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첨가제를 활용한 인분의 지렁이분퇴비화 개선방안

Improvement of Vermicomposting for Human Feces by adding Additives

2020년 8월

서울대학교 대학원

건설환경공학부

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Improvement of Vermicomposting for Human Feces by adding Additives

By

Le Trong Bang

Supervisor: Professor Mooyoung Han

A dissertation submitted in partial fulfilment of the requirements for the degree of Master in Department of Civil and Environmental Engineering.

Department of Civil and Environmental Engineering

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August 2020

Abstract

Improvement of Vermicomposting for Human Feces by adding Additives

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Urine-diverting dry toilets (UDDTs) is one of the sustainable sanitation systems for human excreta management. In UDDTs, feces and urine are collected and treated separately. Currently, UDDTs are facing many problems relating to odor control, feces and urine treatment, and nutrient loss. Among these problems, feces treatment could be highlighted as the primary concern.

Human feces are considered as natural fertilizer due to the large quantity of nutrients contained within feces which is useful for plants growth. Besides the high nutrient levels, high levels of pathogens were also observed in feces, which can cause diseases if exposed. Thus, before the application of human feces to soil as a fertilizer, it has to be treated to meet the maximum allowable limit of E. coli stipulated in the guideline of WHO, 2006 ($< 3 \log_{10}$ cfu/g dry weight).

One sustainable method of decomposing organic wastes is vermicomposting. Vermicomposting could be defined as the use of earthworms and microorganisms in converting organic matter in organic wastes to soil conditioner. Similarly, since human feces contain a substantial amount of

organic matter, this process could be applied to human feces. However, higher composting time (treatment time) has created a barrier in the adaptation of vermicomposting process for decomposition of human feces in large scale. Thus, in this study, sawdust was considered as a catalyst to reduce the treatment time to improve the vermicomposting process.

This study was aimed at evaluating (1) the feasibility of vermicomposting as a treatment method for source-separated human feces from UDDTs, (2) the effect of sawdust on vermicomposting of the feces to reduce treatment time, (3) observation of the changes in nitrogen forms during vermicomposting of human feces with and without sawdust, and (4) optimizing the addition of sawdust in the process. To achieve the target (1), (2) and (3), four reactors consisting bedding material were designed; blank (F) containing the feces only, one containing the feces and earthworm without sawdust (FV), one containing feces and sawdust without earthworm (FA), and another containing feces, sawdust and earthworm (FAV). Three ratios of feces to sawdust; 1 : 0.5, 1 : 1, and 1 : 2 were considered in reaching target (4).

pH was observed to increase rapidly (up to 8.88 - 8.9) in first two weeks in FA and FAV, then decease slowly until 105th day until pH was in the range of 6.79 - 6.87. Contrastingly, only a marginal reduction of pH was observed (from 8 to 7.25) in FV.

The shortest treatment time of human feces in was observed in FAV (90 days), with volatile solids (VS) stabilized around 45% of total solids (TS) after 75th day. Further, E. coli population (2.73 $\log_{10}$ cfu/g dry weight) was below the

WHO guideline after 90^{th} day. Other reactors without earthworms showed higher amount of VS (62.02 – 80.05 % of TS) and E. coli population (4.42 – 6.57 $\log_{10}$ cfu/g dry weight) even after 105 days of treatment.

However, despite the reduction of VS and E. coli population, a significant nitrogen loss was observed during vermicomposting of human feces. Total dissolved nitrogen (TDN) loss was about 85% after 45 days in FAV, while, 44% of TDN was lost after 105^{th} day in FV.

In addition, nitrogen forms have changed from ammonium to ammonia, nitrate and nitrogen gas form during vermicomposting which is indicated by the changes in NH_4^+/NO_3^-. The NH_4^+/NO_3^- ratio in final product in FAV was observed to be in the range of 0.22 – 0.02 after 75 days while, the ratio in FV was 8.75 after 105 days of treatment.

The optimization of feces to sawdust ratio (1:0.5, 1:1 and 1:2) in vermicomposting showed that the reduction of volatile solids (VS) and E. coli population are independent from the sawdust content. VS reduction was about 41.56 – 45.57 % and E. coli reduction ranged from 4.1 log – 4.5 log under all ratios considered. Similarly, no significant difference in the increase of biomass was observed in all ratios considered (52% – 71%). Thus, the ratio (1:0.5) of feces and sawdust could be recommended in vermicomposting of human feces to minimize volume of vermicomposting reactor for the treatment.

Overall, the results of this study suggest that vermicomposting is a better alternative for treating source-separated human feces. Addition of sawdust to

human feces can be recommended in vermicomposting of human feces to reduce the treatment time and volume of reactor.

Keyword: UDDTs; human feces; vermicomposting; sawdust; treatment time; nitrogen loss.

Student number: 2018-20662

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CHAPTER 1. INTRODUCTION

1.1 Current global sanitation situation

In 2017, WHO/UNICEF Joint Monitoring Program for Water Supply, Sanitation and Hygiene (JMP) reported that, approximately 2.0 billion people still do not have basic sanitation facilities such as toilets or latrines, 673 million people still practice open defecation (Organization, 2019). As a result, annually about 827,000 people in low- and middle-income countries die due to issues pertaining to inadequate potable water, sanitation, and hygiene out of which 432,000 people die as a result of poor sanitation (WHO, 2019).

As shown in Figure 1.1. the percentage of people without access to improved sanitation facilities varies significantly around the world. The regions with the poorest sanitary conditions are low and middle-income areas as Sub-Saharan Africa (51%), Oceania (66%), Least Developed Countries (50%) and Landlocked Developing Countries (49%) (Organization, 2019).

The United Nations (UN) has proposed Sustainable Development Goals (SDGs) in 2015 (UN, 2015) among the proposed 17 SGDs, target 6.2 states the: "By 2030, achieve access to adequate and equitable sanitation and hygiene for all and end open defecation, paying special attention to the needs of women and girls and those in vulnerable situations". This means, an additional 2.0 billion people (26% of global population) will need to be served with improved sanitation by 2030, as showed in Figure 1.2.

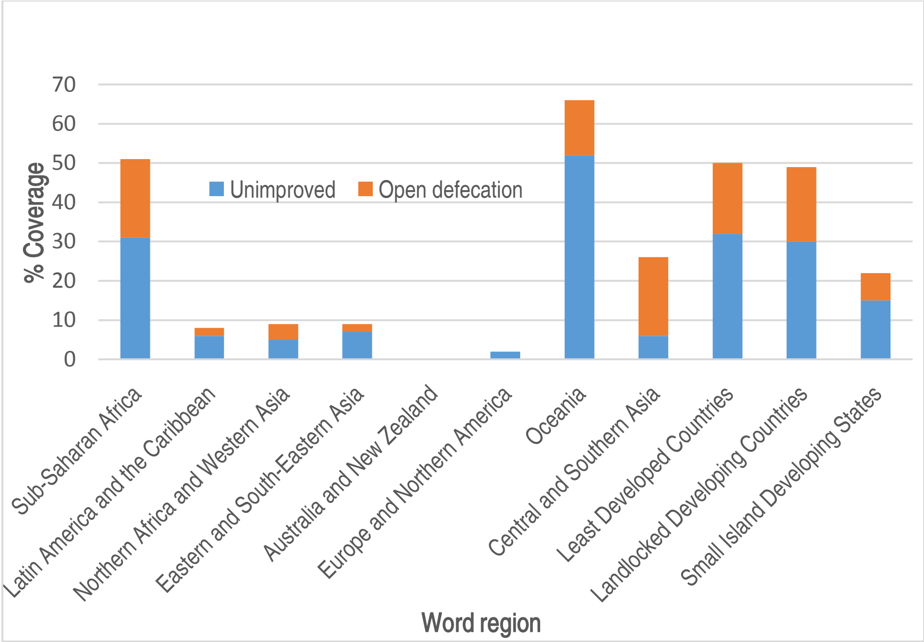

Figure 1. 1 Percentage of people without access to improved sanitation facilities by regions in 2017 (Adapted from WHO/UNICEF, 2019)

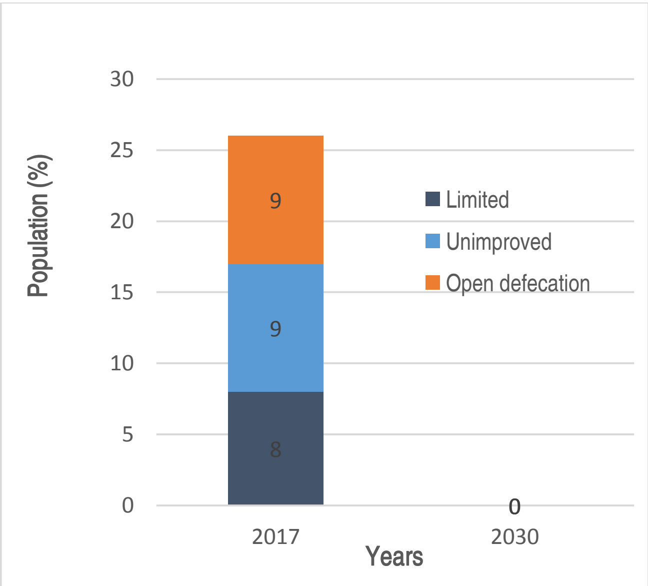

Figure 1. 2 Global sanitation coverage in 2017 and the target for 2030 (Adapted from WHO/UNICEF and UN 2019)

Due to the complexity of the issue, a single approach might not be adequate in the efforts to solve this problem. Combining multiple solutions as centralization and decentralization system, on-site and off-site sanitation are required to achieve this goal. For developing countries, on-site sanitation systems are preferred in solving the sanitation issues in urban and rural areas due to financial and technical feasibility. Septic tank or pit latrines are widely used in developing countries as on-site systems, but do not offer a sustainable solution since such systems could potentially contaminate groundwater.

1.2 Urine-diverting dry toilets (UDDTs)

Urine-diverting dry toilet (UDDT) is one of the sustainable sanitation systems as it provides a multitude of benefits such as waterless operation, low investment cost and operation costs, simple technology for adoptable anywhere (Andreev et al., 2017). In UDDTs, feces and urine are collected and treated separately, and could be easily combined with bulking agents (i.e. sawdust, rice straw, biochar) for on-site co-treatment to recycle nutrients for plants. Population growth, lack access to proper sanitation facilities, and depletion of freshwater bodies and nutrients in soil, have pushed the attention of policy makers and researchers in the application of UDDTs a potential especially, in rural arears of developing countries (Lalander et al., 2013). However, due to issues pertaining to odor control, feces and urine treatment, and nutrient loss, UDDTs were not well received by communities. Among these issues, feces treatment is highlighted by many researches as poorly treated feces could contain of that could cause diseases (Otaki & Kazama, 2019).

(a) (b)

Figure 1. 3 Urine-diverting dry toilets (UDDTs) (a), and sitting toilet (b) squatting toilet

1.3 Treatment objectives of human feces

Treatment method and level to which feces should be treated depend on the end-use of the final product. Generally, the objective is to ensure the protection of human and environmental health (Strande & Brdjanovic, 2014). It is accomplished by reducing of pathogens contained in feces to meet National/International Standard (e.g. WHO (2006); ISO-30500 (2018); NSF/ANSI-41 (2018)) and stabilizing organic matter. In addition, nutrient in the feces needs to be conserved for re-use as fertilizer.

Pathogens die-off: This is the primary objective of treatment of human feces because it contains a lot of microorganisms originating from our digestive system. These microorganisms can be pathogenic for humans if exposed to untreated feces. Thus, human feces need to be treated to a level desirable for the end-use of final product (e.g. discharged to the environment, used in agriculture) (Strande & Brdjanovic, 2014).

Stabilization of organic matter: Most the organic matter in human feces generate odor and are easily decomposed. If the feces are discharged to the environment, it has the potential of contaminating surface water. Thus, stabilization of organic matter in human feces is required, decompose organic matter which decompose slowly (Strande & Brdjanovic, 2014).

Nutrients: Human feces contain significant amount of nutrients, which are essential for plant growth and increasing crop yield. However, if it is not managed properly, it can contaminate groundwater or surface water by infiltration into the soil or due to transport through surface runoff (Strande & Brdjanovic, 2014).

1.4 Current challenges of human feces treatment

Management of human feces could be done on-site and off-site (Buzie-Fru, 2010). On-site sanitation is most preferred in developing countries due to inexpensive nature of the process (Jangam & Pujari, 2019). However, existing on-site sanitation systems have few inherent problems such as lack feces treatment, larger volumes of tanks, high treatment/retention time. According to WHO (2006) recommendations should be stored for at least 1 to 2 years prior to.

The treatment/retention time affects the volume of treatment system (i.e. the volume of feces tank for UDDTs system). Thus, it can affect the feasibility of the system due to substantial initial investment costs. The treatment/retention time is determined by the time required for pathogen die off and stabilization of organic matter. The treatment time required vary with treatment process, as shown in Table 1.1.

Table 1. 1 Treatment time required for different treatment processes

Treatment process	Materials	Treatment time	Reference
Storage	Dry excreta	1 - 2 years	WHO (2006)
Composting (temperature >50ºC)	Sludge + wood chips Manure + sawdust	2.5 – 4 months	Rynk et al. (1992)
Chemical treatment (e.g. Alkaline treatment)	Feces + CaO, pH>9	> 6 months	WHO (2006)
Composting + vermicomposting	Feces + vermicompost + soil	3 – 12 months	Yadav et al., (2012); Yadav et al. (2011)

1.5 Vermicomposting as a sustainable technology for treatment of source-separated human feces

1.5.1 What is vermicomposting?

Vermicomposting is a process in which the organic matter was decomposed to humus by earthworm and microorganisms (Samal et al., 2019). Vermicomposting is considered as biochemical degradation/decomposition process, where organic matter is converted to earthworm biomass and soil (vermicompost). Vermicomposting is a sustainable technology for decomposing organic wastes (Swati & Hait, 2018). This technology is not only rapid, zero waste, and energy saving, but also effective in recycling nutrient and organics (Ahmed et al., 2019; Edwards, 2010; Yadav et al., 2010). Nowadays, vermicomposting has been applied widely in the world because it is safe and (Lee et al., 2018).

As other organic wastes, human feces contain large amount of organic matter which can be degraded by earthworm. The component of vermicomposting of human feces shows as Figure 1.4.

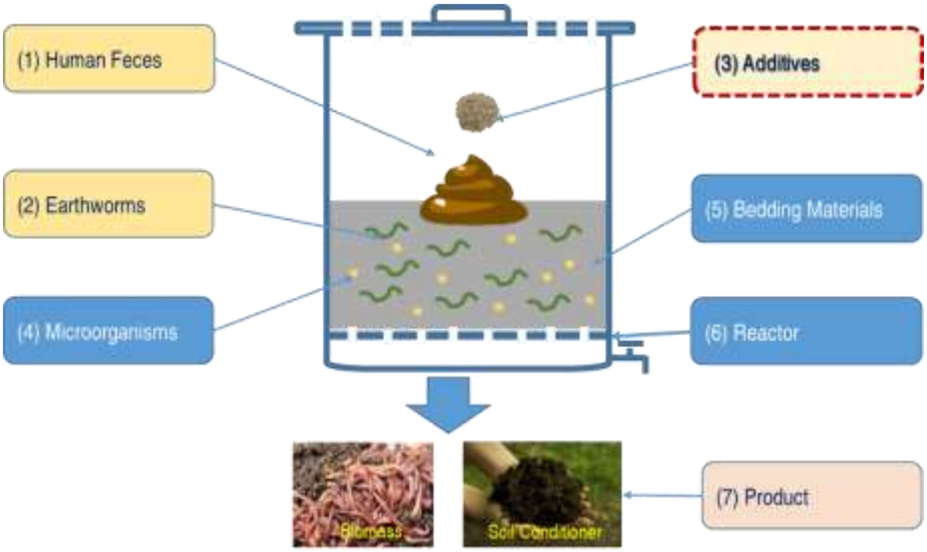

Figure 1. 4 The component of vermicomposting of human feces

1.5.2 Human feces

Human feces are natural organic fertilizers as feces carry nutrients essential for plant growth. Approximately, 130 g (wet weight) of feces on average is produced daily by a person, containing approximately 2.34 g of nitrogen (NH_4^+ -N: 372 mg, NO_3^--N: 126 mg), and phosphorous about 108 mg (Table 1.2) (Funamizu, 2019). Since concentration of heavy metals are relatively low in the feces than in farmyard manure and chemical fertilizer (WHO, 2006; Yadav et al., 2010), it could be used with minimal adverse effects to flora and fauna. However, the pathogens in human feces should be controlled to meet the maximum allowable limit of E. coli (3 log10 cfu/g dry weight) and helminth eggs of treated fecal (1 egg/g dry weight) (WHO, 2006)

Table 1. 2 Characteristics of human feces (Expanded from Funamizu (2019))

Parameter	A average content	Production per capita per day
Moisture	70%	91 g
pH	7.5	-
Total solids (TS)	30%	39 g
Volatile solids (VS)	86.76 %	33.8 g
Total nitrogen (TN)	60.1 mg-N/g dry weight	2.34 g
Total dissolved nitrogen (TDN)	20.69 mg-N/g dry weight	807 mg
Ammonium (NH_4-N)	9.55 mg-N/g dry weight	372 mg
Nitrate (NO_3-N)	3.24 mg-N/g dry weight	126 mg
Phosphate (PO_4-P)	4.5 mg-P/g dry weight	176 mg
E. coli	8.34 log_{10} cfu/g dry weight	325 log_{10}

1.5.3 Earthworm

According to Reynolds and Wetzel (2004), there are more than 8,300 worm species in the world, of which about half are terrestrial earthworms (Dominguez & Edwards, 2011a), only a few are known to be suitable for vermicomposting (Dominguez & Edwards, 2011a; Shalabi, 2006). The species used most commonly in temperate areas are Eisenia fetida and Dendrobena veeta species, while Eudrilus eugeniae and Perionyx excavates are used in tropical areas

(Edwards & Arancon, 2004). Some aspects of the biology of Eisenia fetida and Perionyx excavates species are illustrated on table 1.3., and the life-cycles of Eisenia fetida is showed on Figure 1.5.

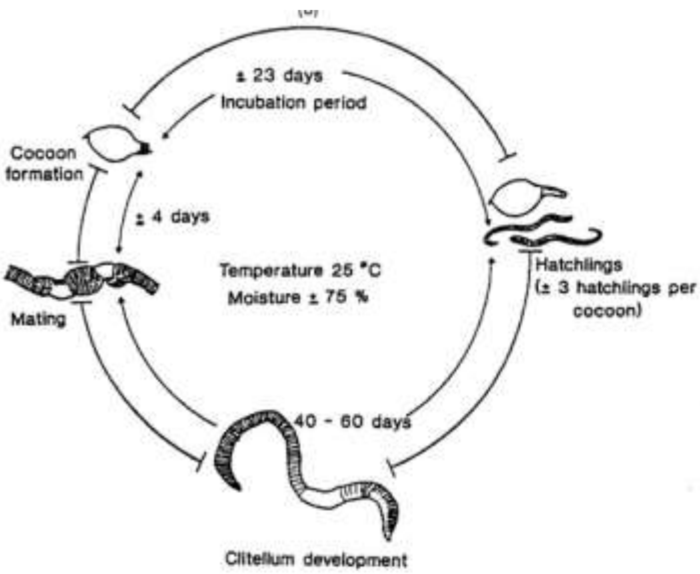

Figure 1. 5 The life-cycles of Eisenia fetida species (Venter & Reinecke, 1988)

Although there is a synergistic effect of earthworms and microorganisms in stabilizing organic matter in vermicomposting, earthworms are observed to be crucial drivers of the process (Dominguez & Edwards, 2011b). The contribution of earthworm in this process is physical/mechanical (i.e. substrate aeration, mixing, as well as actual grinding) and biochemical (i.e. microbial decomposition of the substrate in the digestive system of the earthworms) (Ndegwa & Thompson, 2000).

Table 1. 3 Some aspects of the biology of Eisenia fetida and Perionyx excavates species (Expanded from Dominguez and Edwards (2011a))

Content	Temperate species	Tropical Species
	Eisenia fetida	Perionyx excavates
Photo		
Color	Brown and buff bands	Reddish brown
Size of adult earthworms	4 – 8 mm x 50 – 100 mm	4 – 5 mm x 45 – 70 mm
Mean weight of adults	0.55 g	0.5 g– 0.6 g
Time to maturity (days)	28-30	28 - 42
Number of cocoons day^{-1}	0.35 - 5	1.1 – 1.4
Incubation time (days)	18 -26	18
Hatching viability (%)	73 - 80	90
Number of worms $cocoon^{-1}$	2.5 – 3.8	1 – 1.1
Life cycle (days)	45 - 51	40 - 50
Limits and optimal temperature	0 °C - 35 °C (25 °C)	9 °C - 37 °C
Limits and optimal moisture	70% – 90% (80% – 85%)	75% - 85%

1.5.4 Additives (bulking agent)

Several mineral, organic or biological additives can be used to enhance composting and vermicomposting process (Bernal et al., 2009; Onwosi et al., 2017). Due to abundancy, it is common to use an organic additive in developing countries in UDDs systems. There is a wide range of organic additives such as rice straw, sawdust, mature composts, grass clippings, biochar, etc., When choosing an organic additive, attention must be paid to C/N ratio of the initial mixtures to preserve a safe operating condition for microorganisms and prevent nitrogen leaching during composting/vermicomposting (Barthod et al., 2018; Doublet et al., 2011). The optimum C/N ratio in composting are in the range of 20-30. The amount of additive can be reduced when it contains high C/N ratio initially.

When adding an organic material, the natural microbial community could be enhanced during composting and vermicomposting process through the changes in the aeration, temperature, moisture, pH, etc. (Barthod et al., 2018) (Figure 1.6). Thus, the time of stabilization of organic matter could be reduced.

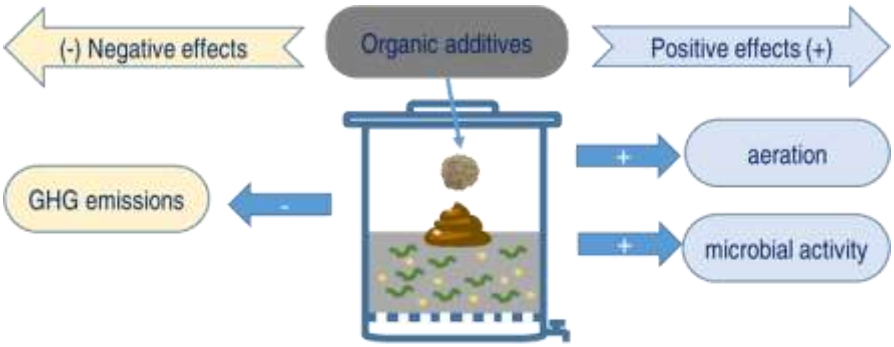

Figure 1. 6 The potential negative and positive effects of organic additives.

1.5.5 Microorganisms

During vermicomposting process, microorganisms support the breakdown of organic matter with earthworm (Gómez-Brandón et al., 2011). Microorganisms create the enzymes to help decompose organic matter, but earthworm are the crucial driver of the process as they are involved in the indirect simulation of microbial populations through fragmentation and ingestion of the substrate. Earthworms reduce overall microbial biomass and activity during vermicomposting. Earthworms' activity helps microbial communities use the available energy efficiently and plays a key role in shaping the structure of the microbial communities in organic waste during the vermicomposting process (Dominguez, 2011). The effects of earthworms on microbial biomass and activity is shown in Figure 1.7.

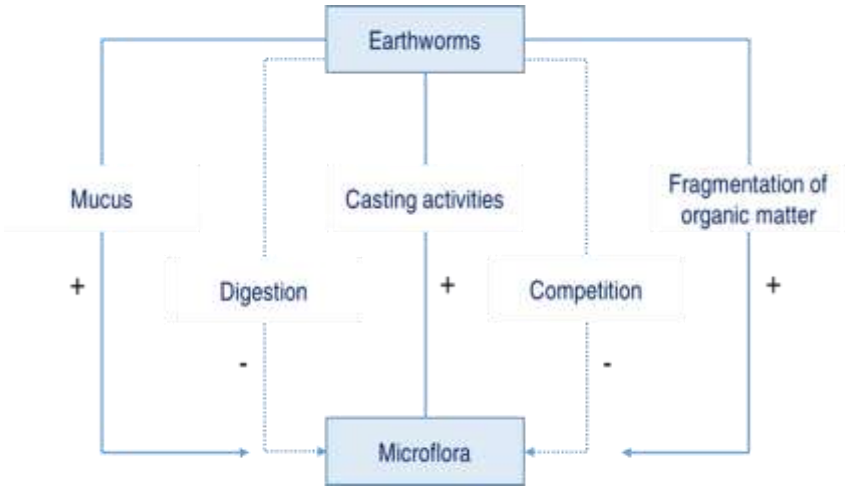

Figure 1. 7 Positive (+) and negative (-) effects of earthworms on microbial biomass and activity (Dominguez, 2011)

1.5.6 Bedding material

Bedding material is needed for enrichment of the diversity of microbial population and enzymatic activities (Yadav et al., 2010). As a result, it is considered as “bio-seed” to support the initial microorganism colonies in the vermicomposting process. In addition, bedding material can support earthworms to survive if extreme environmental conditions rise in the substrate such as high temperature, ammonium, salt, pH, etc. Generally, mature vermicompost is used as a bedding material.

1.5.7. Reactor

Reactor used in vermicomposting process is called “vermi-reactor”, where earthworms live, grow and decompose the organic waste. Proper design of vermi-riactor is crucial to facilitate an optimal environment for earthworms to grow. Volume of storage, method of harvesting product, humidity and temperature control are the factors considered when designing a vermi-reactor.

Figure 1. 8 Vermi-reactor sample

1.5.8 Product

Final product of vermicomposting process includes two parts: biomass (earthworm protein) and soil (considered as soil conditioner). Earthworm protein is used by the animal feed industry, while soil conditioner is used in improving poor soil and plant growth media.

1.6 Review of research issues

Several studies have demonstrated the feasibility of using this technology for source-separated feces. Shalabi (2006) reported the suitability of the feces as a substrate by using different earthworm species. Buzie-Fru (2010) found the ideal moisture content (65% - 80%) and temperature (20 - 25 ^{o}C) for vermicomposting of feces. Yadav et al. (2010) have investigated the feasibility of vermicomposting when vermicompost and soil were used as bulking/supporting material.

Yadav et al. (2010) has highlighted the need of supporting materials through his observation of earthworms dying out within an hour of introducing human feces with without supporting materials. Further, substrate material with high salt and ammonia has observed to affect negatively in vermicomposting (Dominguez & Edwards, 2011a). Therefore, the bulking material should have a lower salt and ammonia content. Sawdust is a common organic bulking agent for composting, and have been used as a bulking agent in vermicomposting a variety of wastes such as sewage sludge and bovine manure (Rockenbach De Almeida et al., 2018; Suleiman et al., 2017). However, no studies could be found assessing the combined effect of sawdust and source-separated feces in vermicomposting.

1.7 Objective of the study

The overall study objective is to investigate the feasibility of applying vermicomposting technology on human feces management. More specifically, the study evaluates the effect of addition of additives (sawdust) on vermicomposting of source-separated human feces, focusing on reducing treatment time, which is investigated observing time required for stabilization of organics matter and die-off of E. coli. Besides that, changes in nitrogen forms during the vermicomposting process was also investigated. The detailed breakdown of objectives of this study are as followings:

(1) Investigate the effect of vermicomposting for human feces treatment

Focusing on:

- The feasibility of vermicomposting for human feces treatment
- Observation of the changes in nitrogen forms during the process.

(2) Investigate the effect of sawdust on human feces treatment by vermicomposting

Focusing on:

- The effect of adding sawdust into the vermicomposting to reduce treatment time
- Observation of the impact of adding sawdust on the changes in nitrogen forms during the process.

(3) The optimization mixing ratio of human feces with sawdust in vermicomposting process

Focusing on:

- Investigate the requirement treatment time for the vermicomposting in different ratio of feces and sawdust (1 : 0.5, 1 : 1 and 1 : 2)
- Survey of biomass of earthworm in the different ratio

1.8 Dissertation structure

This dissertation consists of 5 chapters. Chapter 1 describes the current state of sanitation in the world, the challenges of human feces treatment, current state of vermicomposting of human feces, research objective, and dissertation structure. Chapter 2 shows experimental result observed in this study. Chapter 3 focuses on improving vermicomposting of the feces by adding additives (sawdust in this study) to reducing the treatment time. Chapter 4 investigates the optimum mixing ratio of human feces and sawdust. Chapter 5 summarizes the results of this study and discuss areas that needs further investigation. The structure of this dissertation is shown in Figure 1.9.

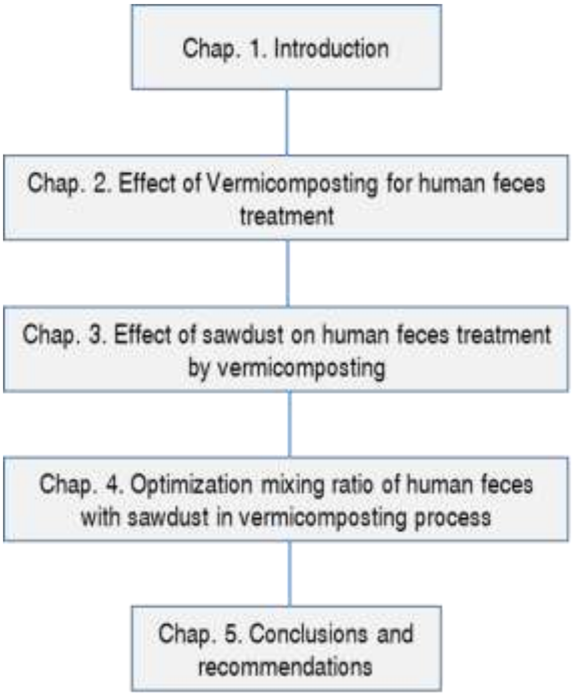

Figure 1. 9 Dissertation structure

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CHAPTER 2. INVESTIGATE THE EFFECT OF VERMICOMPOSTING FOR HUMAN FECES TREATMENT

2.1 Objectives

The objective of this chapter were to prove the feasibility of vermicomposting technology as treatment method for source-separated human feces from urine-diverting dry toilets (UDDTs). The aim was achieved through comparing the operation of three reactors; one contains earthworm and feces, one contains earthworm, feces and sawdust, and other one contains feces only (considered as control). The effect of the process was assessed via the treatment time (i.e. reduction of volatile solids (VS) and E. coli population). In addition, the changes in nitrogen forms during the process was observed.

2.2 Material and methods

2.2.1 Raw substrates, earthworm, sawdust and bedding material

Fresh human feces were collected daily for 2 months from a urine-diverting dry toilet (UDDT) at the rooftop of building 35, Seoul National University, South Korea and were stored in a plastic container at the rooftop. Feces were mixed thoroughly prior to analysis and usage in different experiments.

Earthworm species *Eisenia fetida* were used in this experiment. The earthworms were collected from Earthworm Agricultural Research Center, South Korea. Earthworms were acclimatized for human feces before the commencement of the experiment (2 to 3 months) to avoid sudden change of earthworm habitat by introducing human feces with low organic loading rate and increasing over time. Earthworms were chosen randomly from the culture reactor.

Sawdust was collected from the local farm garden at Seoul, Korea, and was dried at room temperature and sieved using 6mm sieve prior to the experiment.

Bedding material was used as a supporting material for earthworms to survive in case extreme environmental conditions prevail. In addition, bedding material is also needed in enhancing the diversity of microbial and fungal population (Li et al., 2020; Yadav et al., 2010). In this experiment, mature vermicompost was used as bedding material. It was collected from stock culture reactors at Earthworm Agricultural Research Center, South Korea. The composition of bedding material, human feces and sawdust is presented in Table 2.1.

Table 2. 1 Initial physico-chemical characteristics of human feces, sawdust and bedding material in the experiment (mean ± SD, n=4)

Parameter	Human feces	Sawdust	Bedding material
pH	7.45 ± 0.12	6.54 ± 0.23	6.96 ± 0.13
Total solid (%)	30.18 ± 4.49	93.85 ± 1.57	45.13 ± 4.62
Volatile solid (% of total solid)	86.76 ± 0.34	79.27 ± 2.80	25.2 ± 2.48
Total dissolved nitrogen (mg-N/g dry weight)	20.69 ± 4.53	0.18 ± 0.03	0.37 ± 0.06
Ammonium (mg-N/g dry weight)	9.55 ± 2.37	0.09 ± 0.05	0.015 ± 0.007
Nitrate (mg-N/ g dry weight)	3.24 ± 3.49	0.07 ± 0.02	0.30 ± 0.06
E.coli (log_{10} cfu/g dry weight)	8.34 ± 0.25	Not detected	< 2

2.2.2. Reactors

PVC plastic rectangular reactors (vermi-reactor) (25 cm x 25 cm x 15 cm), with 0.038 m^2 top surface area were used for this experiment (Figure 2.1). All the vermi-reactors were covered by plastic lids to maintain a dark environment within the reactor. Several five mm diameter perforations on the lid were made to facilitate ventilation and gas exchange. Also, several five-mm diameter perforations on the bottom of the reactors were made to allow water to be drained if a high moisture content develop in the vermi-reactors. Each vermi-reactor was introduced with a substrate and 1.5 kg of bedding material (wet basic). The substrate and bedding material were separated by a plastic mesh (10mm x 10mm). A geotextile layer was put on the bottom of vermi-reactors to prevent the bedding material breaking throughout with water.

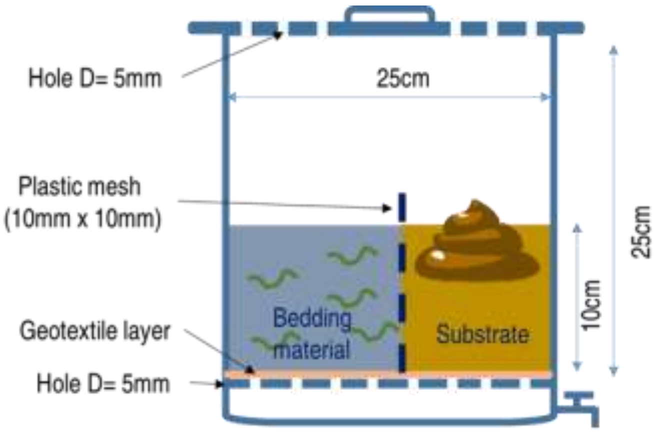

Figure 2. 1 Vermi-reactor used in this study

2.2.3 Experimental setup

Three sets of reactors were prepared namely; F (without earthworm and sawdust), FV (with earthworms and without sawdust), and FAV (with earthworms and sawdust), the composition of which are presented in Table 2.2. F (without earthworm and sawdust) reactors are considered as control of this study. The human feces were mixed with sawdust at a ratio of 1:1 (v/v) and were considered as final substrate for FV and FAV reactor. Then, 100 earthworms, having ≈14.5 g biomass, were released into each reactor (FV and FAV). The day of earthworm injection was considered as day zero.

All the reactors were kept at room temperature during winter (23 ± 3 ºC). The moisture content of each reactor was maintained at 65-80% throughout the duration of the experiment by covering the bin with a fiber cloth layer (Domínguez et al., 2018). Samples from the reactors were collected at 15 days' interval, and each sample was collected uniformly from every reactor. All the analysis was done on wet weight basis. The experiment was repeated three times for each set of reactor.

Table 2. 2 Composition of substance in vermicomposting reactors

Composition	Vermicomposting reactors code		
	F	FV	FAV
Human feces (g)	800	800	800
Earthworms (number)	-	100	100
Sawdust (ml)	-	-	800

(-) without that composition

2.2.4 Physico-chemical analysis

Analyses were done immediately upon sample collection and when this was not possible, samples were stored in the refrigerator at 4 ºC to prevent any changes in the properties of the sample. Analysis for pH of each sample were determined in extracts of 1:10 (w/v) feces soil : distilled water ratios (Yadav & Garg, 2009). The amounts of total solids (TS) were measured after keeping in an oven at 105 ± 2 ºC for 24 h until a constant weight was observed (Mengistu et al., 2017). Volatile solids (VS) was measured using the method described by Yadav et al. (2011). For determination of total dissolved nitrogen (TDN), ammonium-nitrogen (NH_4^+ -N) and nitrate-nitrogen (NO_3^- -N), firstly the diluted samples (1:10 (w/v) samples: distilled water ratio) were mixed using a magnetic stirrer for 2 h at 250 rpm. Then, the solids and liquid was separated by a centrifuge operating at 15,000 rpm for 15 minutes. The liquid part was used for the analysis after filtering it through a 0.45 μm membrane. Finally, the analysis was done using the HUMAS UV/Visible spectrometer, Model HS-3300 (HUMAS, Korea). The concentration of TDN was derived using the measured value by dry weight obtained from TS.

2.2.5 E. coli analysis

The samples were collected and analyzed for E. coli the same day. Two to three grams of samples from each reactor were mixed with 10 ml of distilled water by using magnetic stirrer for 10 minutes at 250 rpm. All the equipment and solutions used for the analysis i.e. backers and distilled water, were autoclaved at 121 ^{o}C for 15 minutes. Serial dilutions (a dilution with 10 times) were made. M-TEC ChromoSelect Agar (Sigma-Aldrich) were used for this analysis (EPA, 2002). Then, all the plates were incubated 35 ± 0.5 ^{o}C for 2 ± 0.5 h, then, transferred to incubator at constant temperature of 44.5 ± 0.2 ^{o}C for 22 ± 2 h. The number of E. coli were expressed as $\log_{10}$ cfu (colony forming unit)/ g dry weight sample and the mean value was calculated.

2.2.6 Statistical analysis

All statistical analyses of the experiment were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). The difference between the reactor and the control samples for observed parameters were marked significant if *P* value was less than 0.05.

2.3 Results and discussion

2.3.1 pH variation during vermicomposting

Initially, the pH of all reactors were similar (7.47 ± 0.20) but, at the end of the process (105th day) pH ranged from 6.87 ± 0.31 to 7.34 ± 0.42 as shown in Figure 2.2. The pH value after 105 days for vermicomposts (6.87 – 7.25) were within the best pH rage for plants (6 -8.5) (Esmaeili et al., 2020), and also for worms (5-9) (Dominguez & Edwards, 2011). During first 30 days, pH in FAV was observed to be significantly different compared to F (*P<0.05).* pH in FAV increase rapidly (up to 8.90 ± 0.21) than F during first 15 days. While pH in FV was increase slightly compared to F. This suggest that the increase of pH could be possibly due to the activity of earthworm and microorganism or the addition of sawdust. The changes in pH could be due to decomposition of organic matters and generation of intermedia products such as ammonium ions (NH4+) during vermicomposting process (Yadav et al., 2010). After 15th day, the decrease of pH from peak pH level in FAV (from 8.90 ± 0.21 to 6.87 ± 0.31, respectively) was significant (P<0.05). The result could be explained by the release of H^+ ion during the nitrification process (Cáceres et al., 2016). Several study have reported similar results as Devi and Khwairakpam (2020); Gupta and Garg (2008); Gusain and Suthar (2020); Komilis and Ham (2006).

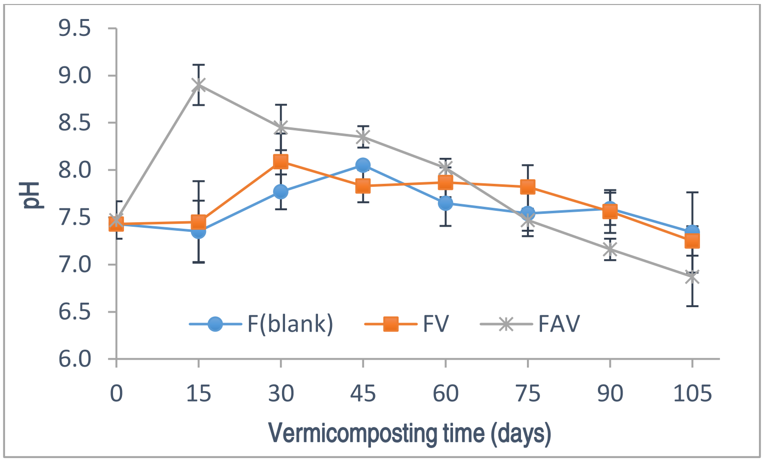

Figure 2. 2 Variation of pH in different reactors during vermicomposting. Mean values (mean ± SD, n=3)

2.3.2 Evaluation of the effect of vermicomposting of human feces to reduce treatment time

2.3.2.1 Volatile solid (VS) changes

Loss of volatile solids (VS) is an important indication for evaluating the decomposition and stabilization of organic matter during vermicomposting process. A significant reduction (P<0.05) in VS was observed in FV and FAV after 105 days of the treatment (Figure 2.3). The percentage reduction of VS in FAV and FV are 41.57% and 27.14%, respectively. This suggest that the organic matter has undergone decomposition and mineralization processes within these reactors (Dastpak et al., 2020). Compared with blank (F), the reduction of VS in FV was significant (*P<0.05*), suggesting that earthworms can considerably increase the decomposition rate of organic matter during vermicomposting process through bioconversion of the substrate material by digestion (Domínguez et al., 2019; Huang et al., 2014; Jjagwe et al., 2019; Suthar et al., 2017). Further, the reduction of VS in FAV were also significant (*P<0.05)* compared to FV, reduced by 1.83 times. Similar results were observed by Hanc and Dreslova (2016). This indicates that the decomposition rate of organic matter during vermicomposting process can be enhanced with the addition of sawdust. Generally, the human feces have been decomposed significantly during vermicomposting process (i.e. in FV and FAV).

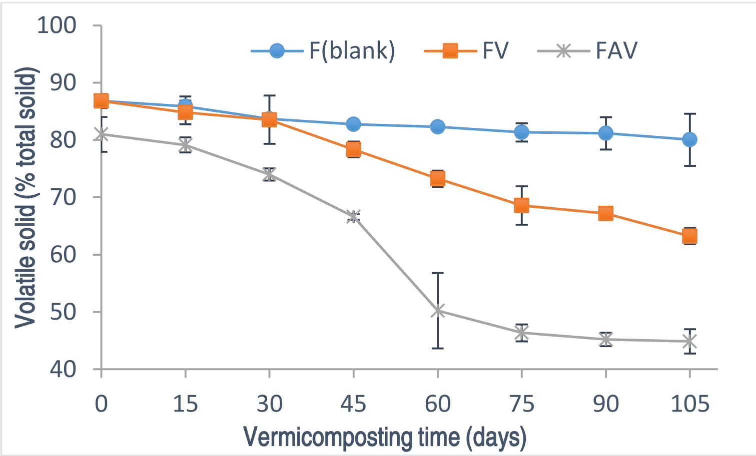

Figure 2. 3 Variation of volatile solid in different reactors during vermicomposting. Mean values (mean ± SD, n=3)

2.3.2.2 E. coli inactivation

WHO (2006) has stipulated a maximum allowable concentration of E. coli in the treated feces must be below than 1000 cfu/g dry weight for safe use in agriculture. E. coli in substrate continuously decreased in all reactors despite the presence of worms (Figure 2.4). The decrease of E. coli after 105 days of treatment in FV was statistically significant (*P<0.05*), decreased by 0.95 $\log_{10}$

cfu/g, in comparison with blank (F). Procházková et al. (2018) by the addition of Eisenia andrei in vermicomposting of apple pomace waste, have demonstrated that E. coli removal could be enhanced. In addition, compared with FV, the decrease of E. coli of vermicomposts in FAV was significant (*P<0.05*), decreased by 2.55 log_{10} cfu/g. This suggest that die-off of E. coli could be propelled by the addition of sawdust in vermicomposting process. The reduction of E. coli during vermicomposting could be attributed to the enzymatic activity of earthworms and the antagonistic interactions of the endosymbiotic microbes by earthworm (Sen & Chandra, 2009; Swati & Hait, 2018). Furthermore, E. coli of vermicomposts in FAV was well under the guideline of WHO (2006) after 90^{th} day of vermicomposting while the population of E. coli of composts in FV was higher than the maximum allowable limit even after 105 days of treatment (Figure 2.4). This suggest that, removal of E. coli in the vermicomposting process was not effective in the vermicomposting reactor with feces only.

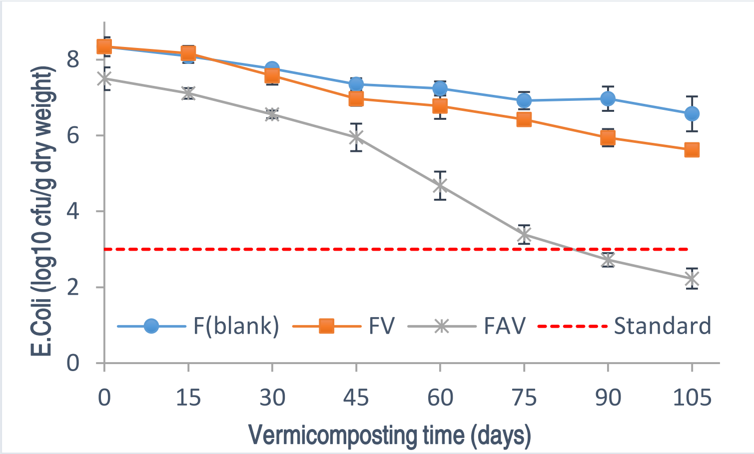

Figure 2. 4 Variation of E. coli concentration in different reactors during vermicomposting. Mean values (mean ± SD, n=3)

2.3.3 Evaluation of the changes of nitrogen forms during vermicomposting of human feces

2.3.3.1 Changes in Total Dissolved Nitrogen (TDN)

The decrease of total dissolved nitrogen (TDN) of the final product in all reactors were significant compared with the initial TDN of in each reactor ($P<0.05$) (Figure 2.5) and reduction of TDN during vermicomposting is a common phenomenon. The highest reduction of TDN was observed in FAV (85.58%) while the lowest was observed in F (25.49%). Compared with F and FV, the decrease of TDN of compost in FAV were significantly larger ($P<0.05$) by a factor of 1.47 – 2.54 times. This indicates that nitrogen in human feces could be significantly reduced by adding sawdust. This might be due to decomposition and transformation of organic nitrogen into inorganic forms which leads to a loss through ammonium evaporation under high pH conditions and denitrification process (Pisa et al., 2020; Yang et al., 2020). This phenomenon could be the underlying reason behind the significant reduction rate of TDN of composts in FAV in first 60 days compared with other reactors. Conversely, after 60 days, no statistically significant (*P>0.05*) increment was observed in TDN of compost in FAV. The rise of TDN after 60 days may be possibly due to decomposition and transformation of insoluble organic nitrogen to either inorganic or soluble organic nitrogen forms through microbial activity and earthworm digestion (Esmaeili et al., 2020; Li et al., 2020). In addition, the decomposition of organics in human feces by joining the activity of earthworm and microorganism also contributed to TDN reduction. This result was demonstrated via significant difference of TDN reduction between F and FV reactor.

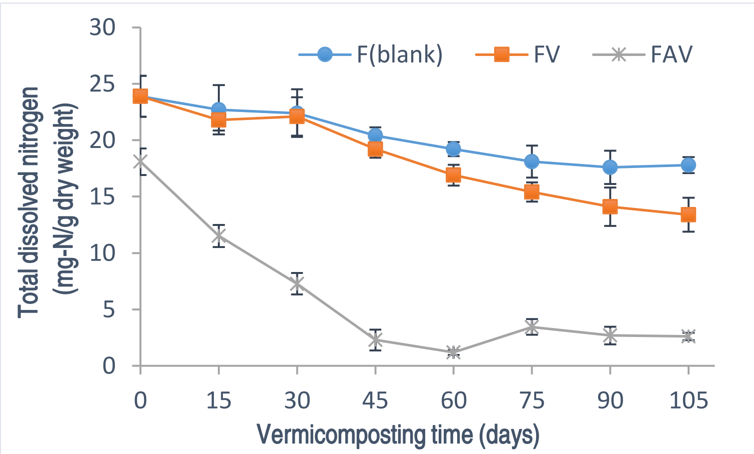

Figure 2. 5 Variation of total dissolved nitrogen concentration in different reactors during vermicomposting. Mean values (mean ± SD, n=3)

2.3.3.2 Changes in NH_4^+:NO_3^- ratio

The initial NH_4^+:NO_3^- ratio of substrate in all reactors were high as the initial NH_4^+ content of the substrate (Figure 2.6) (F ≈FV=11.24 mg-N/g dry weight and FA ≈ FAV= 9.61 mg-N/g dry weight) was greater than NO_3^- content (F ≈FV=0.56 mg-N/g dry weight and FA ≈ FAV= 0.65 mg-N/g dry weight). The reduction of NH_4^+:NO_3^- ratio of composts were statistically significant ($P<0.05$) for all reactors when compared with the initial ratio of each reactor. Furthermore, the reduction rate of NH_4^+:NO_3^- ratio of composts in FAV in first 45 days were significantly higher than other reactors ($P<0.05$). High NH_4^+ concentration in substrate under a high pH environment, could induce NH_4^+ ion loss through NH_3 gas generation (Yang et al., 2020). In addition, the reduction of the NH_4^+:NO_3^- ratio could be due to nitrification reactions in compost and vermicompost product (Yang et al., 2020). The reduction of NH_4^+:NO_3^- ratio in FV was significantly different (P<0.05) compared with bank (F). This indicated that the reduction of NH_4^+:NO_3^- ratio during vermicomposting process could be promoted by earthworm.

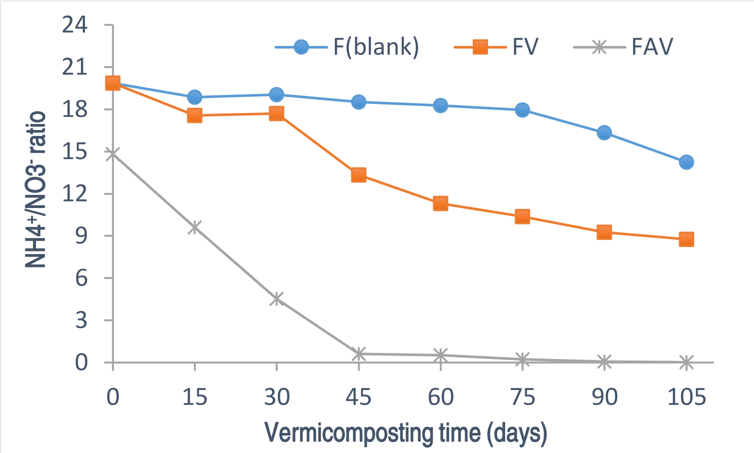

Figure 2. 6 Variation of NH_4^+:NO_3^- ratio in different reactors during vermicomposting. Graph with mean value.

2.4 Summary

In this chapter, the vermicomposting was demonstrated to be efficient techniques for treating source-separated human feces from urine-diverting dry toilets (UDDTs). The primary role of earthworm in the decomposition of organic matters and reduction of E. coli population in human feces during vermicomposting were demonstrated. The experimental results showed that the reduction of volatile solids (VS) in vermicomposting ranges 27% - 44%, while 2.7 log – 7.3 log of E. coli population has been reduced in the process. In addition, significant loss of nitrogen during vermicomposting of human feces was recorded. The formation of nitrate ion (NO_3^-) from ammonium ion (NH_4^+) in final product also was indicated in the experiment.

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CHAPTER 3. INVESTIGATE THE EFFECT OF SAWDUST ON HUMAN FECES TREATMENT BY VERMICOMPOSTING

3.1 Objectives

The objective of this chapter were to investigate, (1) the effect of sawdust on vermicomposting of source-separated human feces, (2) the impact of the addition of sawdust on the changes nitrogen forms during the treatment. Three reactors; one contains feces and sawdust, one contains earthworm, feces and sawdust, and other one contains feces only (considered as control) were prepared in this experiment for accomplishing the aims.

3.2 Material and methods

Raw substrates, earthworm, sawdust and bedding material, reactor, analysis of physico-chemical, E. coli and statistics are described as in chapter 2

3.2.1 Experimental setup

Three sets of reactors were prepared namely; F (without earthworm and sawdust), FA (with sawdust and without earthworm), and FAV (with earthworms and sawdust), the composition of which are presented in Table 3.1. F (without earthworm and sawdust) and FA (with sawdust and without earthworm) reactors are considered as control of this study. The human feces were mixed with sawdust at a ratio of 1:1 (v/v) and were considered as final substrate for FA and FAV reactor. Then, 100 earthworms, having ≈14.5 g biomass, were released into FAV reactor. The day of earthworm injection was considered as day zero.

Table 3. 1 Composition of substance in vermicomposting reactors

Composition	Vermicomposting reactors code		
	F	FA	FAV
Human feces (g)	800	800	800
Earthworms (number)	-	-	100
Sawdust (ml)	-	800	800

(-) without that composition

3.3 Results and discussion

3.3.1 pH variation during vermicomposting

pH was in the ranges of 7.43 ± 0.03 – 7.47 ± 0.20 in initial mixtures, and that changed insignificantly to 6.87 ± 0.31 – 7.25 ± 0.16 at the end of the process (105^{th} day) as shown in Figure 3.1. The pH value after 105 days for vermicomposts (6.87 – 7.25) were within the best pH rage for plants (6 -8.5) (Esmaeili et al., 2020), and also for worms (5-9) (Dominguez & Edwards, 2011). During first 30 days, pH in FA and FAV was observed to be significantly different compared to F ($P<0.05$). pH in FA and FAV increase rapidly (up to 8.88 ± 0.13 and 8.90 ± 0.21, respectively) than F during first 15 days. This suggest that the increase of pH could be possibly due to the addition of sawdust. Sawdust (an organic additive) can stimulate the microbial activity by improving aeration (Barthod et al., 2018), leading to the creation of amines and ammonium during the mineralization and decomposition of organic nitrogen (García-Sánchez et al., 2017; Mengistu et al., 2017) which could increase the pH. After

15th day, the decrease of pH from peak pH level in FA and FAV (from 8.88 ± 0.13 and 8.90 ± 0.21 to 6.79 ± 0.34 and 6.87 ± 0.31, respectively) was significant (*P*<0.05). The result could be explained by the release of H^+ ion during the nitrification process (Cáceres et al., 2016).

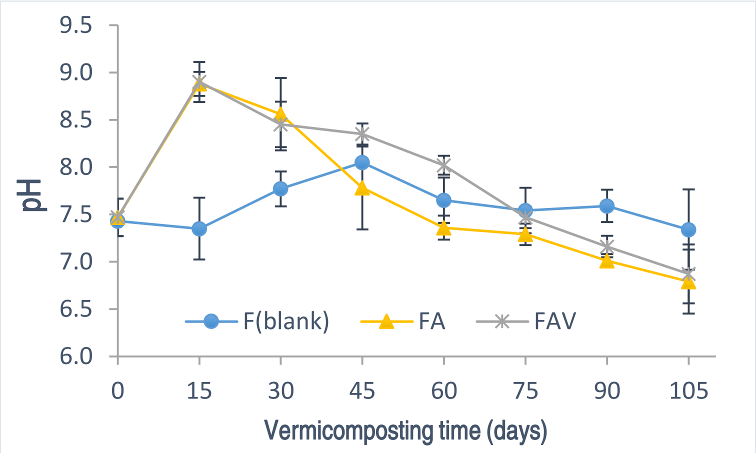

Figure 3. 1 Variation of pH in different reactors during vermicomposting.

Mean values (mean ± SD, n=3)

3.3.2. Evaluation of the effect of adding sawdust on vermicomposting of human feces to reduce treatment time

3.3.2.1. Volatile solid (VS) changes

A significant reduction (*P*<0.05) in VS was observed in FA and FAV after 105 days of the treatment (Figure 3). The percentage reduction of VS in FAV and FA are 41.57% and 23.43%, respectively. This suggest that the organic matter has undergone decomposition and mineralization processes within these reactors. Compared with blank (F), the decrease of VS in FA was significant (*P<0.05*), by 3.07 fold. This illustrates that the addition of sawdust could significantly enhance the decomposition rate of organic matter during composting process by stimulating the microbial activity through improved aeration (Barthod et al., 2018). Further, the reduction of VS in FAV were also significant (*P<0.05)* compared to FA, reduced by 1.77 times. The result suggests that earthworms can considerably increase the decomposition rate of organic matter during vermicomposting process through bioconversion of the substrate material by digestion (Domínguez et al., 2019; Huang et al., 2014; Jjagwe et al., 2019; Suthar et al., 2017). Similar results were observed by Hanc and Dreslova (2016). This indicates that the decomposition rate of organic matter during vermicomposting process can be enhanced with the addition of sawdust.

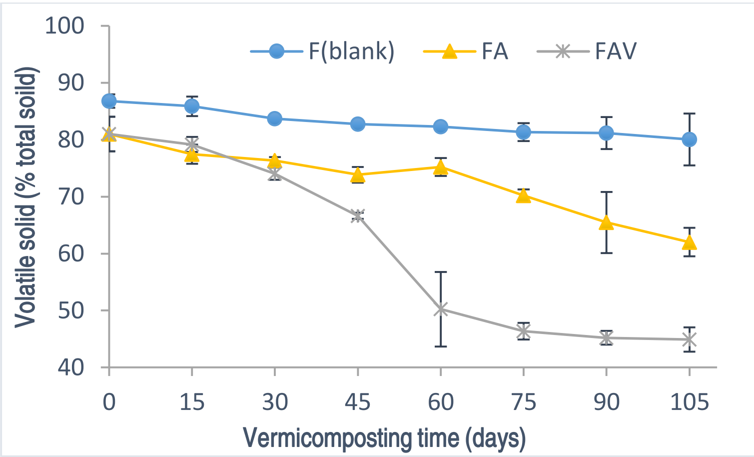

Figure 3. 2 Variation of volatile solid in different reactors during vermicomposting. Mean values (mean ± SD, n=3)

In addition, the reduction of VS in FAV was statistically insignificant from 75th day to 105th day (46.36 ± 1.46%, 45.21 ± 1.19% and 44.90 ± 2.13%, respectively) (*P>0.05*). It could be due to stabilization of VS in FAV. However, the value of VS in other reactors (F and FA) were still high and unstable (81.34 ± 1.59% and 70.19 ± 1.09%, respectively). This suggest that, by adding sawdust, the time required for stabilization of organic matter by vermicomposting could be reduced.

3.3.2.2 E. coli inactivation

E. coli in substrate continuously decreased in all reactors despite the presence of worms (Figure 3.3). The decrease of E. coli after 105 days of treatment in FA was statistically significant ($P<0.05$), decreased by 1.31 $\log_{10}$ cfu/g, in comparison with blank (F). Sossou et al. (2014) by the addition of sawdust, charcoal and rice husk in composting have demonstrated that E. coli removal could be enhanced. In addition, compared with FA, the decrease of E. coli of vermicomposts in FAV was significant ($P<0.05$), decreased by 2.55 $\log_{10}$ cfu/g. This suggest that reduction of E. coli population could be improved by earthworm in vermicomposting process. The reduction of E. coli during vermicomposting could be attributed to the enzymatic activity of earthworms and the antagonistic interactions of the endosymbiotic microbes by earthworm (Sen & Chandra, 2009; Swati & Hait, 2018). Furthermore, E. coli of vermicomposts in FAV was well under the guideline of WHO (2006) after 90th day of vermicomposting while the population of E. coli of composts in F and FA were higher than the maximum allowable limit even after 105 days of treatment (Figure 3.3). This suggest that, removal efficiency of E. coli in the vermicomposting process could be by the adding sawdust.

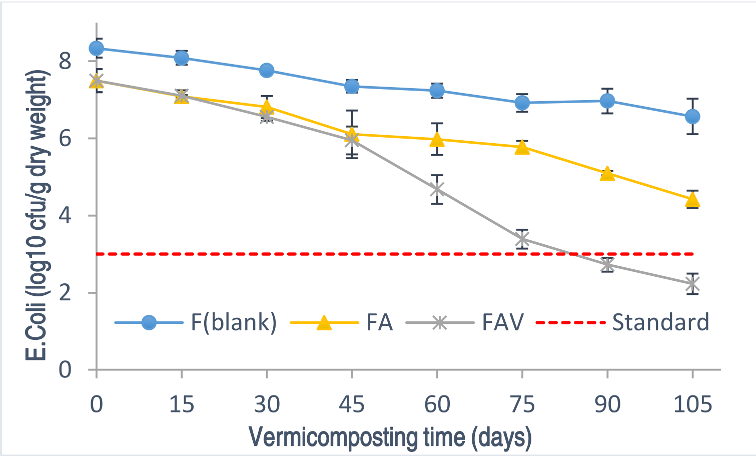

Figure 3. 3 Variation of E. coli concentration in different reactors during vermicomposting. Mean values (mean ± SD, n=3)

3.3.3 Evaluation of the impact of adding sawdust on the changes of nitrogen forms during vermicomposting of human feces

3.3.3.1. Changes in Total Dissolved Nitrogen (TDN)

The decrease of total dissolved nitrogen (TDN) of the final product in all reactors were significant compared with the initial TDN of in each reactor ($P<0.05$) and reduction of TDN during composting/vermicomposting is a common phenomenon. The highest reduction of TDN was observed in FAV (85.58%) while the lowest was observed in F (25.49%). Compared with F, the decrease of TDN of compost in FAV and FA were significantly larger ($P<0.05$) by a factor of 2.52 and 2.32, respectively. This indicates that nitrogen in human feces could be significantly reduced by adding sawdust. This might be due to decomposition and transformation of organic nitrogen into inorganic forms which leads to a loss through ammonium evaporation under high pH conditions and denitrification process (Pisa et al., 2020; Yang et al., 2020). This phenomenon could be the underlying reason behind the significant reduction rate of TDN of composts in FAV and FA in first 60 days compared with other reactors. Conversely, after 60 days, no statistically significant (*P>0.05*) increment was observed in TDN of compost in FAV and FA. The rise of TDN after 60 days may be possibly due to decomposition and transformation of insoluble organic nitrogen to either inorganic or soluble organic nitrogen forms through microbial activity and earthworm digestion (Esmaeili et al., 2020; Li et al., 2020). Overall, most of the nitrogen is lost (85%) during vermicomposting of human feces using sawdust as bulking agent. Hu et al. (2013) reported that 91.66% of nitrogen was lost during hot composting of human feces using fresh sawdust as a bulking agent.

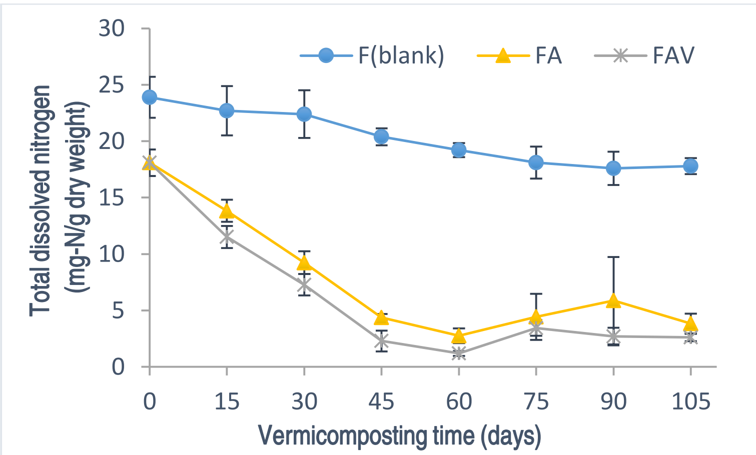

Figure 3. 4 Variation of total dissolved nitrogen concentration in different reactors during vermicomposting. Mean values (mean ± SD, n=3)

3.3.3.2 Changes in NH4+:NO3- ratio

The reduction of NH_4^+:NO_3^- ratio of composts were statistically significant (*P<0.05*) for all reactors when compared with the initial ratio of each reactor. Furthermore, the reduction rate of NH_4^+:NO_3^- ratio of composts in FAV and FA in first 45 days were significantly higher than other reactors (*P<0.05*). High NH_4^+ concentration in substrate under a high pH environment, could induce NH_4^+ ion loss through NH_3 gas generation (Yang et al., 2020). Further, addition of sawdust could encourage the reduction of the NH_4^+:NO_3^- ratio through mineralization of organic nitrogen (García-Sánchez et al., 2017), transforming NH_4^+ to NH_3 gas. In addition, the reduction of the NH_4^+:NO_3^- ratio could be due to nitrification reactions in compost and vermicompost product (Yang et al., 2020). Bernai et al. (1998) and Gong et al. (2018) have suggested that a mature product is achieved when NH_4^+:NO_3^- ratio less than 0.16. In this study, the final vermicompost product in FAV (NH_4^+:NO_3^- =0.02) was mature while FV (NH_4^+:NO_3^- =8.75) and FA (NH_4^+:NO_3^- =0.19) could not attain maturity.

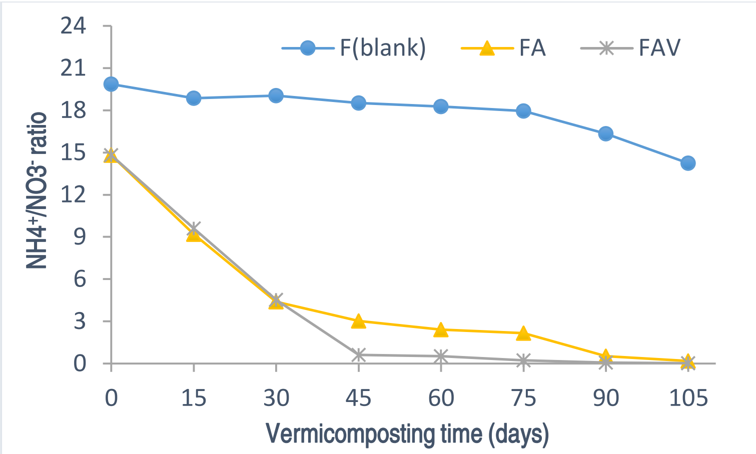

Figure 3. 5 Variation of NH4+:NO3- ratio in different reactors during vermicomposting. Graph with mean value.

3.4 Summary

By adding sawdust into the human feces, the treatment time of vermicomposting process could be shortened (completed after 90 days). Adding saw dust in the vermicomposting process could enhance the VS stabilization by 53%. Further, addition of sawdust in vermicomposting has shown an additional removal of E. Coli by more than 2.5 log magnitudes. In addition, rate of nitrogen loss during vermicomposting of human feces was observed to be controlled by initial ammonium (NH_4^+) content and pH and the nitrogen loss rate in vermicomposting was higher when sawdust was present. Overall, the use of sawdust in vermicomposting process could be recommended to reduce the treatment time of human feces.

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CHAPTER 4. THE OPTIMIZATION MIXING RATIO OF HUMAN FECES WITH SAWDUST IN VERMICOMPOSTING PROCESS

4.1 Objectives

The objective of this chapter was to find the optimum mixing ratio of feces and sawdust in vermicomposting of human feces. The best ratio of feces to sawdust, which can minimize the treatment time and volume of vermicomposting reactor. The reduction of volatile solids (VS) and E. coli population, with the increase of biomass were used for detecting the best ratio for vermicomposting of human feces.

4.2 Experiment setup

Three sets of reactors with different human feces and sawdust ratio (feces: sawdust = 1:0.5, 1:1 and 1:2 (w/v)) were prepared for this experiment, the composition of which are presented in Table 4.1. Human feces were mixed with sawdust at these ratios and were considered as final substrate. Then, 100 earthworms, having approximately 27 g biomass, were released into each bin. The day of earthworm injection was considered as day zero. Samples from the reactors were collected at 0^{th} and 80^{th} day. Each sample was collected uniformly from every reactor. All the analysis was done on wet weight basis. The experiment was repeated two times for each set of reactor.

Table 4. 1 Composition of substance in vermicomposting reactors

Composition	Ratio of human feces and sawdust		
	1 : 0.5	1 : 1	1 : 2
Human feces (g)	800	800	800
Earthworms (weight(g)/number)	27.79/100	27.46/100	27.06/100
Sawdust (ml)	400	800	1600

4.3 Physical analysis

In terms of physical analysis, total solids (TS) and volatile solids (VS) were observed in this experiment. The protocols used in measurement of these parameters are described as in chapter 2.

4.4 E. coli analysis

This parameter was analyzed as described in chapter 2.

4.5 Biomass analysis

All vermicomposting reactors were manually examined at 0^{th} (initial) and 80^{th} (final) day of the treatment for survival and growth of earthworms by total weight and population of earthworms.

4.6 Results and discussion

4.6.1 The effect of different ratio on treatment time of vermicomposting

4.6.1.1 Volatile solids (VS) changes

After vermicomposting, Volatile solids (VS) content decreased significantly in all the reactors (Figure 4.1). Loss in VS content was in the range of 41.56% - 45.57% in vermicomposting reactors with different ratios. Maximum VS loss was observed in the reactor with 1: 0.5 feces: sawdust ratio. The result suggests that the easily biodegradable organic matter was gradually exhausted during the process by joint activity of earthworms and microorganisms (Esmaeili et al., 2020; Sharma & Garg, 2018; Yadav & Garg, 2011). Earthworm create mucus and enzymes that stimulate the activity of microorganisms (Pérez-Godínez et al., 2017).

According to Figure 4.1, the reduction of VS in all the vermicomposting reactors was not statistically significant (*P*>0.05). This indicated that volatile solids in the organic matter may not be significantly increased with the increase of sawdust content into vermicomposting of human feces.

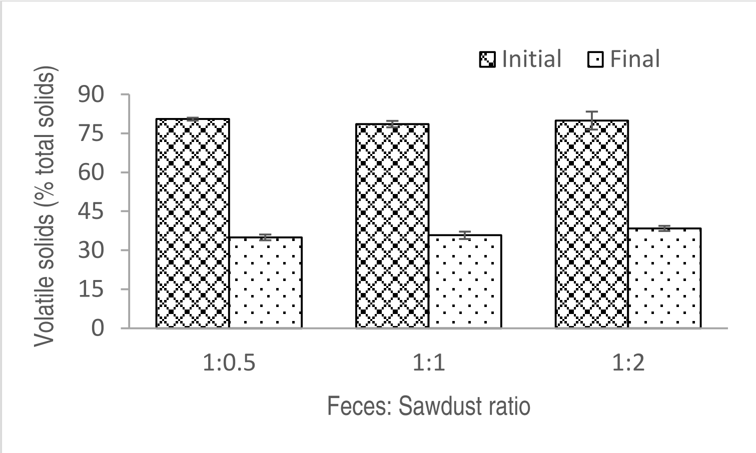

Figure 4. 1 Initial and final volatile solids in different treatment ratios of feces: sawdust (1:0.5, 1:1 and 1:2)

4.6.1.2 E. coli inactivation

E. coli reduced in all vermicomposting reactors compared to initial population (Figure 4.2). There was 4.1 log – 4.5 log reduction of E. coli in reactors by the end of the vermicomposting. The maximum E. coli reduction was found in the reactor which had a ratio of 1: 0.5. The reduction of E. coli in vermicomposts could be attributed to the enzymatic activity of earthworms and the antagonistic interactions of the endosymbiotic microbes by earthworms (Sen & Chandra, 2009; Swati & Hait, 2018).

Also, Figure 4.2 indicate that these reduction of E. coli population during vermicomposting was not statistically significant(P>0.05). This demonstrated that the reduction of E. coli population in final vermicomposting product may not be affected by increasing the sawdust content.

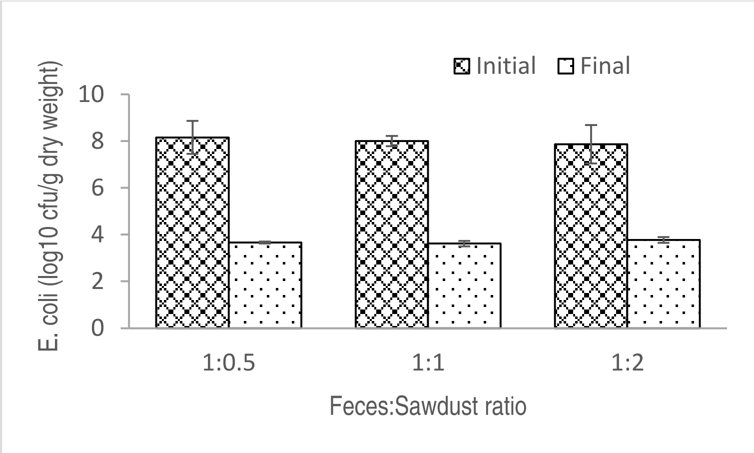

Figure 4. 2 Initial and final E. coli population in different treatment ratios of feces: sawdust (1:0.5, 1:1 and 1:2)

4.6.2 The effect of different ratio on biomass and amount of earthworm

4.6.2.1 Total biomass growth

The initial earthworm weight ranged between 26 and 29 g and increased to 42 – 46 g after 80 days of vermicomposting (Figure 4.3). The weight of earthworms in vermicomposting reactors was in the following order: 1:2 > 1:1 > 1:0.5. Results indicate that increase of earthworm weight about 1.52-, 1.65-, and 1.71-folds in ratios of 1: 0.5, 1:1, and 1:2, respectively. The increase in earthworms' biomass may be explained by the conversion of digested organic fraction of the substrate to biomass (Hanc & Dreslova, 2016; Suthar, 2008; Yadav & Garg, 2019).

In addition, compared with between different vermicomposting reactors, the increase of total biomass of these reactors were insignificant (*P*>0.05). This result suggests that the increase of sawdust content into vermicomposting of human feces may not impact on the increase of earthworm biomass.

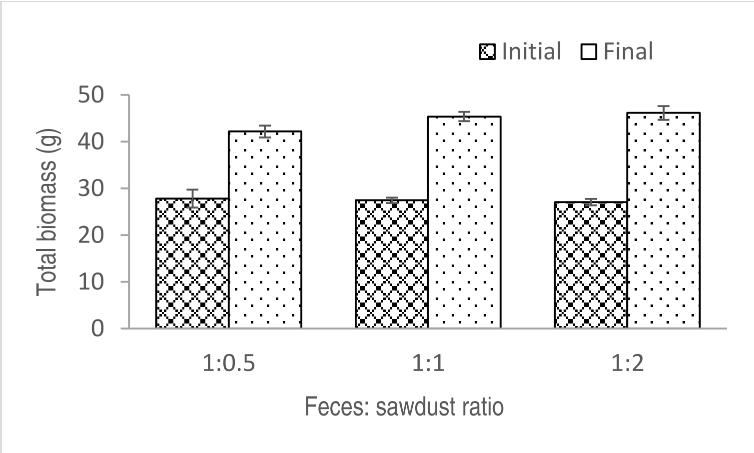

Figure 4. 3 Initial and final total biomass in different treatment ratios of feces: sawdust (1:0.5, 1:1 and 1:2)

4.6.2.2 Population of earthworm

According to Figure 4.4, The population of earthworms in different vermicomposting reactors have significantly increased (P<0.05) compared with the initial population. The maximum earthworm population was recorded in the reactor with the ratio of 1:2 (154) followed by, 1:1 (151) and 1: 0.5 (142). This increment of earthworm population may be due to presence of some earthworms' cocoons in bedding material.

The population at the end showed an insignificant difference among different vermicomposting reactors (P>0.05). This means that the population of earthworms may not be affected by increasing the sawdust content.

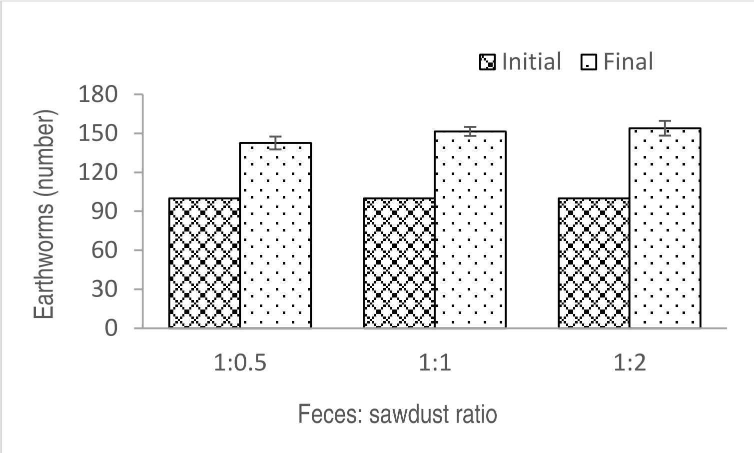

Figure 4. 4 Initial and final earthworm population in different treatment ratios of feces: sawdust (1:0.5, 1:1 and 1:2)

4.7 Summary

The experiment results suggest that the reduction of volatile solids (VS) and E. coli population, and the increase of total earthworms' biomass and population in all vermicomposting reactor, were significant, indicated by the reduction of VS range from 41.56% to 45.57%, E. coli population from 4.1 logs to 4.5 logs while the increase of total earthworms' biomass range between 52% and 71 %, earthworms' population from 42% to 54% in all treatment. However, the effects of increasing the sawdust content into human feces on growth, development, and reproduction of earthworm, and also reducing the treatment time in the vermicomposting process, was not investigated. Thus, the ratio of feces and sawdust (1: 0.5) could be recommended in the vermicomposting of human feces for reducing the volume of the vermicomposting reactor.

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CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Human feces are considered as natural fertilizer due to the large quantity of nutrients contained within feces which is useful for plants growth. Besides the high nutrient levels, high levels of pathogens were also observed in feces, which can cause diseases if exposed. Thus, before applying human feces to the soil, it has to be treated to meet the maximum allowable limit of E. coli as suggested in the guideline of WHO (2006).

One sustainable method of decomposing organic wastes is vermicomposting. Vermicomposting could be defined as the use of earthworms and microorganisms in converting organic matter in organic wastes to soil conditioner.

Human feces are toxic to earthworms due to large amount of ammonium and salt present in feces, which make earthworms die in a short time if worms are introduced directly to fresh feces. Thus, earthworms are acclimatized or the physical characteristics of human feces are modified.

The treatment time of human feces is the amount of time required to stabilize the organic matter and reduce the indicator for pathogens to meet a standard. In this study, volatile solids (VS) and E. coli were used as indicators of stabilization of organic matter, and pathogens die-off in final product.

However, higher composting time (treatment time) has created a barrier in the adaptation of vermicomposting process for decomposition of human feces in large scale. Thus, in this study, sawdust was considered as a catalyst to reduce the treatment time to improve the vermicomposting process.

This study investigates the effect of vermicomposting process for source-separated human feces and improvement of the process by the addition of sawdust. Evaluation of the effect of different ratios of feces to sawdust (1:0.5, 1:1 and 1:2) on reducing the treatment time and the growth of biomass. Moreover, the changes of nitrogen forms were observed in vermicomposting of human feces with and without sawdust.

pH during vermicomposting is a main environmental factor influencing the survival of earthworms and the quantity of the final product. Most of the earthworms are relatively tolerant to pH ranges between 5 and 9. The best pH range for plants was 6 to 8.5.

In the present study, pH increase rapidly (up to 8.9) during first two weeks then decease slowly until 105th day while, pH range of 8 to 7.25 in vermicomposting without sawdust were recorded.

Volatile solids (VS) is a common indicator of stabilization of organic matter. The result suggest that the stabilization of VS is approximately 45 % of TS after 75 days of treatment by vermicomposting with sawdust as a catalyst. Unstable and high VS (63.23% TS) were obtained after 105 days in vermicomposting without sawdust.

E. coli is a key parameter and an indicator for the presence of pathogens. The guideline of WHO (2006) has stipulated a maximum allowable concentration of E. coli in treated. We found that the reduction rate of E. coli in vermicomposting with sawdust faster (2 log cfu/g) than without sawdust. Furthermore, E. coli when vermicomposting with sawdust was well under the guideline of WHO (2006) after 90 days of treatment.

Besides the reduction of VS and E. coli, it was observed that nitrogen loss is significant in vermicomposting of human feces. Specifically, total dissolved nitrogen (TDN) loss was about 85% after 45 days of vermicomposting with sawdust, while, 44% of TDN loss was

In addition, nitrogen forms have changed from ammonium to ammonia, nitrate and nitrogen gas form during vermicomposting which is indicated by the changes in NH_4^+/NO_3^-. The NH_4^+/NO_3^- ratio in final product in FAV was observed to be in the range of 0.22 – 0.02 after 75 days while, the ratio in FV was 8.75 after 105 days of treatment.

Several studies have suggested that a mature product is achieved when NH_4^+/NO_3^- ratio less than 0.16.

The optimization of feces to sawdust ratio (1:0.5, 1:1 and 1:2) in vermicomposting showed that the reduction of volatile solids (VS) and E. coli population are independent from the sawdust content. VS reduction was about 41.56 – 45.57 % and E. coli reduction ranged from 4.1 log – 4.5 log under all ratios considered. Similarly, no significant difference in the increase of biomass was observed in all ratios considered (52% – 71%). Thus, the ratio (1:0.5) of feces and sawdust could be recommended in vermicomposting of human feces to minimize volume of vermicomposting reactor for the treatment.

Overall, the results of this study suggests that vermicomposting is a better alternative for treatment of source-separated human feces. By adding sawdust into human feces at 1:0.5 ratio could be recommended in vermicomposting of human feces to reduce the treatment time and volume of reactor.

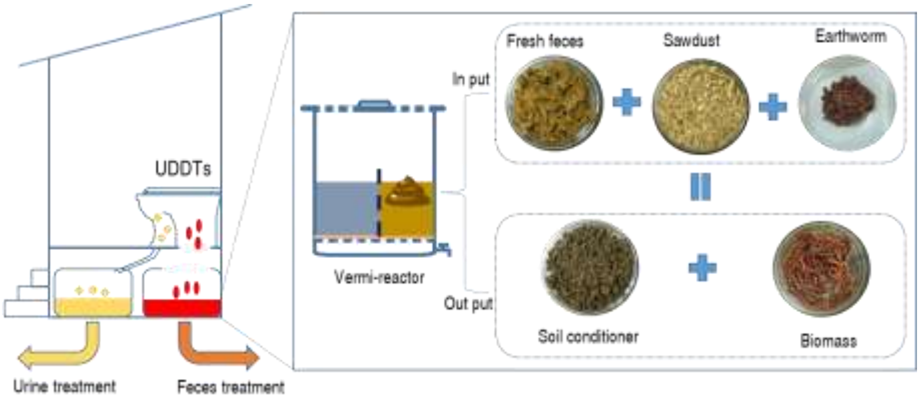

Figure 5. 1 Graphical conclusion of vermicomposting of source-separated human feces with the addition of sawdust

5.2 Recommendation for further study

In this part, several directions and ideas for future research is recommended.

5.2.1 Larger-scale experiments

The lab-scale experiments with a small amount of human feces (600 – 800 mg) per each reactor were performed. Some influencing environmental conditions impacted the results of the experiment such as moisture and temperature loss. It would be better if the experiment could be performed for a longer period with a larger amount of feces (4-5kg) to find out whether the treatment time could be reduced due to the increase in temperature in the first several weeks (after mixing human feces with sawdust). It would be useful to identify how much and for how long the temperature will increase in this period and its potential impact on earthworm?

5.2.2 Size of organic additives

The results of the experiment may be affected by the size of sawdust as it create different environmental conditions when mixed the feces. Activity of earthworms and microorganisms can be impacted. Thus, investigate of different sizes of sawdust such as 0-4 mm, 2-6 mm, 4-8 mm and 6-10 mm is necessary. In the present study, size of sawdust used were in the range 0-6 mm.

5.2.3 Microbial additives

One of the methods to improve vermicomposting of human feces is by the addition of microorganisms. The decomposition of organic matter during the treatment will be enhanced by microorganism. Thus, the treatment time of vermicomposting of human feces could be reduced.

5.2.4 Earthworm density and growth rates

It would be necessary to define the density and growth rates of earthworms in human feces when adding additives. Similar study was conducted before using substrates different from human feces.

5.2.5 Carbon to nitrogen (C/N) ratio

Carbon to nitrogen (C/N) ratio has been reported to be an important factor for stimulating microorganism activity in composting. The C/N ratio of 20-30/1 has been found to ne optimum for optimizing in the process. However, C/N ratio in vermicomposting human feces was not investigated.

5.2.6 Vermicompost quality

One of the main concerns in vermicomposting of human feces is the quality of final product. The addition of some component into human feces during vermicomposting may create a good product for agriculture application. Thus, this matter can be investigated further.

5.2.7 Continuous-flow vermicomposting

Vermicomposting of human feces can be a good on-site treatment method for systems such as urine-diverting dry toilets (UDDT). The vermicomposting reactor can be fed daily or every week and investigate continuous-flow vermicomposting depending the purpose of the study.

국문초록

소변분리건조화장실(Urine-diverting dry toilets)은 사람의 배설물관리를 위한 지속가능한 위생시스템 중 하나이다. UDDT 에서는 대변과 소변을 따로 분리하여 처리한다. 현재, UDDTs 는 냄새 조절, 대소변처리, 영양소 손실과 관련된 여러 문제에 직면해 있다. 그중 대변처리는 우선관심사가 되고 있다.

인분에는 식물성장에 유용한 영양성분이 많이 포함되어 있기 때문에 천연비료로도 알려져 있다. 높은 영양성분이외에도 다량의 병원균들이 있기 때문에 이에 노출되면 질병의 원인이 된다. 따라서 인분을 토양에 비료로 사용하기 위해선 세계보건기구 (WHO) 2006 년 가이드라인에 명시된 대장균의 최대 허용치 (< 3 $\log_{10}$ cfu/g 건량)을 충족하여 처리해야 한다.

유기폐기물을 분해하는 지속가능한 방법으로는 지렁이분 퇴비화가 있다. 지렁이분 퇴비화는 지렁이를 사용하여 유기폐기물의 유기물질을 토양 개량제로 전환시키는 것을 의미한다. 마찬가지로, 인분에는 다량의 유기물질을 포함하고 있기 때문에 이 공정을 적용시킬 수 있다. 그러나 퇴비화 시간(처리 시간)이 길기 때문에 대규모로 인분을 분해하는 지렁이분 퇴비화공정의 적용에 걸림돌이 된다. 따라서 본 연구에서 톱밥을 지렁이분 퇴비화 공정을 개선하기 위해 처리 시간을 단축하는 촉매로 사용하였다.

본 연구는 (1) UDDT 에서 분리되는 인분에 대한 처리 방법으로서 지렁이분 퇴비화의 타당성, (2) 지렁이분 퇴비화에 대한 톱밥의 효과, (3) 톱밥 유무에 따른 인분의 지렁이분 퇴비화 중 질소 형태의 변화, 그리고 (4) 톱밥 첨가의 적정량(optimization)을 평가하기 위한 것이다. 목표 (1), (2) 및 (3)를 달성하기 위해 4 가지 각기 다른 지렁이 서식 상자를 설계하였는데, 이는 대변만을 포함하는 대조 서식상자(F), 톱밥 없이 대변과 지렁이를 포함하는 서식상자(FV), 지렁이 없이 대변과 톱밥을 포함하는 서식상자(FA), 대변, 톱밥, 지렁이를 포함하는 서식상자 (FAV)이다. 목표(4)을 달성하기 위해 대변 대 톱밥의 세 가지 비율, 즉 1 : 0.5, 1 : 1, 1 : 2 가 고려되었다.

pH 는 FA 와 FAV 에서 처음 2 주 동안 급격히 증가(최대 8.88 - 8.9)한 후 105 일까지 서서히 감속하여 pH 가 6.79 - 6.87 범위로 유지되는 것이 관찰되었다. 대조적으로, FV 에서는 pH 의 점진적인 감소만 관찰되었다(8 ~ 7.25).

FAV 에서 가장 짧은 인분 처리 시간(90 일)이 관찰됐으며 75 일 이후 휘발성 고형분(VS)이 전체 고형분(TS)의 약 45%로 안정화됐다. 또한 대장균 개체수(2.73 $\log_{10}$ cfu/g 건조량)는 90 일 이후 WHO 가이드라인 기준을 만족하였다. 지렁이가 없는 다른 서식상자는 105 일의 치료 후에도 VS(TS 의 62.02~80.05%)와 대장균군(4.42~6.57 $\log_{10}$ cfu/g 건중량)의 수치가 다소 높게 측정되었다.

그러나 VS 와 대장균 개체수가 감소했음에도 불구하고, 인분의 대변을 지렁이분 퇴비화 과정에서 상당한 질소 손실이 관찰되었다. 총 용존 질소(TDN) 손실은 FAV 에서 45 일 후 약 85%인 반면, FV 에서는 105 일 후 TDN 의 44%가 손실되었다.

또한, 질소 형태는 지렁이분 퇴비화 과정에서 암모늄이 암모니아, 질산염, 질소 가스 순의 형태로 바뀌었으며, 이는 NH_4^+/NO_3^-의 변화로 나타낼 수 있다. FAV 에서 최종 생산물의 NH_4^+/NO_3^-비율은 75 일 이후 0.22~0.02 범위인 것으로 관찰되었고, FV 에서는 퇴비화 105 일 후 8.75 였다.

지렁이분 퇴비화에서 대변 대 톱밥 비율(1:0.5, 1:1, 1:2)을 최적화한 결과 휘발성 고형분(VS)과 대장균군 감소가 톱밥 함량과는 무관한 것으로 나타났다. VS 감소량은 약 41.56 – 45.57%였으며 대장균 감소량은 4.1 log – 4.5 log 에서 모든 비율을 고려했다. 마찬가지로, 모든 고려 비율(52% – 71%)에서 바이오매스 증가의 유의미한 차이는 관찰되지 않았다. 따라서 인체 대변의 지렁이분 퇴비화에 대변과 톱밥의 비율(1:0.5)을 권장하여 퇴비화를 위한 지렁이분 퇴비화 서식상자의 부피를 최소화할 수 있었다.

결론적으로, 이 연구를 통해 지렁이분 퇴비화가 분리된 인분을 처리하는 더 나은 대안으로 제안하는 바이다. 또한 서식상자의 처리

시간과 부피를 줄이기 위해 인분의 지렁이분 퇴비화에 톱밥을 첨가하는 것이 권장될 수 있다.

주요어: UDDT, 인분, 지렁이분 퇴비화, 톱밥, 처리 시간, 질소 손실

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