similar tendency at all moisture levels. As discussed earlier (in the case of the test series); a sharp decrease during the first 36 days, then a relative stability for the rest of the investigation period, with the values reached at 65, 70 and 75% moisture content being almost the same (20 – 30 mg NH_4^+-N /kg). For the controls, shown in Fig. 4.7b, the decrease was less rapid at all the moisture levels investigated compared to the test series, except the reactors at 65% moisture where a relatively higher decrease rate was recorded.

In this study it was observed that high ammonia (1830 mg/kg) concentrations result in mortality of earthworms, which necessitated the adoption of an adaptation procedure in all the experimental units. This observation is in agreement with the findings of Edwards (1998) which reported that relatively high amounts of ammonia in fresh manure will require aging to values less than 500 mg/kg before they are acceptable to *E. foetida*. Gunadi and Edwards (2003) have demonstrated the inability of *E. foetida* to survive in vegetable wastes at ammonia values within the range of 1880 ± 68 mg/kg.

The results reported here suggest that earthworms had a great impact on nitrogen transformations in the faecal matter, enhancing both ammonia volatilization and nitrification. As high losses of NH_3 has been ruled out in this discussion, it may be concluded that the decrease in NH_4^+-N was mainly because the earthworms produced conditions in the faecal matter that favoured nitrification. This point is clearly reflected in the corresponding increase in NO_3^- -N (Fig. 4.8a).

4.1.7 Water soluble nitrate

The conversion from NH_4^+-N to NO_3^- -N (nitrification) is a two-step oxidation process which involves the formation of nitrite (NO_2^-) as an intermediate. In this study NO_2^--N was recorded but has not been reported. Nitrification requires suitable conditions in terms of oxygen supply, pH and temperature.

Indications for optimum temperature for nitrification vary in the literature (e.g. Kuntze *et al*., 1994: 15-35 °C; Scheffer and Schachtschabel, 1998: 25-30 °C; Beck, 1979: 25-35 °C). The optimum pH for both nitrification steps lies at 8.5 but the steps vary with regard to their tolerance ranges (NH_4^+ oxidation: 7.5 to 9.5; NO_2^- oxidation: 5.5 to 10.5 (Beck, 1979). The latter author has pointed out that the speed of the process is also affected by oxygen availability.

In this study, both the temperature (24 to 25 °C) and pH (5.4 to 8.2) were within the reported optimum range for nitrification. Also, since all reactors had mesh bottoms, and perforated lids, conditions existed for sufficient aeration. Furthermore, Parkin and Berry (1994) have reported that earthworm movements in the substrate make channels thereby creating aerobic pores that enhance nitrification, which was expected to be the case in the test reactors of the present study.

Figures 4.8a and 4.8b show the profiles of Nitrate nitrogen concentrations during the experimental period. Concentrations of NO_3^--N were clearly more in the test reactors (with earthworms), beginning from day 24 up to the end of the experimental period. Earthworms increased the NO_3^--N concentration markedly after 84 days compared to the blanks (without worms) where the increase was only apparent. There was a clear pattern in the nitrate content changes at the different moisture contents in the test reactors. The highest nitrate concentrations were observed on day 84 and day 96 at the 65% and 75% moisture contents in reactors with worms. Limited nitrification has taken place in the blanks at any of the moisture levels, with the first clear signs of nitrate formation observed after day 72. No nitrification was recorded in the blanks at 80% moisture, probably due to the existence of anoxic conditions.

Indications concerning the optimum moisture for nitrification vary in the literature. Sindhu and Cornfield (1967) have reported optimum moisture content of 50% m.w.h.c (maximum water holding capacity) for mineralization and nitrification in soil with earthworms. In the present study, although there was no specific pattern in the NO_3^--N changes at the different moisture contents in the blanks, 65% moisture appeared to be more suitable for nitrification compared to the rest of the moisture levels monitored.

Other researchers (Short *et al*., 1999; Maboeta and Van Rensburg, 2003; Benitez *et a*l, 1999) working on various organic wastes have reported values of NO_3^--N and increased rates similar to those observed in the present study. In addition, Hand *et al.* (1988) have observed rapid increase in NO_3^--N concentration within 14 days of vermicomposting cattle manure, whereas Ayiteh *et al.* (2000) have reported 28-fold increases in NO_3^--N concentrations after 17 weeks, compared to 3 fold increase in controls.

It is important to indicate that part of the high nitrate concentration observed on day 84 and day 96 in this study could have been a result of the mineralization of some of the earthworm biomass, and not due to decomposition of faecal matter. The decrease in nitrate content between day 60 and day 72 respectively at the 75 and 85% moisture contents might have been due to denitrification as a result of high moisture content of the substrate which led to anoxic conditions in part of the vermicompost. Additionally, microbial biomass immobilization of nitrogen cannot be excluded. In the final phase of vermicomposting, nitrification was intensified especially at moisture contents of 65, 70 and 75% (Fig. 4.8a).

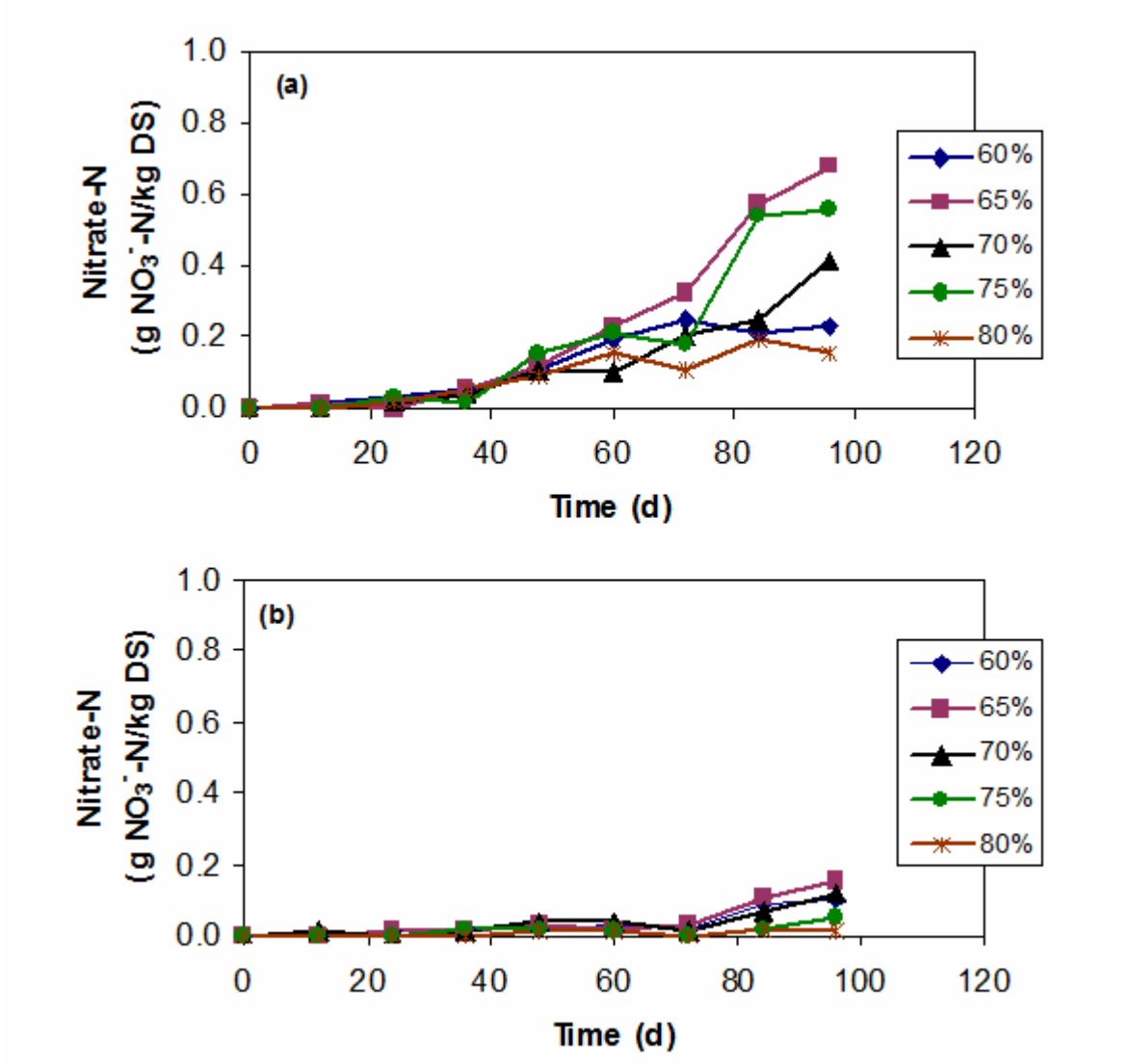


Figure 4.8: Moisture level and earthworm effects on water soluble nitrate.(a): Test (with worms), (b): Blank (without worms)

Figure 4.9 shows the relationship between the pH and water soluble nitrate during the experiment for the test reactors.

The literature suggests that there is a strong relationship between nitrification and pH, but in the present study a weak correlation between the two parameters was found (Fig. 4.9). This was probably because nitrification is controlled by microbial activity, and the microorganisms have pH limits within which they are efficient. Figure 4.9 suggests that at pH values between 7.0 and 7.7, a linear relationship exists between the nitrification process and change in pH. Predicted pH = 7.3 - 1.8 (NO_3-N concentration). At pH values outside this range, the relationship between the two parameters is unclear.

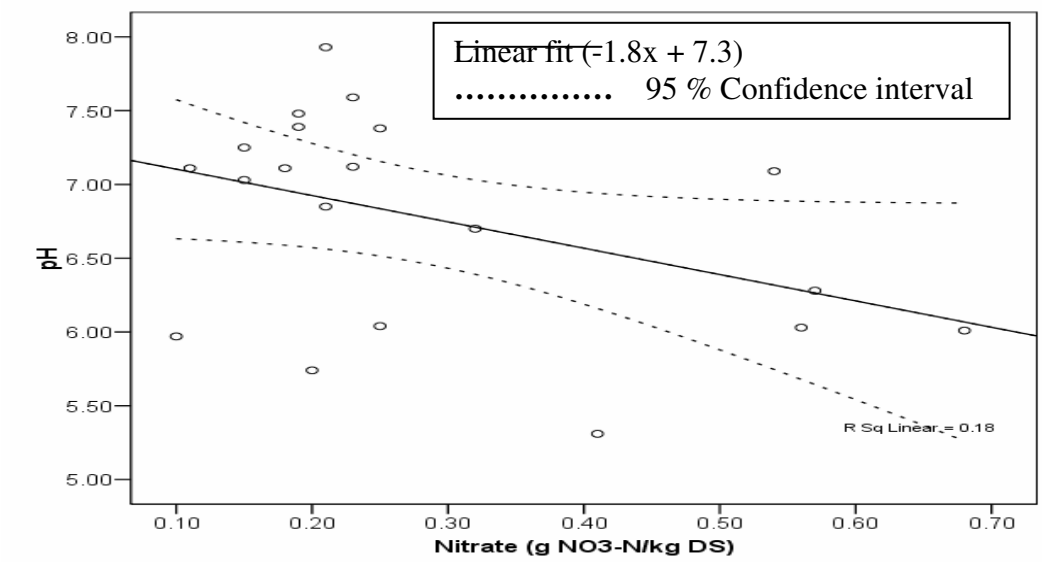


Figure 4.9: Correlation between water soluble nitrate and pH. (Scatter plot showing fit with confidence interval.)

4.1.8 Water extractable phosphorus

Water extractable phosphorus (water extractable-P) content rapidly increased at all moisture contents during the first week of treatment in both test and control reactors (Figs 4.10a and 4.10b). However, the increase rate was less marked in the controls than the test reactors. Also, there was a clear difference between the test and control series in the pattern of phosphorus content change at the different moisture contents. This difference suggests that earthworm mediated phosphorus mineralization.

In this study, the water extractable-P correlated positively with duration of vermicomposting, for the test reactors. The strongest correlations were found at the 65% (r = 0.84) and 75% (r = 0.93) moisture levels. The remaining moisture levels showed weak correlation with duration of vermicomposting. As Figure 4.10a indicates, the increase in water extractable-P was highest at 65 and 75% moisture levels. However, the generated water extractable-P was low compared to the initial content.

For the controls shown on Figure 4.10b, available phosphorus was weakly correlated with treatment duration. The general trend in the controls was: increase in available phosphorus up to day 36, then a plateau throughout the remaining period except at 65% and 80% moisture levels. A similar trend was observed for the test series except that available phosphorus increased after day 72 at all moisture levels, but not for 80% moisture level where a decline was noted.

Lee (1992) have reported that the organic matter that passes through the gut of the earthworm results in some amount of phosphorus being converted to more available forms. In this study, under optimum conditions (75% moisture content) the increase of water-extractable P was about 20%. Significant increase of available phosphorus has been observed by Manna *et al.*

(2003) after composting various organic wastes with three different worm species. Kaushik and Garg, (2004) recorded significant increase in total phosphorus and water extractable-P after composting textile sludge mixed with cow dung and agricultural residues for 11 weeks. Mansell *et al.* (1981) showed that plant litter contained more water extractable-P after ingestion by earthworms and they attributed this increase to physical breakdown of the material by earthworms. Shalabi (2006) have reported increases in available phosphorus at higher temperatures (20-25 °C).

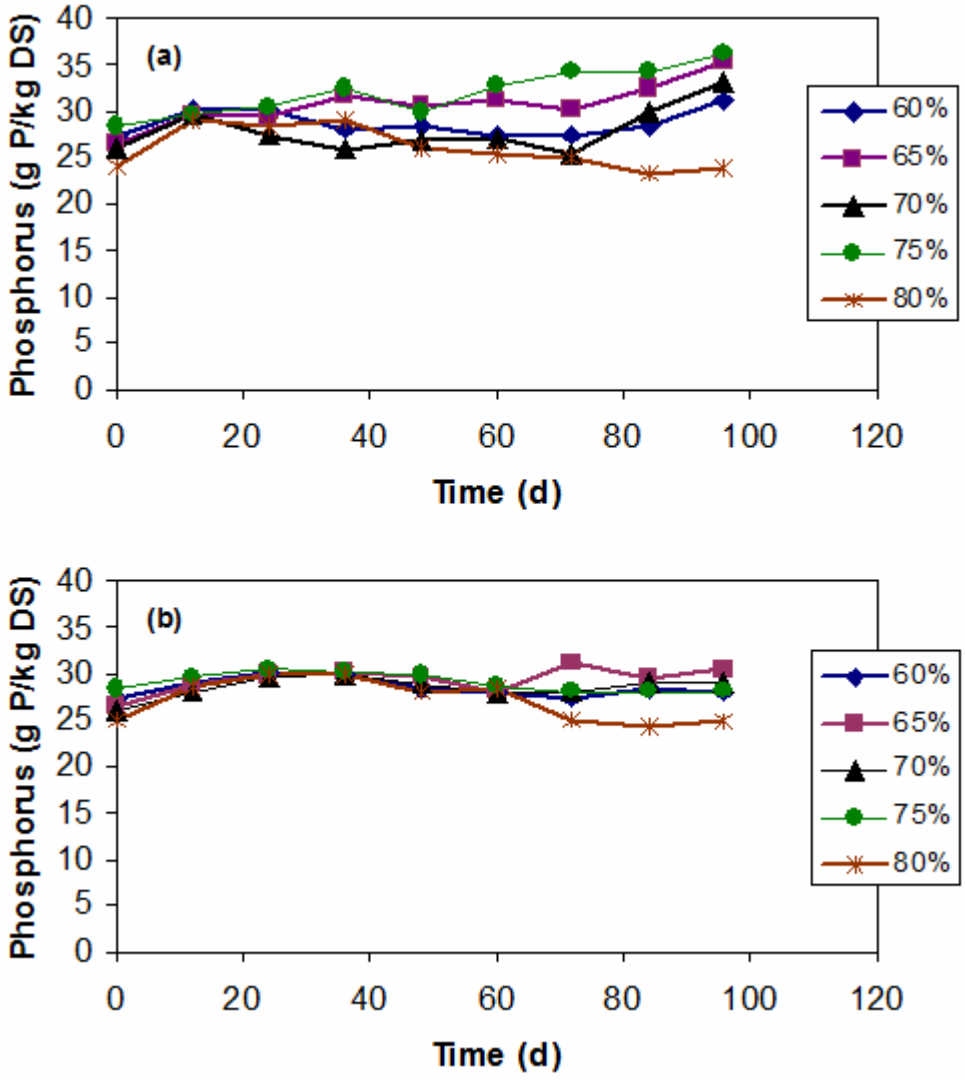


Figure 4.10: Moisture level and earthworm effects on water extractable phosphorus.(a): Test (with worms), (b): Blank (without worms)

Various researchers have provided different explanations for the increase of water extractable-P. Suthar (2006) has observed that the release of phosphorus in available form is partly by earthworm gut phosphatases. After 150 days of composting a mixture of crop residues, farm yard manure and cattle dung, the researcher observed 63-105 % increases in available phosphorus. The investigator suggested that further release of water extractable-P (available phosphorus) may be by P-solubilizing microorganisms in worm casts. This assumption was supported by Vinotha *et al.* (2000) who have observed that microorganisms play an important

role in enhanced phosphatase activity during vermicomposting. The latter authors then concluded that earthworm processed waste material contains high concentration of nutrients due to enhanced microbial activity during the vermicomposting process. Ghosh *et al*. (1999) have explained the increase by suggesting that earthworms take up non-soluble phosphorus as nutrient in their bodies for syntheses and release the remaining phosphorus in a mineralized (available) form.

In the present study, available phosphorus concentrations were far lower (1-33 %) than those reported by previous researchers may be due to the shorter treatment duration (96 days).

The trend reported in this study contradicts the reports of some researchers who have observed decreases in available phosphorus during composting and vermicomposting processes. Ndegwa and Thompson (2001) have reported decreases in available phosphorus between16 and 33 %. Bentiz (1999) has also reported non-detectable levels of available phosphorus after 6 weeks of vermicomposting. The authors explained this observation by suggesting that increased worm biomass might have stimulated microbial metabolism and caused immobilization of free phosphates into worm and microbial tissues. Ghosh *et al*. (1999) noted that mineralized P was quickly fixed to aluminum, iron, or calcium ions.

The available phosphorus concentrations found in this study are supported by the reports of Ndegwa *et al*. (2000) who observed an increase in available phosphorus for materials with initial C/N ratios of 10, 15 and 20, but a decrease in available phosphorus only for the material with an initial C/N ratio of 25. The C/N ratio of the faecal material used in the present study ranged between 10 and 18, which falls within the range reported by the latter authors to be responsible for increasing concentrations of available phosphorus, thus offering support to the trend observed in the present study

4.1.9 Total Kjeldhal nitrogen (TKN)

The TKN profiles shown in Figures 4.11a and 4.11b indicate that TKN increased slightly during the first 12 days of the experiment in the test reactors, whereas in the blanks TKN remained virtually unchanged throughout the experiment. After day 36 there was a clear pattern in TKN change in the test units suggesting that the different moisture levels had influenced TKN concentrations, with the most pronounced influence recorded at 70% and 75% moisture contents. In contrast, no specific pattern was observed for the controls.

Overall, TKN had increased in the test units by the end of the experiment between 4.9 and 7.8 g/kg dry solids at the different moisture contents. The values found in this study were higher than those reported by Sampedro *et al*. (1996) who observed increases from 0.8 to 1.72 g/kg after 90 days vermicomposting of paper sludge. However, the values of the present study are lower than those reported by Shalabi (2006) who observed TKN values between 15000 and 35000 mg/kg dry solids with *E. foetia*. Nevertheless, the latter researcher reported decrease in TKN from initial concentration of 29000 to 15000 mg TKN/kg dry solids when *Dendrobaena veneta* was employed.

TKN profiles similar to those observed in this study have been reported by Hand *et al*. (1988) who found that *E. foetida* in cow dung slurry increased the nitrate-nitrogen content. Several

researchers (Kaushik and Garg, 2004; Tajbakhsh *et al*., 2008, Elvira *et al*. 1996, 1998) have also reported increases in TKN during the vermicomposting process.

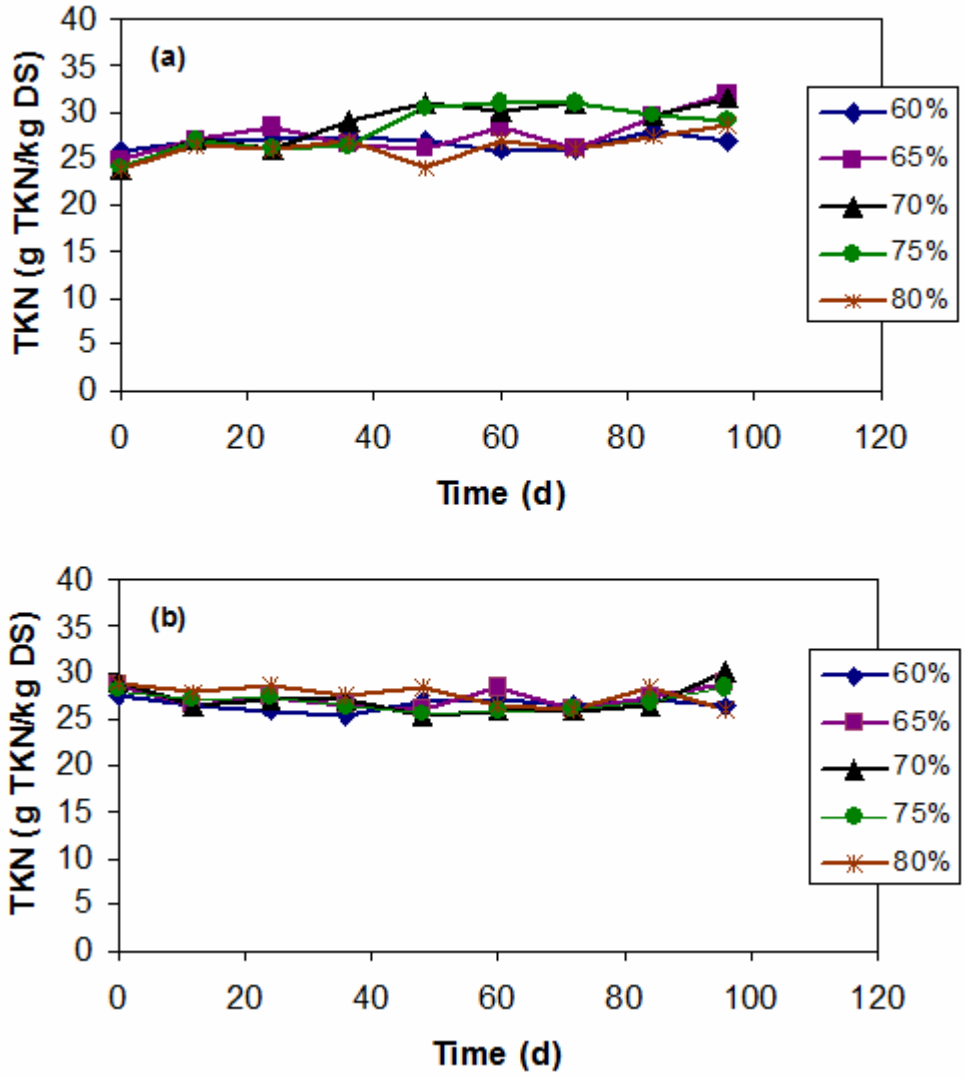


Figure 4.11: Moisture level and earthworm effects on Total Kjeldhal Nitrogen.(a): Test (with worms), (b): Blank (without worms)

Various reasons have been advanced for the increase TKN during vermicomposting. Suthar (2006) has suggested that earthworm activity enriches the nitrogen profile of vermicompost through microbial mediated nitrogen transformation, through addition of mucus and nitrogenous wastes secreted by earthworms. However, the author pointed out that nitrogen enrichment patterns generally depend upon the quality of the substrate material. The rest of the other authors cited above have attributed the increases to mineralization of organic matter.

Two major effects can be discussed for TKN dynamics during vermicomposting; namely degradation of volatile solids (VS) and removal of ammonia. TKN comprises all compounds of nitrogen with the oxidation number -3; that is ammonia (inorganic) and amines (organic)

including amino acids, protease and other substances containing amino groups. When VS decreases from, say 800 to 400 g/kg like in the case of vermicomposting at 70% moisture content (Fig. 4.4a), TKN is postulated to increase by about 100% if there were no degradative or transformative reactions with respect to TKN.
This means that the TKN would be expected to increase from about 25 to 50 g/kg. As the removal of NH_4^+-N in the same experiment was only about 1.7g/kg, the low increase from 23 to 30g TKN/kg can be explained by ammonia removal. It can thus be assumed that a large amount of the nitrogenous organics which occurred as TKN were mineralized forming ammonia and that the predominant part of the formed ammonia instantaneously volatilized. Otherwise, an ammonia accumulation would have been observed.

Despite the high reduction of organic matter reported in this study, there was a weak correlation between volatile solids reduction and TKN change. Shalabi (2006) has explained this phenomenon by suggesting that there could have been a nitrogen uptake by earthworms. He posited that the expected TKN increase was compensated by the NH_4^+-N loss through volatilization or its conversion to NO_3^--N through nitrification. Bernal *et al.* (1996) have suggested that ammonification, NH_3 volatilization and denitrification are responsible for nitrogen decrease during vermicomposting. NH_3 volatilization is not likely to explain the relatively low levels of TKN observed in this study due to the low pH recorded throughout the experiments. Furthermore, reasons other than denitrification can be invoked to account for the low TKN concentrations observed in the present study. A possible reason is immobilization into microbial biomass or due to assimilation of nitrogen by earthworms as suggested by Bansal and Kapoor (2000).

4.1.10 TC/TKN ratio in the solid phase

The C:N ratio-time profile of faecal matter at all moisture levels decreased progressively throughout the experimental period (Fig. 4.12a). C:N ratios decreased rapidly during the first 12 days, and then decreased more gradually for the duration of the experiment, with the exception of the reactors at 70 and 75% moisture where relatively rapid declines were observed after day 36 up to the end of the experiment. The highest reduction in C:N ratio was observed in the reactors at 70% moisture, decreasing from 17.7 on day 0 to approx. 7.1 on day 96 (approx. 60 % reduction). This was close to the C:N value (49 %) recorded in the reactors at 65 and 75 % moisture levels.

C:N reduction rates similar to those found in this study have been reported by Gunadi *et al*. (2003). They monitored the C:N ratio of cattle and pig manures at five moisture contents during vermicomposting using *E. foetida* and observed reduction of C:N ratio of the separated cattle solids from 40 to 22 and a reduction from 15 to 8 for the pig manure solids.

For the blanks (without worms), there was no clear pattern in C:N ratio change which remained relatively in a plateau after 12 days except the reactors at 65% moisture where a relatively higher decrease was observed after day 48. Palsania *et al*. (2008) have reported 66% reduction in C:N ratio (from 57.18 to 19.38) after 48 days of vermicomposting solid waste bargasse using Eudrilus eugeniae, in comparison to only 12.13% reduction in controls (without earthworms). Suthar (2006) has reported decreases in C:N ratios of 67% to 69% during vermicomposting of agricultural wastes (farm yard manure, cattle dung, crop residues) using *Perionyx excavatus.* The author reported that C:N ratio showed strong negative

correlation with vermicomposting duration, which is similar to the observation of the present study.

The decreasing C/N profile observed in this study is due to the reduction of organic carbon (as CO_2) in the process of respiration and the production of nitrogen, as reported by Kale *et al*. (1982), Gajalakshmi *et al.* (2001), Gunadi *et al*. (2002) and Suthar (2009). Nogales *et al*. (1999) have observed little change in C:N ratio of vermicomposted dairy biosolids due to similar extent of decrease in nitrogen and carbon contents, in comparison to that of manure which declined from 24 to 8. For the test series shown in Figure 4.12a, it is clear that such a steady state was not reached in the present study as C:N ratio continued to reduce up to the end of the experiment.

Because decrease in C:N ratio reflects changes in the forms and properties of organic matter during bioconversion (Harada *et al*., 1981), it has become a widely used parameter to monitor the progress of organic matter decomposition. Much relevance was attached to this parameter by Flintoff (1976), Tchobanoglous *et al.* (1993), Ambrose (1983), Ashbolt and Line (1982), among others. These authors have suggested optimum C/N values for composting. Raul *et al*. (1983) advised that the suitable ratio may range from 30 to 50; Flintoff (1976) recommended the optimum C/N ratio to range from 20 to 70 depending on available carbon. These latter authors suggest that if the C/N ratio is kept up to 60 at initial stage of experiment, it will promote bioconversion process and will not exert any disorder during vermicomposting. Much lower cut-off values of C/N ratio for optimum vermicomposting have been suggested by other authors. Ndegwa and Thompson (2000) and Butt (1993) have recommended that the C/N ratio be maintained at approximately 25. As a maturity/stability indicator, Edwards and Bohlen (1996) have suggested a final C/N value of about 20. Chanyasak and Kubota (1981) have observed that the ratio varies from 8 to 29 for a mature/stable vermicompost.

In the present study, the initial C/N ratio range (16 - 18) was very low in comparison to the ranges recommended by the above authors for optimum decomposition, and therefore C/N ratio cannot be used as a reliable indicator of maturity in this study. As expected, the initial C/N range of this study was lower than that (20 - 25) reported by Shalabi (2006) due to the higher carbon content (from toilet tissue) of the faecal solids used in Shalabi's study, which achieved a final C/N ratio of 13 -18. Nevertheless, the ratio (7.1) reached at the end of the present study indicated the near completion of vermicomposting process. However, it needs to be pointed out that much of the scientific literature (Chanyasak and Kubota, 1981; Carvalho et al., 1991; Palsania et al., 2008) have advised against the use of C/N ratio as an absolute indicator of maturity. This is because during carbohydrate decomposition, and the associated ammonification process, the release of carbon and nitrogen fluctuates highly, making the C/N ratio an unreliable parameter to measure the degradation process. This fact is supported by Carvalho et al. (1991) who have pointed out that the C/N ratio is undependable due to its lack of control on the entire vermicomposting process. Woods End (2005) has advised that it is necessary to consider that not all the total carbon is actually available for microbial use. Or, if nitrogen is lost, C/N ratios may go up not down during late stages of decomposition. Although this behaviour was not observed in the present study, it is important to point out that C/N values must be weighed against observed decomposition traits before making any conclusions about product stability based on C/N Figures alone.

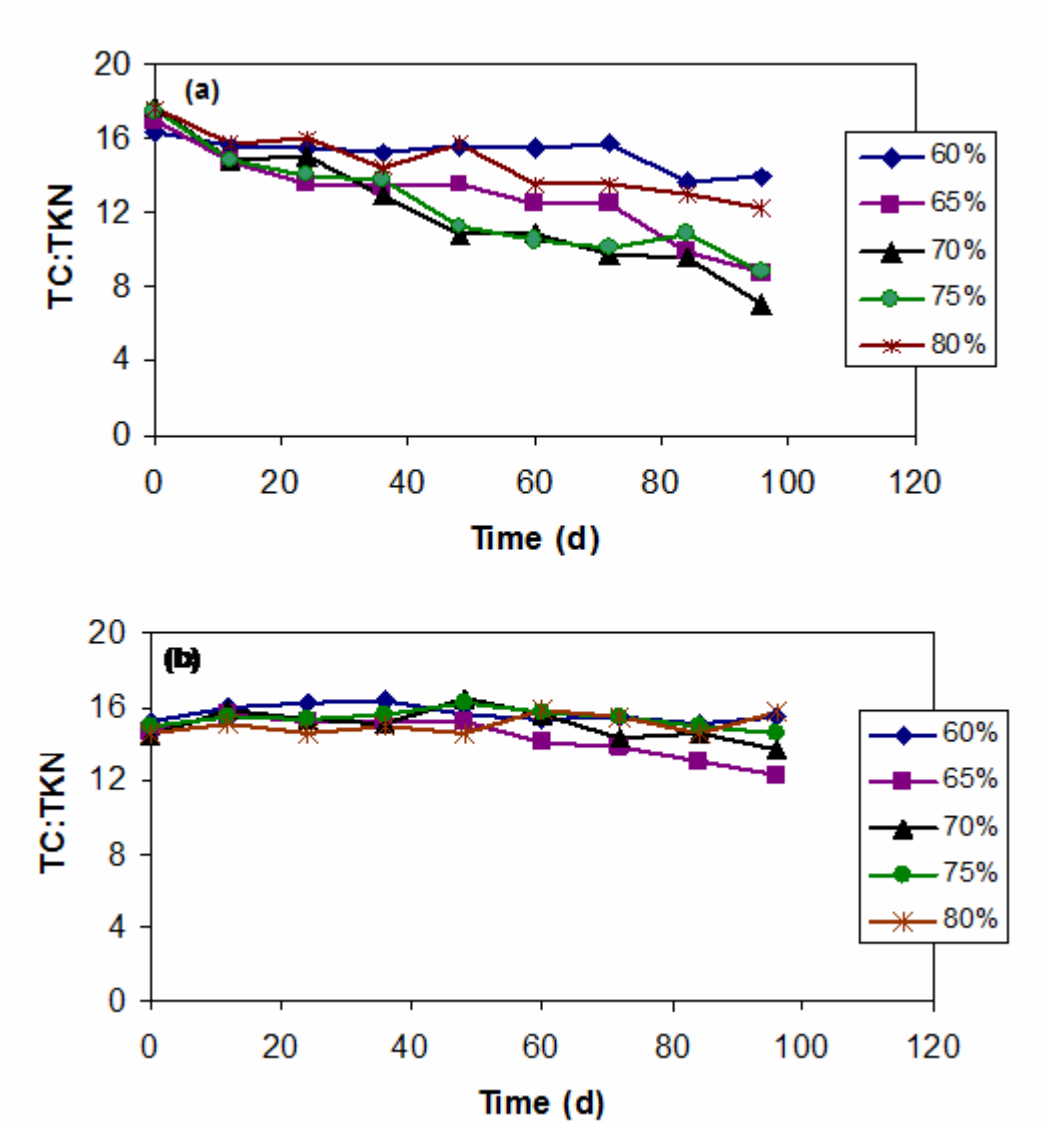


Figure 4.12: Moisture level and earthworm effects on TC/TKN solid phase.(a): Test (with worms), (b): Blank (without worms)

Chanyasak and Kubota (1981) have suggested the ratio of carbon/organic nitrogen instead. They argued that the carbon/organic N ratio is comparatively more dependable than C/N ratio. Morel *et al*. (1985) have recommended that the ratio of final C/N to initial C/N is a reliable indicator to monitor the decomposition process, but the authors did not provide clear limits for this ratio. However, Vourinen and Saharinen (1997) have reported a C/N_{final} to $C/N_{initial}$ of 0.49-0.59 after two months of vermicomposting. The recommendation by Chanyasak and Kubota (1981) may be reliable because, as observed in the present study the TKN has remained relatively unchanged (Fig 4.11a). Thus the decrease in C/N reported in this work is largely due to decline in TOC. Indeed, there was a strong correlation (r = 0.96) between TOC reduction and C/N decline. Taken overall, a C/N_{final} to $C/N_{initial}$ value of 0.40-0.51 was recorded in the present study, which is very similar to that reported by Vourinen and Saharinen (1997).

The C/N ratio profile of this study suggests that the moisture levels ranging from 65 to 75% may be the optimum range for vermicomposting of faecal matter. This result slightly deviates from the findings of Palsania *et al.* (2008) who studied the effect of moisture content variation over kinetic reaction rate during vermicomposting process at different ranges of moisture content, and concluded that the moisture content 75 ± 5% is the optimal range at which the vermicomposting process is fastest. This may be due to the differences in substrate and species of earthworm involved. Palsania *et al.* (2008) have used sugarcane bagasse as substrate and the worm species *Eudrilus eugeniae.*

4.1.11 Evaluation of moisture experiments and concluding remarks

It is clear from the foregoing discussions that the moisture content of human faeces at 25°C influences the activities of earthworms, and changes the dynamics of faecal matter degradation by modifying various chemical and biochemical properties. The following key observations were made:

Carbon (C) concentrations decreased during curing due to the breakdown of easily degradable C by microorganisms. This resulted in a more stable form of C left in the end product. The decreased rate was higher when earthworms participated in the decomposition process.
During curing, total Kjeldahl nitrogen concentrations remained the same or increased slightly.
Available N levels (ammonium-N and nitrate-N) were generally higher in the presence of earthworm than without worms.
C/N ratio is not a useful stability criterion for faecal matter because of low C/N
Increase of available P was a maximum of 20%. Due to essentially the same mass of P being present in decreasing dry matter, a decrease of VS by 50% would have led to 100% increase of phosphorus. But this was not the case, and no explanation can be provided here.

Several studies have demonstrated that there is a strong relationship between the moisture content of waste and the growth, the productivity and activity of earthworms. Reinecke and Venter (1985) showed that a moisture difference of as little as 5% in the substrate has a significant influence on the rate of growth and earthworm activity. According to Loehr *et al.* (1988), both excessive and insufficient moisture can adversely influence growth of earthworms.

In the present study, moisture contents in the range of 65-75% appeared to be optimal for rapid and efficient vermicomposting of faecal matter.

Various researchers have reported optimal moisture contents different from those observed in this study: Dominguez and Edwards (2004) have reported 50 to 90 % moisture content as the optimum range for vermicomposting. Neuhauser *et al.* (1988) and Loehr *et al.* (1988), working on various substrates, showed that the optimum moisture content for vermicomposting is 80 to 90 %. Dominguez and Edwards (1997) have shown that *E. Andrei* cultured in pig manure grew and matured between 65 and 90% moisture content, the optimum being 85%. Edwards (1988) and Sims and Gerard (1985) have observed that *E. foetida* can survive in moisture between 50 and 90% but grows more rapidly between 80 and 90 % in animal wastes. Their observations agree with earlier reports by Dresser and Mckee (1980) who found that moisture contents between 50 and 90 % are the most appropriate for vermicomposting processes.

However, some researchers have reported optimal moisture contents close to those observed in the present study. Liang *et al.* (2003) have reported 60-70% moisture content as the optimum range for maximum microbial activity during vermicomposting of sewage sludge. Similar findings to those of this study have also been reported by Reinecke and Venter (1985) who observed that the optimum moisture content for *E. foetida* was above 70 % in cow manure, but *E. Andrei* cultured in pig manure grew and matured best between 65 and 90 % moisture content, with 85 % being the optimum.

Taken overall, the results of this study suggest that there is no absolute moisture content which is ideal for vermicomposting of faecal matter. The optimal moisture content will depend on the substrate's water holding capacity, which diminishes during decomposition due to loss of organic content, and thus the ideal level of moisture will likewise diminish. Therefore, it can be concluded that the most suitable moisture content will depend on the processing goals and handling technology. For stabilisation of human faecal matter by vermicomposting, with the goal of nutrient recycling, a moisture content of 70% is recommended.

4.2 Effect of loading rate

In this part of the study investigations were carried out in order to:

- *Understand the effects that loading rate have on substrate residence time,*
- *Determine how control of loading rates affects the stabilisation process and the development of suitable physical-chemical conditions,*
- *Identify a loading rate that allows for optimisation of the continuous flow vermicomposting process.*

It is presumed that optimisation of the vermicomposting process depends primarily on the delivery of appropriate levels of substrate to the reactor and the development of optimum physical-chemical conditions for aerobic biodegradation processes to occur. An inadequate substrate loading rate (i.e. the mass and frequency of loading) may lead to inefficient utilization of substrate, resulting in reducing conditions that are insufficient to support complete decomposition of organic substances, thereby increasing the potential for accumulation of intermediate products. Additionally, excessive levels of substrate may lead to heat build-up with adverse effects on worms. Viljoen and Reinecke (1992) have observed that earthworm mortalities occur at temperatures above 30°C. Furthermore, worms are sensitive to low oxygen conditions, and decomposition may proceed slowly or not at all if such conditions are maintained over a certain period of time. Therefore, determining an appropriate substrate loading rate and delivery method are critical design and operational objectives for successful implementation of continuous flow vermicomposting.

In this study, reactors were seeded with mature worms (70g per reactor, wet weight) at different substrate loading rates (6.7, 10.0 and 13.3 kg feed/m^2/day). With twice weekly feeding, this provided feeding rates of respectively 0.82, 1.22 and 1.63 kg-feed/kg-worm/day for the three feed levels, which were designated accordingly as: light, moderate and heavy loadings. In order to date the substrate at each loading, markers (quartz pebbles) were added to the material during pre-processing (homogenisation). A different colour marker was

introduced every week. This procedure allowed dating of the different layers of material in the reactors and determination of the solids retention times. At the end of the experiment each reactor contained distinctly stratified layers of material (distinguished by the markers) with an increasing gradient of age, resembling a soil profile; as described by Aira *et al.* (2007a), from upper to lower layers as follows: 4, 5, 6, 7 …18 weeks. At the end of the experiment, the various layers were carefully separated and the substrate analysed.

In the following sections, variations in chemical characteristics of solids retained in the reactors at various layers for the three feed levels are discussed. The quantities of vermicast produced at various stages during the experimental period are presented in Table 4.2 and the corresponding physical-chemical characteristics discussed in section 4.2.8.

4.2.1 pH

As shown in Figure 4.13, the different loading rates have affected the pH of the solids differently. The pH increased in all reactors during the first 13 days, irrespective of the loading rate, as a consequence of the degradation and mineralisation of organic compounds. As discussed in previous sections, it can be assumed that the initial pH increase is caused by degradation of fatty acids contained in the faecal matter.

A steady decline in pH during the remaining experimental period was observed with the exception of reactors fed at heavy rate in which pH increase was recorded at solids retention time of 78 days up to the end of the experiment. A likely explanation of this is the formation of CO_2 during the degradation of organics and nitrification.

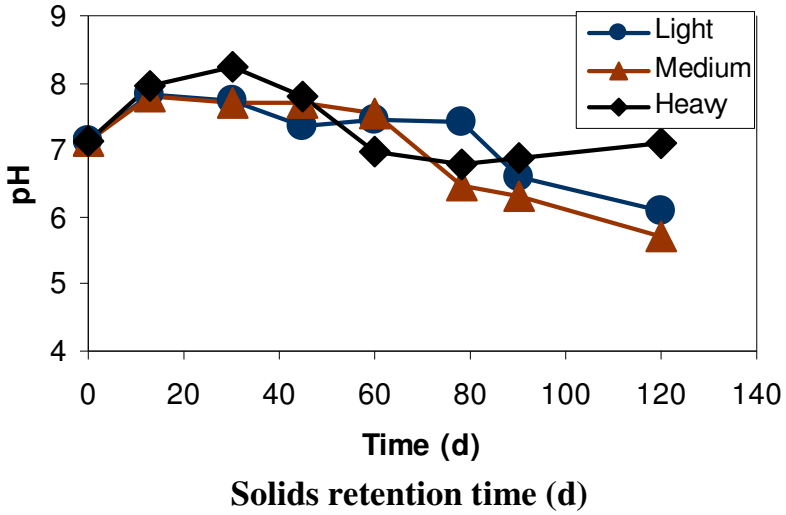


Figure 4.13: The effect of different substrate loading rates on pH dynamics during continuous vermicomposting at different solids retention times.

Observations of the present investigation are in agreement with results offered in this study (section 4.1.1), as well as of Shalabi (2006) and Datar *et al.* (1997), where the pH was found to increase with processing time during the early stages of decomposition, followed by a decreasing trend. The results however, contradict the observations of Ndegwa and Thompson

(2000) whereby the pH shift was found to decrease from the beginning of treatment. The latter authors made the observation while investigating the effects of feeding rate and stocking density on the vermicomposting of biosolids.
In the present study, the lowest pH (5.7, from an initial value of 7.1) was recorded in reactors loaded at moderate rate, which correlated well with the degree of nitrification (Fig. 4.16b) observed at this loading rate.

4.2.2 Volatile solids and total organic carbon

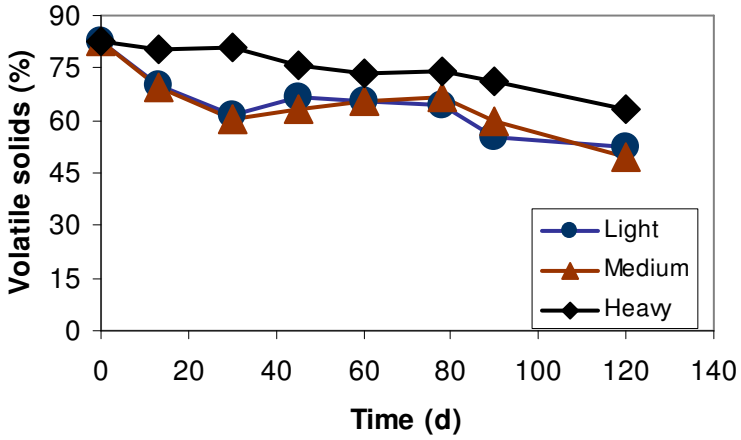
Figure 4.14 summarises the results of both the change in total organic carbon (TOC) and the percent decrease in volatile solids (VS) at the different solids retention times. The mean percent decrease in VS ranged from 23 to 43% while the TOC decrease ranged between 35 and 64%. Graphs for both parameters show parallels. The lower percentage decrease in VS might be explained by higher extents of thermolabile carbonates contained in VS in comparison to TOC.

As shown in Figure 4.14 (a), the light and moderate loading rates have resulted in higher VS reduction compared to the loading at 13.3 kg feed/m^2/day (heavy rate). In fact, there was no clear difference in VS reduction trend in the two sets of reactors fed at light and moderate rates, where the VS contents decreased from an initial value of about 83 to 52 and 43% respectively at the end of the experiment, compared to a final value of 63% in the reactors maintained at heavy loading rate.

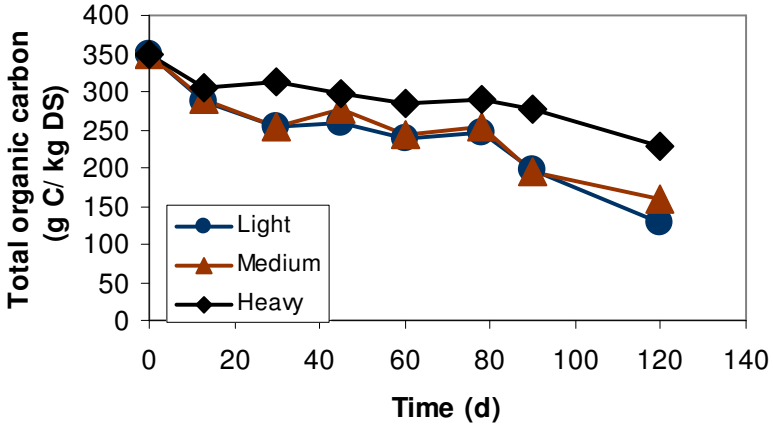
The three feed levels all seem to have resulted in TOC reduction to reasonable extent (Fig. 4.14b). But, as noted previously (with VS), the heavy feeding level had a substantial amount of TOC that was still intact at the end of the experiment, suggesting that longer solids retention time would be required to achieve stability of the product.

In a similar study conducted by Ndegwa and Thompson (2000) with biosolids as substrate, they found out that the different feed levels (0.75, 1.0 and 1.25 kg-feed/kg-worm/day) did not result in significantly different VS reductions. However, the authors observed that different earthworm densities produced significantly different VS reductions. They concluded that for the purpose of biosolids treatment, a feeding level of 0.75 kg-feed/kg-worm/day and a stocking density of 1.60 kg-worm/m^2 are optimal.

In the present study, a feeding level in the range of 0.8 to 1.2 kg-feed/kg-worm/day appears to be optimal for the substrate, faecal matter.



Solids retention time (d)



Solids retention time (d)

Figure 4.14: The effect of different substrate loading rates on volatile solids and total organic carbon dynamics during continuous vermicomposting at different solids retention times. (a) Volatile solids, (b) Total organic carbon.

4.2.3 Water soluble organic carbon

There was a rapid decline in water soluble organic carbon (or dissolved organic carbon – DOC) concentration during the first 30 days of vermicomposting, as the readily soluble DOC was decomposed leaving behind more sTable carbon fractions, such as cellulose and hemicelluloses. DOC then decreased gradually for the duration of the experiment (Fig. 4.15). Concentrations rose slightly at 40 days retention time, and then returned to previous levels at day 60, probably due to a watering event for moisture correction. It may also be due to analytical errors. By day 30, DOC concentrations of the substrate in all feed levels were 60 to 67% lower than in raw substrate.

Contrary to expected results, DOC concentrations at day 120 (end of experiment) in reactors fed at heavy rate were clearly lower than in light and moderately loaded reactors. This observation did not correlate with VS and TOC removal. It was probably due to loss of soluble organic carbon during watering/moistening events. This anomaly may also have been

an artifact of sampling; earthworm activity produced layers of decomposed, partially decomposed, and worm castings, requiring thorough homogenization for the analysis at each sampling date. By the end of the experiment, DOC levels in the light, moderate and heavy feed levels were respectively 85, 84 and 95% lower than in the raw substrate.

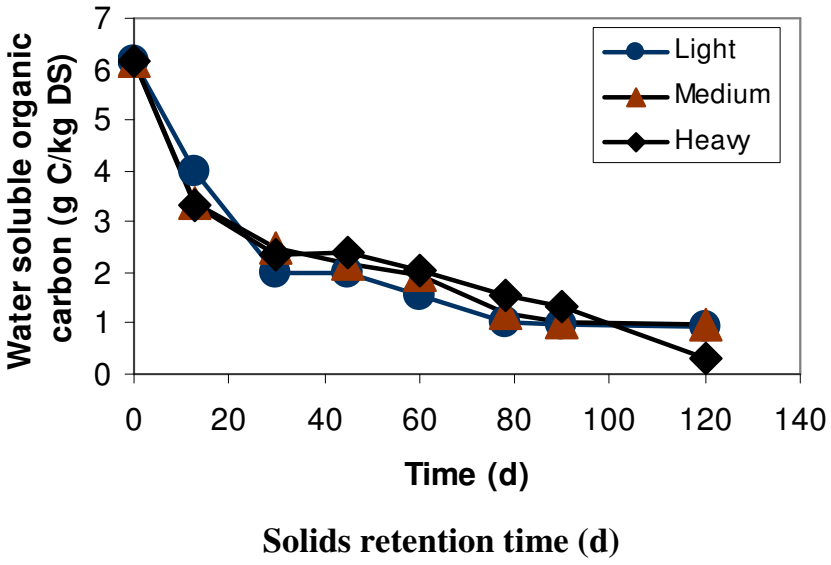
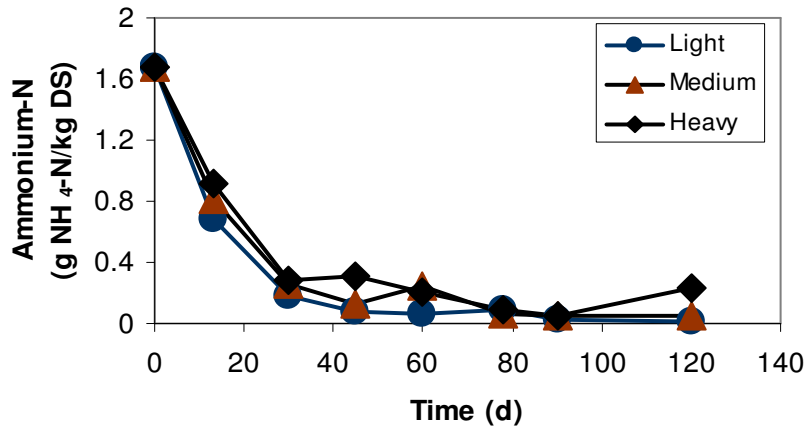


Figure 4.15: The effect of different substrate loading rates on water soluble organic carbon dynamics during continuous vermicomposting at different solids retention times.

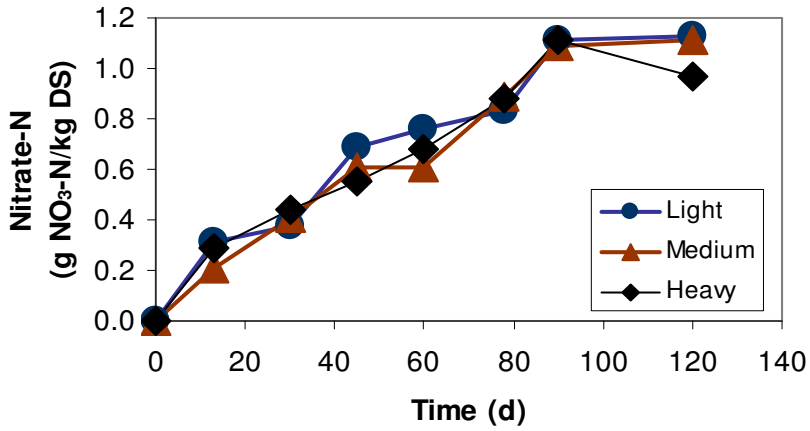
4.2.4 Water-soluble ammonium-N and nitrate-N

Ammonium-N (NH_4^+-N) concentration generally decreased in all feed levels during the first 30 days of vermicomposting. This trend correlated strongly and positively (r = 0.83) with the reduction rate of water soluble organic carbon all three feeding levels.

Overall, NH_4^+-N concentration did not vary clearly between different levels of feed application (Fig. 14.16a). However, averaged over time, NH_4^+-N concentrations decreased more markedly and were lower (9 to 12%) in reactors fed at light and moderate rates at the end of the experiment.



Solids retention time (d)



Solids retention time (d)

Figure 4.16: The effect of different substrate loading rates on ammonium-N and nitrate-N dynamics during continuous vermicomposting at different solids retention times. (a) Ammonium-N, (b) Nitrate-N

As seen in Figures 14.16a and 14.16b, NO_3^--N increased in parallel with the decrease in NH_4^+-N concentration during the vermicomposting process. An examination of the two soluble nutrients indicates that, water soluble ammonium was decreased by about 86 to 97%, that is from 1640 to about 50 mg NH_4^+-N/kg dry matter, while the NO_3^--N increased from 0.0 mg/kg to about 1130 mg/kg dry matter. Concentrations of NO_3^--N in light and moderate feed levels were in a higher range (1000 to 1130 mg/kg dry matter) than in reactors maintained at heavy feed level (900 mg/kg dry matter). Overall, it can be stated that not the entire ammonia was converted to nitrate. Notably, in periods with slightly alkaline pH (see Fig. 4.13), a part of the ammonia can be assumed to be volatilised as NH_3.

These observations suggest, as previously indicated, that for the vermicomposting of faecal matter, feed application in the range of 6.7 to 10.0 kg feed/m^2/day (0.8 to 1.2 kg-feed/kg-worm/day) is optimal.

4.2.5 Water soluble phosphorus (WSP) and potassium

Available phosphorus and potassium contents at the three feed levels were highly variable (Fig. 4.17). Phosphorus content in reactors fed at moderate level decreased markedly at first sampling (day 13). A slight decrease in phosphorus was also observed in reactors maintained at light feed level at third sampling (day 30). Concentrations rose slightly at a retention time of 45 days, and then returned to previous levels at day 90, with the exception of reactors fed at moderate level where phosphorus contents rose continuously from day 13, reaching a sharp peak at day 60 before decreasing gradually to almost level the concentrations at the light and heavy feed rates at day 90. These variations in concentrations may be attributed to errors in retention time determination as well as the artifacts of sampling and analysis.

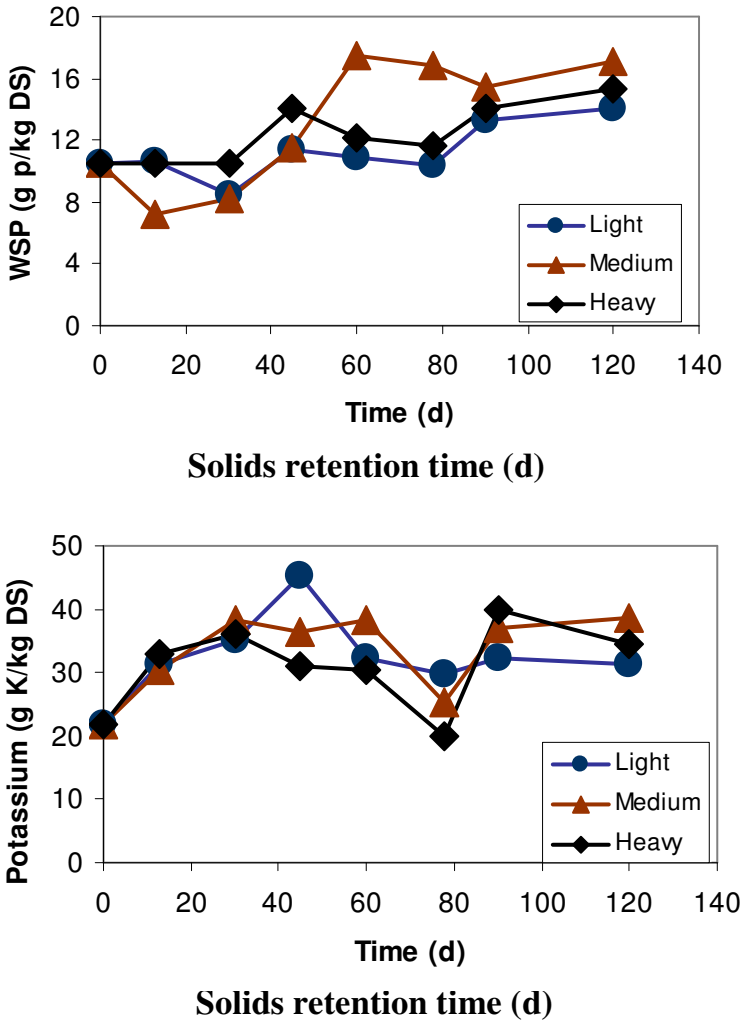


Figure 4.17: The effect of different substrate loading rates on water soluble phosphorus and potassium during continuous vermicomposting at different solids retention times. (a) Water soluble phosphorus, (b) Potassium.

As Figure 14.17a indicates, the increase in available phosphorus at the longest solids retention time was highest in moderately fed reactors (62.5%), followed by heavy feeding (46.2%), and light feeding (34.0%). The available phosphorus in this study also correlated positively (r = 0.78) with solids retention time. The increase in concentration of phosphorus was due to decrease in dry matter mass during vermicomposting. Unlike nitrogen, phosphorus is relatively non-mobile during the decomposition process and hence its mass remains relatively unchanged. Therefore, since the same mass of phosphorus is present in less dry matter at the end of vermicomposting, phosphorus concentration increases.

As shown in Figure 14.17b, the trend for potassium was different in that potassium concentrations rose linearly at all feed levels up to day 30. The highest potassium increase (106%) was registered in reactors fed at light level at a retention time of 45 days, which could possibly be a result of sampling or analytical errors. By day 78, Potassium concentrations at all feed levels had decreased markedly (15 to 45%) relative to the concentration at day 30. This was likely due to sampling error. However, concentrations rose markedly at day 90 and by the end of the experiment, the net increases were respectively 42.9, 76.9 and 56.1% in the light, moderate and heavily fed reactors.

These results suggest that for optimal mineralisation of organic matter during continuous vermicomposting, reactors should be fed with faecal matter at moderate rate (1.2 kg-feed/kg-worm/day).

4.2.6 Total Phosphorus

The amount of total phosphorus (TP) in the substrate increased slightly with treatment from an initial value of 29 g/kg dry matter to about 30 to 33 g/kg dry matter during the first 30 days of vermicomposting. In the reactors fed at heavy rate, an unexpected decline in concentration was observed from day 30 to day 78 (Fig. 14.18), probably due to immobilisation in worm tissue and analytical errors.

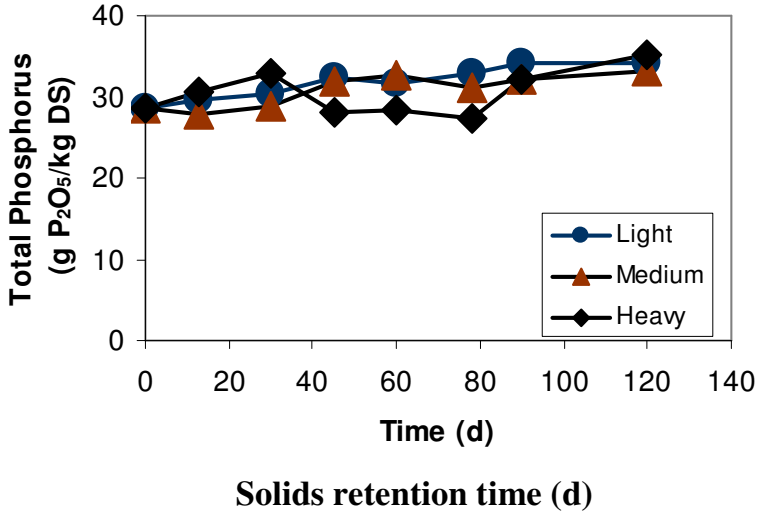


Figure 4.18: The effect of different substrate loading rates total phosphorus dynamics during continuous vermicomposting at different solids retention times.

Besides this observation, differences regarding the effect of feed levels were not detected since the final percentage increase in TP was very similar at the three levels investigated. This was not in accordance with VS removal where the final percentage was clearly lower in reactors fed at heavy rate in comparison to light and moderately fed reactors.

Corroborating Satchell and Martin (1984) who observed negligible increase in TP of paper waste sludge, after worm activity, the increases in TP recorded in the present study are too small to be attributed to the direct action of earthworms.

The results suggest that feed level has no effect on TP concentrations during continuous vermicomposting.

4.2.7 Vermicast production rate, mass balance analysis and characteristics of vermicast

4.2.7.1 Vermicast production rate

The vermicast output from the reactors, studied for 120 days, is summarised in Table 4.2. The vermicast yield consistently decreased with increasing feed application rates – from an average 140.5 mg-vermicast/g-worm/day in the light loading rate reactors to 96.0 mg-vermicast/g-worm/day in the heavy loading rate reactors. Jain *et al.* (2003) have observed that the vermicast production rate depends on the characteristics of the waste, environmental conditions and earthworm species. They found that the vermicast production varied from 0.20 to 0.29g-vermicast/g-worm/day using *E. foetida* with municipal solid waste as substrate. Gajalakshmi *et al.* (2001) have reported 0.20 to 0.21g-vermicast/g-worm/day in low rate digesters fed on a mixture of paper and cow dung using the worm *Eudrilus eugeniae.*

Table 4.2: Temporal variation in vermicast production (grammes) from test reactors operated at 2.3 kg worms m^{-2}

	Production in 90 days			Day 90 to Day 100			Day 100 to Day 110			Day 110 to Day 120		
	Reactor			Reactor			Reactor			Reactor		
Reactor Loading Rate	**I**	**II**	**Mean**	**I**	**II**	**Mean**	**I**	**II**	**Mean**	**I**	**II**	**Mean**
6.7 kg feed/m^2/week	139	142	140.5	43	38	40.5	40	29	34.5	32	43	37.5
10.0 kg feed/m^2/week	113	124	118.5	36	27	31.5	25	24	24.5	29	26	27.5
13.3 kg feed/m^2/week	101	96	98.5	24	27	25.5	13	18	15.5	17	14	15.5

4.2.7.2 Mass balance analysis

Knowledge of the mass balance of inputs and outputs during vermicomposting is important for understanding the decomposition process which is needed for optimising the design and operation of the process. Dry matter mass balance estimates are also useful for calculating nutrient component changes on a mass basis.

Mass losses were expected to occur due to losses in water (from evaporation), carbon (through CO_2 evolution) and nutrients (through volatilization and denitrification). Therefore, mass balances were constructed for the three feed application rates and dry matter and moisture losses were determined at the end of the experiment. The overall reduction in mass of input substrate, due to volatile solids losses, was determined and is shown in Table 4.2.

The results showed that approximately 40% of the substrate deposited in the moderately fed reactors was removed during the vermicomposting process through moisture and volatile solids losses (Fig. 4.19), equivalent to 0.24 kg of the input mass. This was clearly greater than the blanks (without worms) where the reduction was a mere 7.9%. Mass balance also indicated that higher (21.7%) removals of moisture and solids occurred in reactors fed at light rate than in those subjected to heavy rate feed application where the losses were marginal (15.8%). These results suggest that increasing the proportion of faecal matter supplied to the reactors may lower the overall rate of mass removal. It should be recalled that there was no vermicast production from the reactors without worms. Therefore, for the blanks, dry matter was estimated by sampling the oldest (bottom) layer of the material for each loading rate.

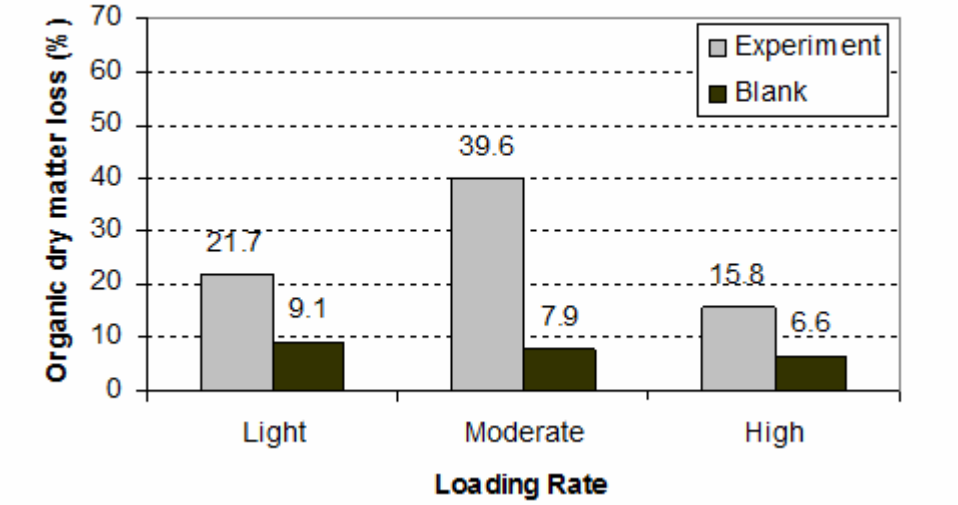


Figure 4.19: Loss of dry matter during vermicomposting

4.2.7.3 Physical-chemical characteristics of vermicompost

In the following Table and sections, the chemical parameters of vermicast harvested at different periods during the experiment are presented and briefly discussed.

Table 4.3: Temporal variation in vermicast chemical parameters

Parameter	Raw Value	Day 90			Day 90 to Day 100			Day 100 to Day 110			Day 110 to Day 120		
		A	B	C	A	B	C	A	B	C	A	B	C
pH	7.1	6.9	7.1	6.8	5.9	6.2	7.2	7.0	5.9	7.0	6.1	5.7	7.1
Salt (g/kg DS)	14.3	14.3	18.0	17.0	18.6	15.9	20.2	18.8	23.1	19.1	26.4	24.1	20.1
VS (%)	83.1	74.3	77.2	79.9	70.0	63.9	67.4	65.9	51.9	58.2	61.4	43.5	67.3
TC (g/kg DS)	367	289	281	289	247	239	288	213	216	293	192	171	289
TOC (g/kg DS)	353	276	274	280	235	227	278	207	203	281	167	158	237
DOC (g/kg DS)	6.2	2.4	2.5	3.7	2.0	2.0	3.6	1.5	1.2	3.3	1.2	1.1	3.1
NH4-N (g/kg DS	1.61	0.9	0.8	1.0	1.0	0.7	1.1	0.8	0.6	0.9	0.2	0.3	0.4
NO3-N (g/kg DS)	n.d	0.3	0.2	0.3	0.3	0.6	0.2	0.7	0.7	0.4	1.3	1.0	0.8
C/N ratio	14.1	7.5	4.7	3.9	7.4	9.2	6.0	7.0	10.6	6.2	7.5	5.7	4.7

A: 0.80 kg-feed/kg-worm/day, B: 1.20 kg-feed/kg-worm/day, C: 1.63 kg-feed/kg-worm/day

As shown in the Table 4.3, the raw substrate had a pH of 7.1, and at the first vermicast production (roughly 90 days) the pH varied only slightly at the three feed application rates, but displayed clear differences in the subsequent vermicasts harvested. The lowest pH of 5.7 was recorded in the reactors fed at moderate rate (1.20 kg-feed/kg-worm/day), suggesting a greater degradation of organic matter and higher nitrification rates at this feed application rate.

There was an increase in salt content in all the vermicasts harvested. The highest increase (84.6%), at the end of the experiment, was observed in the reactors fed at light rate (0.80 kg-feed/kg-worm/day), followed by the moderately fed reactors with 68.5% increase in salt concentration. This increase is due to loss of organic carbon.

The volatile solids (VS) were reduced from 83.1% in the raw substrate to 43.5% in the final vermicasts from the moderately fed reactors, and the reduction rate was clearly higher than that achieved in the reactors fed at light and heavy rates which had final VS values of 61.4 and 67.3% respectively. The VS reduction did not correlate well with the observed changes in salt content, suggesting that besides carbon loss other factors are contributing to the increase in salt content. The moisture content of the vermicasts could be one such factors. The moisture content reduced steadily in all the vermicasts produced, from 75% (raw substrate) to about 18 ± 3% (final vermicast).

It can be seen from Table 4.3 that total carbon (TC) reduced from 367g/kg to 171g/kg dry substance, and total organic carbon (TOC) dropped from 353 to 158 g/kg dry substance in moderately fed reactors, at the end of the experiment. Reduction of TC and TOC in the light-rate-fed reactors compared favourably with the former, whereas the heavy-rate-fed reactors displayed minimal TC and TOC decreases (Table 4.3). A similar trend, as for TC and TOC, was observed at the different feed application rates for dissolved organic carbon (DOC). Generally; TC, TOC and DOC decreased steadily in all the vermicasts produced.

Ammonium-nitrogen decreased in the vermicasts, with the highest reduction (87.5%) recorded in the reactors fed at light rate, followed by moderately fed reactors (81.4%). This was well correlated with the observed increase in nitrate-nitrogen concentrations. Carbon to nitrogen ratio decreased in all vermicasts, mainly due to loss of organic carbon.

4.2.8 Assessment of the effect of loading rate

The results show that the chemical properties of faecal matter change differently during continuous vermicomposting at different loading rates. The following main conclusions can be made:

Volatile solids (VS) and carbon concentrations decreased due to the decomposition of easily degradable C by worms and microorganisms. However, even after 17 weeks into the vermicomposting process, the reduction in VS was still low (approximately 23% to 40%) at the three feed levels investigated, suggesting that the material was not fully stable and continued treatment was desirable.
Soluble nutrient contents (nitrate-N, phosphorus and potassium) increased whereas ammonium-N content decreased
Available nutrient levels were generally higher in reactors fed at 0.8 kg-feed/kg-worm /day (light feed level) and 1.2-feed/kg-worm /day (moderate feed level) than reactors fed at 1.63 kg-feed/kg-worm /day (heavy feed level).
Total phosphorus concentrations increased slightly during vermicomposting. Feed application rate had no clear affect on concentrations of total phosphorus.

These observations are consistent with the general hypothesis that earthworms accelerate the rate of carbon mineralization. Dominguez *et al*. (2003), Atiyeh *et al.* (2000), and more recently Aira *et al.* (2006) and Shalabi (2006) reported similar reductions in volatile solids and total organic carbon in vermicomposting experiments with the earthworm *E. foetida* with separated faecal solids. Furthermore, Aira and Dominguez (2007a) have demonstrated that organic carbon loss is greatly enhanced by the presence of earthworms. The authors investigated the effects of rate of manure application on carbon loss and microbial stabilization and concluded that the rate of feed application has a strong effect on microbial-earthworm relationships. They recommended the use of low application rates of manure when the objective is the microbial stabilization of the waste.

In the present study, although feed application rates had an overall strong effect on faecal matter degradation during vermicomposting, this effect was more evident in reactors fed at light (0.8 kg-feed/kg-worm/day) and moderate rates (1.2 kg-feed/kg-worm/day). These observations are consistent with the findings of Aira and Dominguez (2007a), as it seems that at loading rates higher than the above range, volatile solids and carbon reduction were no longer affected by the rate of substrate application.

Atiyeh *et al.* (2001) has noted that one of the greatest challenges to vermicomposting is to assure that the feedstock does not attain a temperature high enough to begin the thermophilic process of decomposition, as this would kill the worms. Fredrickson and Ross-Smith (2004) has suggested that temperature must be kept below 35°C during the vermicomposting process. Heat is likely to be generated in the system if feed accumulates faster than worms can process.

Therefore, the feeding rate is a critical design criterion for the vermicomposting systems. The common approach is to apply waste frequently in thin layers, a few centimetres thick, to reactors containing earthworms in order to prevent overheating and help keep the waste aerobic.

In the present study it was found that faecal matter can be effectively processed by vermicomposting in continuous-flow systems when it is applied at a maximum rate of up to 10.0 kg feed/m^2/day, with a worm density of 2.33 kg-worm/m^2 or 1.2 kg-feed/kg-worm/day.

4.3 Worm Introduction Density

Does high earthworm introduction density accelerate the rate of volatile solids reduction and increase the faecal matter stabilization rate?

Three main environmental factors influence the survival, reproduction and the performance of earthworms in organic wastes. These are temperature, moisture content and feed/worm ratio. Shalabi (2006) demonstrated that even when environmental conditions such as temperature and moisture are optimum, problems such as worm mortality and inefficient substrate decomposition can develop due to overcrowding.

The influence of environmental conditions and population density on worm processing activity has not been well documented in earthworms. Until now studies on earthworm stocking density have focused more on its influence on the growth and reproduction of earthworms than on actual vermicomposting of the respective substrates (Ndegwa, 2000).

It is likely that amount of reactor space would alter earthworm behaviour and influence growth, reproduction and performance of individuals. Frederickson *et al.* (1997) and Dominguez and Edwards and Lofty (1977), have reported significant reduction in growth rate and reproduction of earthworms as population densities increased. This finding is corroborated by the observations of Garg *et al.* (2008) who reported that overcrowding of worms affect the efficiency of a vermicomposting system, in terms of substrate processing rate, even when all parameters have been optimized.

Focusing research on adequate space requirements (stocking densities) may lead to management practices that could improve survivability and performance.

This part of the study was undertaken to determine the optimum introduction density of earthworms in continuous vermicomposting of faecal matter. Before presenting the results, mention needs to be made that as no flow of decomposed material from the mesh bottom occurred in the blanks (reactors without earthworms) throughout the experiments, only the oldest layers were assessed morphological (colour, odour and texture). No analyses were performed on the substrate in the blanks. Hence there are no time profiles of the blanks in Figures 4.20 to 4.26.

4.3.1 pH

As shown in Figure 4.20 there was no clear pattern in pH change at the three stocking densities. In the first 8 weeks pH dropped from an initial mean value of 7.2 to 5.8. This decrease correlated with the trend in VS reduction, and was likely due to the release of CO_2 during the decomposition process. After this period (8 weeks) pH increased markedly up to week 12, probably due to breakdown of intermediary products such as organic acids and production of some ammonia. Then it remained relatively stable until week 24. Afterwards, pH decreased until week 32, likely due to nitrification, and rose again towards the end of the experiment.

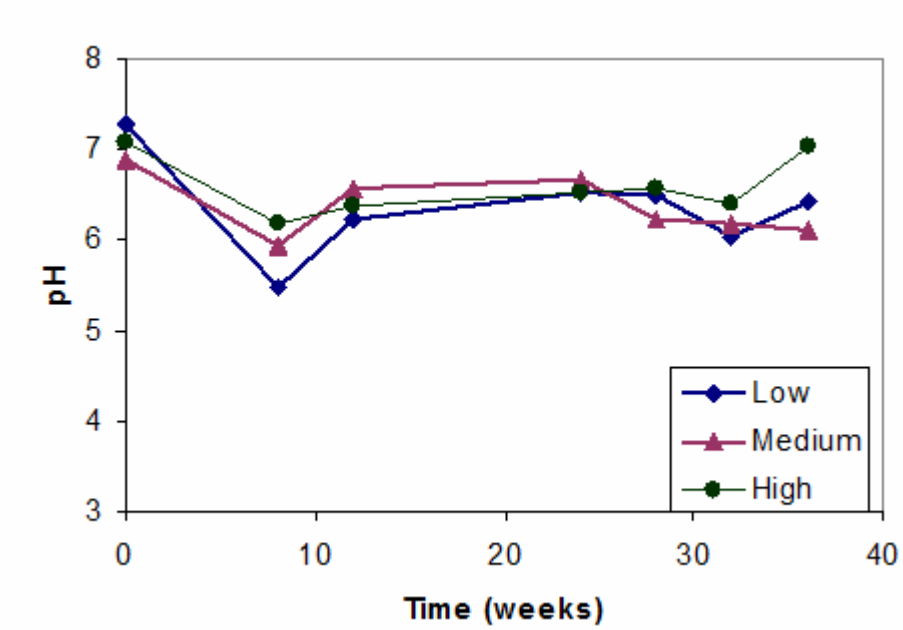


Figure 4.20: pH of the initial substrate and of the harvested vermicast at different worm stocking densities during continuous vermicomposting

4.3.2 Volatile solids

Figure 4.21 shows that the percent reduction in volatile solids (VS) increased with stocking densities. The mean VS reduction in the low, medium and high stocking densities were respectively 38.6, 42.4 and 40.4 %, of the initial value of 84.5 % dry solids. Most of the reduction occurred during the first 8 weeks of the experiment and this can be explained by the decomposition of readily biodegradable organic matter. Elvira *et al.* (1998) have explained this by suggesting that during earthworm gut transit fragmentation of organic matter takes place which significantly increases the percentage of fine particles. This increases the substrate surface area for microbial degradation. This view is supported by Hartenstein and Hartenstein (1981) who observed that this mechanical action of the worms increased the surface to volume ratio, thus increasing the microbial activity in the substrate.

In this study, there was a clear difference in VS reduction rates between the lowest and the highest stocking densities up to week 12, with the most pronounced reduction tendency being observed at the medium and high densities. At the low stocking density, lowest mean VS reduction was found. Between week 12 and week 24, VS reduction was stable at the three

stocking rates. After week 24, VS reduction leveled off, remaining almost in a plateau until the end of the experiment, suggesting that the reactors had attained equilibrium in worm densities. Beyond a threshold density limit, worms compete with each other for space and food, and such intra-specific competition is more pronounced under conditions of overcrowding. This can be explained by the fact that worms grow and reproduce during vermicomposting, and under low crowding conditions they tend to multiply more rapidly than at higher densities. It is then obvious that the populations approach equilibrium at some point, hence the similar VS removal rates. The VS reduction trend observed from week 32 to the end of the experiment might be interpreted as establishment of a stationary phase in the continuous vermicomposting.

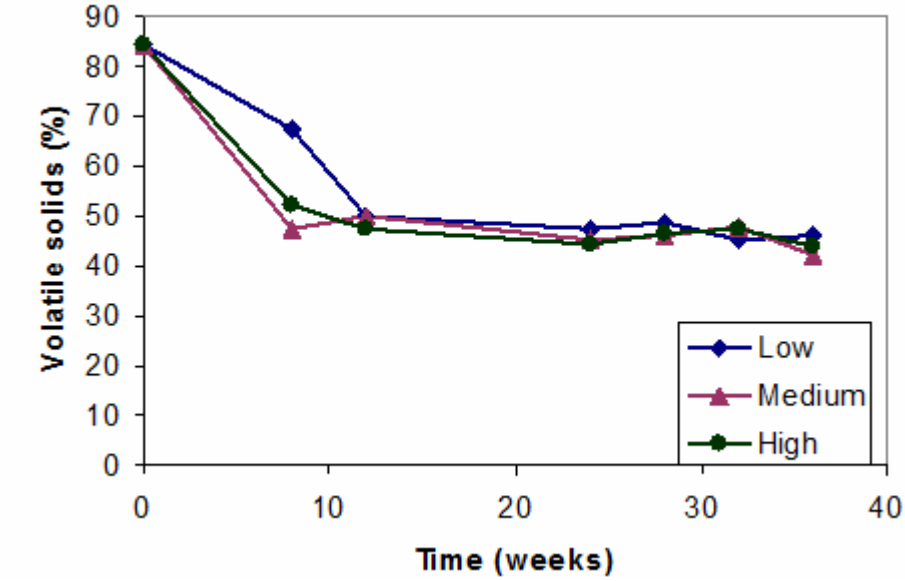


Figure 4.21: Percent change of substrate volatile solids at different worm stocking densities during continuous vermicomposting

4.3.3 Total organic carbon

For the total organic carbon (TOC), an initial reduction pattern similar to that of volatile solids was observed as shown in Figure 4.22. The worm densities, “medium” and “high” have resulted in clearly higher initial reductions. TOC reduction was apparent between week 28, and this was likely due to the fact that the most readily degradable substrate had been diminished. The overall TOC level of the three densities was rather similar at the end of the experiment. There was a striking difference between VS TOC concentrations: While VS exhibited a plateau between week 12 and end of experiment, TOC concentrations continuously decrease. This may indicate some general differences between the two parameters; while VS certainly includes thermally labile carbonates and bicarbonates, TOC was probably more limited to organic compounds. So, the general mineralization products (bicarbonates) can be assumed to contribute to VS more pronouncedly that to TOC. However, TOC analysis of solids usually also affected by TIC like carbonates and bicarbonates unless they have been removed by agitating the solids with acids prior to analysis. Therefore, the given hypothesis for the differences in TOC and VS time courses is questionable.

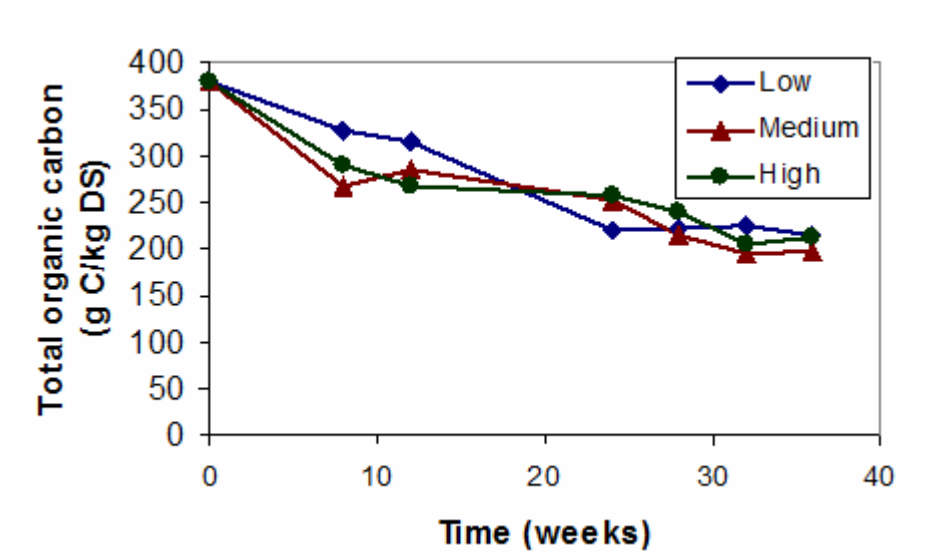


Figure 4.22: Total organic carbon of the initial substrate and of the harvested vermicast at different worm stocking densities during continuous vermicomposting

4.3.4 Water soluble ammonium

The different worm densities have produced almost similar effects on the ammonium-nitrogen (NH_4^+-N) concentrations (Fig. 4.23). NH_4^+-N concentrations generally decreased during the experimental period. After week 24, NH_4^+-N reduction was relatively stable at all densities except an unexpected peak at the low density at week 28, which cannot be explained. At the end of the experiment, similar concentrations of NH_4^+-N were observed for all stocking densities.

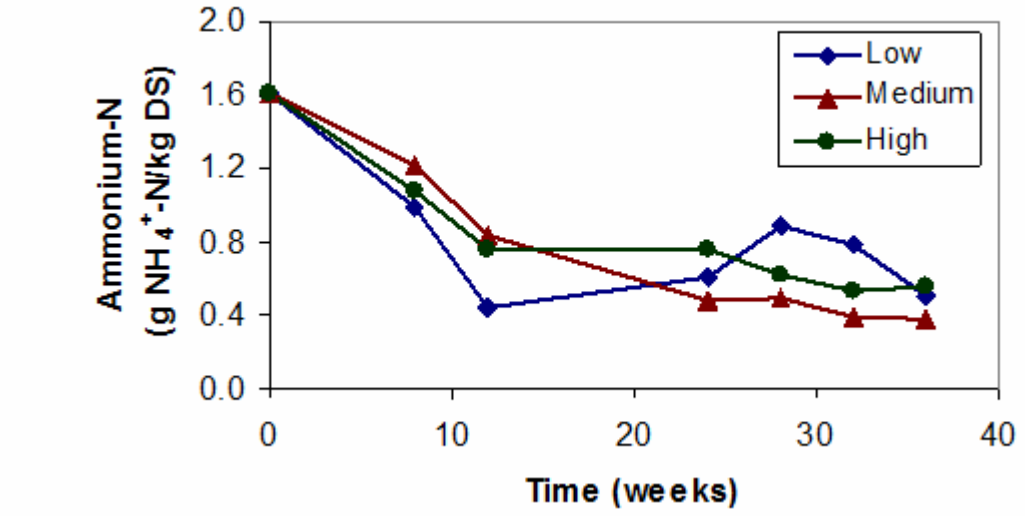


Figure 4.23: Ammonium-N concentrations of the initial substrate and of the harvested vermicast at different worm stocking densities during continuous vermicomposting

4.3.5 Water soluble nitrate

As shown in Figure 4.24, nitrate nitrogen (NO_3^--N) concentrations increased steadily between week 0 and week 12, which correlated with decreases in NH_4^+-N concentrations during this period. In fact, during the rest of the experimental period, the pattern of NO_3^--N increases followed a similar trend to the change of NH_4^+-N concentrations, except at the low worm density where two transient peaks were observed at weeks 28 and 36, probably due to in-homogeneity of sampling or analytical errors. It is reasonable to assume that unlike VS reduction, the different stocking rates have produced little differences in the nitrification rate, although the rate of conversion of NH_4^+-N to NO_3^--N was slightly higher at the low density (1.60 kg worm/m^2) at the end of the experiment. The transient increase in NH_4^+-N concentration observed at this between weeks 24 and 28 can be explained as follows: death of some earthworms, rapid decomposition of the dead organisms with increased ammonification, followed by rapid nitrification that increased NO_3^--N concentrations. Decrease of ammonium removal rate reflects a phenomenon like slower biological decomposition.

For the control, concentration of NO_3^--N increased from week 0 up to week 4 and gradually reaches a plateau with curing time (data not shown).

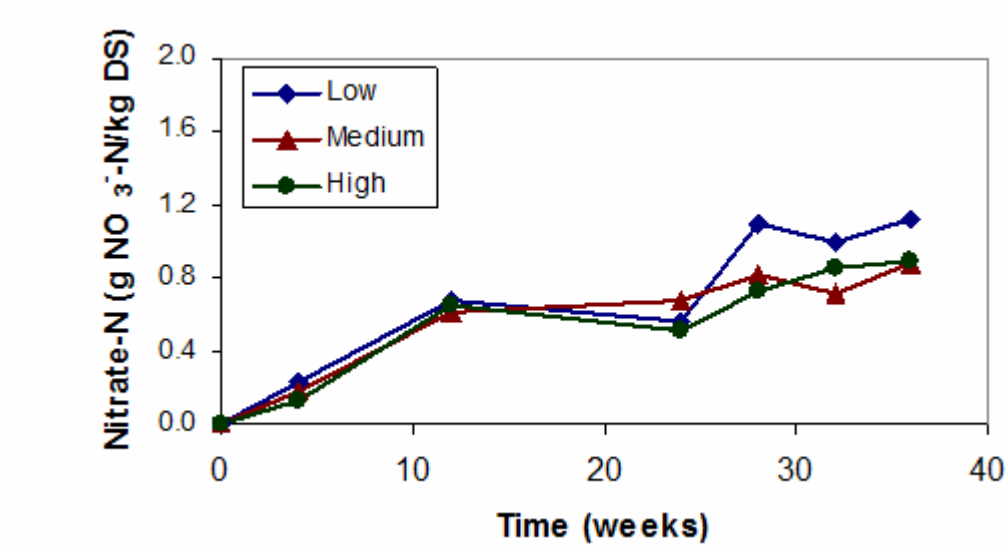


Figure 4.24: Nitrate-N concentrations of the initial substrate and of the harvested vermicast at different worm stocking densities during continuous vermicomposting

4.3.6 TKN

In general, TKN content of the material increased in all the stocking densities (Fig. 4.25). The mean percent TKN increase was 29.5, 46.7 and 20.9 % at the low, medium and high stocking densities respectively. TKN increase can be attributed to organic matter degradation as explained in previous sections.

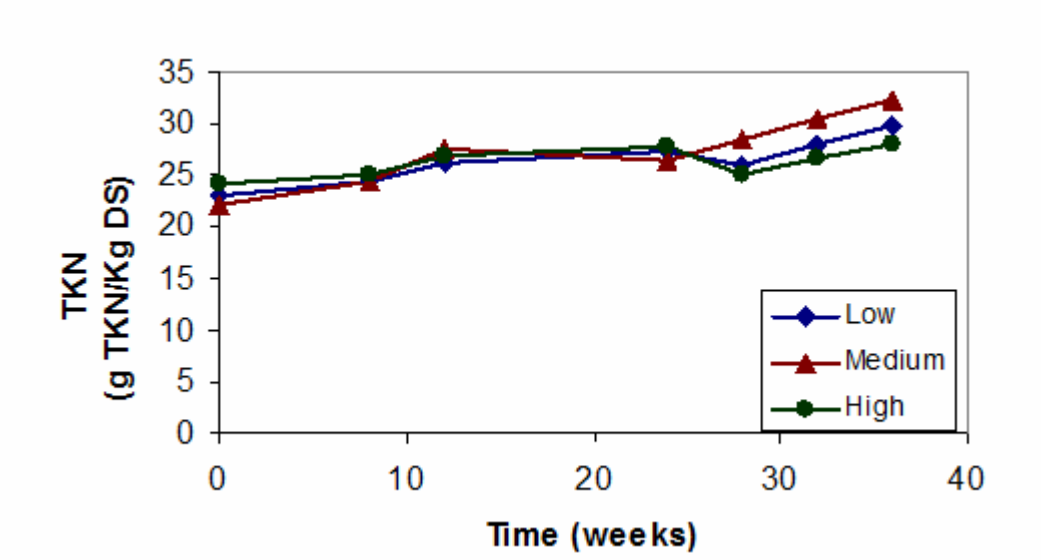


Figure 4.25: TKN concentrations of the initial substrate and of the harvested vermicast at different worm stocking densities during continuous vermicomposting

4.3.7 Total Phosphorus

During the experimental period concentrations of total phosphorus (total P) slightly increased linearly in all stocking densities (Fig. 4.26). The highest total P increase was registered at the low worm density (18.6%), followed by the medium density (8.5%) and the high density (5%). Total P showed high positive correlations with curing period for the low and medium densities (r = 0.98 and r = 0.84), whereas the high stocking density correlated poorly (r = 0.28) with vermicomposting period due to low P increase with time. The increase in total P can be explained by the fact that continuous reduction of organic carbon ultimately results in greater concentration of nutrients as virtually the same amount of nutrients are analysed in smaller dry mass.

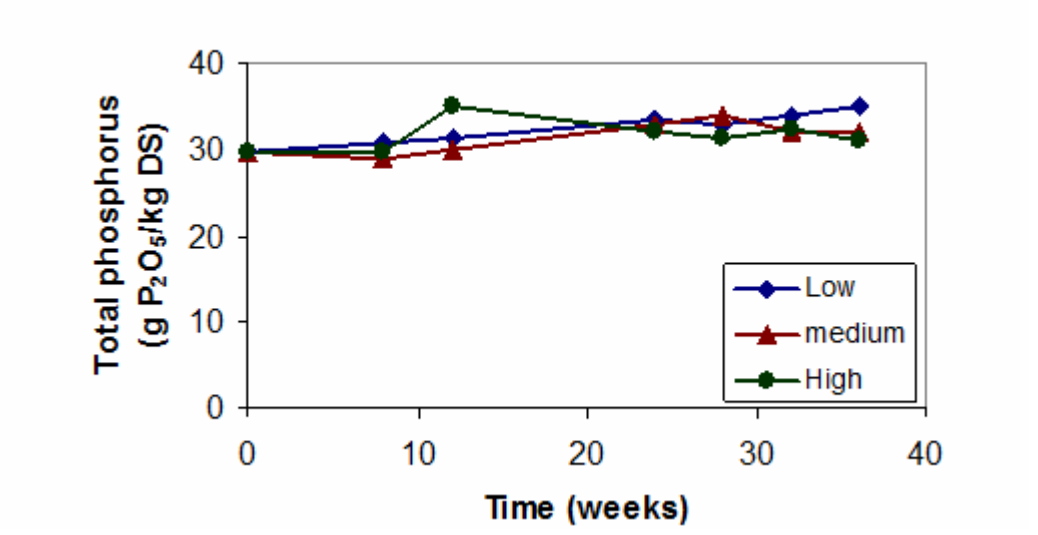


Figure 4.26: Phosphorus of the initial substrate and of the harvested vermicast at different worm stocking densities during continuous vermicomposting

4.3.8 Evolution of heavy metal concentrations during vermicomposting

Heavy metals are not biodegradable. Only transport processes can reduce their concentration in soils. They may enter the food chain when vermicompost are land applied; therefore, their concentrations in the final product deserve consideration. It needs to be mentioned that the solubility, mobility and toxicity of heavy metal compounds differ from that of the elemental heavy metals which were not particularly analysed in this study. Concentrations of heavy metals were predicted to rise due to mass reduction resulting from organic matter degradation (organic carbon loss) and mineralization.

The evolution of five heavy metals, including Ni, Cd, Pb, Zn, and Cu were analysed across all stocking densities via composite samples. The results are summarised in Table. 4.4.

Table 4.4: Evolution of heavy metal concentrations during continuous vermicomposting of faecal matter

Heavy metal concentrations (mg/kg dry substance)						
	Solids retention time					
Metal	8	12	24	28	32	36
Ni	255.0	235.0	144.3	104.7	93.7	81.7
Pb	27.6	20.0	9.3	4.1	2.97	2.31
Cd	0.21	0.20	0.20	0.20	0.21	0.20
Cu	70.2	65.6	76.7	61.3	49.6	38.7
Zn	260.67	251	242.3	211.6	193.4	102.1

Table 4.4 show that heavy metal concentrations in the vermicompost samples decreased with increasing solids retention time in the continuous reactors. Reductions of Pb and Ni concentrations with increasing retention times from week 8 to week 36 were higher (92 and 68 % respectively) than t hose of Cd, Cu and Zn. Heavy metal concentrations were well correlated with vermicomposting period, indicating that increasing the duration of treatment would result in increased reductions.

The metal concentrations in the vermicasts of this study were comparable to the values determined by Fricke *et al.* (1996) who have reported 0.5, 40, 17 and 86 mg/kg (dry weight basis) for Cd, Cu, Ni and Pb, respectively, in "green" waste compost. With regards to biosolids, the heavy metal concentrations that can be found in the literature vary considerably, among the metals as well as among different publications.

It was expected that since heavy metals were not degradable their concentrations will increase (if they do not leave the system), as a consequence of the carbon losses by mineralization during the decomposition process. With the exception of evaporation (transport to the gaseous phase) none of the transported processes indicated above could have caused heavy metals to leave the system. Harms and Bunke (2002) have advised that under normal conditions the evaporation of heavy metals can be neglected, except for elemental mercury and for $(CH_3)_2Hg$ that have high vapour pressures and for that reason, a tendency to evaporate.

Different earthworm species deal with heavy metals in different ways: immobilisation (in fatty cells of the gut wall), accumulation/storage (in waste nodules formed within the body

cavity) and excretion (through the calciferous glands) being the most common, according to Andersen and Laursen (1982). Thus, accumulation in the worms is the only plausible explanation for the decreasing concentrations observed in this study. Similar trends to those observed in this study have been reported by Shahmansouri *et al*. (2005) who recorded significant decrease in heavy metals concentration during vermicomposting of sewage sludge with *E. foetida*. Leonard *et al*. (2001) have reported similar results after investigating the uptake of heavy metals by earthworms in relation to the availability in soil and the intestine. Furthermore, numerous authors as reviewed by Beyer and Hensler (1987) and Ireland (1983), have reported that earthworms can accumulate heavy metals from contaminated and non-contaminated environments. Ireland (1983) states that Cd does not appear to concentrate in earthworm tissues indefinitely, and the concentration factor or storage ratio (the ratio of a metal in worm tissue to that in the substrate) decreases with increasing Cd concentrations, unlike Pb which appears to accumulate continuously.

Graff (1981) investigated the accumulation of heavy metals in *Eisenia foetida* and *Eudrilus eugeniae* before and after feeding on compost made from municipal solid waste. The heavy metal contents (in ppm dry weight) before and after feeding were: for E. *foetida*, Cu 4 to 29, Zn 140 to 640, Pb 3 to 14, Cd 2to 9, Hg 0.1 to 14; for *Eudrilus eugeniae*, Cu 17 to 55, Zn 165 to 360, Pb 10 to 72, Cd 4 to 6, Hg 1 to 15. These data indicate earthworms are extracting heavy metals from organic waste and concentrating them in their tissues.

However, the results of the present study do not agree with the findings of Barrera (2001) who reported increasing concentrations of heavy metals during vermicomposting of sewage sludge, and of Carter *et al*. (1983) who found that in *Lumbricus rubellus*, the Cd levels in casts tended to increase as contaminated sludge concentration increased up to a certain level.

It can be suggested that the pathways of accumulation and elimination of heavy metals by earthworms vary between.

The German regulations on the use of sewage sludge in agriculture (AbfKlärV) give limits for 7 different heavy metals which are considered to be most relevant. Five of them are shown in Table. 4.5, along with the European Community (EC) and US-EPA limits for comparison purposes.

Table 4.5: Comparison between heavy metal concentrations and guidelines (mg/kg DS)

	Ni	Pb	Cd	Cu	Zn
Vermicompost	81.7	2.3	0.2	38.7	102.1
AbfKlärV	200	900	10	800	2500
European Community	350	975	30	1375	3250
US-EPA	420	480	85	4300	7500

Sources: US-EPA (1999); European Community (2000)

As Table 4.4 shows, the heavy metal contents at the solids retention times investigated decreased to lower values than for very low retention times. Except for Ni, all heavy metal concentrations measured were below the guidelines even at the lowest tested solid retention time. The relatively high concentrations of Ni cannot be explained, however, it may be due to the artefacts of sampling and analysis.

These results suggest that application of vermicompost derived from faecal matter on land may be considered safe with respect to heavy metals. However, the long-term effect of the accumulation of heavy metals in soils needs to be carefully considered.

4.3.9 Assessment of the impact of worm starter density on vermicomposting process

Earthworm introduction density had a marginal influence on physico-chemical parameters during the vermicomposting process.

However, earthworm density had a small effect upon rates of faecal matter stabilization and mineralization from the beginning up to the mid stages (approx. week 12) of

vermicomposting. After this period, there were no clear differences in performance at the different seeding rates. As discussed earlier, it is reasonable to assume that worms in the less crowded reactors grew more rapidly, and after a certain time period (about 10 weeks), the different populations had reached a steady state or equilibrium whereby the influence of earthworm density on the decomposition rates followed a similar trend. Thus, it suffices to conclude that as far as earthworms remain small in numbers, the decomposition and stabilization processes are controlled by population density.

Similar conclusions were reached by Giraddi (2007) during 90 days vermicomposting of crop residues; soybean waste and millet straw. The results of those experiments showed that 100 worms appear optimum for a 1.0 x 1.0 x 0.5 m^2 vermibed holding 30 kg substrate. Dominguez and Edwards (1997) and Frederickson *et al.* (1997) have reported that individual worms grew more and faster at the lowest stocking density; the total biomass production was more at the higher population density. Kale and Bano (1988); Reinecke and Viljoen (1990

) and Hegde *et al.* (1997) have reported similar observations after working on the effect of stocking density on growth and reproduction of earthworms. The authors concluded that the density of earthworms during vermicomposting is related to the rate of waste processing and if vermicompost production is the main aim then it is recommended to maintain a high density of mature earthworms. But, high earthworm densities will eventually reduce the number of earthworms produced, by regulating growth and reproduction. Hence, if the main objective is to produce a net surplus of earthworms, relatively low densities of juvenile earthworms should be used. Equally, regular harvesting of earthworms and cocoons should be carried out to maintain this low density at all times.

Other authors have investigated the stocking density in relation to feed loading rates. Garg *et al.* (2008) have reported that a worm population of 27–53 worms per kg of feed is the most favourable stocking density. Hartenstein and Hartenstein (1981) in their lab-scale experiments on vermicomposting of activated sludge observed that approximately 1 g worm could convert

4 g of activated sludge in 5 days. Ndegwa *et al.* (2000) reported that the best stocking density for vermicomposting of biosolids with paper mulch is 1.60 kg/m^2. The results of those studies showed that a feeding rate of 1.25 kg-feeds/kg-worm/day resulted in the highest bioconversion of the substrate into earthworm biomass while the best vermicompost was obtained at the same stocking density and a feeding rate of 0.75 kg-feeds/kg-worm/day.

In the present study, although when *E. fetida* was seeded at different stocking rates earthworms processed the faecal material more rapidly at higher stocking densities, it was observed that the processing activities of earthworms were not correlated directly to their stocking density over the range 1.60 to 2.80 kg worm m^{-2}.

In this study, an appropriate stocking density seems to be 2.20 kg worms m^{-2} which resulted in 42.4 % reduction in VS. In the same stocking density, percent change of TKN and total organic carbon were 42.4 % and 43.3 %, respectively.

4.4 Earthworm Avoidance-response

Experiments were conducted in this part of the study to determine whether there are electrical conductivity levels correlated with avoidance response of earthworm in the substrate faecal matter.

The electrical conductivity preferences of the earthworm Eisenia foetida is not known, particularly in relation to the substrate faecal matter, but it is now well known that the different species of earthworm exhibit different toxicity tolerance to salts.

Electrical conductivity, which is a measure of soluble salt content, influences the availability of water for earthworms. Previous work (Edwards, 1988) has shown that rapid worm death occurs when feedstock with an electrical conductivity in excess of 4 mS/cm are applied to a vermiculture system.

In the present study, *E. foetida* was exposed to five salt concentrations in five independent experiments aimed at determining whether earthworm avoidance response occurs consistently and may be useful in determining the effect of electrical conductivity on worms. Fischer and Molnar (1996) have observed that osmolarity and ionic composition of body fluids in earthworms fluctuate following changes of external salinity. This leads one to inquire whether worm acceptance and behaviour in faecal matter are correlated with the latter's electrical conductivity properties. Changes in behaviour such as substrate avoidance have an important implication on the vermicomposting process design and control. It was expected that high electrical conductivity resulting from dissolved salts will directly affect earthworms by causing behavioral change (avoidance response) and reduced activity.

Average earthworm avoidance responses at the five salt concentrations studied are presented in Figure 4.27. For each concentration, the bar shows the average distribution (response). The results were essentially the same for the five trials, suggesting that earthworm response was unaffected by the salt concentrations used in this study, except at very low concentration (1 g salt/kg DS) where worm response was significantly different from responses at higher concentrations (7g salt/kg and above). High salt concentrations caused unexpectedly low earthworm avoidance. The data indicate that, contrary to common knowledge, *E. foetida* can

tolerate organic wastes nearly one quarter as salty as sea water (salinity; 3.1 to 3.8%). This level of acceptance/tolerance agrees well with the findings of Piearce and Piearce (1979) who noted that immersion in a solution half as salty as sea water was quickly fatal to several species of earthworms, whereas immersion in a solution one quarter as salty as seawater was tolerated. However, it has to be pointed out that there is a difference between submerging worms in a salt water solution for an acute test and subjecting them to different salinity gradients in an organic substrate. For the latter, the worms may adapt to the new environment.

Other studies (Dominguez and Edwards, 2004) conducted on the avoidance response of Earthworms have suggested that both ammonia and inorganic salts have sharp cut-off points between toxic and non-toxic concentrations. In the present study, although clear avoidance-response boundaries could not be established with the range of salt concentrations investigated, when viewed with the data presented, faecal matter may be acceptable to *E. foetida* at salt concentrations above 28 g/kg, and to a limited extent also tolerable to concentrations as low as 1 g/kg. Findings here suggest that *E. foetida* have sufficient tolerance toward salts to merit use in vermicomposting of faecal matter. However, in other part of this work (section 3.2.2), behavioural observations led to the conclusion that *E. foetida* requires a period of captive acclimation if they are to be used for faecal matter treatment. Findings of the present study support the claim that factors other than high salt levels account for worm avoidance of raw faecal matter. The very low C:N ratio (6:1 - 14 :1) of faecal matter recorded throughout this work suggests that the excess nitrogen contained in the feed stock gradually volatilizes as ammonia, and this is likely responsible for the worm avoidance of the substrate.

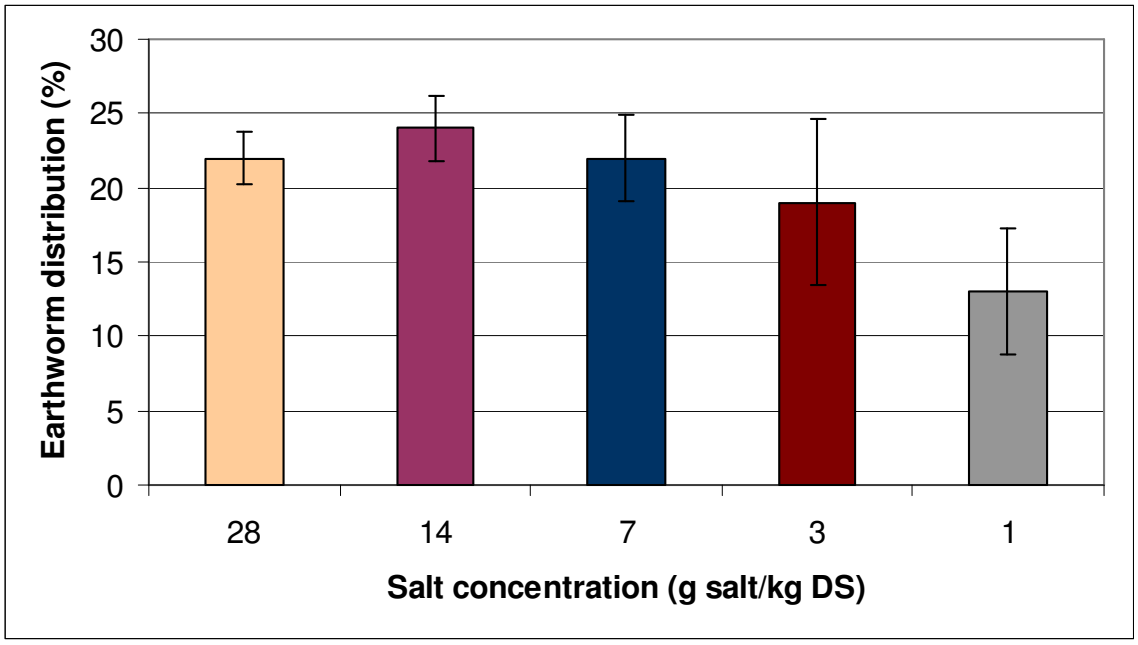


Figure 4.27: Earthworm percent response obtained for 5 salt concentrations. Vertical T bars represent standard error of the mean

Investigation of the tolerance of *E. foetida* to salts as well as optimum concentration of salts for growth and reproduction of this species will be useful if they are to be employed in practical faecal matter management. Further investigation of the avoidance response of this

organism in faecal matter using wide ranges of salt concentrations will be needed. As stated above, trials during the early part of this work have demonstrated conclusively that worms will die if directly fed raw faecal matter. Are there substances in this substrate that are acutely toxic to *E. foetida?* Toxicity testing and field studies are called for to answer this question.

4.5 Hygienic parameters

In this part of the study, investigations were performed in order to answer the following two questions:

- *Does faecal matter treatment by vermicomposting significantly increase or decrease pathogen concentrations?*
- *Is there a difference in the dynamic change of pathogen concentrations for vermicomposting and composting (without earthworms) conditions?*

The results of two lab-scale systems, vermicomposting and microbial composting (without earthworms), will be discussed with respect to time and changes in sanitation indicator bacteria (SIB) populations.

Microorganisms, especially bacteria, are responsible for most of the mineralization of organic matter. They release enzymes that catalyse the oxidization of the organic compounds. The oxidation reactions release energy and organic metabolites, which micro-organisms need to survive.

Therefore, from the point of view of waste treatment, microorganisms are the most critical factor in controlling decomposition, and hence the rate of stabilisation of the organic matter. It has been determined that passage of organic material through the gut of an earthworm can reduce numbers of some microorganisms and increase numbers of others (Satchell, 1983). Eggs of some nematode parasites of birds and mammals, e.g. *Ascaris lumbricoides*, *A. suum* and *Ascaridia agall*i, are not destroyed following passage through the earthworm *Lumbricus terrestris* (Hartenstein and Mitchell, 1978). Microbial pathogens are of greatest concern in waste management for material recycling.

Faecal indicators were monitored in order to obtain information about their evolution during the experimental period, as well as the factors that influence the variations observed. Classical indicators of faecal pollution such as, *Enteroccocus faecalis*, *E. coli*, *faecal coliforms*, Salmonella, Shigella and *enterobacter spp*. were monitored. *Salmonella* and *E. coli* are mentioned in the European and US-EPA regulations. Faecal coliforms survive long in the environment/soil (Table 2.2) and therefore are a good addition to *E. coli* and the other bacteria as an indicator.

Monitoring of the population of some microorganisms is important because changes in their population profiles may reflect the overall quality of the maturing vermicompost. Along with standard chemical and physical indices, dynamic change in microbial indicators will allow conclusions to be made about the rate and extent of stabilisation.

Populations of indicator pathogens in the initial substrate and the changes during the investigation period are shown in Figures 4.28 to 4.33. The mean microbial populations and

the dynamic change during the experimental period are shown in Tables 4.5 to 4.10. All microbial numbers have been reported on a dry weight basis.

4.5.1 Escherichia coli

Analysis of samples for *Escherichia coli* showed an initial concentration of 1.86E+07 CFU/g, for the test row and 1.76E+07 CFU/g for the control/blanks (without earthworms) (Table 4.5). The population developed as shown in Figures 4.28 and 4.29. After 9 days, control samples averaged 0.14-log increase, which equates to 36.5 % increase (percent based on actual pathogen count and not logarithmic numbers) while test samples averaged a 1.71-log reduction, corresponding to a 98.0 % reduction. In the following days, *E. coli* populations decreased in both control and test reactors and after day 19 values of 2.91-log reduction (99.9 %) (in control) and 1.46-log reduction (96.5 %) (in test) were achieved. After 58 days, when the experiments were terminated, the control reactor samples averaged a 0.26-log reduction (45.5%), while the test reactor samples averaged a 3.74-log reduction (99.9%). As a consequence of the logarithmic scale, the *E. coli* elimination by earthworms seems to accelerate during week 4. On the linear scale (Fig. 4.29), it is obvious that the elimination in the test reactors is very high in the beginning, exhibiting intermittent negligible re-growth on certain occasions (days 19 and 58), compared to the blanks. These increases might be due to analytical errors. No re-colonization occurred in the test reactors towards the end of the experiment, which lends support to the claim of Edwards (1988) and Edwards and Bohlen (1996) that earthworms include microorganisms in their diet as food source and can digest them selectively. The results lead to the conclusion that in less than 60 days an almost complete elimination of *E. coli* by earthworms can be expected.

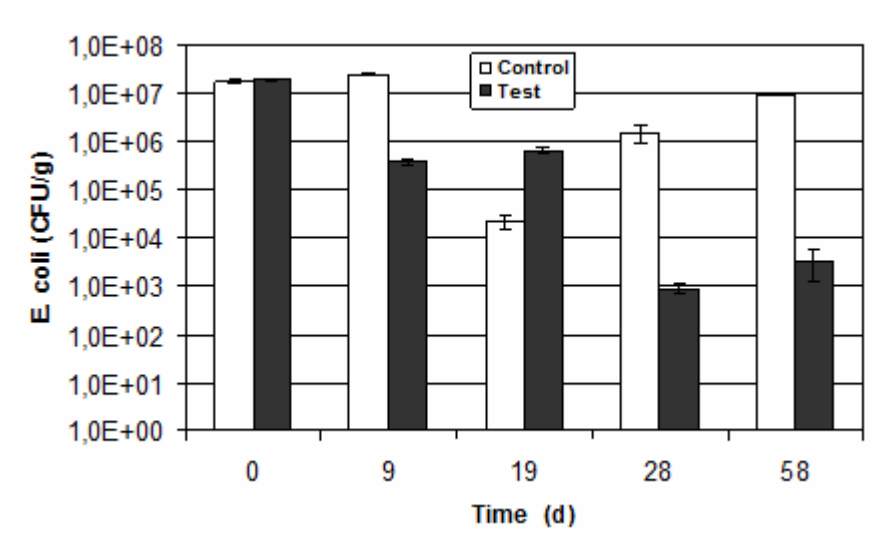


Figure 4.28: *E. coli* average decrease (shown in logarithmic scale) in the control and test reactors during 58 days of treatment. Vertical T bars represent standard

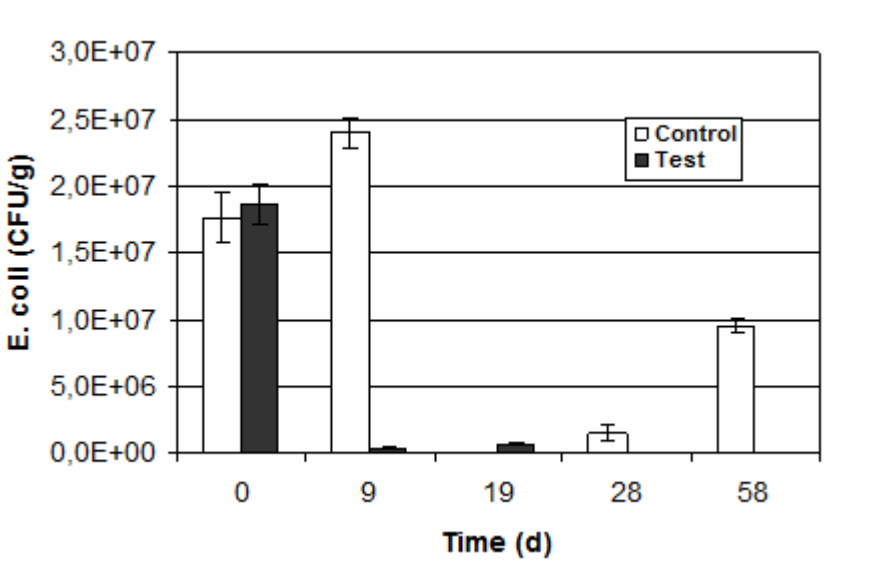


Figure 4.29: *E. coli* average decrease (shown in linear scale) in the control and test reactors during 58 days of treatment. Vertical T bars represent standard

Table 4.6: Mean number of *E. coli* in initial substrate and the dynamic change during the experimental period

Sample Day	*Sample Results (CFU/g)*	
	Control	**Test**
Day 0	1.76E+07 ± 1.96E+06	1.86E+07 ± 1.52E+06
Day 9	2.41E+07 ± 1.14E+06	3.66E+05 ± 3.69E+04
Day 19	2.18E+04 ± 6.40E+03	6.50E+05 ± 8.30E+04
Day 28	1.50E+06 ± 6.23E+05	8.80E+02 ± 2.32E+02
Day 58	9.62E+06 ± 5.41E+05	3.40E+03 ± 2.22E+03

4.5.2 Fecal coliforms

The size of the population of faecal coliforms exhibited a similar pattern to that of *E. coli* except slight variations on days 19 and 28 where, unlike *E. coli*, the populations of faecal coliforms in the test reactors exhibited a marked increase (day 28). When aerobic culture methods are used, *E. coli* is the dominant species found in faeces (Karmali *et al*., 1983). Moreover, according to the American Public Health Association (1992), more than 90% of faecal coliforms are *Escherichia spp*. Therefore, it is understandable that the results obtained are quite similar to the ones for *E. coli*.

The Faecal coliforms samples collected showed an initial average concentration of 2.02E+07CFU/g in the test reactors and 1.89E+07 CFU/g in the controls (Table 4.6). After 9 days, the control reactor samples averaged a 0.88-log reduction, which corresponds to 86.9%. The test reactor samples averaged a 0.09-log reduction (19.2%). Once again the temperature effect on the reduction of pathogen populations was clearly observed. Whereas this trend was

not continuous in the controls, test populations continued to decrease until day 28 when an increase was observed (Figs. 4.30 and 4.31). By day 54 when the trials were terminated, control reactors had attained a 0.29-log reduction (49.3%) whereas a 5.0-log reduction (99.999%) was observed in the test units. From these results, an influence of earthworms on the reduction of Faecal coliforms can be supposed.

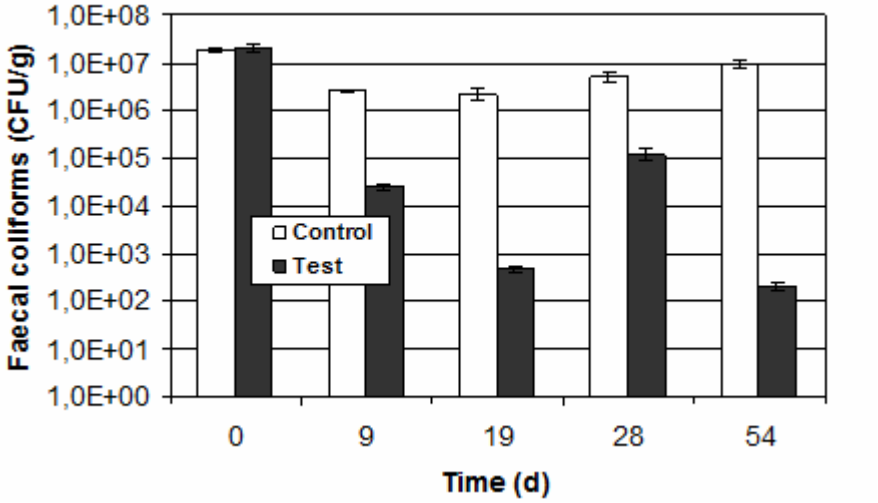


Figure 4.30: Faecal coliforms average decrease (shown in logarithmic scale) in the control and test reactors during 54 days of treatment. Vertical T bars represent standard errors

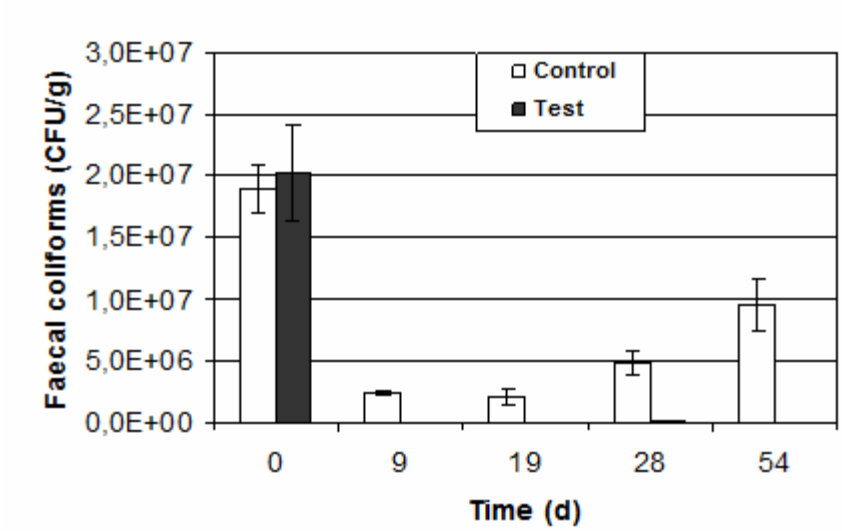


Figure 4.31: Faecal coliforms average decrease (shown in linear scale) in the control and test reactors during 54 days of treatment. Vertical T bars represent standard errors

Table 4.7: Mean number of faecal coliforms in initial substrate and the dynamic change during the experimental period

Sample Day	Sample Results (CFU/g)	
	Control	**Test**
Day 0	1.89E+07 ± 1.96E+06	2.02E+07 ± 3.95E+06
Day 9	2.47E+06 ± 1.16E+05	2.46E+04 ± 2.85E+03
Day 19	2.18E+06 ± 6.40E+05	4.80E+02 ± 8.56E+01
Day 28	4.84E+06 ± 9.50E+05	1.16E+05 ± 3.23E+04
Day 54	9.58E+06 ± 2.06E+06	2.00E+02 ± 4.08E+01

The results are mean ± SE (n = 3)

4.5.3 Enterococcus faecalis

The analysis of collected samples *for Enterococcus faecalis* (*E. faecalis*) showed an average baseline concentration of 7.04E+07 CFU/g (Table 4.7). As shown in the Table, the initial number of *E. faecalis* was the same in both the test and control reactors. After about four weeks (26 days), the control reactor samples averaged a 1.34-log reduction, which equates to a 95.5% reduction (percent is based on actual pathogen count and not logarithmic numbers), (Figs. 4.32 and 4.33). The test reactor samples averaged a 3.10-log reduction (99.9%). After day 35 (end of the fourth sampling set), reductions continued in the test reactors until a 5.22-log reduction (99.999%) was achieved on day 58 when the experiments were terminated; whereas the control reactors showed a reverse trend, averaging a 0.10-log increase (24.72 %) at the end of the experiment. The mean number of *E. faecalis* in the test reactor was significantly lower than that of the control at the end of the experiment (Table 4.7). This observation is illustrated in Figure 4.40b which shows the difference in *Enteroccocus faecalis* elimination between the test and control units in one of the dilution series.

In the present study, counts of *E. faecalis* decreased in the blanks, probably due to the development of thermophilic conditions, but increased again towards the end of the experiment, likely due to temperature declined (Figs. 4.32 and 4.33). It needs to be indicated here that temperature within the substrate was not measured to ascertain if indeed thermophilic conditions existed. However, the observed trend is similar to literature accounts of changes during composting. Sidhu *et al.* (2001) and Garrec *et al*. (2003) have reported decreases in microbial populations during the early stages (first 4 weeks) of composting; supporting the claim that temperature increase is the main factor responsible for the reductions. However, as reported by Golueke (1992) and Dumontet *et al*. (2001), other factors are also involved in the inactivation, like changes in pH, presence of metabolic antagonistic compounds produced by the indigenous microflora, accumulation of toxic NH_3 and microbiological competition for nutrients. Compared to the test reactors where a high removal of *E. faecalis* was achieved, the population trend exhibited by this organism in the controls suggest that there is a strong tendency of postprocessing colonization in the composting system. Other researchers in composting studies have observed similar resurgent growth of thermotolerant coliforms (Hassen, *et al*. 2001) during composting of biowaste and have attributed this to recolonization or recomtamination combined with temperatures that permit growth. This result suggest that although proper management during the thermophilic phase (0-26 days) of composting could bring about significant reduction of pathogens, the hygienic safety of the final product cannot be guaranteed.

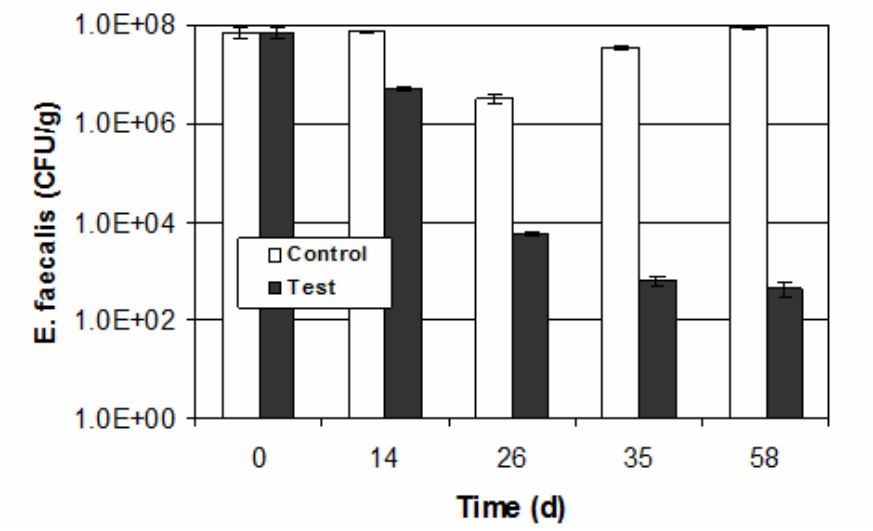


Figure 4.32: *E. faecalis* average decrease (shown in logarithmic scale) in the control and test reactors during 58 days of treatment. Vertical T bars represent standard errors

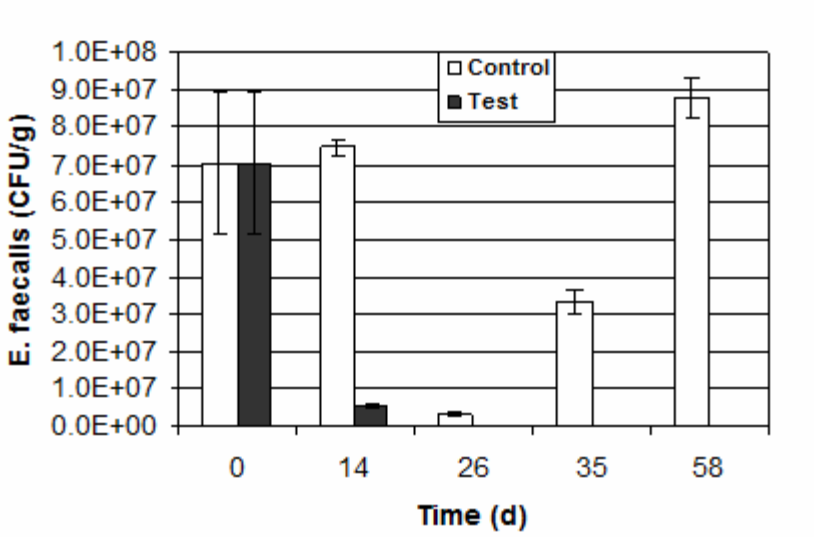


Figure 4.33: E. faecalis average decrease (shown in linear scale) in the control and test reactors during 58 days of treatment. Vertical T bars represent standard errors

Table 4.8: Mean number of *Enterococcus faecalis* in initial substrate and the dynamic change during the experimental period

	Sample Results (CFU/g)	
Sample Day	**Control**	**Test**
Day 0	7.04E+07 ± 1.89E+07	7.04E+07 ± 1.89E+07
Day 14	7.46E+07 ± 1.94E+06	5.18E+06 ± 4.72E+05
Day 26	3.20E+06 ± 6.75E+05	5.54E+03 ± 5.22E+02
Day 35	3.34E+07 ± 3.26E+06	6.20E+02 ± 1.32E+02
Day 58	8.78E+07 ± 5.33E+06	4.20E+02 ± 1.38E+02

The results are mean ± SE (n = 3)

4.5.4 Salmonella spp.

The *Salmonella spp.* samples collected showed an initial average concentration of 7.00E+08 CFU/g in both control and test reactors (Table 4.8). After 19 days, the control reactor samples averaged a 0.18-log reduction, which equates to a 34.3% reduction. The vermicomposting reactor samples averaged a 1.60-log reduction (97.5%). After 59 days, when the experiments were terminated, the control reactor samples averaged a 0.59-log reduction (75.6%), whereas the test reactor samples averaged a 5.58-log reduction (99.999%). According to Eastman *et al.* (2001), researchers working in wastewater treatment facility in the City of Ocoee (Florida) have successfully used vermicomposting to reduce human pathogens, notably *Salmonella spp.* and *faecal coliforms* to well below US-EPA accepted levels in domestic wastewater sludge. In deed, *Salmonella* appeared to have been completely inactivated in the test reactors within 28 days of vermicomposting (Figs. 4.34 and 4.35). As temperature is the main sterilising factor in composting systems, the consecutive decreased in salmonella populations in the control reactors (from day 0 to day 19, to day 28 and to day 36) suggest that this organism has a high sensitivity to temperature. The unexpected rise in salmonella numbers in the control after day 36 can be attributed to the development of mesophilic conditions within the system that favoured growth. These observations are in agreement with the findings of Kumar *et al.* (2004) who investigated the role of earthworms in microbial modification during vermicomposting of mixtures of sewage sludge, rice straw and cattle dung. The authors reported that enteric bacterial populations such as *Salmonella*, *Shigella* and *Escherichia spp.* reduced dramatically and declined to nil within 35 days. According to these authors, there was no re-occurrence of the pathogens on further period.

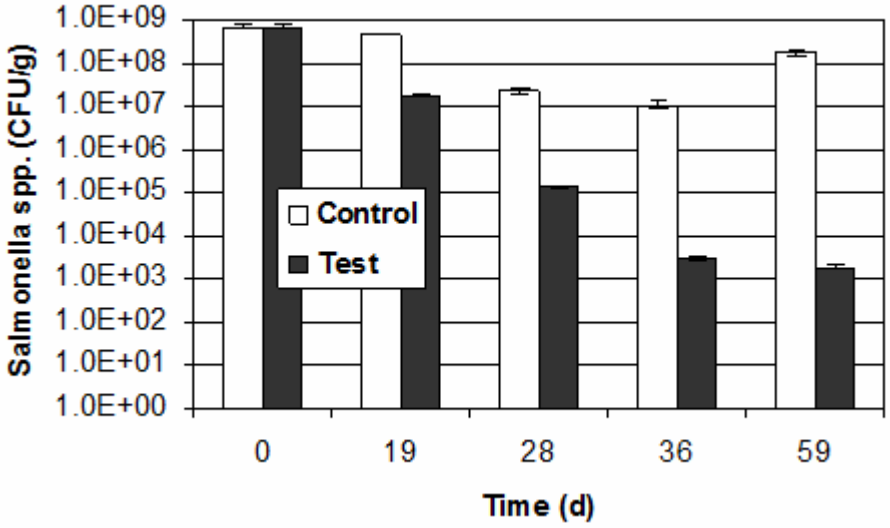


Figure 4.34: ***Salmonella spp.*** **average decrease (shown in logarithmic scale) in the control and test reactors during 59 days of treatment. Vertical T bars represent standard errors**

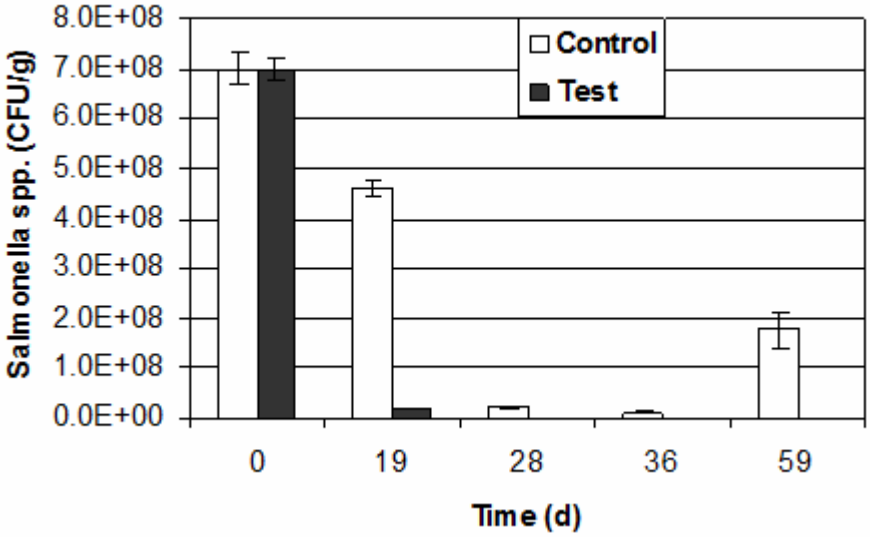


Figure 4.35: ***Salmonella spp.*** **average decrease (shown in logarithmic scale) in the control and test reactors during 58 days of treatment. Vertical T bars represent standard errors**

Table 4.9: Mean number of ***Salmonella spp.*** **in initial substrate and the dynamic change during the experimental period**

	Sample Results (CFU/g)	
Sample Day	**Control**	**Test**
Day 0	7.00E+08 ± 3.14E+07	7.00E+08 ± 2.26E+07
Day 19	4.60E+08 ± 1.29E+07	1.78E+07 ± 5.88E+05
Day 28	2.26E+07 ± 2.14E+06	1.27E+05 ± 1.24E+04
Day 36	1.08E+07 ± 2.32E+06	3.02E+03 ± 2.43E+02
Day 59	1.78E+08 ± 3.64E+07	1.88E+03 ± 1.55E+02

The results are mean ± SE (n = 3)

4.5.5 Shigella spp.

Analysed samples showed a baseline concentration for *Shigella spp.* of 2.28E+08 CFU/g in the control and 2.20E+08 CFU/g in the test reactors (Table 4.9). Thus baseline concentration of Shigella was not significantly different between the two systems. After 19 days, the control reactor samples averaged a 0.57-log reduction, which equates to a 72.8% reduction. Test reactor samples averaged a 1.09-log reduction (91.9%). Both control and test reactor populations exhibit a gradual reduction after day 19 to the end of the experiment, with the rate of decrease slightly higher in the test reactors. On day 59 when the experiments were terminated mean number of Shigella in the test reactors (2.50-log reduction or 99.7 %) was clearly lower than in the controls (1.54-log reduction or 97.11 %) (Table 4.9). Shigella showed a slightly different behaviour from the other pathogen indicators in that it exhibited no tendency to regrow neither in the test nor control reactors. This suggests that if temperature/time profiles are optimized composting could be efficient in eliminating the

pathogens. However, the relatively faster rate of reduction in the test system as implied by Figures (Figs. 4.36 and 4.37) suggests that with earthworms, a shorter time may be required to achieve a complete reduction of Shigella. Figure 4.40a exemplifies the extent of Shigella removal in the controls and test units. The Figure shows a clearly higher eliminnation in the test reactors compared to the controls.

Overall, the slow reduction trends observed in both the controls and test reactors is an indication that Shigella was more resistant than the other indicators studied.

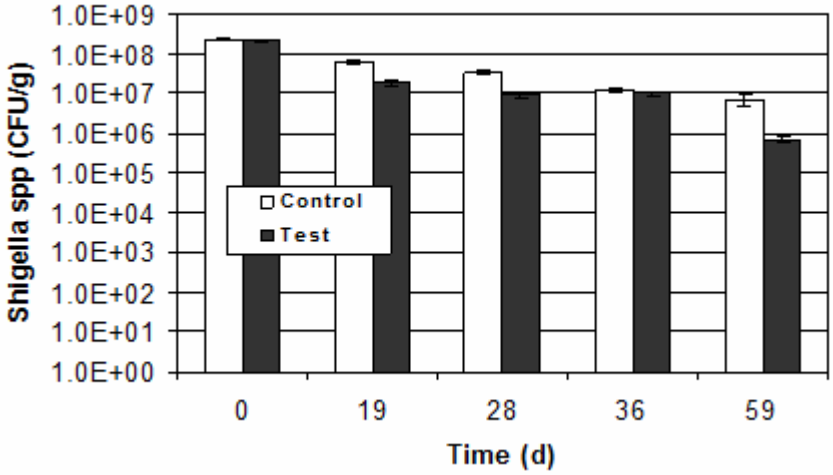


Figure 4.36: ***Shigella spp.*** **average decrease (shown in logarithmic scale) in the control and test reactors during 59 days of treatment. Vertical T bars represent standard errors**

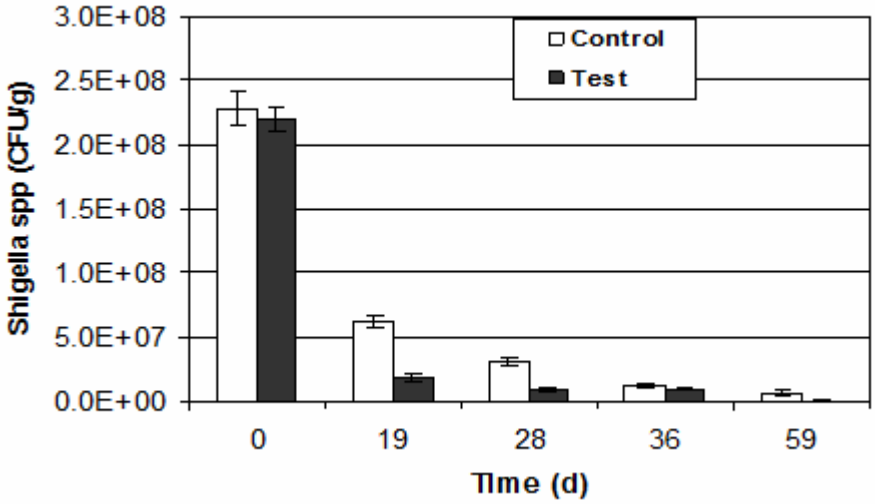


Figure 4.37: ***Shigella spp*****. average decrease (shown in linear scale) in the control and test reactors during 58 days of treatment. Vertical T bars represent standard errors**

Table 4.10: Mean number of *Shigella spp.* in initial substrate and the dynamic change during the experimental period

Sample Day	Sample Results (CFU/g)	
	Control	Test
Day 0	2.28E+08 ± 1.38E+07	2.20E+08 ± 9.13E +06
Day 19	6.20E+07 ± 4.08E+06	1.85E+07 ± 2.43E+06
Day 28	3.14E+07 ± 3.28E+06	8.88E+06 ± 1.39E+06
Day 36	1.22E+07 ± 1.70E+06	1.00E+07 ± 1.47E+06
Day 59	6.60E+06 ± 2.02E+06	7.00E+05 ± 1.29E+05

The results are mean ± SE (n = 3)

4.5.6 Enterobacter

Analysis of samples for *Enterobacter spp.* showed an initial concentration of 9.40E+06 CFU/g (Table 4.10). As shown in the Table, the initial number of *Enterobacter spp.* was the same in the control and test reactors. After 19 days, the control reactor samples averaged a 1.15-log reduction, which equates to a 92.9 % reduction. The vermicomposting reactor samples averaged a 0.83-log reduction (85.1%). The control reactor samples averaged a 1.89-log reduction (98.7%) on day 28, while the test reactor samples averaged 1.64-log reduction (97.7%). After 59 days, when the experiments were terminated, the control reactor samples averaged a 0.36-log reduction (56.8%), while the vermicomposting reactor samples averaged a 3.77-log reduction (99.9%) (Figs. 4.38 and 4.39).

Vermicomposting appeared to have been less efficient in the elimination of enterobacter during the first four weeks of treatment (days 0 to 28) as the rate of population reduction was lower in the test reactors during this period. As with most of the other indicator pathogens enterobacter populations decreased progressively to almost nil toward the end of the experiment, whereas the controls displayed a tendency for regrowth. As explained earlier, this might be due to the fact that the controls entered a mesophilic phase after week 4 rather than maintaining the thermophilic conditions that would have progressively eliminated the organisms.

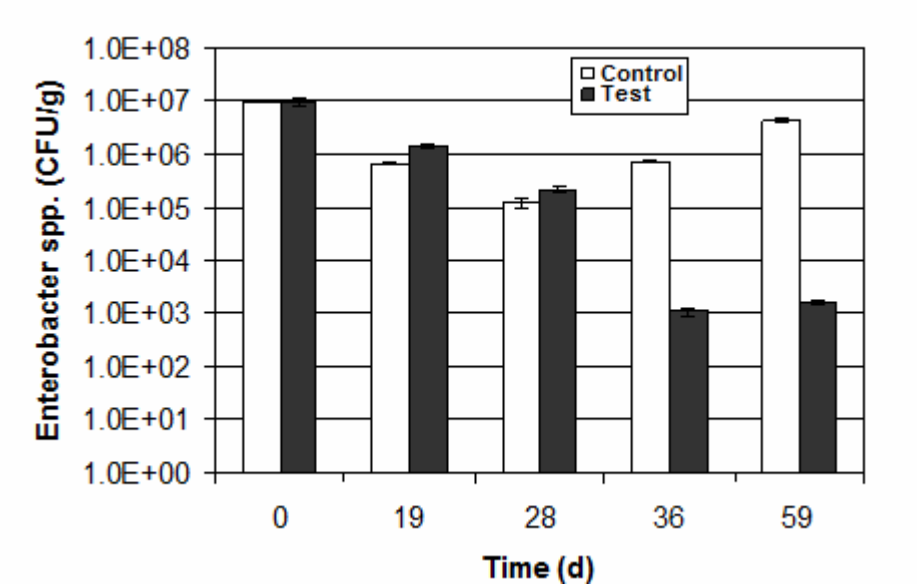


Figure 4.38: ***Enterobacter spp.*** **average decrease (shown in logarithmic scale) in the control and test reactors during 58 days of treatment. Vertical T bars represent standard errors**

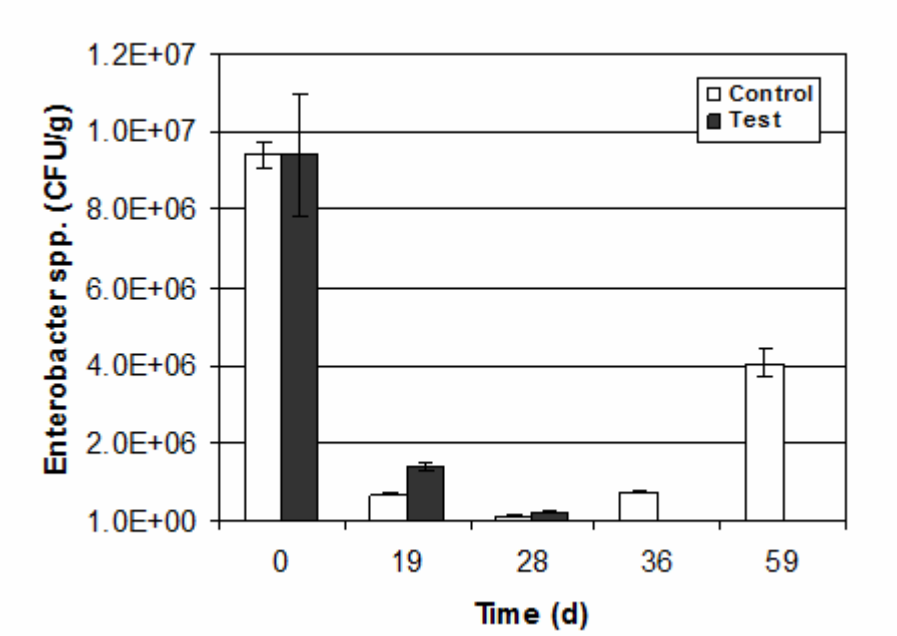


Figure 4.39: ***Enterobacter spp.*** **average decrease (shown in linear scale) in the control and test reactors during 58 days of treatment. Vertical T bars represent standard errors**

Table 4.11: Mean number of *Enterobacter spp.* in the raw substrate and the dynamic change during the experimental period

Sample Day	Sample Results (CFU/*g*)	
	Control	Test
Day 0	9.40E+06 ± 3.16E+05	9.40E+06 ± 1.56E+06
Day 19	6.64E+05 ± 4.27E+04	1.40E+06 ± 9.13E+04
Day 28	1.22E+05 ± 2.56E+04	2.16E+05 ± 2.70E+04
Day 36	7.24E+05 ± 3.43E+04	1.08E+03 ± 1.80E+02
Day 59	4.06E+06 ± 3.28E+05	1.58E+03 ± 1.49E+02

The results are mean ± SE (n = 3)

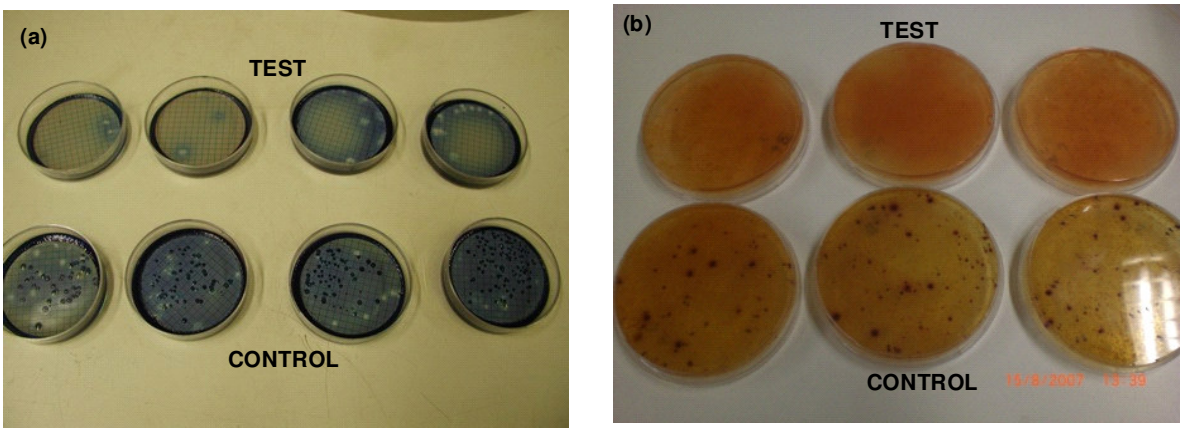


Figure 4.40: Photograph example of SIB obtained on days 36 and 35 for *Shigella spp.* (a) and *Enteroccocus spp.* (b), respectively

4.5.7 Assessment of earthworm influence on SIB populations.

The fate of pathogens during vermicomposting of faecal matter is of much significance in the discussion about reuse-oriented sanitation concepts.

Monitoring of the population sizes revealed that the hygienic effect of stabilizing faecal matter with earthworms and without earthworms both result in reduction of pathogens. The decreasing trend of pathogens during most of the treatment period was in agreement with other works (García *et al.,* 1992; Klamer and Baath, 1998); reporting results obtained with different biomass quantification methods (fumigation-extraction, substrate-induced respiration, ATP content, total phospho-lipid, fatty acid content). The results also corroborate the findings of Eastman *et al*. (2001) who employed quantification methods similar to those of this study to four human-pathogen indicators and observed clearly a higher reduction of pathogens in test rows (earthworm-seeded) within 144 hours compared to controls (without worms).

Although both systems, in the present study, exhibited reductions for all the bacteria analyzed, the reduction was more important when earthworms participated in the composting process: *Escherichia coli* (99.9% vs. 45.3% reduction), Faecal coliforms (99.999% vs. 49.3%

reduction), *Enterococcus faecalis* (99.999% reduction vs. 24.7% increase), *Salmonella spp* (99.999 % vs. 75.6% reduction), *Shigella spp* (99.7% vs. 97.1% reduction), and *Enterobacter spp* (99.9% vs. 56.8% reduction). Similar results were reported by Flack and Hartenstein (1984). In an extensive study on the growth of *E. foetida* on microorganisms and cellulose, the authors demonstrated that this earthworm grows similarly on gram-negative and gram-positive bacteria, and thus concluded that *E. foetida* is able to destroy human pathogens as well as non-pathogenic microbes by decomposing bacteria cell wall with a wide variety of polysaccharides. The results also agree with the conclusions of Bohlen and Edwards (1995) which provide scientific evidence that pathogens do not survive the vermicomposting process.

However, with the exception of Shigella where there was no significant difference between the control and test reactors, the results of this study conflict with the findings of Hand *et al.* (1988), who found no significant difference in bacterial or fungal populations in cow slurry treated with and without earthworms. Also, the results do not corroborate with the findings of Burge *et al.* (1987) and Millner *et al.* (1987), who demonstrated that treatment without earthworms also reduces pathogen concentrations in sludge to very low levels. However, the latter authors did not show whether or not post-colonization occurred. The observations regarding low reduction rates in the controls and reoccurrence of bacteria are supported by the works of various authors who report the ability of several bacteria, notably salmonella, to survive for long time after composting in different organic wastes including raw sludges (Dudley, *et al.*, 1980), sewage sludge composts (Hussong, *et al.*, 1985; Droffner et al., 1995) and commercial compost-based products (Skanavis, *et al*., 1994). Furthermore, Burge *et al.* (1987) and Millner *et al.* (1987) investigating the behaviour of microorganisms during composting concluded that thermophilic composting of solid wastes brings about stabilization of the organic matter and reduces pathogen concentrations to very low levels, however, absolute removal of pathogens is difficult to achieve and many survive the composting process.

Previously obtained data suggest that *E. foetida* requires microorganisms for growth (Neuhauser *et al.*, 1980). Also, as indicated above, most workers suggest that vermicomposting will destroy pathogens faster than thermophilic composting. The data of this study also suggest, but does not prove, that earthworms (*E. foetida*) are capable of eliminating pathogens. The results show the elimination of *Salmonella*, *Enterobacter*, faecal coliforms, *Enteroccocus faecalis* and *E. coli* within 38 days of vermicomposting.

Several international standards for meeting pathogen limits through composting and hence minimise the transfer of pathogens from soil to fresh produce are available (US-EPA, 1993). The US-EPA designates biosolids as Class A (application to lawns, home gardens or golf courses) or Class B (agricultural use or landfills) in regards to pathogen. These classifications indicate the density (numbers/unit mass) of pathogens in biosolids where applicable. Class A biosolids criteria are either a faecal coliform density of less than 1,000 MPN/gTS or a *Salmonella spp*. density of less than 3 MPN/4 gTS. To meet class B criteria, faecal coliform density must be below $2.0*10^6$ MPN/gTS or $2.0*10^6$CFU/gTS. To achieve US-EPA Class A biosolids with composting as treatment method, temperatures must be maintained at >55 °C for a minimum of 3 days. This requirement can hardly be attained uniformly in the substrate during faecal matter composting. In the present study, the requirement for a class B biosolids was attained in the vermicomposting system, but not in the blanks.

These results suggest that vermicomposting really does have an effect on the survival of pathogens, and significantly reduce human pathogens while digesting organic matter. However, the *Shigella spp*. exhibited a tendency to be more resistant. It may thus be assumed that earthworms are selective in the removal of microorganisms. This finding has important implications in the quest to understand how the earthworms – microbial relationship functions.

The findings of this study suggest that vermicomposting may be a feasible option for complete elimination of pathogens in faecal matter as no reoccurrence of sanitation indicator pathogens was observed towards the end of the experiment in the test reactors in contrast to the controls that exhibited a tendency to re-colonise the end-product. Further investigations of the end-product (vermicompost) by variation or prolongation of processing and storage times would generate interesting data and shed more light regarding long-term microbial survival, regrowth and safety of worm-processed human faeces. More in-depth studies are necessary to understand the factors behind the resurge in microbial populations after composting. Important to note is that, no initial data was obtained on the SIB pathogen levels present in the vermicasts substrate applied as bedding at the beginning of the experiment.

5 Proposed Continuous Flow Vermicomposting Toilet for Stand-alone Household Iimplementation and Integration with Terra Preta Sanitation

5.1 Introduction

The aim of this chapter is to propose ways of field-testing the continuous-flow vermicomposting system by incorporating the technology into on-going pilot sanitation programmes. The purpose will be to demonstrate the novel excreta management approach and to show that the technology can contribute to public health protection and food security. For this, Burkina Faso, a developing country located in West Africa has been chosen.

Until recently, implementation of low cost sanitation systems based on ecological sanitation (EcoSan) principles in developing countries has been hampered by various factors including inadequate education, training and participation in decision-making. However, during the last few years a lot of research has been carried out dealing with the aspects of technology selection, participatory planning, capacity building and sustainability of implemented solutions. Evidence in the literature indicates that on-site sanitation systems (OSS) – predominantly, urine diverting dry toilets (UDDTs) are beginning to gain greater penetration as main sanitation options in developing countries, especially sub-Saharan Africa (SSA).

One of such regions in SSA is Burkina Faso, where pilot OSS systems based on the sustainable sanitation concept are currently being implemented in several cities by the organisation CREPA (West African Centre for Lowcost Water Supply and Sanitation).

Between 2006 and 2009 CREPA, in cooperation with the project NETSSAF (Network for the development of sustainable approaches for large scale implementation of sanitation systems in Africa), identified eleven regions from six NETSSAF-targeted countries as showing typical characteristics relevant for designing and planning of sustainable OSS systems. One of such typical settlements was Loumbila in Burkina Faso, and has been chosen as the target demonstration area of the continuous-flow technology.

5.2 Background information on Loumbila

Loumbila is located in the centre of Burkina Faso and is accessible at 20 km from the capital city, Ouagadougou. It is a semi-urban settlement with about 26,500 inhabitants. The settlement is famous for the dam called Loumbila built for the Ouagadougou drinking water supply. Around the dam, horticulture for marketing is practiced during the dry season by many farming associations. Reducing levels of soil fertility are compelling more poor rural people, who traditionally rely on farming for a living, to move into the town looking for employment. As a result, unplanned slum and squatter settlements without services, notably water and sanitation are growing.

It is worth mentioning that Burkina Faso lies within the expanding Sahel where a trend of diminishing rainfall amounts have meant longer, more intense droughts and flooding during large rain events, and that these climatic patterns have an important implication on the implementation of sanitation facilities. Attention to develop the sanitation sub-sector had been largely omitted from Burkina Faso's poverty reduction strategy. Just over half of the population has access to clean water, while less than 15% have access to sanitation.

5.3 Current sanitation practice in Loumbila

Less than one quarter (19%) of households have basic sanitation (such as septic tanks, VIP latrines, pour flush toilets or double vault latrines), about two thirds (61 %) have a simple, shallow pit toilet and the rest (20%) have no toilet. The city has no regular pit desludging services, resulting in a wide range of sanitation problems. Two types of UDDTs were implemented in the area, single vault toilets and double vault toilets. Studies show that single vault UDDTs present significant management challenges. Also, the double vaults which were built out of adobe bricks are not a reliable technical option due to problems during the rainy period.

There are sections of Loumbila town that are congested settlements where land is not available for digging pits and households either share a single latrine for common use or go out for open defecation.

Sanitation is a relatively recent activity of the national water utility, ONEA (National office of water and sanitation). The main focus has been on promotion of autonomous household installations. ONEA subsidise private installations with materials corresponding at 25-30 % of installation costs. Only improved latrines (type VIP or better) are recognised as appropriate installations eligible for subsidies. Average costs are around 100 euro.

Loumbila is selected as 'typical' area for demonstration of the new technology because it is at the interface of urban and rural (district centre, for example, but essentially a semi-urbanized community in a predominantly rural/agricultural area), it has its own values, beliefs and attitudes, therefore vermicomposting technology will provide more understanding of ecological sanitation implementation in this developing region.

5.4 Proposed continuous-flow vermicomposting toilet system for household application

Three scenarios are proposed for designing, costing and small-scale demonstration of the vermicomposting technology in Loumbila. Small-scale piloting of the technology is necessary as studies have shown that there is often initially negative attitude of communities to ecological sanitation systems. Therefore, as for any EcoSan system, an essential step for introduction of the continuous-flow system will be a proper orientation of users by providing good and honest information about the EcoSan philosophy, technical and health issues as well as safe re-use. The three scenarios are as described below.

One scenario will be the low income settlements which have neither septic tanks nor pour flush toilets but are currently using unsatisfactory OSS systems. The aim here would be to determine, through pilot demonstrations in the settlement, whether the safest and least costly solution is the vermicomposting technology or whether the existing OSS system can be up-graded to UDDTs with vermicomposting.

A second scenario will be the areas where households have been served with UDDTs; to retro-fit fifty percent of the facilities to continuous-flow vermicomposting systems. The

purpose here would be to determine whether the vermicomposting technology is more suitable, that is, if it is safer, takes less space and easier to manage than conventional UDDTs.

A third scenario is an area of the city where no basic sanitation facilities exist (open defecation). This scenario proposes to integrate the continuous-flow technology with the emerging Terra Preta Sanitation (TPS) approach. The coupling of lactofermentation and vermicomposting in the TPS approach has been described by Factura *et al.* (2010). Here the purpose will be to determine the feasibility of the TPS toilets and whether it is possible and economically more appropriate to combine the continuous-flow concept and the TPS approach in a wholly novel way.

For each scenario, it is proposed to build five pilot continuous-flow vermicomposting toilet systems, operated under the supervision of CREPA for at least six months.

5.5 Description of the proposed system

Continuous-flow vermicomposting toilet system is designed for households with 5 to 7 members. The toilet is built such that urine is collected and diverted from the front area of the toilet, while faeces fall through a hole into a substructure (a vault – reactor chamber). The toilet can be designed to suit both sitting and squatting cultures and to cope with the use of water for wet-anal cleaning culture. Specially designed urine diversion squatting pans are needed for squatting cultures, and urine diversion seat risers for sitting cultures. These elements can be produced from plastics, porcelain or concrete. For squatters, prefabricated elements with holes for separate outlets of urine and faeces would be more suitable. A third outlet would be necessary for anal cleansing water. The water would be diverted into a small constructed wetland where the water, together with the grey water, is applied to a soil-sand filter planted with crops or grass (Figure 6.1). The constructed wetland will be designed to allow clusters of ten or less adjoining homes to share one treatment unit whose cost and maintenance are also shared. For this kind of wetland, roughly 2 m^2 per capita will be required for BOD removal. The dimensions of the wetland will be designed to be 32 x 6m, and a depth of 0.6m. The volume of the reactor will depend on the number of users. For a household size of 5 to 7, a reactor approximately 80 L with a 1cm screened/mesh bottom and a 25 L plastic container for urine collection is recommended. For odour and insect pest control, a ventilation system is installed. The ventilation consists basically of a pipe that leads from the collection chamber to the outside. The wind can then draw moist air and odours from the chamber through the pipe. The pipe outlet would be sealed with a mesh to trap flies. The ventilation effect can be using a T-shaped attachment at the top of the pipe, or by wind-propelled or electric fan.

As rapid breakdown of faecal matter is required in the reactor, toilet paper or similar organic material are to be handled separately. The system can be self-built by the users totally or partially using commercially available squatting pans or toilet seats. The toilet will have to be built entirely above ground to allow easy access to the reactor chamber for monitoring. The mesh bottom should be elevated around 20 cm above ground level to facilitate collection of out-flow (processed) material.

An important condition for the success of the continuous flow vermicomposting toilet is that sufficient user commitment to the operation and maintenance can be provided. Regular maintenance includes, checking for urine pipe blockages, controlling the moisture levels by reducing or increasing the ventilation. The required working time will not exceed a few hours per week. Neglected maintenance can quickly lead to malfunctioning of the toilet and may severely impair the condition (appearance) and hygiene of the toilet. Therefore, although construction, as well as operation and maintenance of the system are easy to learn, sound training is required.

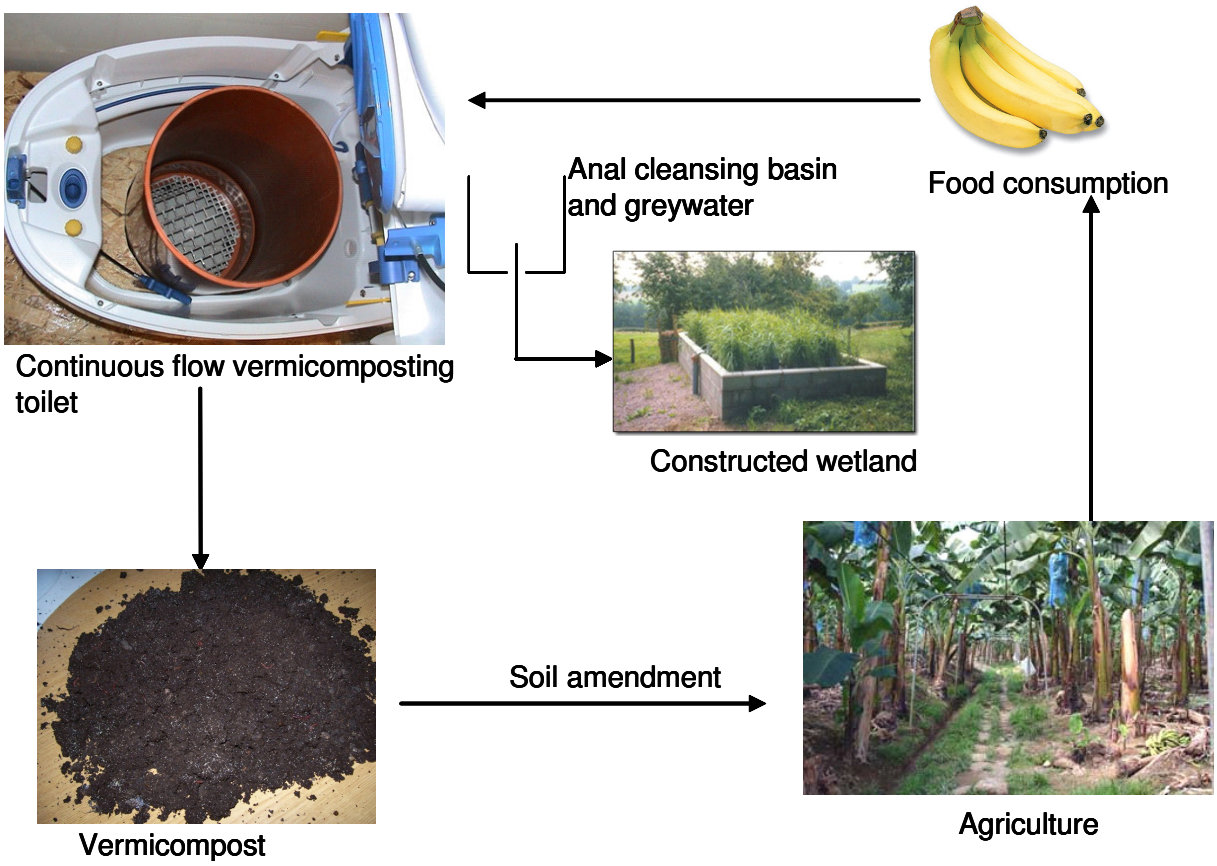


Figure 5.1: Loumbila proposed excreta management concept

5.6 Costs estimate and analysis of the proposed system as applicable to the different scenarios

For scenarios one and three, a complete free-standing unit will be built. Construction can be done with locally available materials and labour. The vermicomposting toilet will have similar construction cost as a double vault UDDT toilet or a VIP latrine (Table 6.1) but it is expected to take up less space and management requirements than existing systems. It is assumed that the municipality will pay for the substructure (collection, treatment chamber and pipes), and users pay for the superstructure. The cost estimates provided in Table 6.1 are mainly based on data gathered and presented by GTZ GmbH in its EcoSan sector project (GTZ, 2006).

Table 5.1: Components and costs of the proposed sanitation system

Component	*Costs in Franc CFA*	Costs in EUR
Chamber (reactor)	6,500	10.00
Toilet seat with UD, polished concrete (GTZ, 2005)	9750	15.00
Urine chamber	1,500	2.00
Pipes	2000	3.00
Foundation	32,500	50.00
Steps	13,000	20.00
Walls	52,000	80.00
Roofing	26,000	40.00
General fitting	13,000	20.00
Labour	65,000	100.00
Total	**221,250**	**340.00**

Each household is required to make a contribution in kind, such as locally available construction materials and unskilled labour, to the construction of the toilet. The municipality is expected to provide subsidy of approx. EUR 272 per household (equals 80% of the total construction cost). It is important to note that the above table provides rough estimates only, as the superstructure can be built from any material, depending on the users' preference and local availability. Thus, the real investment costs for a vermicomposting toilet will vary depending on location and materials used.

Scenario two involves adaptation of existing UDDTs to continuous flow systems, thus the costs will be comparatively lower. If the toilet is to be installed in-house, the investment costs for a functioning and comfortable unit would be approx. 20,000 CFA (EUR 30) if local materials and labour are used.

6 Conclusions and Recommendations

6.1 Conclusions

The bench-scale vermicomposting trial examined the feasibility of treating human faeces in a flow-through sanitation system by investigating two important design criteria and controlling two main environmental variables. Important indicators of system performance were monitored, and characteristics of the final product were analysed due to the increasing interest to recycle materials in human excreta in agriculture. The main conclusions are outlined in the following paragraphs.

Application of the "continuous-flow" vermicomposting technology for the treatment of faecal matter in dry sanitation systems is feasible. However, the technique may initially present a significant challenge as faecal matter alone – without toilet paper – tends to have an incorrect carbon-to-nitrogen ratio and moisture content for earthworm growth.

Human faeces is toxic to earthworms. Unless worms are acclimatized or the physical characteristics of the material are modified, faecal matter; even when pre-treated, cannot be effectively processed by vermicomposting. This problem is linked to the high nutrient content of faecal matter, the production of ammonia during decomposition and the tendency for anaerobic conditions.

Consequently, direct application of worms to vermicomposting systems with raw faecal matter as substrate should be avoided. For continuous-flow vermicomposting, a critical step is to adapt the organisms.

The moisture content and temperature of the substrate are the two main environmental factors influencing the vermicomposting process. For smooth functioning of the process and to produce a stable product in a relatively short period of time, the temperature should lie in the range between 20 and 25° C (Shalabi, 2006).

In the present study, 70% moisture content was found to be most favourable for continuous vermicomposting of faecal matter resulting in 79.6% organic carbon (OC) reduction within a 96 day treatment period. The 65 and 75% moisture contents were found to be adequate with respectively 73.7 and 53.3% OC reduction.

However, there is no absolute moisture content which is ideal for vermicomposting of faecal matter. The optimal moisture content will depend on the material's water holding capacity, which diminishes during decomposition due to loss of organic content, and thus the ideal level of moisture likewise diminishes. A high organic matter substrate will have a high water holding capacity and a high ideal moisture content. Thus, the most suitable moisture content will depend on the processing goal and the handling technology.

Monitoring of water content is crucial for the success of the vermicomposting process. In the present study, low VS reduction (3.5% and 2.9% respectively) were recorded at 60 and 80% moisture levels, compared to 29.7, 34.7 and 26.7% VS reduction measured respectively at 65, 70 75% moisture, indicating that low and high substrate moisture were unfavourable for earthworm activity. In a pre-trial study to establish the minimum moisture requirement of *E.*

foetia, it was found that at moisture levels of 40%, 45%, 50%, and 85% all worms had died by day 3.

Feedstock application rate and initial earthworm inoculation density influenced the vermicomposting process. The vermicasts yield consistently decreased with increasing feed application rates. Earthworms tend to stabilize the substrate slowly at higher stocking densities. Even when the relevant environmental conditions of moisture and temperature are optimal, problems can arise due to overcrowding.

A stocking density of 2.20-kg worms/m^2 and a feeding rate of 1.2 kg-feed/kg-worm/day were found to optimize performance in the present study. It may have been possible to achieve higher loading rates had the reactors been stocked with the maximum density of worm biomass per square meter of loading surface area, and/or if worms were allowed a longer period to acclimatize to the substrate.

An evaluation of various chemical parameters (electrical conductivity, volatile solids, and contents of organic carbon, ammonium, nitrate, and phosphorus) indicated that faecal matter was not fully stabilized after 13 weeks vermicomposting.

Levels of electrical conductivity (soluble salts) increased during the decomposition of faecal matter. The progressive increase in salt contents was generally greater in the presence of worms than without worms. Electrical conductivities (EC) observed in the eluates ranged from 3.2 to 5.4 mS/cm. Salt tolerance limits of the most tolerant plants is approximately 3 mS/cm. Therefore, from a salinity consideration, vermicompost from faecal matter may not be suitable for agricultural application, unless diluted with soil material.

The volatile solids reduction (34.7%) obtained after 13 weeks solids retention time in the continuous vermi-composter falls short of the target (38%) set by the US-EPA as threshold for stability and vector attraction reduction. This suggests that the vermicompost obtained in this study was not stable and required extended curing time.

Ammonium-nitrogen decreased and nitrate-nitrogen contents increased progressively during continuous vermicomposting. This confirms that earthworms increase the transformation of organic nitrogenous compounds to plant-available forms.

Another parameter that is commonly used for assessing compost stability is the carbon-to-nitrogen (C:N) ratio. In this study the C:N ratio was not used as an index of stability. Much of the scientific literature has advised against the use of C:N ratio as an absolute indicator of maturity because during carbohydrate decomposition the release of carbon and nitrogen fluctuates highly, making the C:N ratio an unreliable parameter to measure the degradation process. Consequently, values of C:N ratio need to be weighed against observed decomposition traits before making conclusions about product stability based on C:N figures.

In addition, the evolution of the total content of the heavy metals; zinc, copper, cadmium, nickel and lead during the vermicomposting process was studied, because these are problematic in biosolids. As a consequence of the carbon losses by mineralization, increases in the total amounts of the heavy metals during the vermicomposting process was expected.

However, the amounts of heavy metals consistently decreased. This study found a decrease of between 45 % and 90 % of heavy metals in thirty-six weeks.

The vermicomposts obtained in this study can be classified as suitable for soil amendment because the heavy metal concentrations were lower than the maximum permitted limits in biosolids composts for their final disposal, according to US-EPA (US-EPA, 1999) and the European Community (EC, 2000) standards limits. However, it needs to be pointed out that the concentrations of heavy metals analysed were low in the raw substrate, and complied with the US-EPA and European Community's limits.

Earthworms have an effect on the populations of bacteria during stabilisation of faecal matter. Bacterial pathogens appear to be eliminated as they enter the earthworm's food chain.

In the present study, Sanitation Indicator Bacteria (SIB) were detected at levels that would grade the vermicompost as hygienisation Class B, according to the US-EPA guidelines for use and disposal of biosolids. Therefore, there is a scientific basis to support consideration of vermicomposted human faeces for soil amendment, from a health-safety stand-point.

All the vermicomposts produced in this study would be classified as "restricted use"–meaning that the compost can only be used in forestry, agriculture, and soil and site rehabilitation, but not on home lawns and gardens, public contact sites and for urban landscaping, as required by US-EPA standards.

Overall, the results of this study suggest that the continuous-flow vermicomposting approach is a promising low-cost alternative for on-site application. As dry sanitation systems based on urine separation are spreading rapidly in the developing world, the flow-through approach could provide a simple, yet reliable solution for solving the problems of collection, treatment and transportation of faecal matter especially in densely populated areas.

Whilst this study has demonstrated the potential of the continuous-flow approach, the findings are limiting to performance under controlled bench-scale units. The results illustrate the importance of a well managed process. Thus, field application will depend on diligent management of the system. Constant monitoring of water content, reactor temperature, worm behaviour and oxygen levels is required to ensure smooth functioning of the system.

6.2 Recommendations for further research

Several directions of future research can be followed to better understand the continuous-flow vermicomposting process, as discussed below.

6.2.1 Reactor design and geometry

Research needs to be conducted to establish a suitable surface-to-volume ratio of the reactor. This ratio is important as it has an effect on heat retention and air exchange, both of which strongly affect worm survival and activity.

Vermicomposting process takes place within the temperature range of mesophilic composting, and as previously stated, temperature is one of the important factors affecting the process.

Heat released during microbial decomposition of organic matter is the primary source of temperature increase within the reactor. The larger the surface area, the higher is the surface-to-volume ratio, which compromises the heating up of the system during microbial decomposition. Although this might be an advantage in hot climates as it allows heat to dissipate, it is a disadvantage in cold climates, as external heat supply may be needed. Therefore, for each climatic situation, an optimum surface-to-volume ratio needs to be established.

Secondly, as *E. foetida* are surface-dwelling organisms, with their feeding, reproduction and resting activities mostly confined to the topmost 10 cm of the vermicomposting system where new feed is present, it is possible the reactor geometry may be influencing the loss of efficiency per worm. A high cross-sectional area of the reactor might improve worm access to the substrate, thereby enhancing growth and vermicast production per worm, especially in crowded reactors.

6.2.2 C:N ratio

C: N ratio has been reported to be the most important factor determining substrate palatability in epigeic (surface-dwelling) earthworm species. Previous studies have suggested efficient vermicomposting C: N ratio of 20-25:1 parts carbon to every one part of nitrogen, on a dry matter basis. C:N ratio measured in the raw substrate of the present study was consistently lower than the recommended range. The current study did not attempt to raise the C:N ratio of the substrate, neither did it investigate the effect of the narrow C:N ratios on earthworm performance. There, further study should be carried out with various C:N values to determine the optimum carbon-to-nitrogen range of *E. foetida* for the substrate, faecal matter.

6.2.3 Microbiological aspects

A major issue in the recycling of materials in faecal matter is the fate of pathogens during treatment. Protozoa, in particular, have been reported to be resistant to various treatment procedures, notably to chemicals in wastewater treatment plants, and to be able to survive for long periods in the soil. In the current study, no measurements were made on the fate of protozoa during vermicomposting. Research is needed to evaluate the survivability of these pathogens, and to gain a deeper understanding of the range of bacteria pathogens that can be eliminated during vermicomposting, as well as the elimination mechanisms.

6.2.4 Integration with Terra preta sanitation

A new direction in the management of human faeces is the Terra preta sanitation (TPS) approach. TPS holds a promising solution to the avoidance behaviour exhibited by earthworms in faecal matter and as the intermediate ‘lacto-fermentation step’ along with the use of additives may render faecal matter amenable for earthworms processing. TPS additives have also been reported (Factura *et al.*, 2010) to solve the odour problems, thus eliminating the need for ventilation. It is recommended to investigate the possibility of integrating the TPS approach and the continuous-flow technique, after the ‘lacto-fermentation step’. The integrated system will provide the benefits of labour and space requirement savings.

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Appendix: The Prototype Single Chamber Continuous Verimcomposting Toilet with Urine Diversion

Prototype (The Separett Villa, originally designed by Ecovita)

Collection and treatment chamber

Ventillation system

Processed material collection

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EDUCATION

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Although the potential benefits of human excreta treatment by vermicomposting for rapid stabilisation and for recovery of useful materials have already been demonstrated, the aspects of the technology design, operation and engineering have not been investigated in detail.

This study focused on designing and optimizing the performance of a continuous flow vermicomposting process that enables an accelerated rate of volatile solids reduction in dry toilets thereby increasing the stabilization rate. The continuous flow process can be integrated in urine diverting dry toilet systems provided two environmental parameters can be managed: temperature and moisture levels. A new sanitation technology for management of human organic waste based on flow-through vermicomposting has been developed.

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