CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical Agrisciences

Influence of vermicomposting on the content of pathogenic microorganisms in sewage sludge

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Declaration of Authorship

I, Bc. Šárka Procházková, declare that this thesis by the title Influence of vermicomposting on the content of pathogenic microorganisms in sewage sludge, submitted in partial fulfillment of the requirements for the degree of M.Sc. in the Faculty of Tropical Agrisciences of the Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced or acknowledged.

April 26th, 2013

Bc. Šárka Procházková

Abstrakt

Biologické procesy rozkladu organické hmoty reprezentují složitou kooperaci procesů mezi vyššími a mikro-organismy v podmínkách vyhovujících jejich bytí. Kompostování představuje nenahraditelnou součást koloběhu živin v přírodě a vytváří stabilní produkt vhodný pro vylepšení stavu půdy jakož i pro bezpečnou likvidaci. Vermikompostování využívá specifických druhů žížal, které posílí proces rozkladu materiálu a tudíž i biologickou stabilizaci. Různé experimenty dokázaly, že vermikompostování může stabilizovat nebezpečné odpady; mnoho z nich je zaměřeno na kaly. Autoři vidí biologickou nestabilitu kalů jako potencionální problém, zvláště v případě vysokého obsahu patogenů. Byly založeny čtyři vermikompostéry, dva jako kontroly ($V_{C15\%}$ a $V_{C20\%}$) obsahující 15 % a 20 % kalu, zbývající jako experimenty ($V_{15\%}$ a $V_{20\%}$) se stejným obsahem kalu a sto padesáti žížalami po dobu devadesáti dnů. Umístěny uvnitř ve stabilní pokojové teplotě, vermikomposty byly udržovány na 40 – 60 % vlhkosti a testovány na přítomnost oxidu uhličitého a amoniaku pro stanovení zralosti. Podíl kalu byl zkoumán každých 14 dní na přítomnost Koliformních bakterií a *Escherichia coli*. Výsledky ukazují, že vermikompostování nemělo značný vliv na pokles těchto organismů v testované směsi, avšak podpořilo zrání, jelikož vermikompost $V_{15\%}$ dosáhl plné zralosti ke konci experimentu.

Klíčová slova: vermikompostování, biologická stabilizace, kal, *Escherichia coli*, Koliformní bakterie

Abstract

Biological processes of organic matter decomposition represent a complex cooperation of processes between higher and micro-organisms in conditions favoring their existence. Composting represents an irreplaceable part of nutrient cycle in nature and is able to produce a stable product fit for soil conditioning purposes as well as a substance safe for disposal. Vermicomposting utilizes special types of earthworms which enhance the process of decomposition and biological stabilization. Dangerous wastes have proven to be stabilized through vermicomposting through various experiments, most of them involving sludges. Authors see biological instability of sludges as potentially dangerous especially in terms of high pathogen content. Four vermicomposts were set up, 2 of them being controls ($V_{C15\%}$ and $V_{C20\%}$), containing 15% and 20% of sludge respectively, other 2 representing experiments ($V_{15\%}$ and $V_{20\%}$) with same content of sludge and 150 earthworms each, for 90 days. Held indoors in stable room temperature, the vermicompost mixture was kept at 40 – 60% moisture and tested for presence of carbon dioxide and ammonia to determine level o compost maturity. Sludge portion was examined every 14 days for presence of Coliform bacteria and *Escherichia coli*. Results show that vermicomposting did not have a substantial influence on the decrease of these microorganisms in the tested mixture; however it supported the maturation, as vermicompost $V_{15\%}$ reached full maturity by end of experiment.

Keywords: vermicomposting, biological stabilization, sludge, *Escherichia coli*, Coliform bacteria

Preface

Composting is a set of physical and chemical processes, which transform waste of organic origin into a mature and biologically stable product termed compost. Organic waste after this transformation process is harmless to the environment. In fact, when released back to nature, products of composting quickly become its part. Moreover, compost has been proven to have positive effect on the soil conditions, stabilizing the soil-water regime, suppressing undesired weeds, supplying essential elements for plant growth in an accessible form for the roots' uptake. The agronomic potential of composting has already been recognized by our ancestors and has become an important part of organic waste management, especially in agricultural sector.

Vermicomposting is based on the composting methods, except for a small difference: vermicomposting is the decomposition of organic matter with the use of earthworm species. Earthworms are an indispensable part of nature's organic matter cycle. As they eat through the biomass, they modify it physically, increasing the surface area on which soil microbes can flourish. Moreover, the earthworms' castings contain essential microorganisms which induce the chemical decomposition process of the biomass.

It is undoubtedly true that waste of anthropogenic origin is generated in vast amounts on daily bases and consists of complex and hazardous matter. This matter must undergo managed treatment before being released back into the environment. Same concept is applied to wastewater or sewage treatments, where biologically unstable sludge high in pathogenic bacteria is generated. Sludge cannot be discharged into an open environment without being properly treated, stabilized and cleared from pathogenic bacteria.

Sustainability in terms of agricultural and rural development is the key to ecological coexistence of humans and nature without overly ballasting the environment with energy-intensive processes and dangerous waste. Why use artificial chemical processes, when same results can be obtained using processes which naturally occur in the environment?

Presented Thesis is focused on composting (vermicomposting) as a sustainable alternative for organic waste management, particularly the untreated sewage sludge. The effect of composting and vermicomposting on biomass was assessed theoretically by literature review (in the first chapter of this Thesis, Introduction) and experimentally by a set of trials with vermicomposters. Literature review was produced in order to obtain as many scientific secondary data as possible about characteristics and aspects of composting and vermicomposting. Gaining knowledge by this assessment, own vermicomposting experiments

were set up to fulfill the aim: to measure the interim results of vermicomposting sewage sludge and perceiving the effect of this process on the biomass maturity and pathogen content. Can vermicomposting generate a mature, pathogen-free vermicompost and thus demonstrate its sustainability and feasibility as a component of waste treatment management process? May this Thesis bring denouement to this question.

Content

1. Introduction

1.1. Basic Composting Characteristics

Composting is an aerobic decomposition process where raw material is transformed into a stable humic end-product under controlled conditions by the help of soil microorganisms, bacteria and higher organisms such as fungi or earthworms (Cooperband, 2000; Suthar, 2008; Singh et al., 2011). Raw material, in this case, stands for bio-degradable matter. In nature, this matter deteriorates and decays away continuously on ground levels or in the soil – it is a naturally occurring process where organic material is reprocessed. Composting works on the same principal, only under controlled circumstances and in an enclosed space.

This transformation of the bio-degradable matter is carried out by the 'decomposers': facultative and obligate aerobic bacteria, yeasts, fungi and larger organisms, such as springtails, ants, nematodes and oligochaete worms. These decomposers play a crucial role in the efficiency and speed of compost formation and without them, degradation of the organic matter could not take place. Similarly, controlled conditions of the composting process play an equally important role in generating a mature end-product.

The final product of the composting process is compost – a substance which has undergone restricted thermophilic and aerobic biological breakdown and is both mature, non-phytotoxic and stable, being impervious to any further breakdown (Suthar, 2008; Singh et al., 2011; Wichuk and McCartney, 2011). Raviv (2005) mentions two basic agricultural uses of the compost: soil amendment and an ingredient in container media. Correspondingly, Singh et al. (2011) states, that compost can be used in landscaping, agriculture or horticultural works.

There are many advantages results from compost application. For example, compost has the benefits of enhancing the soil by providing organic material, fighting plant diseases, decreasing fertilizer needs and improving water retention. Of course, the kind and characteristics of the generated compost are dependent upon the amalgamation of input materials and the composting method used (Raviv, 2005). Similarly, characteristics of the compost, such as the content of plant nutrients (N, P, K, Mg, Ca) and other less-occurring elements (Pb, As, Hg) specify the compost utilization (Barker, 1997).

Utilization of composting is as old as agriculture itself and can therefore be perceived as an ancient technology (Cooperband, 2000). References for composting techniques can be found in biblical and old Roman texts as well as in records originating from successive centuries (Rynk, 1992). In those cases, compost was used principally as soil amendment for replenishing soils' organic matter so that stably high yields were guaranteed. In terms of science, composting

was first investigated by Sir Albert Howard in India, who observed composting of organic materials piled up in a heap in his so-called 'Indore method' (Howard, 1943). These principles form the basis for present-day composting techniques.

The abundant use of compost as a fertilizer was interrupted by the World War II and subsequent mechanization of agricultural practices. Typical manure and compost gave way to synthetic fertilizers and composting practices declined (Cooperband, 2000). Their comeback was initiated by ingenuities conducted at the end of 20th century, which revealed new utilizations of composting technique in terms of waste management. Composting is now perceived as a sustainable and undemanding alternative for management of organic waste (Suthar, 2008).

1.1.1. Biology of Composting Process

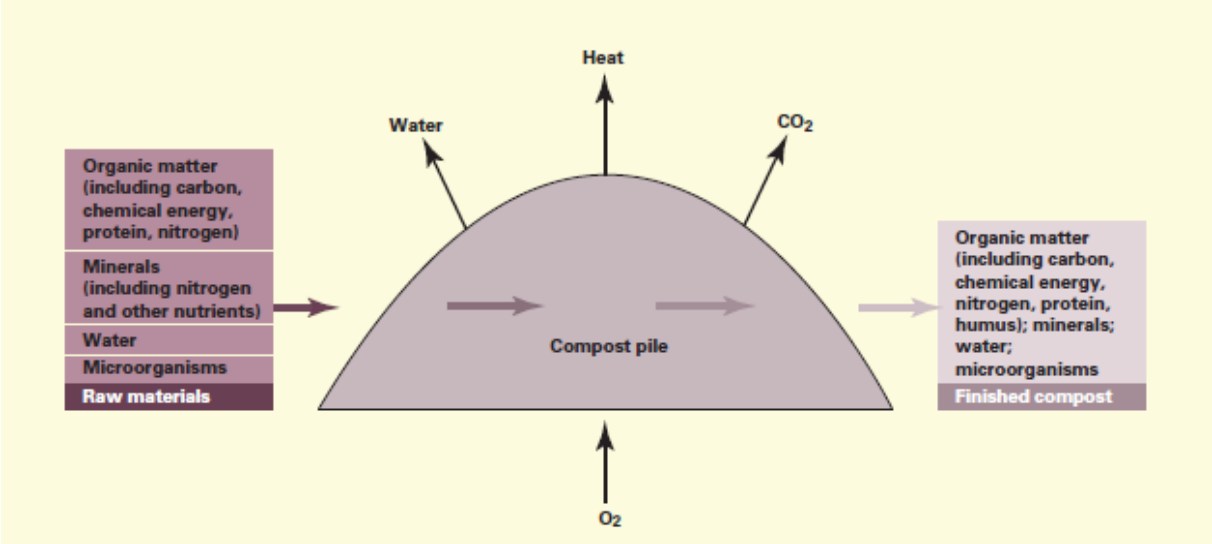


Figure 1.1: Scheme of composting system. Source: Rynk (1992)

Figure 1.1 shows a typical composting pile. Raw materials of organic origin contain elements such as carbon and nitrogen, compounds such as protein, minerals, water and energy stored in chemical bonds of these substances. Mesophilic microorganisms, which flourish at temperatures of 10 (25) to 45 °C, are the first to occupy the composting heap (Cekmecelioglu et al., 2005; Cooperband, 2000) and initiate the first composting stage, the mesophilic stage.

Conditions at start of composting must be compatible with the living conditions of microorganisms involved in the composting process. Commonsensically, it is important that these microorganisms increase their numbers quickly in order to engage in the decomposition process efficiently as possible. Mesophilic organisms (including protozoa, bacteria and fungi) initiate the decomposition (and composting) process by simply obtaining energy to support their

existence and thus performing aerobic oxidation of the organic matter – raw material. By-product of this oxidation is generation of carbon dioxide and heat. Notice the oxygen in figure 1.1 and the arrow indicating its uptake by the pile and similarly, carbon dioxide and heat going out of the pile.

Oxidation eventually results in the build-up of temperature, which can reach 45 to (60) 75 °C within 24 to 72 hours (Cekmecelioglu et al, 2005; Cooperband, 2000). However, Jenkins (1999) mentions that temperatures as high as 70 °C are neither usual nor required in backyard compost. The pile enters the 'active phase' of composting, the thermophilic phase, and retains this temperature for days to weeks, depending on the time of the year, pile shape and size, the C:N ratio and breakdown speed of carbon (Hong et al., 1982). Mesophilic organisms no longer occupy the pile and instead are replaced by thermophilic organisms, which can withstand and thrive in temperature ranges of 45 to 75 °C. They continue in organic matter decomposition, breaking down simpler compounds with intensive demand for oxygen, while the increased temperature has a cleansing effect, killing human and plant pathogens, nematodes, weeds and disease vectors (Cekmecelioglu et al., 2005; Alexander et al., 2002). The increased demand for oxygen must be satisfied by aerating the heap, usually by turning or through active aeration.

Simplest way of determining the onset of active phase is by observing steam coming up from the hot pile. Evaporation is very high during this phase and thus water must be adequately supplied. Insufficient water content would not only slow the decomposition process and cause deterioration of thermophilic organisms, but also increases risk of self-ignition of the pile. Evaporation of water accompanied by decomposition of organic matter result in reduction of mass of the pile by 50 % of the original mass (Singh et al., 2011).

Active phase is followed by the cooling phase, where temperature decreases to some 38 °C and is maintained as the heap enters the curing phase, the last stage of the composting process. Mesophilic organisms are able to re-occupy the heap as before the active composting phase. They are able to break down organic materials of more impervious character (Jenkins, 1999). Decrease in temperature also reduces the demand for oxygen and therefore, turning or forced aeration of the composting pile becomes unnecessary. Alburquerque et al., (2006) states that forced aeration should be stopped for the curing phase duration. In curing phase, organic material is transformed into biologically stable humic substances; generation of these substances foreshadows the forthcoming maturity of the whole compost. Curing period is of various lengths with no given time span; commercial compost can cure for 1 to 4 months, while home-made compost for 6 to 9 months (Cooperband, 2000). Alexander et al., (2002) state curing period duration having 30 to 90 days. Curing period is essential in order for quality and mature compost

to form – equally, conditions during curing must be severely controlled to ensure efficient and compete maturity.

After curing period, finished compost is generated, containing somewhat less carbon, protein, water and chemical energy than in raw material (Rynk, 1992). Mature compost is ready for utilization or in case of commercial composting, for packing and transport.

1.1.2. Elementary Composting Parameters

As described in previous chapters, composting process takes place under specific and controlled conditions. Overall, the composting pile goes through four distinct composting stages: mesophilic phase, thermophilic phase, cooling phase and curing or maturation phase (Purnomo et al., 2010). It is the curing stage which is responsible for the formation of humus-like structures making up the compost.

Each of these conditions is essential for the composting biomass to be odorless and uninviting for flies and other animals, while at the same time generating applicable, stable soil conditioner for landscaping, agricultural and horticultural works (Singh et al., 2011). According to Cooperband (2000), there are three important aspects for creating adequate compost:

- Chemical composition of input material – the quantity and quality of minerals and carbon as well as pH
- Porosity of the compost pile – given by size and shape of input material
- Organisms included in the composting process – from micro to macroorganisms

Putting the above three aspects simply, the most important conditions are: temperature, moisture content, ratio between carbon and nitrogen content, pH and aeration. These conditions are crucial for the formation of stable and quality compost.

1.1.2.1. Temperature

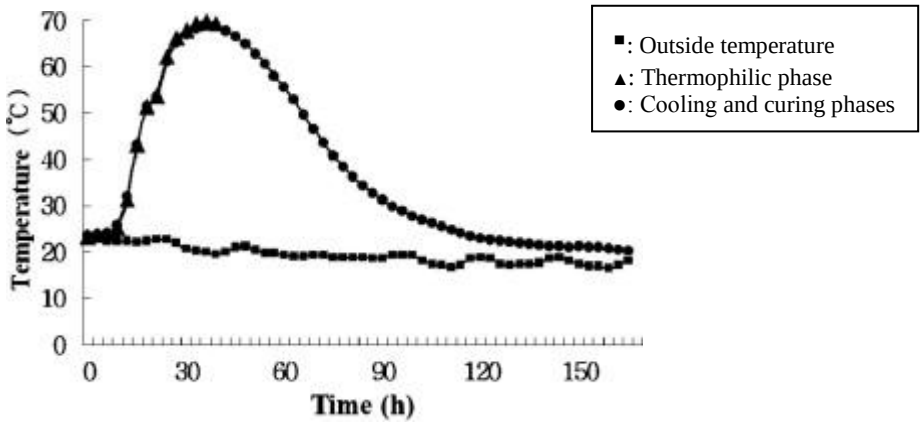


Figure 1.2: Temperature changes during the four composting phases. Source: Purnomo et al. (2010)

The temperature curve, shown in figure 1.2, is very typical for all composting processes, although it is more apparent in batch cultures than in continuous composting methods (Jenkins, 1999). Figure 1.2 illustrates the four composting phases in comparison with the outside temperature. Temperature measurements can therefore be used as confirmable source of identifying the stage of composting the pile is currently going through.

Temperature changes dramatically throughout composting and by-produces heat as a result of the oxidation process. Heat emission is so vigorous scientists have conducted researches to investigate the potential utilization of this heat for underground heating of certain constructions – for example, greenhouses (Hong, Park and Sohn, 1997). Authors observed that while the outside ground temperature was between 6 and 11.9 °C, greenhouse maintained temperature at 17.5 to 32.5 °C.

Temperature seems to affect loss of organic matter during composting, as Adams et al. (2008) imply, observing this phenomenon in the windrow composting method. Authors also found no indications of the composting heaps 'acclimatizing' to deviations of outside temperature.

Authors have diverging opinions on the maximum temperature reached during the thermophilic process (as can be seen in previous section) as there are many factors influencing this maximum. For example, Lashermes et al. (2011) say that reactors of 10 to 300 liter-volume spawn temperatures of 60 °C and higher.

Higher temperatures are crucial for the inactivation of pathogenic microorganisms, such as *Escherichia coli* and *Salmonella* spp., especially when biosolids are composted for later land application. In United States, according to Environmental Protection Agency (EPA), temperature of 55 °C must be kept for at least 3 days for in-vessel and aerated static pile composting and for 15 days for windrow composting in order to kill pathogens; Switzerland demands higher temperatures than 55 °C and for at least 3 weeks; Denmark requires same temperatures for at least 2 weeks and Germany requires higher temperatures than 60 °C for in-vessel composting to be for one week (Cekmecelioglu et al., 2005).

1.1.2.2. Moisture

Demand for moisture changes not only with the phases of the composting process but also with the kind (and characteristics) of material to be composted. For example, Durbin (2008), states that for composting of human fecal matter, initial moisture of 65 % is adequate, due to high loss of water to microorganisms and heat generated by composting, resulting in 20 to 30 %

water loss during first composting week. Similarly, Alexander et al., (2002) recommends having 50 – 60 % moisture content in the initial mix of composting biosolids. Lashermes et al. (2012) on the other hand, used moisture content of 66 % of original mix, containing sewage sludge, grass and hedge trimmings, leaves and branches. Overall, it is recommended to have moisture content between 35 and 60 % by weight. Water provides a medium for nutrient flow, organism movement and life and chemical reactions occurrence; therefore, having moisture content outside of this range may cause harms to the process and its constituents (Yadav, 2011). Moisture of too low content, resulting in drying out of the composting heap, results in total seizure of the biological processes, dying out of composting organisms and a total stop of metabolic processes. Composting process cannot be fulfilled to a mature level, instead dust issues become apparent. With moisture content being too high, porosity of the heap decreases spectacularly, altering aerobic conditions into anaerobic ones. This completely influences the composting process as well as living conditions for composting aerobic organisms, which can no longer engage in the composting process. Runoffs may be produced, with biologically unstable and immature nutrients leaking away into the environment. Just as in the opposite extreme of low moisture, high moisture content disables the completion of composting process to mature levels.

1.1.2.3. C:N Ratio

The C:N ratio, or ratio between carbon and nitrogen of composting material must be balanced in order for these elements to be fully utilized and their losses reduced to minimal amounts. Carbon forms the bases of living matter and nitrogen is the building block of proteins, thus the C:N ratio must be in given ranges to sustain life of the fauna in the composting pile (Durbin, 2008). As for composting of biosolids, authors Alexander et al. (2002) mention the best C:N ratio of initial mix to be 25:30-1. Durbin (2008) justifies the 30:1 ratio as ideal, because it enables microorganisms to exploit substantial amounts of nitrogen. According to him, range of 20:1 to 35:1 is acceptable; while C:N ratio of lower value consents votalization of nitrogen in form of ammonia, higher ratio (meaning the mix contains too much carbon-source material) limits the growth of microbe population by the quick consumption of available nitrogen and generally delays the whole process of composting. Different organic materials have different C:N ratios (as can be seen in table 1.1), however all-purpose rule states that wet and green material comprise mostly nitrogen, while dry and brown material comprises mostly carbon; to

reach a desired C:N ratio, it is recommended to generate a mixture of various materials (Schaub and Leonard, 1996).

Table 1.1: C:N ratios of urban waste products. Source: Jenkins (2005); Durbin (2008)

Carbon: Nitrogen Ratios of Urban Waste Products	
Material	**C:N Ratio**
Cardboard	400-563 : 1
Fruit	40 : 1
Raw Garbage	15-25 : 1
Humanure	5-10 : 1
Newspaper	398-852 : 1
Paper	100-800 : 1
Telephone Books	772 : 1
Urine	15-18 : 1
Vegetables	20-30 : 1

1.1.2.4. pH

The initial pH of the composting mix should be within the range of 6 to 8 (Hong et al., 1982; Schaub and Leonard, 1996; Alexander et al., 2002). Coopeband (2000) also approves this range, mentioning that it is favored by both bacterial and fungal decomposers - bacteria favor pH of 6 to 7.5 and fungi 5.5 to 8.

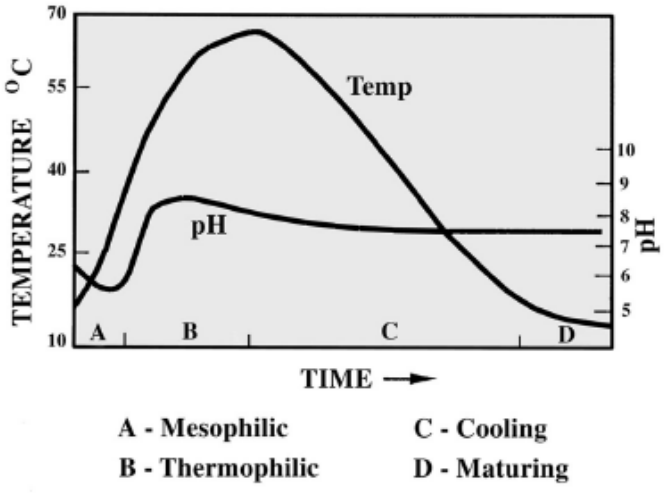


Figure 1.3: Compost temperature and pH variation with time. Source: Ministry of Agriculture and Food (1996)

A curve of pH development over all four temperature phases during composting are shown in figure 1.3. As compost enters the first phase and the temperature increases, pH falls to level of 6 to 5 (or even lower according to the composting material) from the initial pH; with the increase of temperature towards second phase pH increases to levels of 7 to 8 (9); as temperature falls towards the third phase, pH stabilizes and stays within the range of 7 to 9 (Hong et al., 1982). Finished, mature compost should have a pH level of or close to neutral.

The level of pH affects the rate at which the composting organisms transform organic matter. For example, if pH falls to under 7.5, ammonia easily escapes from the composting pile in form of ammonia (Coopeband, 2000) as described by the following equation by Bernal et al. (2009):

$$NH_4^{+}{}_{(compost)} \leftrightarrow NH_{3\ (compost)} + H^{+} \leftrightarrow NH_{3(gas)} \leftrightarrow NH_{3\ (atmosphere)}$$

Authors add that this is the main pH limit to be watched; overall, most of the raw material indented for composting has a pH within the recommended range. A correct range may be reached by mixing different materials.

1.1.2.5. Aeration and Oxygen

Composting process is an aerobic process – the presence of air (and oxygen), its content and adequate distribution in the composting heap (whether passive or active) is decisive for accurate progress and efficient composting process (Alburquerque et al., 2008). Excess compaction should therefore be avoided and porosity maintained; if raw material does not allow doing so, measures must be taken to enable free circulation of air in the composting pile. These measures include adding bulky materials to the mix, prodding holes in heaps or pushing PVC pipes into the pile, or in the case of commercial compost production, mechanically turning compost or utilizing blowers (Durbin, 2008). Another way of using perforated PVC is to lay them at the base of compost pile and force air through them by blowing (Alburquerque et al., 2006).

As for correct terminology, Alburquerque et al. (2008) uses the specific term Free Air Space (FAS), referring to inter-particle air spaces available for gas transfers, as not all air comprised by the heap can be considered as available. Similarly, total air space (TAS) consists of both inter-particle and intra-particle air spaces, while unavailable air spaces (UAS) are the intra-particle spaces. According to Eftoda and McCartney (2004):

$$FAS = TAS - UAS$$

FAS remains critical for raw materials with high moisture and low porosity, however even in less precarious situation, failing to comply the right amount can result in either prolonging the composting (inadequate FAS) or in extreme losses of heat (superfluous FAS) (Kulcu and Yaldiz, 2007). Solely to provide an example, Alexander et al. (2002) mention a 5 to 15 % concentration of oxygen in the initial mix of biosolids.

1.2. Composting Methods

The main product, compost, can be obtained by various composting methods. Main types of composting methods can be, according to Alexander et al., (2002) and Durbin (2008) typically categorized as windrow, static pile and in-vessel composting. Jenkins (1992) also mentions another important category comprised of batch and continuous composting. The three composting techniques above all fall into the batch composting category. Choice of the composting system should reflect characteristics of the waste to be composted, accessible manpower and economic circumstances (Cekmecelioglu et al., 2005).

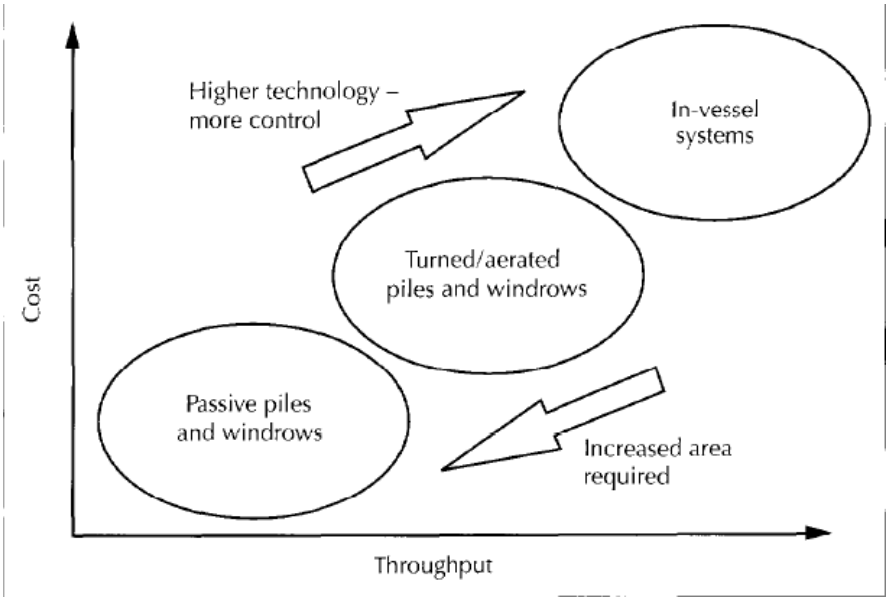


Figure 1.4: Scheme of composting methods comparison. Source: Schaub and Leonard (1996)

Schaub and Leonard (1996) compared these methods in a rough scheme shown in figure 1.4 above. The scheme illustrates a qualitative assessment in terms of investments, area, technology and control of the process. It is visible that access to innovative and better technology bring higher cost, but at the same time better control of the systems – in such case, in-vessel systems are most convenient. In case of lower investments and extensive approach to the

composting process, passive piles and windrows are most appropriate, even though this means having less control over the process and being in demand for more area.

It is also important to mention that aeration and supply of oxygen are the key aspects for any composting methods and turning still remains as one of the traditional kinds (Imbeah, 1997).

1.2.1. Batch and Continuous composting

Figure 1.5 underneath shows an example setup of batch composting method. Batch composting is composting with limited duration. Raw input material is mixed and added all at once, at the beginning of the process and removed in form of compost at the end. Then the process can be repeated. The batch composting method is ideal for commercial, municipal and large-scale production, because it can deal with large quantities of waste at single point in time. The four composing stages are very distinct in this system (Jenkins, 1999).

On the other hand, continuous composting system, (as the term implies) functions unendingly. As soon as the heap or bin is started, raw materials can be added endlessly whenever they become available. Therefore is it an ideal solution for small-scale owners. Typical examples are compost bins or piles in the garden or vermicomposting bins. In this technique, the composted matter is removed after some time and used. The missing portion of volume is refilled by new feedstock. The four composing phases, however, are not very apparent in the continuous system; because raw materials are added at different times they do not decay in synchronization and all stages occur concurrently rather than sequentially (Jenkins, 1999).

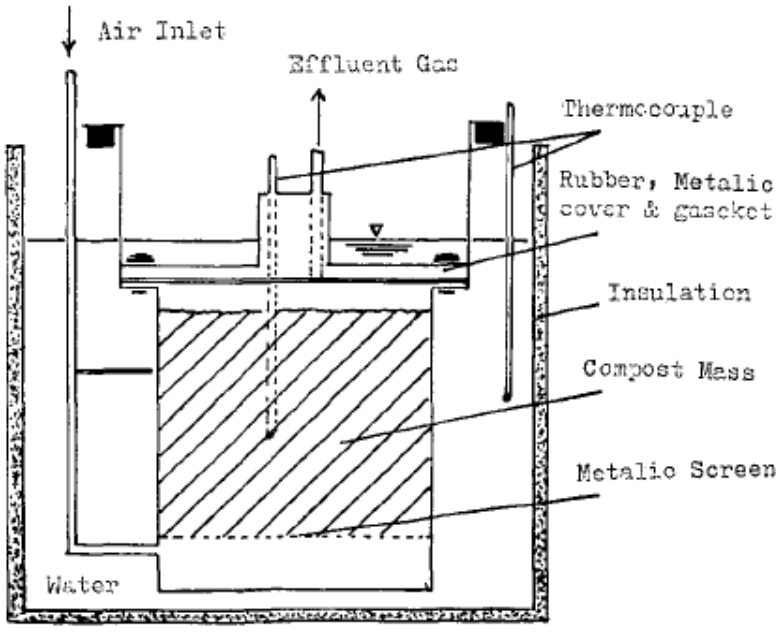


Figure 1.5: Scheme of batch composter. Source: Hong et al. (1982)

1.2.2. Windrow composting

In windrow composting system, the composting mass is positioned into long windrows (rows), which can be up to 2.5 m high, depending upon the turning method. Durbin (2008) gives proportions of 1.5 m in height and 3.5 to 4 m in width stating, that such size and shape of pile are adequate for the decomposer bacteria to proliferate easily and quickly. Another reason is also the question of aeration, because oxygen concentration is a function of depth in the windrow system – see figure 1.6 (Diaz et al., 2005). Notice the large amount of material with minimal oxygen concentration in middle of the pile with height of 1.5m. Windrows must be turned mechanically – this provides the necessary aeration (Imbeah, 1997).

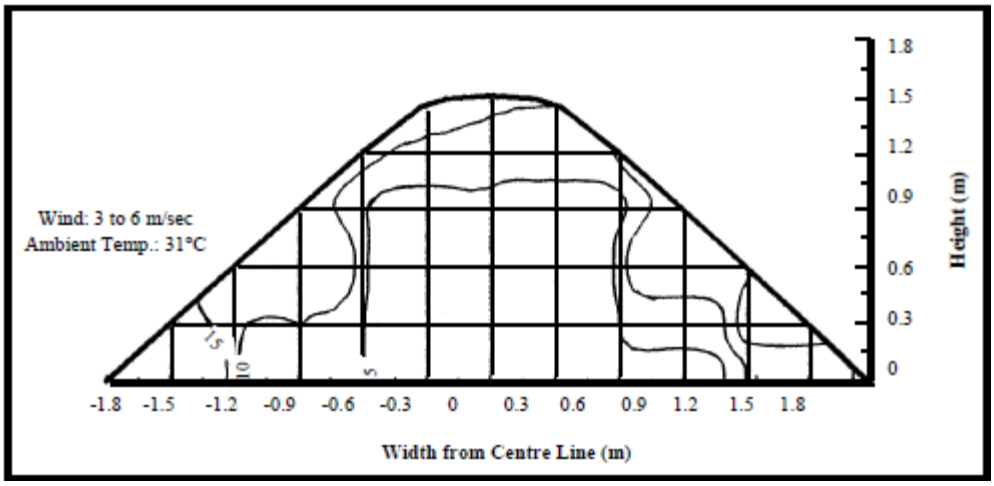


Figure 1.6: O_2 concentration within windrow. Source: Diaz et al. (2005)

Windrows should be positioned in such as manner for the turning machinery to easily go aside when aerating the pile. This makes the windrow composting method less space efficient, however this negative factor is exceeded the positive aspect of windrow systems being able to compost large amounts of material at once (Alexander et al., 2002). Authors also praise the windrow method for generating good and mature product with small investments needed. However, authors also add, that they have observed weather to be an influential factor of the composting efficiency (especially winter), which can reduce the windrows' temperatures. An example open windrow system can be seen in figure 1.7.

Figure 1.7: Picture of open windrows composting system. Source: Alexander et al. (2002)

1.2.3. Static pile composting

Static pile or aerated static pile (ASP) composting is a composting method, where the raw material is placed over aeration pipes, trenches or plenum which aerate the pile by either pulling (negative aeration) or pushing (positive aeration) the air (Alexander et al., 2002). This method consists of three different techniques of aeration: utilizing bulking agents, passive air piping and forced aeration piping (Durbin, 2008). In static pile composting, the compost is not turned at all throughout the composting phases. The material can also be concealed using a layer of matured compost, a biofilter which acts as isolation protection and reduces the odors (Imbeah, 1997; Durbin, 2008). Please see figure 1.8 with a scheme of this composing technique.

Overall, this technique has lower demands on area than windrow systems (but higher compared to in-vessel systems). Alexander et al., (2002) also mention other advantages: capital costs are relatively low; the pathogen elimination is efficient; management of odor is superior to windrow systems. As negative aspects, the authors mention greater odor generation than in-vessel systems, influence of climate fluctuations on the process – but this can be easily diminished by housing the static pile system.

In order to reduce the costs, Imbeah (1997) recommends aerating the piles passively by using different measures such as perforated pipes. Overall, static pile composting achieves identical results as windrow composting at lower costs but with higher labor inputs.

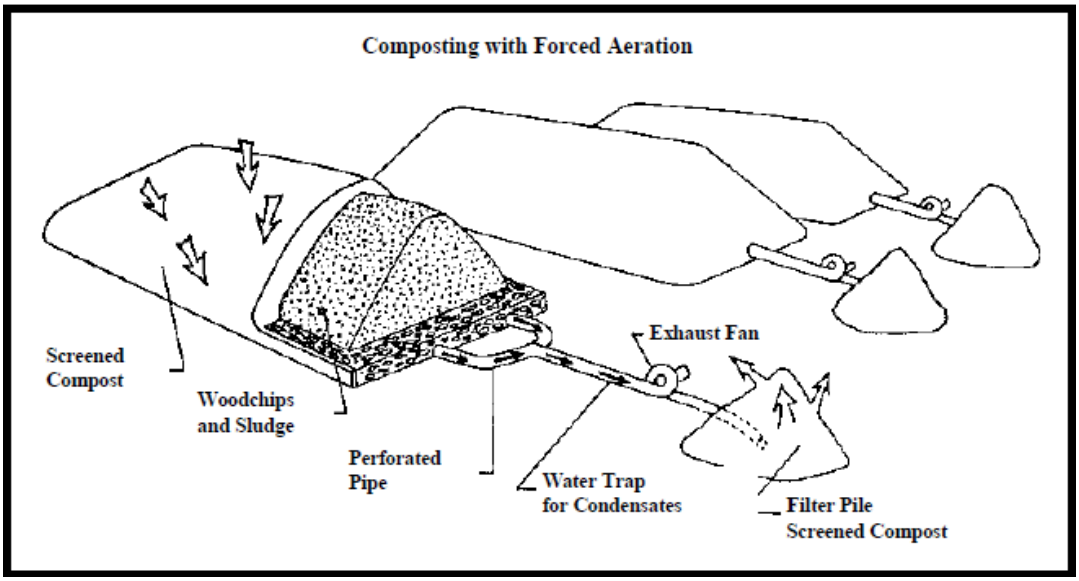


Figure 1.8: Scheme of forced aeration static piles. Source: Diaz et al. (2005)

1.2.4. In-vessel composting

In-vessel composting, according to Cekmecelioglu et al. (2005) has the following advantages: less space is required; system enables better regulation of leachate and gaseous emissions; mesophilic and thermophilic stages have shortened duration and overall the process generates better final product containing lowest number of pathogens. This corresponds to Laio et al. (1993) who found that in-vessels with no aeration had longer thermophilic phases than the aerated ones. As a negative aspect, Cekmecelioglu et al. (2005) mention high initial costs.

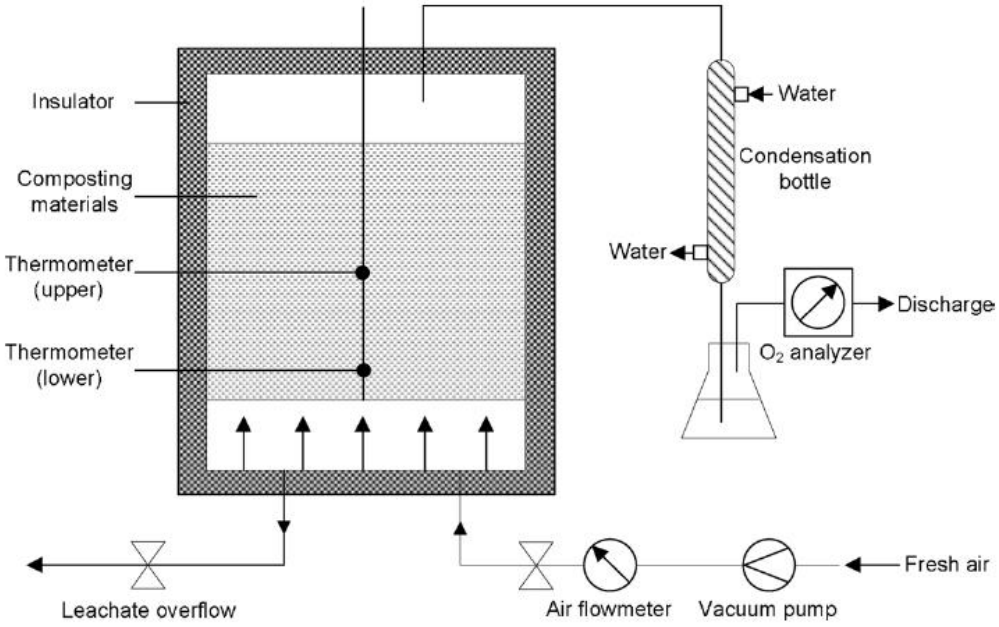


Figure 1.9: Example of in-vessel composting system. Source: An et al. (2012)

Figure 1.9 shows a diagram of an in-vessel composting system used in laboratory conditions for assessment of composting of uric acid and coal ash (An et al., 2012). The choice of this composting system can be justified by looking back at the diagram shown in figure 1.4. It is apparent that this composting method enables very good control (and therefore study) of the composting process itself. An et al. (2012) used forced aeration in form of vacuum pumps. However, Imbeah (1997) warns before extraordinary levels of aeration (when using the continuous system of aeration), as this could result in an undesirable effect of cooling the compost. Overall, in-vessel composting systems have been constructed in order to speed up the composting process through conservation of desired conditions and to minimize negative consequence upon ambient surroundings (Diaz et al., 2005).

In-vessels composting system is in fact a modified aerated static pile method, but the input composting material is positioned into a container. This allows must better control of the

composting process aspects, such as moisture and aeration (Durbin, 2008). In-vessel composting also allows addition of enhancement to the mix by adding decomposer organisms or worms to the bin – vermicomposting functions according to this principal. Durbin (2008) states, that this method has become very popular, even over higher costs; time, space and process efficiency exceeds disadvantageous aspects of this technique.

1.3. Vermicomposting

Vermicomposting is in fact an in-vessel composting method with decomposer earthworms added to the containers in order to enhance the process; stabilization of organic matter through bio-oxidation of raw material is achieved by cooperation between the earthworms and microorganisms (Aira et al., 2007). The effect of earthworms on fertility of soil has long before been recognized (Bansal and Kapoor, 1999). Vermicomposting works on the same principal as a composting system; as an eco-biotechnological procedure, vermicomposting converts organic matter into mature and stable humic product, the vermicompost (Suthar and Singh, 2008). Vermicompost is an excellent organic manure and soil conditioner (Ndegwa and Thompson, 2000; Yadav and Garg, 2011). Cooperation of microorganisms and earthworms is crucial, as earthworms 'drive' the process by defragmenting and digesting the organic material, preparing the substrate biologically for the microorganisms to break down in biochemical manner, which is also accompanied by exogenous hydrolytic enzymes from earthworm gut-associated microflora (Dominguez, 2004; Suthar, 2010). According to Vinceslas-Akpa and Loquest (1997), earthworms have an inevitable effect on the telluric microflora, influencing it by its excreta (vermin casts) and by its movement through the raw material profile; overall this has an accelerating effect on the conversion of raw material into vermicompost.

Vermicomposting can be done is boxes designed for this purpose (vermicomposters) which are usually kept indoors nearby the generation of organic waste, or in case of large-scale vermicomposting, outdoor composting piles can be used (Jenkins, 1999). Earthworms favor darker and moist environment, eating in upper levels and usually moving towards bottom to excrete the undigested food in form of vermicasts (however this habit is not perpetual). Vermicasts can be used directly used as soil conditioner or fertilizer.

Vermicomposters are continuous-feeding and if the environment is pleasurable for the earthworms, they will thrive in it and reproduce. The earthworm population is then able to renew itself and thus the composting process can be labeled as almost endless.

Thriving of earthworms in the composting material is directly connected to thriving of the microorganisms and vice versa. Moreover, places occupied by earthworms have been found to

encourage and have positive effect on the growth of microorganisms. Aira et al. (2007) discovered this phenomenon when vermicomposting pig slurry. They reported that earthworms enhanced microbial biomass carbon by 1.3 times more in comparison to vermicomposting reactor with no earthworms.

Vermicomposting earthworms have also been found to positively effect on mineralization as well as transformation of important elements into accessible forms for plants. Bansal and Kapoor (1999) reported an increase in nitrogen mineralization when using nitrogen-poor raw materials as feedstock. Nitrogen mineralization is in fact very well enhanced in vermicomposting process, mainly due to large generation of vermin casts (which contain a lot of nitrogen) or due to the decay of dead earthworms' bodies, also right in nitrogen (Singh et al., 2011). Suthar and Singh (2008) also found that vermicomposting increased the total nitrogen content by 137.7 – 67.8 % and available phosphorus by 107.9 – 16.9 % while decreasing pH by 67.0 – 15 %, organic carbon by 46.1 – 28.4 % and C:N ratio by 72.2 – 57.1 %. The effect of vermicomposting on organic carbon, total nitrogen, C:N ratio, pH and availability of certain plant-crucial elements such as phosphorous is therefore undeniable.

Vermicomposting is able to handle various types of organic materials (please see section 1.3.1.) and some epigeic earthworms have proven to stabilize dangerous wastes such as sewage sludges of urban and industrial localities (Suthar, 2010). This is where huge potential of vermicomposting technique is seen, as some earthworms have been seen to lower the amount of heavy metals, pathogenic bacteria and viruses while eliminating odors and stabilizing biologically unsteady wastes (such as biosolids, fresh human or animal faces).

Overall, vermicomposting is a very labor-extensive, undemanding enhanced composting method with high sustainability and economic feasibility. Vermicomposting is ideal for small-scale and large-scale organic waste treatment. On large-scale basis, these characteristics make it very suitable for areas with low levels of technological and economic development, but high levels of generated organic waste which is not (or cannot) be properly treated and bio-stabilized in recycling manner. Engaging and sustaining such process in one's home, village or town brings one closer to understanding the Nature's sustainable nutrient cycle.

1.3.1. Basic Vermicomposting Parameters

Earthworms can break down various types of organic materials. Table 1.2 shows types of wastes proven through experiments as vermicompostable.

Table 1.2: Proven vermicompostable types of wastes. Source: Yadav and Garg (2010)

Type of Waste	Reference
Sewage sludge	Sinha et al., 2008 Gupta and Garg, 2008 Khwairakpam and Bhargava, 2009
Cattle wastes	Loh et al., 2005 Plaza et al., 2007
Poultry waste	Ghosh et al., 1999 Garg and Kaushik, 2005
Crop residues	Suthar, 2009
Bagasse	Pramanik, 2010 Kumar et al., 2010
Industrial sludge/waste	Sen and Chandra, 2006 Sangwan et al., 2008 Yadav and Gard, 2009 Subramanian et al., 2010
Human faces	Yadav et al., 2010

Next to the materials shown in table 1.2, vermicomposting can also handle wastes such as solid paper mill waste, petrochemical sludge, food industry sludge, solid textile mill sludge, textile industry sludge, winery waste, olive oil industry waste, pressmud, sugar industry sludge and industrially produced woodchips (Yadav and Garg, 2011). This makes vermicomposting a truly versatile technique of handling dangerous industrial wastes and sludges.

When initiating a vermicompost, one should follow some basic parameters. First of all, it is important to select the right earthworm species. Those species, which easily colonize the organic material, have high rate of consumption and ingestion, reproduce quickly (and in high numbers) and are relatively stress-resistant represent the best choice in vermicomposting (Dominiquez and Edwards, 2004). Most frequently used earthworms for vermicomposting are *Eisenia fetida, Eisenia andrei, Lumbricus rubellus* and *Perionyx excavatus* (Singh et al., 2011).

Initially, it is important to sustain the vermireactor environment in such a way that favors the living conditions of the earthworms. This mainly involves moisture, temperature, and pH.

Moisture levels should be maintained at 40 to 55 % (Singh et al., 2011), however Zajonc (1992) mentions 70 to 80 %, stating that worms can withstand very moist conditions for number of days without any harm. Temperature is relevant to the vermicomposting specie being used – exotic (tropical) species such as *Eudrilus eugeniae* from West Africa flourish at 24 to 29 °C and die at 10 °C; *Eisenia fetida*, a widely used specie in temperate zones, thrives in temperatures between 15 and 25 °C and dies at 35 °C and higher or 5 °C and lower (Zajonc, 1992). As for pH, it should be kept neutral or near this level. However, earthworms are able to adjust the pH of their surroundings to certain extend towards the alkalic range. Moreover, the process of vemicomposting seems to spontaneously change the pH towards neutral level, due to mineralization of nitrogen and phosphorous (Yadav and Garg, 2011).

Another important parameter is the raw material being fed to the earthworms. This can be any organic material except oily, spicy, salty and hard waste including matter having high alkaline or acid levels (Singh, 2011). This is the reason why citrus fruits or carrots should not be fed to worms. Also, it is not recommended to feed them with goods such as eggs, meat and dairy products. C:N ratio is recommended to be at 22:1 to 30:1 (Zajonc, 1992; Singh, 2011), particle size of the feeding material should be 25 to 30 mm in size.

Earthworms have proved to be very picky in choosing their diet. Therefore, it is recommended to mix the vermicomposting material with some organic waste they favor. An example can be vegetable and fruit waste, coffee grounds, tea bags or animal manure. This is especially crucial for wastes listed in table 1.2, where bulking agents are necessary.

If one is not sure, whether his/her mix of waste is suitable for the earthworms to thrive in, Zajonc (1992) recommends a 24-hour test method. Earthworms are placed into a small container holding the homogenous mix of waste and left there for one day. After this period, earthworms are removed and checked. If they survive without any visible harm (injuries on skin, extremely softened bodies) and they react responsively to touch, the mix is compatible with their living conditions. If earthworms die or appear harmed, pre-composting is necessary. This is especially true with some types of organic wastes (Pandey et al., 2008).

1.3.2. Types of Vermicomposting

Zajonc (1992) divides vermicomposting systems into two categories: outdoor and indoor. Composting outdoors means composting in field conditions. The vermicomposting piles, which should not be higher than 60 cm and wider than 2 m are placed on concrete basis or plastic foil. It is important to have a source of water nearby. It is also possible to construct windrows – their

orientation should be identical with the orientation of prevailing winds. Outside conditions bring advantageous aspects such as easy access to the piles with mechanization and large area. Disadvantages are apparent in winter times, when low temperatures can threaten the earthworms and therefore insulation of the piles with hay or similar material becomes crucial.

Composting indoors, according to Zajonc (1992) enable intensification of the process, as ideal conditions (such as temperature) may be kept at the same and ensure vermicompost product whole year round. He recommends using containers with bedding material with total area of 2 m^2. Such container can hold up to 1.2 t of raw material. Container are then stacked above each other, forming 3 to 4 levels. Zajonc (1992) likens this method to indoor cultivation of mushrooms.

Pandey et al. (2008) categorize vermicomposting methods into four basic types: windrow system; wedge system; beds and bin systems; and reactor system. Windrow systems can be used in both indoor and outdoor conditions. They are quite area-demanding and are very various in construction – the most common kinds are static pile windrows and top-fed windrows. Static pile windrows or a batch type are intermittent, where piles of raw material are stacked, then inoculated with earthworms and left until vermicompost is generated. Top-fed windrows are continuous; bedding containing earthworms is covered with new layer of organic material about 10 cm thick repeatedly. Vermicompost is then removed with partially decayed bedding in the bottom layers.

In the wedge system, consecutive layers of organic material to be vermicomposted are added in 45° angle from vertical removable barrier (Edwards and Aracon, 2010). The wedge system should be placed on solid plane and its maximal height is 1.5 m, containing the vermicomposting earthworms in depth of about 15 cm. Finished vermicompost is removed every 3 to 4 months by the help of removable barrier and front load machinery (Edwards C.A. and Aracon N.Q., 2010).

Beds and bin systems can be top-fed or stacked. Top-fed bins are very similar to top-fed windrows just to the fact that it is a continuous-flow process and the vermicomposting process takes place in enclosed space; stacked bins consist of an enhancement in the form of adding vertical partitions to the bins (Pandey et al., 2008). This process can be either continuous or batch.

Finally, the reactor system is continuous and takes place in a contained space (usually box) where material is added at the top of the reactor. After being decomposed by the earthworms, the vermicompost falls through the grid placed near the bottom of the reactor, where it can be collected. Another category of vermicomposting is based upon the number of

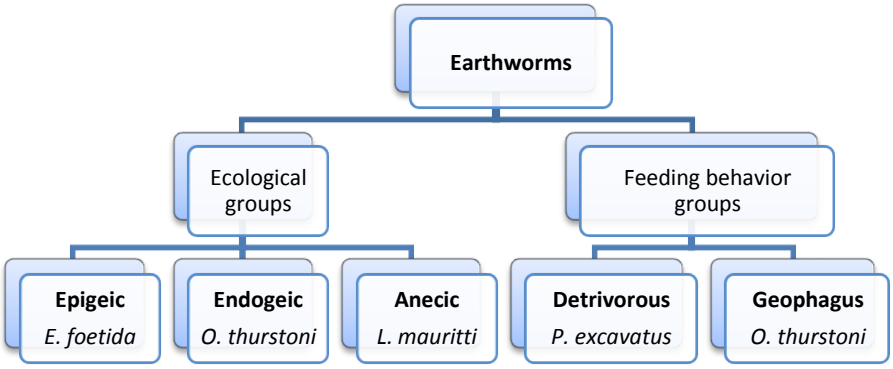


Figure 1.10: Classification of earthworms. Source: Padney et al. (2008)

different ecological groups used in one reactor. Suthar and Singh (2008) compared the traditional monoculture reactors with polyculture ones and found that the later tend to be more efficient in the vermicomposting process as the different ecological species complement each other in the decomposition process.

1.3.3. Types of Vermicomposting Worms

'Vermicomposting' originates from Latin word 'vermis' meaning worms (Singh et al., 2011). At 330 BC, Aristoteles labeled worms as 'the intestines of the soil' and Charles Darwin in his book "The Formation of Vegetable Mould through the Action of Worms with Observation on their Habits" characterized worms as 'ploughs of the earth' (Yadav and Garg, 2011). One way or another, the cycle created by the relationship between organic matter and earthworms is as old as Earth itself and is extremely important in terms of recycling once living matter back into soil so that new living matter can be created. Zajonc (1992) describes a connection between plants, animals, earthworms and vermicompost as a closed relationship which enables the movement of energy and nutrients in between the components of this cycle.

Earthworms are invertebrates with thread-like long, cylindrical and soft body covered with rings along the length; their size (*Microscotex phosphoreus* grows up to 20 mm while *Drawida grandus* can have 1 m) and color can vary as well as their ecology and feeding behavior (Padney et al., 2008). Figure 1.10 below the typical classification.

Epigeic earthworms or 'litter dwellers' inhabit 3 to 10 cm of the soil; they mainly feed on rotting organic matter (such as leaf litter or animal excretes) and have a reduced burrowing habit, remaining mostly in the litter layers (Bouche, 1977 ;Dash and Senapati, 1986). These earthworms are phytophagous, short-lived but with high rate of reproduction; they construct non-permanent burrows in soil and usually do not reach lower soil layers (distribution of nutrients by

these species is only in the litter layers) (Yadav and Garg, 2011). Earthworms which belong to the epigeic category are *Eisenia fetida, Eisenia Andrei, Eudrilus eugeniae, Drawida modesta* and *Perionyx excavates*.

Endogeic earthworms live in 10 to 30 cm of soil, creating horizontal burrows because of their developed burrowing habit and feed on organic portion in the soil (Bouche, 1977; Dash and Senapati, 1986). These earthworms are geophagous with low reproduction rate and feeding on the subsurface soil they create horizontal burrows and thsmix and aerate the soil (Yadav and Garg V.K., 2011). *Octochaetona trhurstoni, Allolobophora caliginosa, Allolobophora rosea* and *Drawida barwelli* belong to this group.

Anecic earthworms have the most developed burrowing habit, going down as much as 30 to 90 cm of the soil and making vertical burrows; they feed on litter and soil (Bouche, 1977; Dash and Senapati, 1986). Anecic earthworms burry into the soil during day and at night come up in search for decaying organic matter. Depth of the burrows are dependent upon ambient conditions, however anecic earthworms fulfill an important role in distribution of organic matter, thus benefiting the nutrient recycling of soil (Yadav and Garg, 2011). Anecic earthworms are: *Lampito mauritii, Lumbricus terrestis, Aporrectodea trapezoides* and *Aporrectodea longa*.
Other interesting aspects which characterize the above described groups are shown in table 1.3. It is generally believed that earthworms can consume 100 to 300 mg/g of their body weight daily (Yadav and Garg, 2011).

Table 1.3: Characteristics for earthworm classification. Source: Dash and Senapati(1986); Bouche (1977)

Characteristics	Epigeic	Endogeic	Anecic
Body size	Small	Medium	Large
Sensitivity to light	Low	High	Moderate
Reproductively	Highest	Low	Moderate
Mobility	Rapid	Slow	Moderate
Efficiency in waste recycling	Well established	Well established in some species	Efficiency data not available
Maturation	Rapid	Slow	Moderate
Casting activity	Surface casting, loose, granular	Mostly underground, thick and long casts	Surface casting, loose and granular

As it was already mentioned, vermicomposting combines the action of earthworms, which mechanically grind the organic matter and microorganisms, which decompose it is biochemical manner. Earthworms modify biological, chemical and physical status of the matter they thrive in, while increasing the surface area of the matter they digest to the microorganisms to act upon it (Yadav and Garg, 2011). By burrowing and twining through the litter, earthworms aerate it and thus substantially speed up the organic matter transformation and stabilization (Suthar and Singh, 2008).

Exotic vermicomposting earthworm species should also be mentioned here. Warmer zones of USA use *Eudrilus eugeniae* for large-scale vermicomposting; this species originates from West Africa. South-east Asia hosts *Perionyx excavatus*, which is very similar to *Eisenia fetida*. Other species can be: *Pherettima hawayana* and *Amynthes rodoricensis*. All these exotic species are very demanding in terms of temperature, which should be in range of 24 to 29 °C.

1.3.4. Utilization and Benefits

According to Suthar (2008), vermicomposting provides a better quality product compared to the traditional composting methods. In contrast to composting, vermicomposting does not require thermophilic stage; vermicompost matures earlier and has finer texture next to being heavy-metal and pathogen free (Singh et al., 2011). Number of bacteria increase faster in the vermicompost and stays at high levels throughout the process. This is because bacteria increase their numbers in the earthworm's gut after being ingested (Zajonc, 1992). This is why vermicomposting results in faster mineralization and nutrient release, such as phosphorus, potassium and magnesium. Next to that, vermicomposting is able to reduce number of pathogenic microorganisms in the finished product. Mitchell (1978) and Arunugam et al. (2004) have denoted reduction in populations of *Salmonella, Escherichia coli* and total elimination of *Shigella* and other fecal coliforms when vermicomposting sewage sludge. Equally, Yadav et al. (2010) have confirmed that vermicomposting has entirely disabled total coliforms in source-separated human faces. Another benefit of vermicomposting is the ability of worms to accumulate heavy metals in their bodies and therefore, resulting product may consist of lower amounts of these elements (Singh et al. 2011). Gupta and Garg (2007) see this 'cleansing' phenomenon as potentially very advantageous, because vermicomposting not only reduces contaminants (resulting from increased urbanization) but also converts waste into a valuable product in an eco-friendly and economically feasible manner.

The greatest benefit of the finished vermicompost is its ability as fertilizer medium. Vermicompost has been found to contain important substances enhancing plant growth

(gibberellins, cytokinins and auxins); it positively effects the growth of root system and flowers; it accelerates maturation of fruits by 1 to 2 weeks, which have an increased content of vitamin C; and overall it increased yields and suppresses diseases (Zajonc, 1992). Ndegwa et al. (2000) also add that vermicompost has an increased accessibility of nutrients, which are crucial for plant growth and that vermicompost is able to hold onto these elements for longer time thanks to its unique structure. Next to these aspects, vermicompost exerts all positive effects which traditionally produced compost has.

1.4. Untreated Sewage Sludge

1.4.1. Sludge Generation

Water-based sewage system has been introduced with the discovery of the first water flushing toilet in 1775 by Alexander Cummings' invention of S-trap toilet, where air sealed the toilet and prevented rotten air from escaping the sewer; by late 19th century, water was widely utilized in all of Britain, which eventually led to foundation of water-carried waste systems which carried waste to centralized wastewater treatment plants (Durbin, 2008)

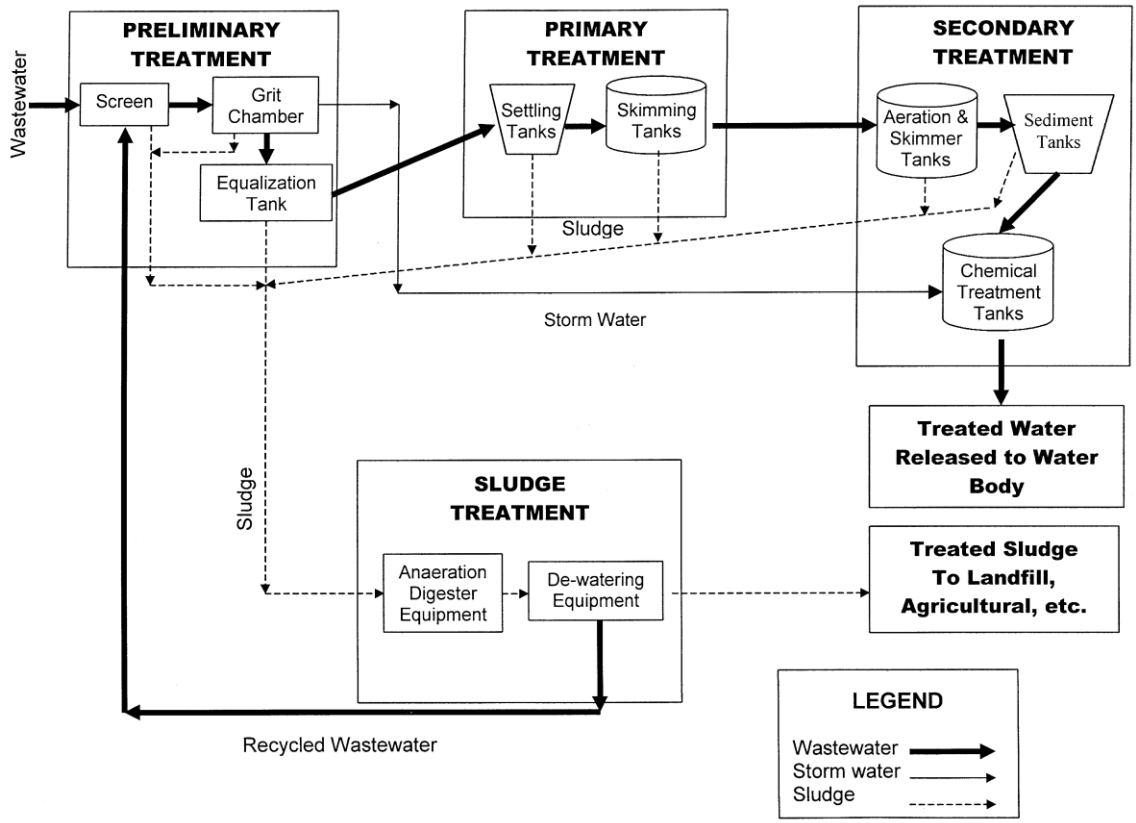
Sludge is a concentrated residue left after water cleansing process of water. This dark substance resembling mud represents a mixture of biosolids which leave households, institutions, streets (as runoffs), medical facilities and industries as water-carried wastes through drain systems of sewers and join other wastewaters in the form of sewage (Harrison et al., 2006; Durbin, 2008). Sludge is generated when polluted municipal wastewaters are deprived off dissolved and suspended impurities by chemical and biological processes of wastewater treatments, so that water may be returned back into environment without causing any contamination. Therefore, waste water treatment process is extremely important and plays a key role in sustaining clean and unpolluted aqua-environment.

Sludge consists of semi-solid and solid remains as well as many other substances removed from wastewaters. This is why these residues of wastewater treatment must be treated properly prior to its disposal (UNEP, 2005).

Wastewater treatment plants have been developed to match the scale and pollution of wastewater they intend to clean. Equally, many different types of wastewater treatments exist, from large intensive plants in populous cities as industrial complexes to extensive constructed wetlands in rural outskirts, utilizing basics of phytoremediation. Nevertheless, wastewater treatment plants must follow basic stages of treatment to remove all impurities from waters: preliminary treatment; primary treatment; secondary treatment; sludge treatment. All these stages can be seen in figure 1.11 below. Preliminary treatment, consisting of screens and grit removal, remove large objects which could damage other parts of the plant. Primary treatment is very typical by its settling and skimming tanks – here, suspended solids settle to bottom of tanks and are eventually removed while clean water overflows the tanks to secondary treatment. Here, aerobic bacteria are introduced to the tank along with air injections. In such environment, aerobic bacteria thrive and feed on dissolved nutrients in wastewater and eventually remove them by thorough consumption. Processed biosolids are then removed in sedimentation tanks. Water can

subsequently be subjected to chemical treatments including chlorination or treatments by O_3 before being released to environment. Sludge is created by sum of fractions of waste generated throughout the treatment from preliminary to secondary as shown in figure 1.11. Sludge is conducted to common sludge reservoir and consequent treatment.

Figure 1.11: Wastewater treatment plant scheme. Source: West and Mangiameli (2000)



1.4.2. Characteristics of Untreated Sludge

Wastewater entering the wastewater treatment plant contains pollutants of various origin, type and characteristic. Apart from the most-occurring pollutants, wastewater may contain organic impurities, pathogens and metals (Harrison et al., 2006). What is removed from wastewater is consequently found in the sludge. According to Pescod (1992), sludge contains substantial amounts of nitrogen, phosphorus and organic matter, which make it fit for agricultural use and land application to return these nutrients back into soil; however, sewage sludge is also abundant in pathogens such as bacteria, viruses, protozoa and parasitic helmiths. These pathogens must be removed before land application of sludge as they represent a potential health risk.

Sludge in its raw stage is a nutrient rich, biologically unstable and pathogenic substance and cannot be disposed off without proper treatment (Pescod, 1992; UNEP, 2005). In order to

minimize environmental effects, handling of sludge must be suspected to proper management techniques. According to UNEP (2005), sludge should not only be managed well, but all activities should eventually lead to minimization of its volume, as shown in table 1.4.

Table 1.4: Practices for reducing and handling sewage sludge. Source: UNEP (2005)

Practices for Reducing and Handling Sewage Sludge	
Prevent generation of large sludge volumes	Separation of sewers and storm drainage systems.
Land application	Contents of metal, salt, nitrogen are tested frequently and levels are within limits.
Drying, liming, composting	Methods to return organic waste back to land; co-composting with yard wastes.
De-watering and disposing in landfills	Dewatering sludge as much as possible to avoid overproduction of leachate.

Other techniques of handling raw sludge include sludge pasteurization, mesophilic and thermophilic anaerobic digestion, composting or dewatering and storage (Pescod, 1992). In case of sludge application to land, European Countries are prohibited to use sludge with characteristics outside the range given by Council Directive No. 86/278/EEC (Pescod, 1992). This directive gives acceptable ranges of dry matter, organic matter, copper, nickel, nitrogen, phosphorus, zinc, cadmium, lead, mercury, chromium, pH and pathogens.

2. Objectives

Objective of this Thesis was to assess the influence of vermicomposting on biological stabilization of untreated sewage sludge in terms of bacterial content of thermophilic microorganisms, specifically Coliform bacteria and *Escherichia coli*. Coliform bacteria and *Escherichia coli* are both used for evaluation of total and fecal contamination of water, respectively.

Goal of this experiment was to evaluate, whether vermicomposting could accelerate decomposition process of untreated sewage sludge resulting in faster biological stability and whether vermicomposting could be used as an alternative in stabilization and pathogen elimination of untreated sewage sludge and subsequent generation of environmentally acceptable end product.

3. Materials and Methods

In order to fulfill the objectives of this Thesis, vermicomposting experiments were set up. Experiment took place indoors under controlled conditions and was periodically assessed for composting maturity (by Solvita® tests) and pathogen content (by 3M™ Petrifilms™). Untreated sewage sludge was vermicomposted together with other biodegradable wastes using special vermicomposting earthworms suitable for temperate zone conditions. Components, arrangement and conditions of this experiment are thoroughly described in the following subchapters, along with methodology of monitoring activities. Experiment, where sludge and other biodegradable wastes were vermicomposted, was set up in indoor conditions and periodically monitored for CO_2 and NH_3 emissions. Coliform bacteria and *Escherichia coli* were tested for from acquired samples of the sludge fraction in the vermicomposts. These tests took place in laboratory conditions of CULS, Department of Microbiology, Nutrition and Dietetics.

3.1.1. Materials

Components of the vermicomposting experiment were: special vermicomposting earthworms, untreated sewage sludge, biodegradable waste as amendments and self-made vermicomposting bins.

3.1.1.1. Sludge

Untreated sewage sludge was provided by a wastewater treatment plant of Hostivice city (ČOV Hostivice), managed by Technical Services Hostivice. This middle-sized wastewater plant consists of all three stages of wastewater treatment. The scheme of primary treatment sedimentary tank can be seen in figure 3.1 (OMS SIMPLEX 2000-6000 EO). This technology has been developed solely for middle-sized plants with foundations in greater depths. Pneumatic sucker, which is mounted on a swivel arm, removes surplus sludge and sends back to bottom of the tank, while treated water flows towards the drains by entering perforated rods in the central cylinder.

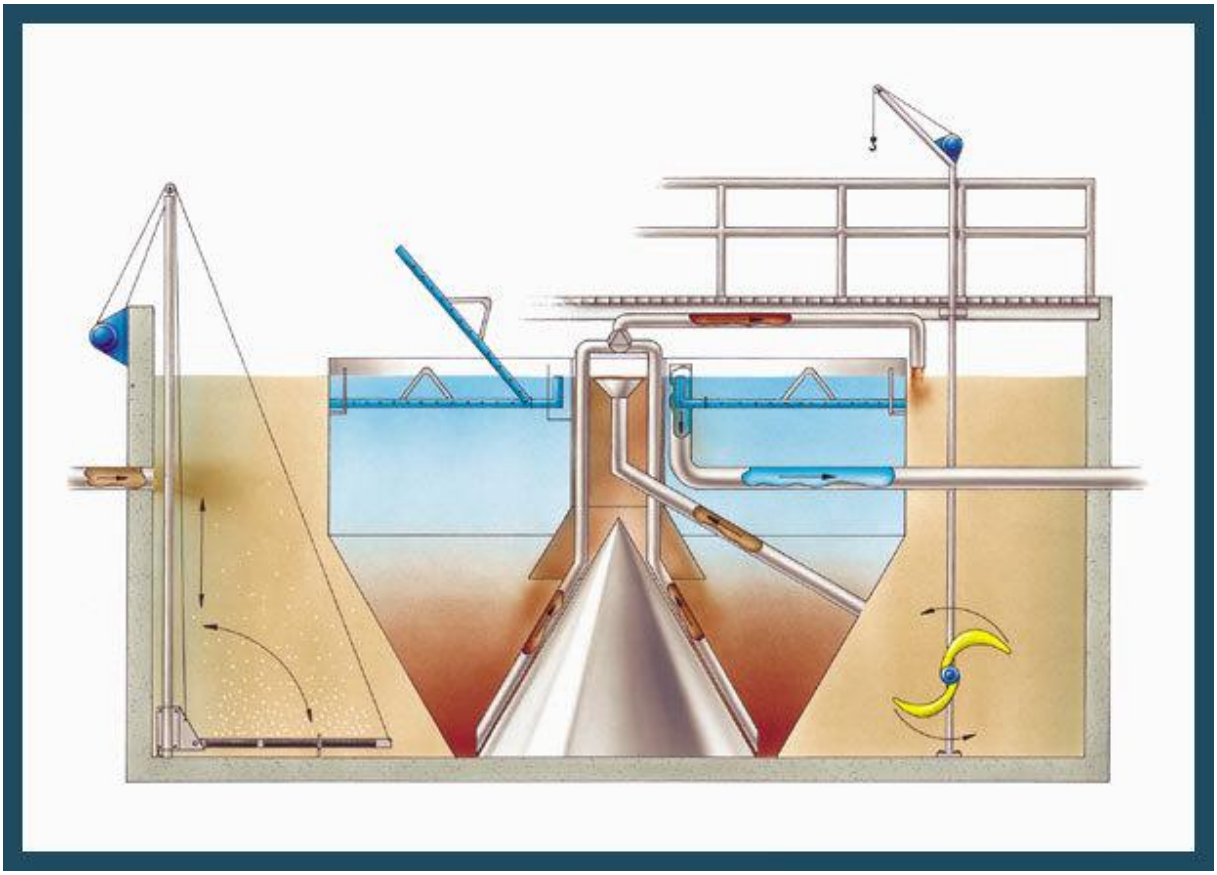


Figure 3.1: Primary Sedimentation Tank SIMPLEX 2000-6000 EO. Source: OMS Walter spol., s.r.o. (2013)

Pre-treatment and secondary treatment is of conventional kind, construction and function corresponding to that described shown in figure 1.11 in chapter 1.

Untreated sludge for the vermicomposting experiment was obtained in thickened, dewatered form. Manager of ČOV Hostivice proclaimed that sludge did not contain any heavy metals.

The following characteristics of the sludge were measured shortly after obtaining the sludge: pH, conductivity, amount of bacteria (Coliform bacteria and *Escherichia coli*), CO_2 and NH_3 emissions. Data were then comprehended as rudimentary output data; they are shown in 4.1 in the result section**.**

3.1.1.2. Amendments

Various kinds of biodegradable waste were added to the untreated sewage sludge as amendment in the vermicomposting experiment. Earthworms have proven to be very sensitive to gases releasing from untreated sludge when burying inside (see chapter 3.1.2.). Pure sludge with no other amendments could not create conditions compatible with life for the earthworms; therefore biodegradable waste, soil, paper and grass were used.

Biodegradable waste included coffee grounds, extracted tea bags, vegetable leftovers (carrots, green salad, potatoes, onion peels) all which were proven to be the most favorite of foods for *Eisenia fetida*. Vegetables leftovers were obtained from one particular household, while tea bags and coffee grounds were obtained by courtesy of institutions (Unicorn System a.s. and Klub Cestovatelů, respectively). Biodegradable waste did not contain any salts, herbs, sugar, milk, eggs or meat as well as any other types of condiments, flavor enhancers or dressings which are not edible or suitable for *Eisenia fetida*.

Soil and cut grass was obtained in Protected Landscape Area Tuchoměřice. Protection of this area ensures an uncontaminated environment of flora and fauna. Soil and grass (identified as *Poa pratensis L.*) also served for equalization of volume during the set up of vermicomposting bins and establishment of vermicomposting mixtures.

Finally, earthworms require bedding, which was provided by shredded paper. No ink was found on this paper, that having no other than white color. Volumetric amount of paper corresponded to 3 – 5 cm of bedding in height of the vermicomposting bins.

3.1.2. Earthworms

Eisenia fetida was used in this experiment. This vermicomposting worm is perfectly adapted to temperate zones. Quantity of 300 earthworms was obtained from Ekodomov, o.s. (based in V Podbabě 29b/2602, Prague 6), a civil association which focuses and promotes sustainable lifestyles based on effective composting methods.

Role of earthworms and their immense importance in the vermicomposting process is described in the Introduction chapter. Vermicomposting biomass had to be modified to create environment compatible with living conditions of these earthworms.

3.1.3. Vermicomposting Bins

Vermicomposting bins for small-scale household utilization do exist and are widely available; however they assume continuous utilization, which also corresponds to their construction. This makes conventional vermicomposting bins unavailable for batch experiment such as one described here. Own vermicomposting bins were developed from plastic containers sized 39×28×14 cm with total volume of 11 l by drilling holes in bottom and lids (see figure 3.2). These were placed onto a deeper tray which could hold water effluent. It was planned that only 10 l of the vermicomposting bins volume was to be utilized.

Figure 3.2: Self-made batch vermicomposting bins.

3.1.4. Test of Earthworm Adaptability

Near the beginning of this experiment it was discovered that *Eisenia fetida* cannot survive in sewage sludge without any amendments, which would provide it with easy foods and resemble its natural environment. Many authors have vermicomposted sludges before (see table 1.2), however amendments, mostly cow or other manures, were always used next to pre-composting treatments of sludge. There are limited scientific works describing vermicomposting of sewage sludge without using cow dung, pig slurry or other manures.

Experiment here described used raw untreated sludge and amendments, which resembled a typical household and back-yard waste. To ensure suitability of mixture for *Eisenia fetida* in terms of living conditions, endurance test was set up. This consisted of 6 containers, each having 1 l in volume, and different mixtures of sludge with soaked hay having maximum 1 cm in length. Pots contained 10 – 35 % of sludge together with 90 – 65 % of soaked cut hay, respectively. Mixtures in these pots are shown in table 3.1 below. Five *Eisenia fetida* were placed into each pot and carefully watched first every 15 minutes for 1 hour, then every hour for 5 hours, finally every 12 hours until end of test, totally 180 hours.

Assessment of *Eisenia fetida* took place as following: all earthworms were taken out of every pot and examined for signs of life by light touching or poking. If they reacted to contact, they were proclaimed as living and returned back to pot. Un-reactive or visibly damaged earthworms were recorded as dead and disposed of. After appraisal of results (see chapter 4.1), it was decided to use 15 % and 20 % concentrations of sludge for main experiment. These

concentrations have proven to be the most adequate for earthworms since most of earthworms survived by end of endurance test.

Table 3.1: Constituents of *Eisenia fetida* endurance test. Source: own measurements

Container	**Worms**	**Sludge (% ; g)**		**Soaked Hay (% ; g)**	
1	5	10	100	90	900
2	5	15	150	85	850
3	5	20	200	80	800
4	5	25	250	75	750
5	5	30	300	70	700
6	5	35	350	65	650

3.1.5. Feedstock Composition

After determining the sludge concentration to 15 % and 20 %, contents of vermicomposting bins were designed (see table 3.2).

Vermicomposting bins $V_{15\%}$ and $V_{20\%}$ served as experiments, as they contained both contained 150 earthworms each. Vermicomposting bins $V_{C15\%}$ and $V_{C20\%}$ served as controls and comparisons to previous vermicomposting bins.

Table 3.2: Material in vermicomposting bins and percentage of volume.

	Vermibins	**Sludge**	**Bulking material – Amendments**					
			vegetable waste	tea bags	coffee grounds	cut grass	soil	paper
Material in Composters (% of volume)	$V_{15\%}$	15	10	10	10	15	20	20
	$V_{20\%}$	20	10	10	10	10	20	20
	$V_{C15\%}$	15	10	10	10	15	20	20
	$V_{C20\%}$	20	10	10	10	10	20	20

In setting up vermicomposting bins, shredded paper was placed at the very bottom. This acted as bedding for the earthworms (see figure 3.3). Bedding is very important for earthworms especially when introduced to new environment.

Figure 3.3: Shredded paper as bedding on bottom of vermicomposting bins.

All amendments (coffee, tea bags, vegetable waste, soil and grass) were cut into smaller pieces; dangerous objects were removed (e.g. metal clip on tea bags). Waste was then thoroughly homogenized and wetted. Layer of this mixture was then placed upon the bedding to height of 4 cm. Measured amount of sludge was then spread in thin layer upon this mixture. This was done to ease acquirement of sludge samples during the experiment. Rest of amendment mixture was then placed on top of this sludge layer.

Vermicomposting bins $V_{15\%}$ and $V_{20\%}$ were then enhanced 150 *Eisenia fetida* each. Controls ($V_{C15\%}$ and $V_{C20\%}$) remained without earthworms.

3.1.6. Experimental Procedure

Vermicomposts were set up for 90 days, testing for Coliform bacteria and *Escherichia coli* were done every 15 days, tests for carbon dioxide and ammonia on the 0^{th}, 45^{th} and 90^{th} day.

Internal conditions such as pH and conductivity linked to untreated sludge were measured at beginning of experiment by conductivity and pH meter. Humidity was measured throughout experiment by hygrometer. Moisture was kept at 40 – 60 %; water was sprinkled upon all mixtures once a week with excess water running out the bottoms of perforated vermicomposting bins.

All four vermicomposting bins were kept indoors in small room where near to constant temperature was kept throughout the experiment. Outside temperature did not fall beyond the range of 17 – 20 °C. Vermicomposting bins were at all times covered by plastic lids to protect the content from outside influences such as dust.

During the experiment, all four vermicomposting bins were periodically controlled by Solvita® tests for maturity index, given by CO_2 and NH_3 emissions. Content of bacteria was determined by Petrifilm™ tests with selective medium for Coliform bacteria and *Escherichia coli*.

3.1.7. Compost Maturity Tests

Compost maturity was estimated by Solvita® test. Solvita® provides tests which enable evaluation of composts' maturity index based on measuring respiration rates of CO_2 and ammonia (NH_3). While high CO_2 levels point to potential overheating of compost, high ammonia levels signify potential phytotoxicity of compost or uneven C:N ratio. Overall, CO_2 and NH_3 values may be interpreted into compost conditions and maturity index levels.

Analysis consists of 2 paddles with gels of different sensitivity, where one is sensitive to CO_2 respiration, the other to NH_3 and a plastic container with screw-on airtight lid. During the test, a sample of mixture is placed inside the plastic container and stuffed gently to avoid too much porosity, up to a line marked on this container; on average, this volume represented 40g (Haney et al., 2008). Narrow surface of testing material should be attained. Both paddles are carefully inserted into the mixture with gel-sides not touching the tested substance or each other. Container is then sealed with lid and placed into a dark place with adequate room temperature for 4 hours (see figure 3.4).

Figure 3.4: Detail of CO_2 and NH_3 paddles during compost maturity tests.

Paddles with sensitive gels are colorimetric, utilizing the basics of Beer-Lambert's law ***(http://solvita.com/compost)***, results are therefore obtained by reading color changes which correspond to levels of gases respired by examined substance. Color keys can be seen in figures 16 for CO_2 and 17 for NH_3. Defining numerical results of CO_2 and NH_3 respirations enables researcher to identify compost maturity and general compost conditions (table 3.3.).

Figure 3.5: Color key for compost maturity according to carbon dioxide. Source: Solvita (2013)

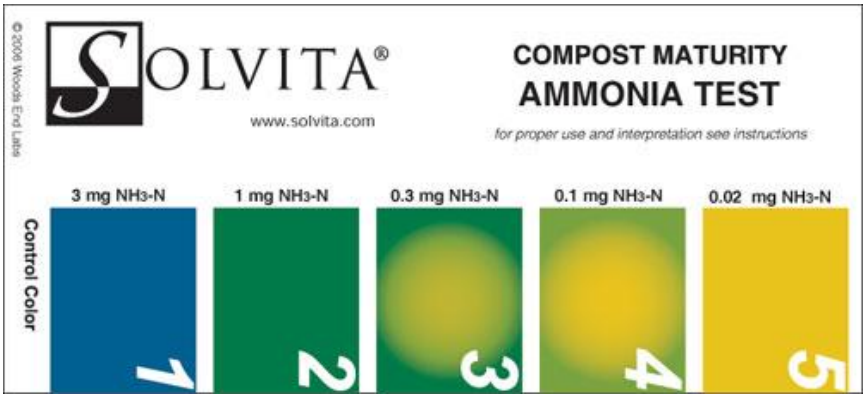
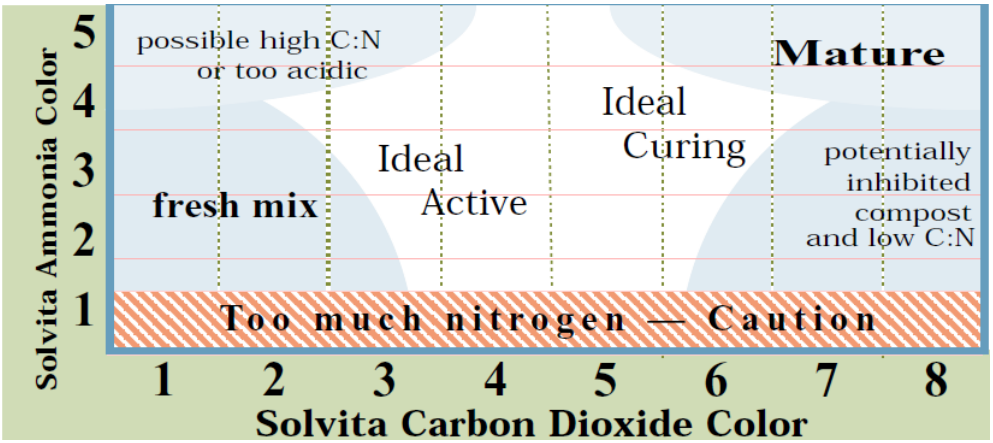


Figure 3.6: Color key for compost maturity according to ammonia. Source: Solvita (2013)

Results on paddles were interpreted according to the color keys. Solvita® tests were carried out 3 times during the vermicomposting experiment; primarily to assess untreated sludge before experiment, secondly after 45 days near middle of experiment, finally at end of vermicomposting. Results are shown in chapter 4.

Table 3.3: General compost conditions. Source: Solvita (2013)



3.1.8. Microbiological Tests

Petrifilm™ tests represented the main means in determining content of pathogenic microorganisms, particularly Coliform bacteria and *Escherichia coli*, which indicate presence marking disease causing organisms. Selective medium on these films enables only certain microorganisms to grow. Colonies are then counted, transferred to Colony Forming Units (CFU) or log CFU/g as pleasured.

Films used in this Thesis are the 3M™ Petrifilm™ *Escherichia coli*/Coliform Count Plates. They have a circular growth area of 20 cm^2 and contain gel with Violet Red Bile (VRB), nutrients which react to glucoronidase activity and form blue colonies, indicating presence of *Escherichia coli*; gel also contains pH indicator, which reacts to acids generated by growing Coliform bacteria colonies and verifies its presence changing into dark red color (3M Petrifilm Interpretation Guide). The see-through top film traps produced gases, so that gas bubbles surround the developed colonies and facilitate the counts.

To test certain medium for above mentioned microorganisms, 1ml of solution containing the medium to be examined is applied on 3M™ Petrifilm™ *Escherichia coli*/Coliform Count Plates growth area. Since 3M™ recommends the counting limit of colonies to 150, results of plates containing more colonies can either be estimated (by counting one sectional square and multiplying it by 20) or dilution of tested medium has to be made. Colonies which lie partially or completely on border of growth area are not counted.

To appraise sewage sludge in this experiment, dilution series were used. These were conducted in laboratory thanks to courtesy of Department of Microbiology, Nutrition and Dietetics located on Faculty of Agrobiology, Food and Natural Resources. Sewage sludge samples were taken from the vermicomposting bins. The attempt was to sample only sludge out of the entire vermicomposting material by hitting the layer of sludge in the vermicomposting bin (i.e. chapter 3.1.2.2.). In laboratory, 1g of sample was weighed and then dissolved in 10 ml physiological saline glass test tube (see figure 3.7). Dilution series were then made by removing 1 ml of solution with syringe and placing it into subsequent flask, containing 9 ml of physiological saline. This produced dilution of 10^{-1}. By repeating this methodology, desired rarefaction was reached from which 1ml of solution was removed and placed in circular motion onto the test area of Petrifilms^{TM} (see figure 3.8). Petrifilms^{TM} were then placed into thermostat, having temperature of 35°C for 24 hours.

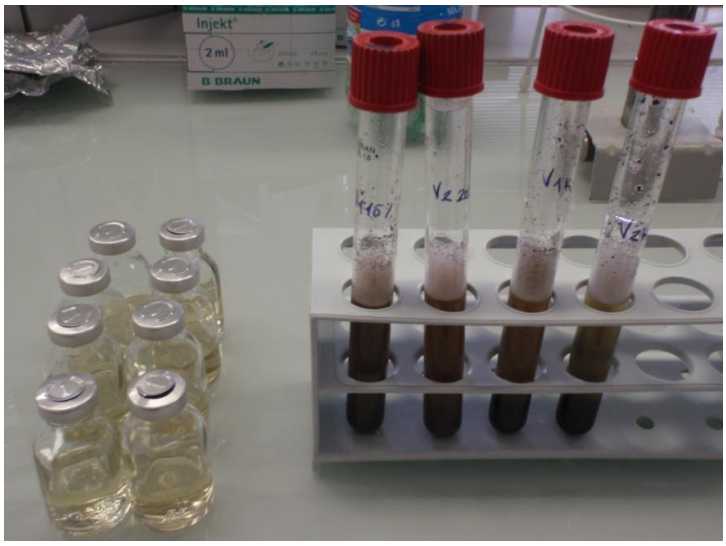


Figure 3.7: Glass test tubes with samples diluted in physiological saline

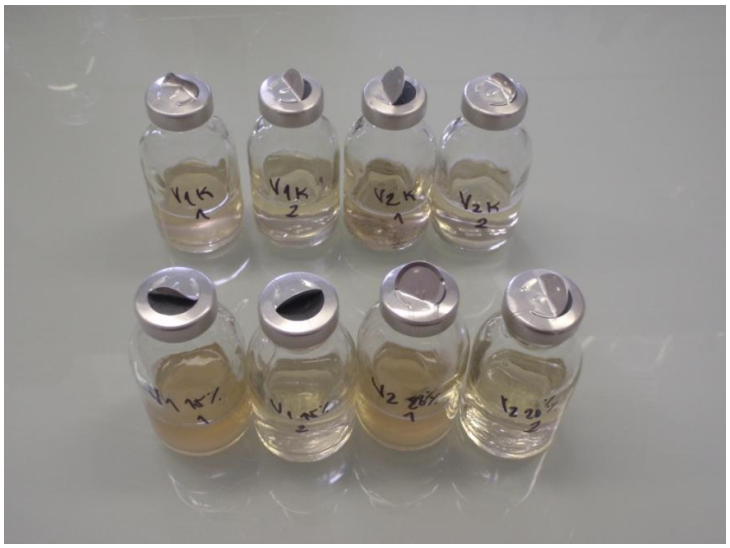


Figure 3.8: Dilution sequences, 1=10^{-1}, 2=10^{-2}

After incubation, Petrifilms^{TM} were interpreted visually by counting colonies developed in the growth area, their sums being converted as following:

Colony Forming Units / ml (CFU/ml) = No. of colonies $\times {}^{1}/_{\text{dilution used}} \times 10$

and

logCFU / g = log (CFU)

Petrifilm^{TM} tests were done to assess obtained sludge and 6 times during vermicomposting, every 14 days. Results were then graphically expressed with data from experimental vermicomposting bins ($V_{15\%}$ and $V_{20\%}$) being compared to control vermicomposting bins ($V_{C15\%}$ and $V_{C20\%}$). Results are expressed in chapter 4.3.

4. Results and Discussion

4.1 Characteristics of Sludge

The following table 1.4 shows measured characteristics of in table 4.1 below. Amounts of Coliform bacteria and *Escherichia coli* as well as levels of carbon dioxide and ammonia served as default data to which results throughout the experiment were compared.

Table 4.1: Sludge parameters, rudimentary data.

Sludge Parameters						
pH	**Conductivity**	**Coliform bacteria**	***Escherichia coli***	**CO_2**	**O_2**	**NH_3**
7.9	1711S	1.6×10^7 CFU	1.6×10^6 CFU	8% produced	8% consumed	8000 ppm produced

4.2. Earthworm Adaptability Results

As described in chapter 3.1.2.1, a trial was carried out where five *Eisenia fetida* were placed into pots with different ratios of sewage sludge and wet hay. This experiment was carried out in order to assess limiting amount of sewage sludge in which earthworms would survive. Results are shown in figures 4.1, 4.2, 4.3 and 4.4:

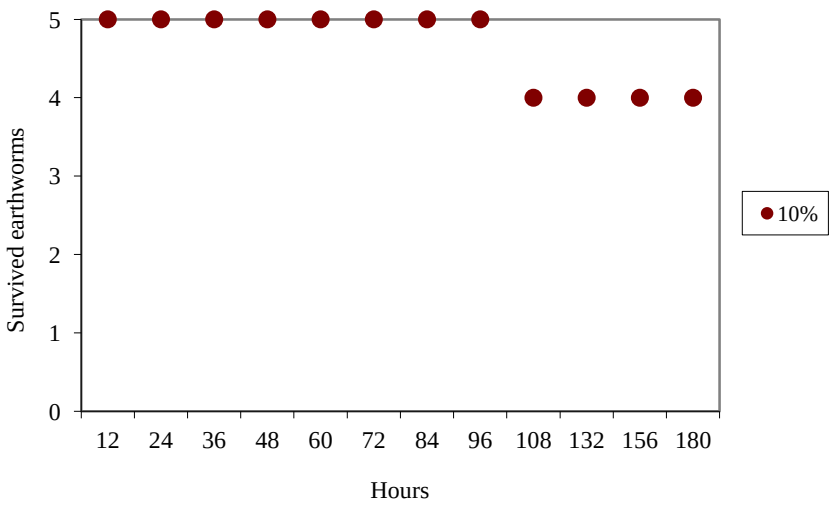


Figure 4.1: Rate of survival of *Eisenia fetida* in 10% sludge concentration.

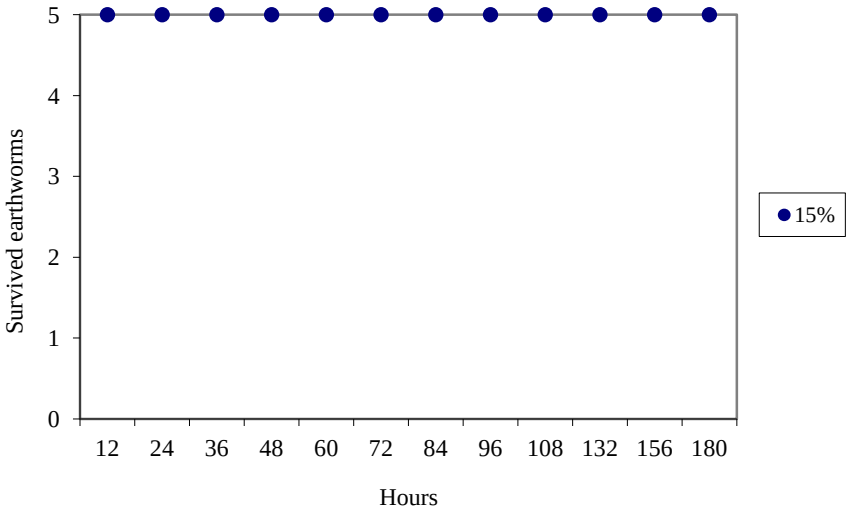


Figure 4.2: Rate of survival of *Eisenia fetida* in 15% sludge concentration.

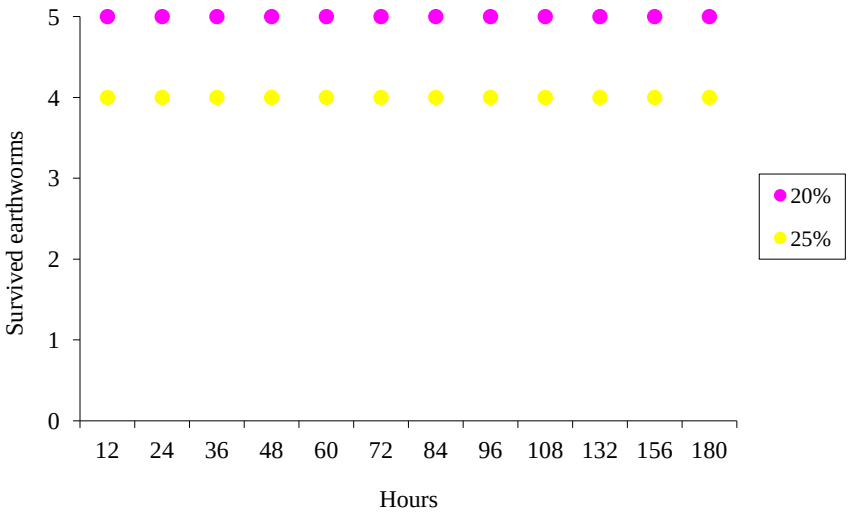


Figure 4.3: Rate of survival of *Eisenia fetida* in 20% and 25% sludge concentration.

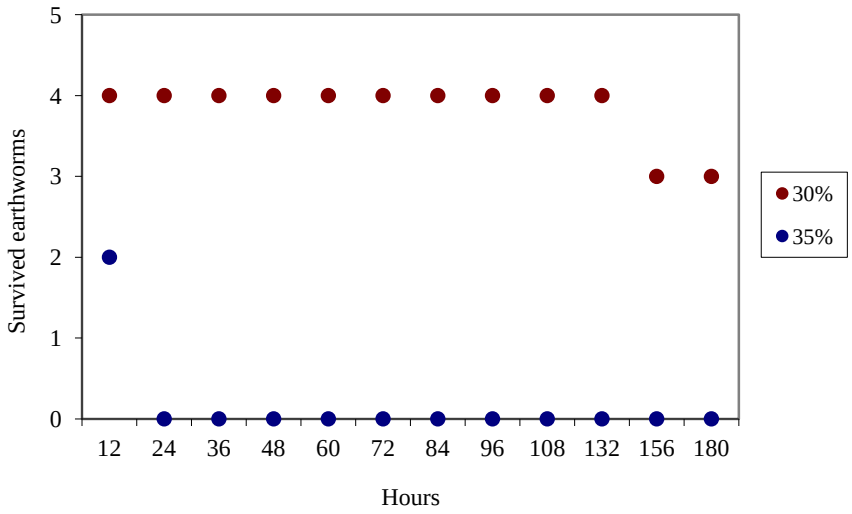


Figure 4.4: Rate of survival of *Eisenia fetida* in 30% and 35% sludge concentration.

Results here shown indicate that 30 % and higher fraction of sludge content becomes unsuitable for *Eisenia fetida*; for 30 %, only three earthworms were alive by end of experiment, while 35 % of sludge became lethal for earthworms within first 24 hours of experiment.

Concentration of 25 % appeared as appropriate in first 24 hours of experiment, however at the 36-hour earthworm control, *Eisenia fetida* seemed fatigue, weak, refused to move and responded very slowly. This appearance sustained until end of experiment. It was therefore concluded that 25 % concentration of sludge is also inappropriate for *Eisenia fetida*; earthworms do survive from 80 % (4 out of 5), however their lack of activity and paralyzing fatigue might have devastating effects on rate of consumption of vermicomposting mix.

Concentrations of 20 %, 15 % and 10 % has a somewhat similar trend, when regarding graphical illustrations in tables above with 20 % being the limiting concentration of sludge appropriate for vermicomposting. It was therefore decided to use 20 % and 15 % concentrations for the main vermicomposting experiment, concluding that 10 % concentration is unsuitable since too little sludge would be vermicomposted.

The acceptable level of sludge content in vermicomposts varies among works of scientific researchers. Tharakan et al. (2006) used 0 %, 10 %, 25 %, 50 % and 75 % of sludge content in vermicomposting bioreactors, where *Eisenia fetida* survived in all bioreactors. However it is important to mention that in this case authors used concentrated and dried sludge together with sterile potting medium, which in comparison with wet fresh sewage sludge used in this experiment must have provided more tolerable environment for the earthworms even in higher sludge concentrations. Similarly, higher ratios of sludge can be endured by earthworms when being pre-composted and/or mixed with cow dung. Ludibeth et al. (2012) have in this

sense achieved tolerable ratios of 70:30, 80:20 and 90:10 of sludge and composted cow dung respectively. Similarly, Ressetti et al. (1999) have pre-composted mixtures of sludge and sawdust (ratios of 1:1, 1:2 and 2:1 of sludge:sawdust) as well as Yadav and Gard (2009) who have studied mixtures of sludge and cow dung; Abida and Krishna (2010) who have vermicomposted sludge and *Eichhornia crassipes* or Singh et al. (2010) who have composted tannery sludge with cattle dung in ratios of 0:100, 10:90, 25:75, 50:50 and 75:25 (Singh et al., 2010). It would be interesting to know what levels of sludge fractions would authors be able to use whilst having fresh untreated sewage sludge such as in case of this Thesis. Pre-composting is a very frequently used technique prior to vermicomposting experiments.

Amendments are crucial because they create an acceptable feed for the earthworms (Yadav et al., 2009). Among these, most frequently used are soil, mature vermicompost, mature compost and most importantly, cow dung. Cow dung has become very favorable among scientific researchers as it appears to be a very useful amendment in enhancing environmentally unfamiliar media to earthworms, reducing the process time and improving the final form of composted waste (Vig et al., 2011). Almost all authors designing vermicomposting experiment with wastes such as paper-pulp sludge, paper mill sludge, food industry sludge, solid textile mill sludge, sugar industry sludge, pressmud, distillery industry sludge, leather industry sludge, dairy sludge, spent mushroom waste (Yadav and Garg, 2011) or source-separated human faces (Yadav et al., 2009) use cow dung or manure to make the waste accessible. However cow dung is not always available in adequate amount and hygienized form, which was also the case of experiment described in this Thesis. It was thus decided to use other wastes, which were known to be preferable to *Eisenia fetida* in terms of food and at the same time were seen as largely available. In fact, specific amendments provide the vermicompost with not only specific product but also specific environment during vermicomposting. Amendments of tea bags, coffee grounds, soil, grass and vegetable kitchen waste provided such environment in which 15 % and 20 % of untreated and fresh sewage sludge could be vermicomposted without causing harm to the process or *Eisenia fetida*. It is an object of speculation whether higher volumetric fractions of sludge could have been achieved by using cow dung or manure with fresh sewage sludge or by pre-composting the mixture. In fact, this could be a matter of future research in terms of best and highly available amendment and its pre-treatments.

4.3. Compost Maturity Results

Table 4.2 shows results of Solvita® tests of 1st (primary tests for sludge characteristics), 2nd (day 45 of experiment) and 3rd (day 90 of experiment) sampling trials. All tests were done as described in the Solvita® brochure. Values, which were read of color keys shown in figure 3.5 and 3.6 above.

Table 4.2: Solvita test results

Days of vermicomposting; Samples	**$V_{15\%}$**		**$V_{20\%}$**		**$V_{C15\%}$**		**$V_{C20\%}$**	
	NH_3	**CO_2**	**NH_3**	**CO_2**	**NH_3**	**CO_2**	**NH_3**	**CO_2**
0 / 1	5	3	5	3	5	3	5	3
45 / 2	5	4.5	5	4	5	4	5	4
90 / 3	5	5.5	5	4.5	5	4.5	5	5

Sample 1 was conducted before the experiment was begun; values show measured levels of pure sludge. This is why they are the same across all vermicomposting bins, since the very same sludge was used in the consequent mixes. Levels 5 and 3 for NH_3 and CO_2 respectively can be interpreted according to table 3.2 as "possible high C:N or too acidic" slowly transiting into "active" compost conditions. This is not surprising, since sludge is the result of biological decomposition processes; therefore is it susceptible to rotting and decay and moves into active compost conditions.

Sample 2 represents levels measured half-way through the experiment, where a representative sample of vermicompost from each vermicomposting bin was examined. While vermicomposting bins $V_{20\%}$, $V_{C15\%}$ and $V_{C20\%}$ show same levels of CO_2 and move into an ideal active compost condition, vermicomposting bin $V_{15\%}$ shows higher levels, particularly 4.5 and is on the verge of ideal curing compost condition. *Eisenia fetida* examined in this vermicomposting bin were found to be more active than those in $V_{20\%}$; their activity might be the reason for faster maturation of this mixture. NH_3 levels remained the same for all mixtures.

Sample 3 was also conducted as representative samples of whole vermicompost. Content of vermicomposting bin $V_{15\%}$ with CO_2 levels of 5.5 has moved from curing to mature compost condition. Vermicomposted mixture in this vermicomposting bin was at this point at verge of biological stabilization. Vermicomposting bins $V_{20\%}$ and $V_{C15\%}$ moved to same level of 4.5 for CO_2, while $V_{C20\%}$ moved to 5.

To interpret these results, $V_{15\%}$ exerted processes of biological and chemical character, which were able to stabilize the mixture within 90 days of experiment. *Eisenia fetida* in this

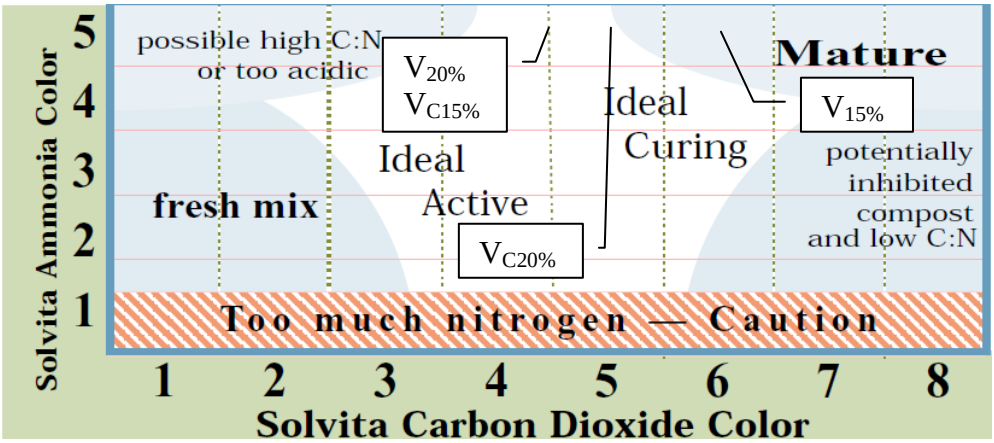


Figure 4.5: Interpretation of Solvita test results on general compost conditions table

mixture showed increased activity with cocoons appearing within four weeks of experiment setup. Indeed reproduction of earthworms is one of the best indicators in assessment of vermicomposting process (Vig et al., 2011). Clearly, the combination of 15 % sludge and amendments was favored over the 20 % sludge and amendments by earthworms, where cocoons appeared in later stages and worms lacked native active behavior. Interestingly enough, when Begum and Krishna (2010) vermicomposted sewage sludge with pieces of *Eichhornia crassipes*, they indicated best results for 20 % sludge concentration, including highest number cocoons, earthworm population and weight gain of earthworms. However, authors have pre-composted the mixture for 30 days before inoculating with earthworms.

Vermicomposting container $V_{C20\%}$ holding 20 % sludge and no earthworms has managed to reach the level of ideal curing, nearly mature while $V_{20\%}$ with earthworms remained in ideal active level of compost maturity. In this case therefore, earthworms did not have any positive effect onto the mixture in terms of biological stabilization. This again might be due to decreased activity of earthworms in the mix due to less adequate environment created by 20 % of sludge. Non-palatability and toxicity of sludge undeniably represents a great problem in many vermicomposting cases (Vig et al., 2011). $V_{20\%}$ attained the same levels of maturity as $V_{C15\%}$ without earthworms. Regarding the controls $V_{C15\%}$ and $V_{C20\%}$, the difference between their achieved levels of maturity is not substantial (see figure 4.5).

Solvita ® enables the assessment of maturity of a medium on the basis of ammonia and carbon dioxide gas emissions. Other techniques in assessing maturity of vermicomposting end-product exist, such as determining organic matter content, total nitrogen content, C:N ratio (Yadav and Gard, 2011) or simply the rate of production and number of worms in the

vermicomposting containers (Vig et al., 2011). In connection with C:N ratio, Sen and Chandra (2007) concluded that the decrease in C:N ratio in vermicomposted mixture indicates higher organic mixture decomposition and therefore can be regarded as an indicator of biological stabilization. Such trends in C:N decline were also observed by many other researchers such as Sangwan et al. (2008), Suthar (2010), Elvira et al. (1996), Kaushik and Garg (2003) or Garg et al. (2006).

Time, in which biological stabilization in vermicomposted mix was achieved in experiment conducted in this Thesis, was 90 days for $V_{15\%.}$ However, time needed for biological stabilization is reliant and changes with many biotic factors, such as stocking density of earthworms in mix, microorganisms represented in the mix and enzymes secreted by the earthworms (Yadav and Garg, 2011). Nonetheless these factors vary according to waste being vermicomposted. Ndegwa et al. (2000) reported an ideal stocking density of 1.60 kg of worms per m^3 in vermicomposting of biosolids, Dominquez and Edwards (1997) propose eight earthworms per 43.61 g of dry matter for pig manure and Singh et al. (2010) achieved bets results with inoculation of 25 g/kg of beverage industry bio sludge. Microorganisms represented in vermicomposted material depend on the composition of default material (Yadav and Gard, 2011). Finally, enzymes which are emitted by earthworm gizzard and intestine, cause biochemical conversions of cellulosic and proteinaceous substances (Hand et al., 1988) while fulfilling metabolic purposes such as decomposition and detoxification (Nannipieri and Bollag, 1991). Various combination of these factors, together with a fact that earthworm eats between 100 and 300 mg of feed per gram of its body weight, results in fluctuating time durations in achievement of biological stability. Moreover, pre-composting of the mix may reduce the total vermicomposting time by up to 20 days (Shweta et al., 2010). Singh et al. (2010) received a stabilized product within 110 days, Nahrul Hayawin et al. (2010) in 84 days, Subramanian et al. (2010) in 45 days (though pre-treating the mix for three weeks) and Maboeta and Rensburg (2003) vermicomposted for 84 days.

4.4. Microbial Results

Petrifilm™ tests were done in order to assess levels of *Escherichia coli* and Coliform bacteria in vermicomposted sludge. Both microorganisms are seen as indicators of fecal contamination, which sewage sludge consists of in very high levels. As mentioned in chapter 3.1.4.2., results were obtained after 24 hours of incubation time; colonies of *Escherichia coli*

were blue while Coliform bacteria were red. Dilution series were done to avoid results labeled as TNTC – too numerous to count. Acquired data was recalculated into CFU/ml and logCFU/g. Results are presented graphically below. To comprehend, whether it was vermicomposting and *Eisenia fetida* activity that caused the decrease in *Escherichia coli* and Coliform bacteria, results of vermicomposting experiments ($V_{15\%}$, $V_{20\%}$) were compared with controls ($V_{C15\%}$, $V_{C20\%}$).

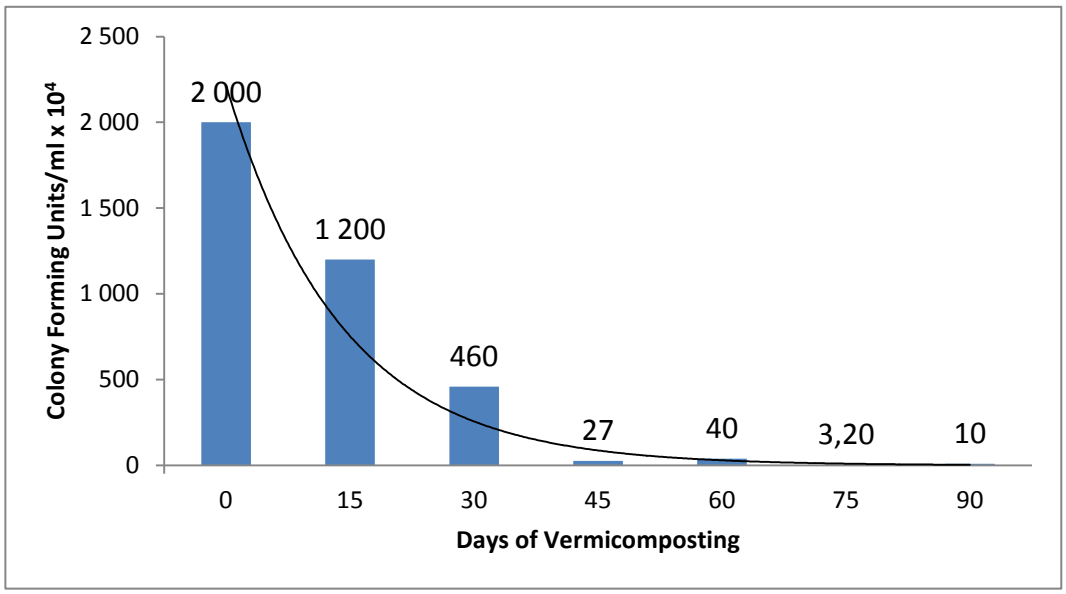


Figure 4.6: Coliform bacteria trend in vermicompost $V_{15\%}$

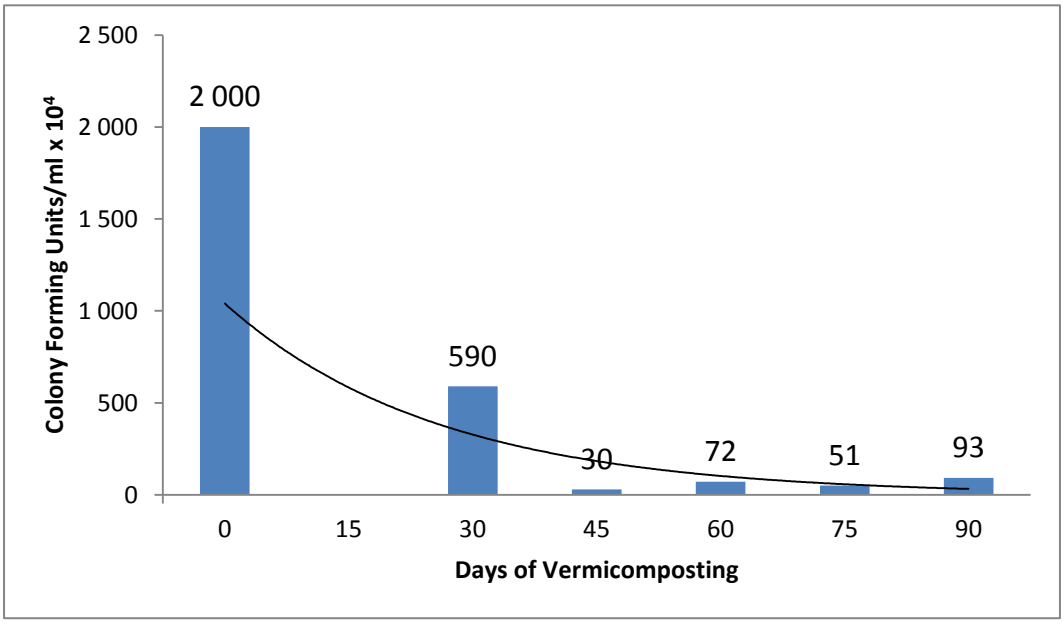


Figure 4.7: Coliform bacteria trend in vermicompost $V_{C15\%}$

It should be hereby mentioned that result for vermicompost $V_{C15\%}$ day 15 has been excluded from the scatter plot data due to its surprising value. Another factor worth mentioning

are the values measured and a fact, that they are not always decreasing (notice figure 4.6 and values acquired from days 45 to 90) even though the overall trend is to decrease. This tendency is visible throughout the rest of the figures exerting these results. One of possible explanations for this phenomenon is that bacteria were not distributed throughout the sludge equally. In fact, bacteria are hardly ever found as individual cells; instead they are aggregated in medium in forms of aggregated flocs or clumps (McDowell et al., 1986; Jamieson et al., 2002). Another explanation includes *Eisenia fetida,* which did not move throughout the substrate equivalent ways but instead developed their burrows in random character and affect the colonies of microorganisms unevenly. Finally, it was extremely difficult to acquire a representative sample of pure sludge, even though it was distributed in homogenous layer just above paper bedding. Within the third sampling of sludge, the mixture in vermicomposters $V_{15\%}$ and $V_{20\%}$ have attained a dark brown color, almost identical to the color of sludge. All these factors could have caused the samples taken to contain not only sludge but also partially amendment mixture fractions.

Surprisingly enough, values of Coliform bacteria seem to decrease in very similar manner in vermicomposting bins $V_{15\%}$ and $V_{C15\%}$. Even though levels of Coliform bacteria in $V_{C15\%}$ days 45, 60, 75 and 90 are higher than in $V_{15\%}$, the difference is not substantial.

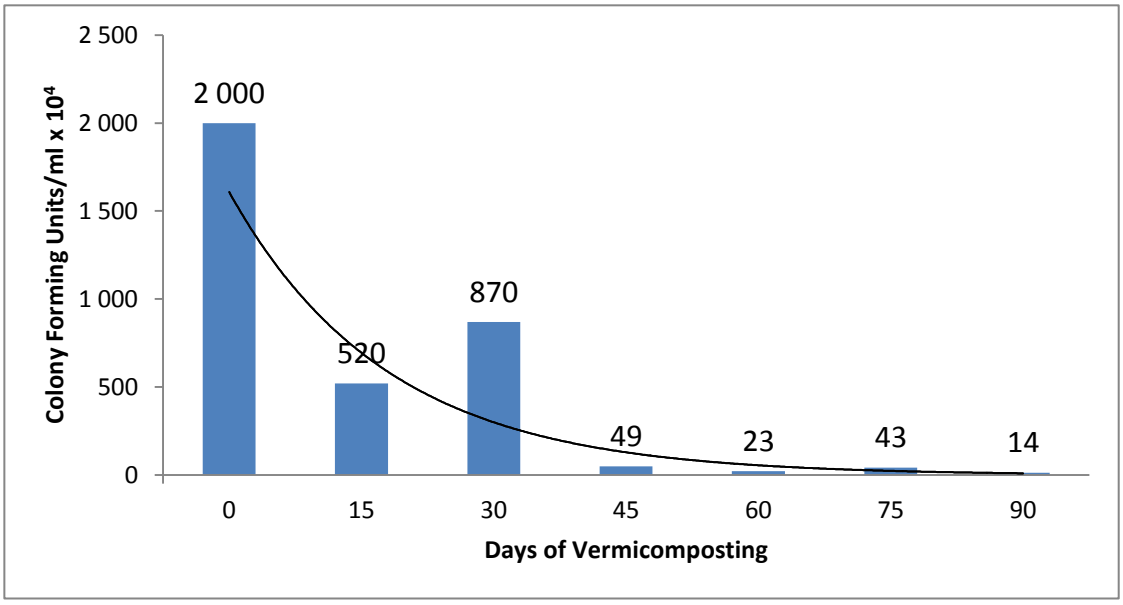


Figure 4.8: Coliform bacteria trend in vermicompost $V_{20\%}$

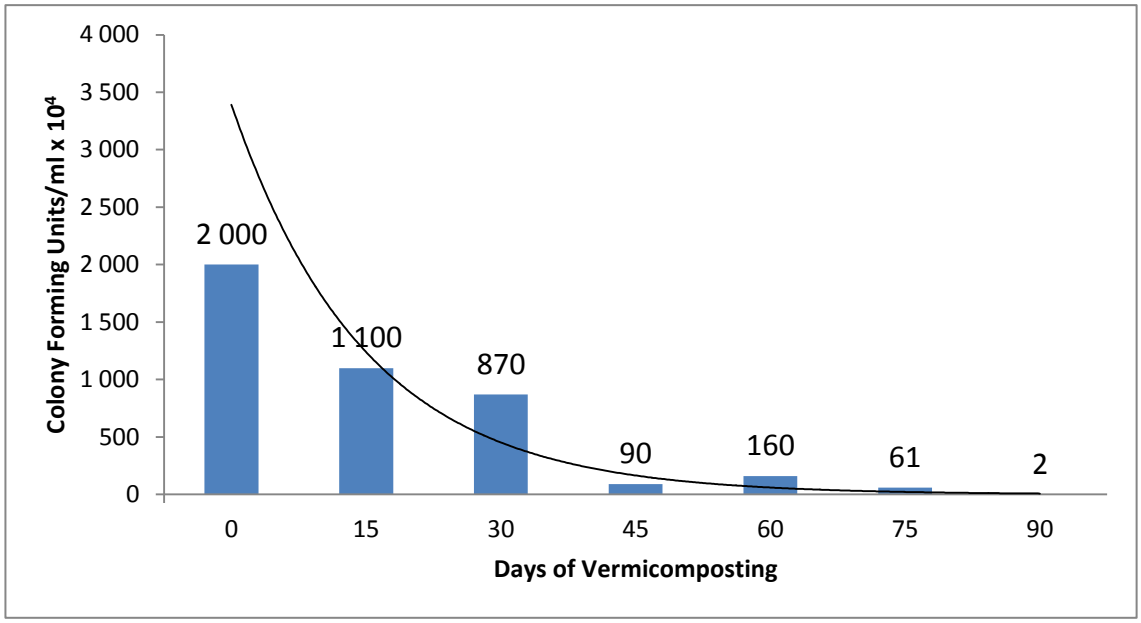


Figure 4.9: Coliform bacteria trend in vermicompost $V_{C20\%}$

Same development in decrease of Coliform levels can be seen in results obtained from vermicomposts $V_{20\%}$ and $V_{C20\%}$ in figures 4.8 and 4.9.

Vermicompost $V_{C20\%}$ shows higher levels of Coliform bacteria on days 15, 45, 60 and 75 compared to $V_{20\%}$; levels from testing on day 30 were identical for both vermicomposts. However, the differences in Coliform levels between experiment and control are not significant, demonstrating that decrease in Coliform bacteria was not considerably affected by vermicomposting. Figures 4.10 and 4.11 show *Escherichia coli* levels in $V_{15\%}$ and $V_{C15\%}$.

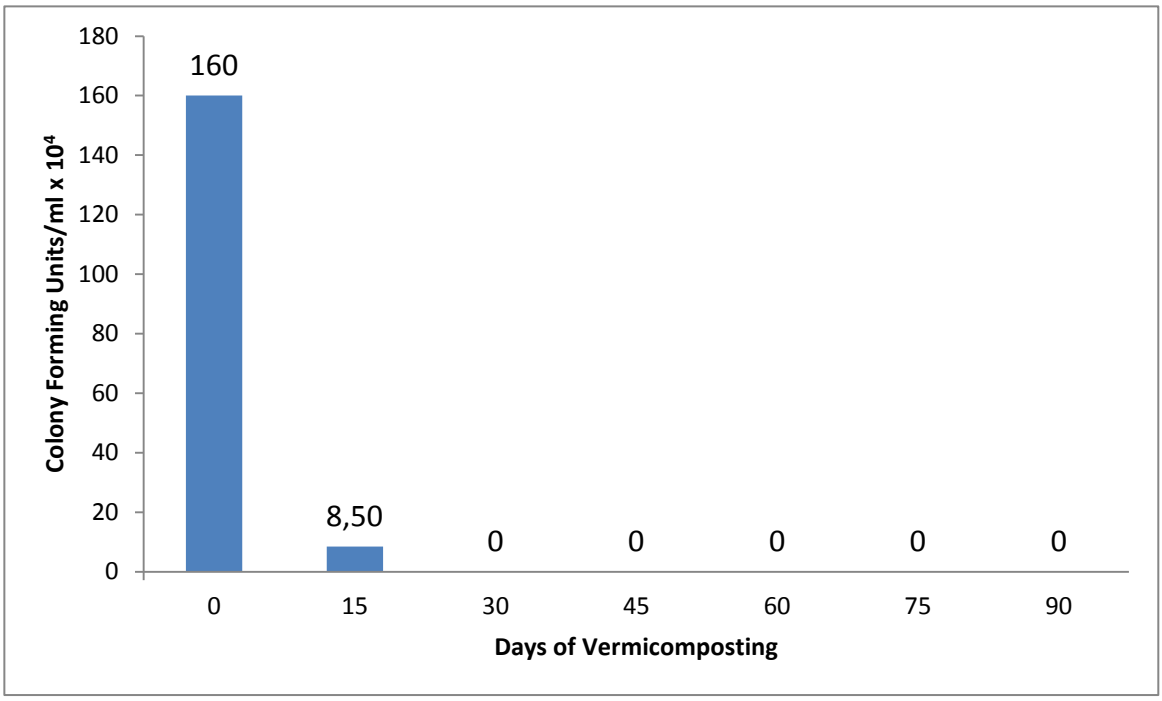


Figure 4.10: *Escherichia coli* trend in vermicompost $V_{15\%}$

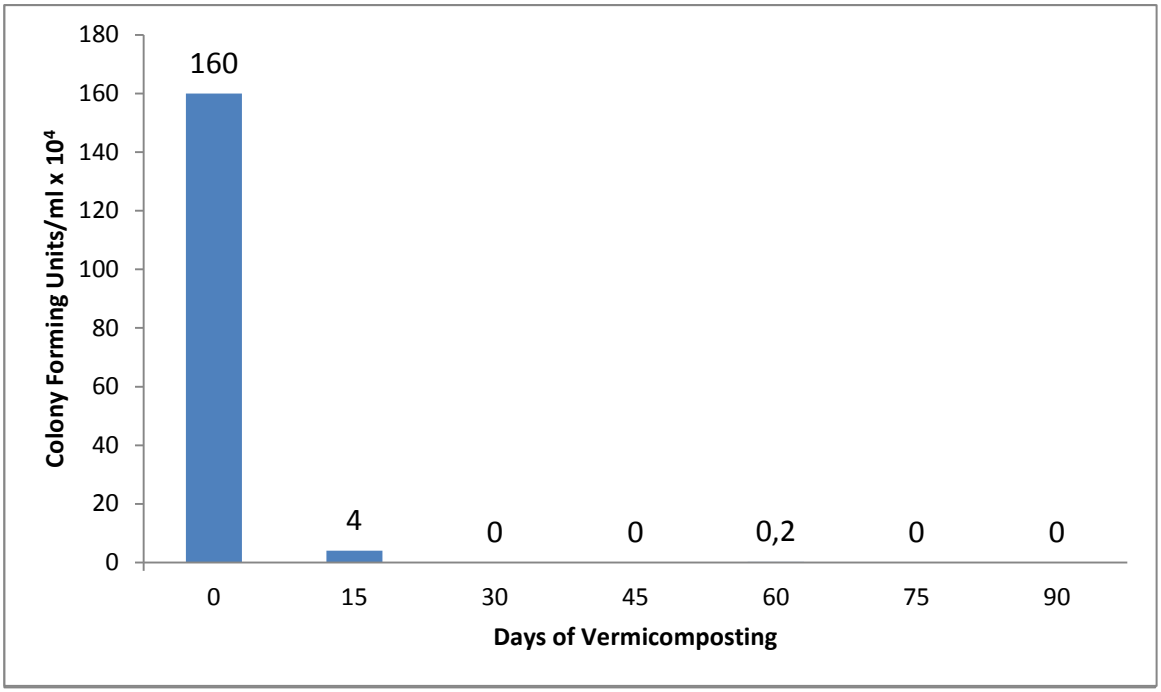


Figure 4.11: *Escherichia coli* trend in vermicompost $V_{C15\%}$

In comparison, *Escherichia coli* decreased more rapidly in the vermicomposts than Coliform bacteria. This may be due to a fact that *Escherichia coli* does not survive for long times outside its fitting environment, such as fresh faces or tract of digestive system.

Greatest decrease in *Escherichia coli* numbers were recorded within the first 15 days of experiment. Vermicompost $V_{15\%}$ showed smaller amount of *Escherichia coli* than $V_{C15\%}$, however the difference is not significant when observing graphical illustrations. In day 60, vermicomposting $V_{C15\%}$ showed 0.2 CFU/ml $\times 10^4$ of *Escherichia coli*; on the other hand, $V_{15\%}$ did not have any positive results on the presence of *Escherichia coli* until end of experiment. Therefore, it can be said that vermicompost $V_{15\%}$ was free of *Escherichia coli* more rapidly than $V_{C15\%}$ that is by day 30, while $V_{C15\%}$ still showed presence of *Escherichia coli* 30 days later.

Escherichia coli levels in $V_{20\%}$ and $V_{C20\%}$ are shown in figures 4.12 and 4.13. Vermicompost $V_{C20\%}$ showed higher levels of *Escherichia coli* in day 15; however by day 30 levels in both vermicomposts were measured to be the same. Trend of decrease of *Escherichia coli* in first 15 days for $V_{20\%}$ is almost identical to decrease in $V_{15\%}$ with difference of 0.5 CFU/ml $\times 10^4$. Both $V_{20\%}$ and $V_{C20\%}$ showed presence of *Escherichia coli* in day 30, while vermicomposts $V_{15\%}$ and $V_{C15\%}$ showed none or close to none *Escherichia coli*. Overall, the difference between $V_{20\%}$ and $V_{C20\%}$ was not evaluated significantly different, since difference between these vermicomposts in day 15 was over only one decimal place.

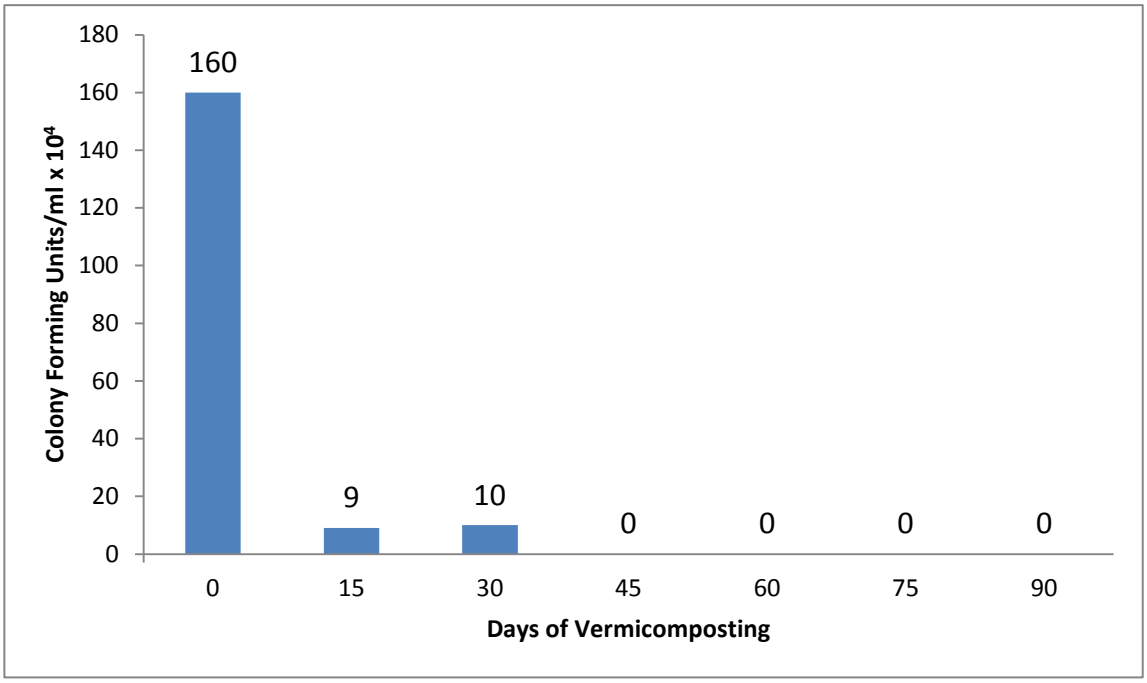


Figure 4.12: *Escherichia coli* trend in vermicompost $V_{20\%}$

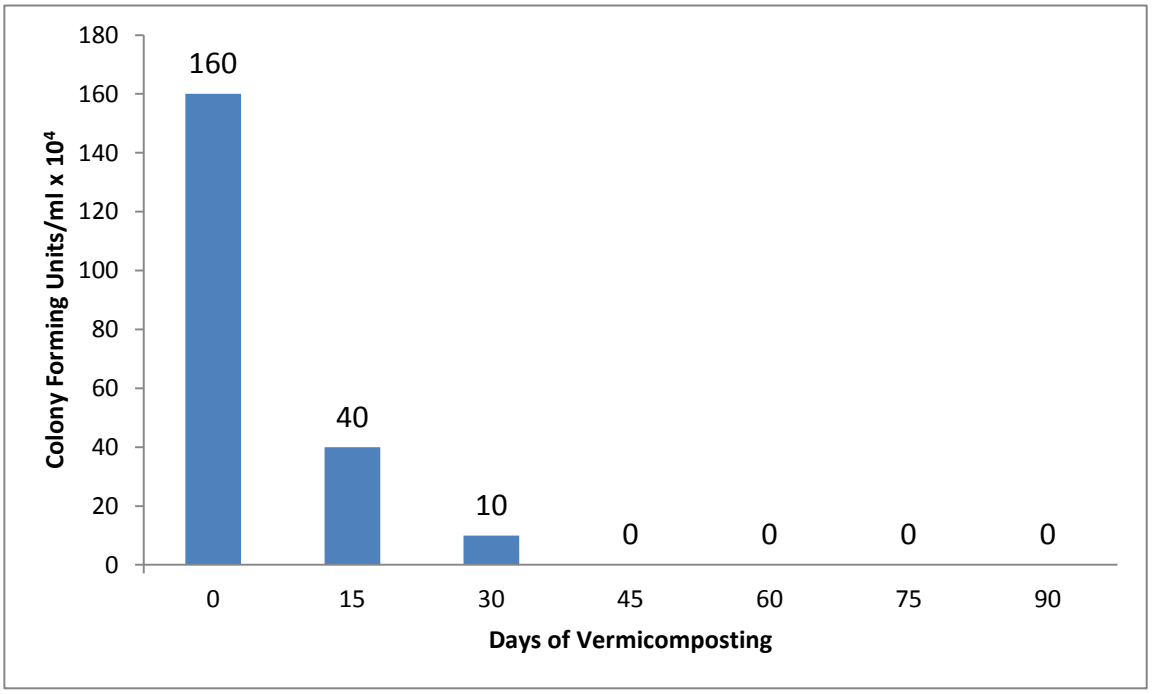


Figure 4.13: *Escherichia coli* trend in vermicompost $V_{C20\%}$

Since many authors describe and emblazon vermicomposting as an effective method in removing pathogens from the mixture, it was surprising to find that results of controls ($V_{C15\%}$, $V_{C20\%}$) and experiments ($V_{15\%}$, $V_{20\%}$) were not statistically significant. Yadav et al. (2009) have vermicomposted source-separated human faces and concluded that vermicomposting completely eliminated total coliforms, receiving value of no total coliforms in end-product while having 5.0

$\times 10^9$ MPN/g levels of total coliforms in default mix. However, the default mix had to be pre-composted in presence of amendment material to make the feed suitable for *Eisenia fetida*, as authors themselves state in conclusion. Pre-composting itself lowers the amounts of pathogenic microorganisms and is very often conduced for this purpose, as Begum and Krishna (2010) did in their experiment. As mentioned in chapter 4.2, many authors use pre-composting pretreatment to stabilize the mixture and make it acceptable for the earthworms, on the other hand this pretreatment lowers the amount of pathogens in default mixture with earthworms completing the pathogen elimination. This is precisely the case of Ressetti et al. (1999) who vermicomposted raw aerobic sludge with sawdust; experiment was initiated by composting stage with *Eisenia eugeniae* being added in maturation stage. Composting stage in this experiment removed pathogens by 93% subsequent vermicomposting resulted in 100% elimination. Thus is can be concluded, that vermicomposting does have an effect on pathogenic elimination, however this effect is not substantial not significant regarding high number of pathogens in the default mix.

Eisenia fetida are said to make the environment more incompatible to pathogens (Kadam et al., 2008). Micro fauna of substrate are affected by transit of the substrate through gut of earthworms through the action of intestinal enzymes (Monroy et al., 2008; Rodríquez-Canché et al., 2010). However authors have verified these facts based on laboratory testings in small-scale experiments and usually with the pre-composting phase included. Aira et al. (2011) mention this phenomenon in their experiment, designing a continuous-feeding vermireactor for cow manure hygienization. They have concluded that earthworms had little effect on total coliforms, yet have reduced *Escherichia coli* to acceptable levels. However authors have stated that effects on pathogens were generally small in terms of physical changes in substrate. This might be the case of experiment conducted for this Thesis, where differences between $V_{15\%}$ and $V_{C15\%}$ and $V_{20\%}$ and $V_{C20\%}$. The overall decrease trend of coliforms and *Escherichia coli* shown in figures 25, 26, 27 and 28 can be explained by a fact, that bacteria of this type have very short life spans in foreign environment and consequently their amounts decrease (Durbin, 2008). In connection with generally little effect of earthworms on pathogenic microorganism described by Aira et al. (2011), little statistical differences between controls $V_{C15\%}$ and $V_{C20\%}$ and experiments $V_{15\%}$ and $V_{20\%}$ respectively in terms of similar decrease of total coliforms and *Escherichia coli* is explicable.

5. Conclusion

Considering all factors of the experiment, combination of amendments, stocking density and characteristics of sewage sludge, it was observed that vermicomposting had little effect on decrease of Coliform bacteria and *Escherichia coli* in specific mix in comparison to controls. Therefore, prior treatment of sewage sludge in terms of pathogen reduction could represent a suitable option to accomplish pathogen stabilization. Various studies have proven this technique to be viable.

Further, it was observed that the maximal concentration of untreated sludge in the substrate for optimal growth of *Eisenia fetida* is 20 %. *Eisenia fetida* could thrive in such mixes without the need of prior treatment of sludge. Vermicompost with 15% of sludge reached biological stabilization in terms of carbon dioxide and ammonia in 90 days unlike the other vermicomposts, generating an environmentally acceptable end-product. However, it is questionable whether this result could be generalized for all sewage sludges. Characteristics of sludge, their content and chemical makeup alternate with contents of wastewater.

It was also observed that process of vermicomposting of sewage sludge is affected by many factors, which combine together to create various outcomes. It is therefore tough to precisely state the conditions and characteristics of these outcomes, considering the complexity of vermicomposting as biological process. Recommendations therefore could be to conduct thorough experiments and observe their outcomes, dependency and effects of all factors and on vermicomposting as a whole.

Overall it was proven that vermicomposting is a technologically-liberated process where biological stabilization of hazardous waste can be achieved. It’s utilization in cases of large-scale treatments could be the goal of future researches.

6. Rereferences

Abida B, Hari K. 2010. Management of Municipal sewage sludge by vermicomposting technique. International Journal of ChemTech Research, 2 (3): 1521-1525.

Adams JDW, Zennaro M, Frostick LE. 2008.Composting of green waste: Observations from windrow trials and bench-scale experiments. Environmental Technology, 29 (11): 1149-1155.

Aira M, Monroy F, Dominquez J. 2007. Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermicomposting independently of the application rates of pig slurry. Science of the Total Environment, 385 (1-3): 252-261

Aira M, Monroy F, Dominquez J. 2007. Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermicomposting independently of the application of pig slurry. Science of the Total Environment, 385 (1-3): 252-261.

Alburquerque JA, Gonzalvez J, Garcia D, Cegarra J. 2006. Composting of solid olive-mill by-product ("alperujo") and the potential of the results compost for cultivating pepper under commercial conditions. Waste Management, 26 (6): 620.626.

Alburquerque JA, McCartney D, Yu S, Brown L, Leonard JJ. 2008. Air space in composting research: a literature review. Compost Science & Utilization, 16 (3): 159-170.

Alexander R, Szmidt R, Cruz P. 2002. Composting biosolids: Factsheet [online]. Glasgow: REMADE Scotland. Available at: www.remade.org.uk (accessed on 12th December, 2012).

An CJ, Huang GH, Yao Y, Sun W, An K. 2011. Performance of in-vessel composting of food waste in presence of coal ask and uric acid. Journal of Hazardous Materials, 203: 38-45

Barker AV. 1997. Composition and uses of compost. ACS Symposium Series, 668: 140.162

Bernal MP, Alburquerque JA, Moral R. 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresource Technology, 100 (22): 5444-5453.

Bouche MB, 1977. Stratégies Lombriciennes. Soil Organisms as Components of Ecosystem, p 122-132.

Cekmecelioglu D, Demirci A, Graces RE. 2005. Application of optimised in-vessel food waste composting for windrow systems. Biosystems Engineering, 91 (4): 479-486.

Changa CM, Wang P, Watson ME, Hoitink HAJ, Michel FC. 2003. Assessment of the Reliability of a commercial Maturity Test Kit for Composted Manures. Compost Science and Utilization, 11 (2): 127-145.

Cooperband LR. 2000. Composting: Art and science of organic waste conversion to a valuable soil resource. Laboratory Medicine, 31 (5): 283-289

Dash MC, Senapati BK. 1986. Vermitechnology, an option for organic waste management in India. In: Dash MC, Senapati BK, Mishra PC (eds) Verms and vermicomposting. Orissa. School of Life Sciences, Sambalpur University, p. 157-172.

Diaz LF, Savage GM, Eggerth LL. 2005. Alternatives for the treatment and disposal of healthcare wastes in developing countries. Waste Management, 25 (6): 626-637.

Dominguez J. 2004. State-of-the art and new perspectives on vermicomposting research. In: Edwards CA (ed). Earthworm ecology. London. CRC Press, p. 401-424.

Dominquez J, Edwards CA. 1997. Effects of stocking rates and moisture content on the growth and maturation of *Eisenia Andrei* in pig manure. Soil Biology and Biochemistry, 29 (3-4): 743-746.

Dominquez J, Edwards CA. 2004. Vermicomposting organic wastes: A review. In: Mikhail WZA, Shakir SH (eds). Soil Animals and Sustainable Development. Cairo, p. 369-395.

Drew D. 2008. Batch composting of human excrement with urban waste products. Senior Honors Thesis. Brown University. Providence.

Edwards CA, Aracon NQ. 2010. The science of vermiculture: The use of earthworms in organic waste management. Ohio: Ohio State University. Available at: http://www.slocountyworms.com/wp-content/uploads/2010/12/THE-SCIENCE-OF-VERMICULTURE.pdf

Eftoda G, McCartney D. 2004. Determining the critical bulking agent requirement for municipal biosolids composting. Compost Science & Utilization, 12 (3): 208-218.

Elvira C, Goicoechea M, Sampedro L, Mato S, Nogales R. 1996. Bioconversion of solid paper-pulp mill sludge by earthworms. Bioresource Technology, 57 (2): 173-177.

Garg P, Gupta A, Satya S. 2006. Vermicomposting of different types of waste using Eisenia fetida: comparative study. Bioresource Technology. 97 (3): 391-395.

Hand P, Hayes WA, Frankland JC, Satchell JE. 1988. The vermicomposting of cow slurry. Pedobiologia, 31 (3-4):199-209.
Hand P, Hayes WA, Frankland JC, Satchell JE. 1988. The vermicomposting of cow slurry. Pedobiologia, 31 (3-4): 199-209.
Haney RL, Brinton WH, Evans E. 2008. Estimating Soil Carbon, Nitrogen, and Phosphorus Mineralization from Short-termed Carbon Dioxide Respiration. Communications in Soil and Plant Analyses, 39 (17-18): 2706-2720.

Harrison ET, Oakes SR, Hysell M, Hay A. 2006. Organic chemicals in sewage sludges. Science of the Total Environment, 367 (1-2): 481-497.

Hong JH, Matsuda J, Ikeuchi Y. 1982. Composting characteristics of mixed dairy manure with bulking agent. Journal of Faculty of Agriculture, 61 (1): 13-43.

Hong JH, Park KJ, Sohn BK. 1997. Effect of composting heat from intermittent aerated static pile on the elevation of underground temperature. Applied Engineering in Agriculture, 15 (5): 679-683.

Howard A. 1943. An Agricultural Testament. Oxford. Oxford University Press, 228p.

Imbeah M. 1997. Composting piggery waste: A review. Bioresource Technology, 63 (3): 197-203.

Jamieson RC, Gordon RJ, Sharples KE, Stratton GW, Madani A. 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. Canadian Biosystems Engineering, 44: 1.1-1.9.

Jenkins J. 1999. Manure Handbook: Second edition. Grove City. Chelsea Green Publishing, 308 p.

Joseph J. 2005. The Manure Handbook: A guide to composting human manure (Third Edition). Grove City. Chelsea Green Publishing, 257 p.

Kadam AM, Oza GH, Nemade PD, Shakar HS. 2008. Pathogen removal from municipal wastewater in constructed soil filter. Ecological Engineering, 33 (1): 37-44.

Kaushik P, Garg VK. 2003. Vermicomposting of mixed solid textile mill sludge and cow dung with epigeic earthworm Eisenia fetida. Bioresource Technology, 90 (3): 311-316.

Kulcu R, Yaldiz O. 2007. Composting of goat manure and wheat straw using pine cones are bulking agent. Bioresource Technology, 98 (14): 2700-2704.

Laio PH, Vizcarra AT, Chen A, Lo KV. 1993. Composting separated solid swine manure. Journal of Environmental Science and Health. Part A, Environmental Science and Engineering, 28 (9): 1889-1901.

Lashermes G, Barriuso E, Le Vollio-Poitrenaud M, Houot S. 2011. Composting in small laboratory pilots: performance and reproducibility. Waste Management, 32 (2): 271-277.

Maboeta MS, Van Rensburg L. 2003. Vermicomposting of industrially produced woodchips and sewage sludge utilizing Eisenia fetida. Ecotoxicology and Environmental Safety, 56 (2): 265-270.

McDowell-Boyer LM, Hunt JR, Sitar N. 1986. Particle transport through porous media. Water Resources Research, 27 (4): 1901-1921.

Ministry of Agriculture and Food. 1996. Composting Factsheet (online). Abbotsford. Available at: http://www.agf.gov.bc.ca/resmgmt/publist/300Series/382500-2.pdf (accessed 3rd January, 2013).

Mitchell MJ, Brown BA, Swift BL. 1978. Effects of Oniscus asellus feeding on bacterial and nematode populations of sewage sludge. Oikos, 30 (1): 90-94.

Monroy F, Aira M, Domínquez J. 2008. Changes in density of nematodes, protozoa and total coliforms after transit through the gut of four epigeic earthworms. Applied Soil Ecology, 39 (2): 127-132.

Nahrul Hayawin Z, Abdul Khalin HPS, Jawaid M, Hakimi Ibrahim M, Astimar AA. 2010. Exploring chemical analysis of vermicompost of various oil palm fibre wastes. Environmentalist, 30 (3): 273-278.

Nannipieri P, Bollag JM. 1991. Use of enzymes to detoxify pesticide-contaminated soil and waters. Journal of Environmental Quality, 20 (3): 510-517.

Ndegwa PM, Thompson SA. 2000. Effect of C-to-N ratio on vermicomposting of Biosolids. Bioresource Technology, 75 (1): 7-12

Ndwega PM, Thompson SA, Das KC. 2000. Effects of stocking density and feeding rate on vermicomposting of biosolids. Bioresource Technology, 71 (1): 5-12.

Pandey A, Soccol CR, Larroche C. 2008. Current developments in solid-state fermentation. New York. Springer Science+Business Media, p.517.

Pescod MB. 1992. Wastewater treatment and use in agriculture. Rome. Food and Agriculture Organizations of the United Nations, p. 254.

Purnomo AS, Koyama F, Mori T, Kondo R. 2010. DDT degradation potential of cattle manure compost. Chemisphere, 80 (6): 619-624.

Raviv M. 2005. Production of high-quality composts for horticultural purposes: A mini-review. HortTechnology, 15 (1): 52-57

Ressetti RR, Soccol T, KasKantzis NG. 1999. Application of vermicompost to control pathogens in composted sewage sludge. Senare, 12 (12): 61-70.

Rodríquez-Canché L, Vigueros LC, Maldonado-Montiel T, Martínez-Sanmiguel M. 2010. Pathogen reduction in septic tank sludge through vermicomposting using *Eisenia fetida*. Bioresource Technology, 101 (10): 3548-3553.

Rynk R. 1992. On-farm composting handbook. Ithaca, New York. Northeast Reagional Agriculture Engineering Service, 204p.

Sangwan P, Kaushik CP, Garg VK. 2008. Vermiconversion of industrial sludge for recycling the nutrients. Bioresource Technology, 99 (18): 8699-8704.

Schaub SM, Leonard JJ. 1996. Composting: An alternative waste management option for food processing industries. Trend in Food Science & Technology, 7 (8): 263-268.

Sen and Chandra, 2007. Chemolytic and solid-state spectroscopic evaluation of organic matter transformation during vermicomposting of sugar industry wastes. Bioresour Technol 98:1680-1683

Shweta KR, Singh BL, Deepshikha V. 2010. Integrating microbial composting and vermicomposting for Effective utilization of by-products of sugar cane-processing industries. Bioremediation Journal, 14 (3): 185.

Singh J, Kaur A, Vig A, Rup PJ. 2010. Role of *Eiseni fetida* in rapid recycling of nutrients from bio sludge of beverage industry. Exotoxicology and Environmental Safety, 73 (3): 430-435.

Singh RP, Embrandiri A, Ibrahim M, Esa N. 2011. Management of biomass residues generated from palm oil mill: Vermicomposting a sustainable option, 55 (4): 423-434

Singh RP, Singh P, Aujo ASF, Ibrahim MH, Sulaiman O. 2011. Management of urban solid waste: Vermicomposting a sustainable option. Resources conservation and recycling, 55 (7): 719-729.

Solvita. 2002. Guide for Solvita testing for Compost Maturity Index. Mt Vernon: Woods End Research Laboratory. Available at: http://www.solvita.co.uk/downloads/solvita_man3.5.pdf (accessed 13th February, 2013).

Solvita. 2013. Compost Maturity Determination. Mt Vernon: Woods End Research Laboratory. Available at: http://solvita.com/compost (accessed 13th February, 2013).

Subramanian S, Sivarajan M, Saravanapriya, S. 2010. Chemical changes during vermicomposting of sago industry soil waste. Journal of Hazardous Materials, 179 (1-3): 318-322.

Suthar S, Singh S. 2008. Comparison of some novel polyculture and traditional monoculture vermicomposting reactors to decompose organic wastes. Ecological Engineering, 33 (3-4): 210-219.

Suthar S. 2008. Vermicomposting of vegetable-market solid waste using *Eisenia fetida*: Impact of bulking material on earthworm growth and decomposition rate. Ecological Engineering, 35 (5): 914-920

Suthar S. 2010. Pilot-scale vermireactors for sewage sludge stabilization and metal remediation process: comparison with small-scale reactors. Ecological Engineering, 36 (5): 703-712.

Tharakan J, Tomlinsons D, Addagada A, Shafagati A. 2006. Biotransformation of PCBs in Contaminated Sludge: Potential Novel Biological Technologies. Engineering in Life Sciences, 6 (1): 43-50.

UNEP. 2005. Solid Waste Management. Osaka. UNEP International Environmental Technology Centre, 524 p.

UNEP. 2005. Solid Waste Management. Rome. Food and Agriculture Organizations of the United Nations, p. 560.

Vig AP, Singh J, Wani SH, Dhaliwal SS. 2011. Vermicomposting of tannery sludge mixed with cattle dung into valuable manure using earthworm *Eisenia fetida* (Savigny). Bioresource Technology, 102 (17): 7941-7945.

Vinceslas-Akpa M, Loquest M. 1997. Organic matter transformations in lignocellulosic waste products composted or vermicomposted (Eisenia fetida andrei): Chemical analysis and 13C CPMAS NMR spectroscopy. Soil Biology and Biochemistry, 29 (3-4): 751-758-

West D, Mangiameli P. 2000. Identifying process conditions in an urban wastewater treatment plant. International Journal of Operations & Production Management, 20 (5): 573 – 590

Wichuk KM, McCartney D. 2011. Compost stability and maturity evaluation – a literature review. Canadian Journal of Civil Enginerring, 37 (11): 1505-1523

Yadav A, Garg VK. 2009. Feasibility of nutrient recovery from industrial sludge by vermicomposting technology. Journal of Hazardous Materials, 168 (1): 262-268.

Yadav A, Garg VK. 2011. Recycling of organic wastes by employing Eisenia fetida. Bioresource Technology, 102 (3): 2874-2880

Yadav A., Garg VK. 2011. Industrial wastes and sludges management by vermicomposting. Reviews in Environmental Science and Biotechnology, 10 (3): 243-276.

Yadav KD, Tare V, Ahammed MM. 2010. Vermicomposting of source-separated human faeces for nutrient recycling. Waste Management, 30 (1): 50-56.

Zajonc I. 1992. Chov žížal a výroba vermikompostu. Povoda. Animapress, p. 59.

Zucconi F, Pera A, De Bertoldi Forte M. 1981. Evaluating toxicity of immature compost. BioCycle, 22 (2): 54-57.

Appendix A – List of Figures and Tables

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