DOKUZ EYLUL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

INVESTIGATION OF COMPOST PRODUCTION FROM WASTEWATER TREATMENT SLUDGE BY VERMICOMPOSTING

by
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İZMİR

INVESTIGATION OF COMPOST PRODUCTION FROM WASTEWATER TREATMENT SLUDGE BY VERMICOMPOSTING

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We have read the thesis entitled **"INVESTIGATION OF COMPOST PRODUCTION FROM WASTEWATER TREATMENT SLUDGE BY VERMICOMPOSTING"** completed by **AHENK ÖZÜM** under supervision of **PROF. DR. İLGİ K. KAPDAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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ABSTRACT

Vermicomposting is an innovative technology used for stabilization of organic wastes. It has got higher nutrient value than conventional composts and also it is cost-effective and requires short processing period. Earthworms and microorganisms work together in order to product vermicompost that can be used for natural fertilizer. Earthworms condition the substrate, initiate microbial activity which provide biochemical degradation of organic wastes.

The aim of the study was to convert wastewater treatment sludge to environmentally friendly product as natural fertilizer. For this purpose, yeast industry wastewater treatment sludge was used as organic waste for vermicomposting. Waste sludge mixed with cow dung (CD) as a co substrate and *Eisenia foetida* was the worm in vermicomposting. The vermibed was prepared by mixing cow dung and sludge in different ratios. The sludge content of vermibed was varied between 20 percent and 100 percent in order to determine optimum sludge content in vermibed. Variation of pH, total organic carbon (TOC) and total nitrogen (TN) content of bed were monitored every 15 days. The changes in carbon/nitrogen (C/N) ratio throughout the operation were calculated. A decrease in C/N ratio was observed during the vermicomposting. Maximum decrease in C/N ratio was achieved at 20 percent sludge content at which the highest enrichment of nitrogen was observed.The percent decrease in C/N ratio was in the order of 20 percent> 30 percent> 40 percent sludge contens. The decrease in C/N ratio was in parallel to nitrogen enrichment. But, the best condition for vermicomposting of sludge can be suggested as 30 percent sludge and 70 percent cow dung and 60 days of operation.The product obtained at the end of process was in good quality in terms of C/N ratio and can be used as natural fertilizer.

Keywords: Vermicompost, organic waste, C to N ratio

VERMİKOMPOST YÖNTEMİ İLE ARITMA ÇAMURLARINDAN KOMPOST ÜRETİMİNİN ARAŞTIRILMASI

ÖZ

Vermikompostlama organik atıkların stabilizasyonu için kullanılan yenilikçi bir teknolojidir. Vermikompost, klasik kompostlardan daha yüksek besin değerine sahiptir, ayrıca uygun maliyetli ve hızlı bir metottur. Bu metotta, solucanlar ve mikroorganizmalar doğal gübre olarak kullanılabilecek bir vermikompost üretimi için birlikte çalışırlar. Solucanlar substratı uygun hale getirip, biyolojik aktiviteyi başlatırken, mikroorganizmalar organik atıkların biyokimyasal bozunmasını sağlar.

Tez çalışmasında, arıtma çamurlarının vermikompost yöntemi ile gübreye dönüştürülmesi amaçlanmıştır. Bu amaçla, maya endüstrisi arıtma çamuru ve inek gübresi substrat olarak ve *Eisenia foetida* solucan kültürü olarak kullanılmıştır. En uygun çamur konsantrasyonunu belirlemek için, arıtma çamuru miktarı yüzde 20 ile yüzde 100 arasında değişen karışımlar hazırlanmıştır. pH, toplam organik karbon (TOC) ve toplam azot (TN) değerleri her 15 günde bir izlenmiştir. Karbon/ Azot (C/N) oranındaki değişimler vermikompostlama süreci boyunca hesaplanmıştır. Karışım oranına bağlı olarak karbon azot oranında önemli azalmalar gözlenmiştir. C/N’deki en önemli azalma, en çok azot zenginleşmesinin de gerçekleştiği yüzde 20 çamur içeriğinde elde edilmiştir. Karbon azot oranındaki düşüş, azot zenginleşmesinde olduğu gibi yüzde 20 > yüzde 30 > yüzde 40 şeklinde olmuştur. Ancak vermikompostlama için en uygun koşullar, 60 gün sonunda durağan hale ulaşmasından dolayı yüzde 30 çamur, yüzde 70 gübre içeren karışımda elde edilmiştir. Prosesin sonunda doğal gübre olarak kullanılabilen, karbon azot oranı açısından iyi kalitede bir ürün elde edilmiştir.

Anahtar sözcükler: Vermikompost, organik atık, karbon azot oranı

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INTRODUCTION

Over the last few years, one of the biggest problems is management of solid wastes. Major environmental (contamination of soil and ground water and odors) and disposal problems occur due to the produciton of organic wastes (Garg, Gupta, &Satya, 2006). The increase in the amount of waste is one of the environmental problem, in*global development.* Rapid urbanization encroachment of fertile area and increasing population cause the generation of large quantities of waste (Aalok, Tripathi, & Soni, 2008).

There are several, chemical, physical and microbiological methods to remove the organic solid wastes are currently in use. However these methods are not cost effective and they are time consuming (Garg et al., 2006). Beside,dumping or sanitary land filling, other disposal methods of solid waste can be incineration and composting (Aalok et al., 2008).

Composting is the natural process of decomposition of organic matter by microorganisms under controlled conditions. Organic materials such as crop residues, animal wastes, food garbage, some municipal wastes and suitable industrial wastes, enhance their suitability for application to the soil as a fertilizing resource, after having undergone composting. In other words, composting is an accelerated biooxidation of organic matter passing through a thermophilic stage (45 to 65 ºC) where microorganisms (mainly bacteria and fungi and actinomycetes) liberate heat, carbon dioxide and water (Mupondi, 2010).

Five factors that are critical for composting process are: temperature, air, moisture, carbonnitrogen ratio, and pH levels. Temperature is a major factor in determining the type of microorganisms, the rate of metabolic activities, and the rate of biodegradation of the organic waste (Mupondi, 2010). Finished compost is a more stable product made up of microbial residues and the more resistant organic compounds from the raw materials (Mupondi, 2010).

In this regard vermiculture is an alternative technique for composting. Vermiculture biotechnology is an innovative method. An important part of waste recycling is the breeding and propagation of earthworms and the use of its castings. We can say about vermiculture, earthworms are used as natural bioreactors for cost-effective and environmentally sound waste management (Aalok et al., 2008).

Vermicompost plants have entered industrial and domestic marketing in different countries. Specially USA, Canada, Japan and Italy. In ABD, a company about earthworms began a farm in 1978-1979 with about 500 tones capacity per month. A company in Japan has three 1000 tones per month plants that processing wastes from pulp and food industries. Also, there are about 3000 other vermicomposting plants in Japan with 5-50 tones capacity per month. It has also started in Italy and in the Philippines (Aalok et al., 2008).

1.1 What is Vermicomposting

Vermiculture (derived from the Latin *vermis* meaning worm) involves the mass production of earthworms for waste degradation, and composting with 'vermicast' production. Earthworms are a major soil fauna on Earth, constituting 80 percent of the soil invertebrate population in many ecosystems, specially in the tropical ecosystems (Sinha, Herat, Agarwal, Asadi, & Carretero, 2002).

Earthworms and microorganisms work together in the vermicomposting in order to stabilize the organic material. While microorganisms provide the biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and starting the biological activity (Suthar, 2009).

The vermicomposting present a better quality product than the other common composting systems. Also vermicompost is a stabilized product. It is high nutritional value food for the plants. It is easy in the way of taken up by the plants (Suthar, 2009).

The vermicompost has more proper nutrients per kg weight than the organic material from that it is produced. The biological activity of earthworms provides nutrient rich vermicompost for plant growth thus facilitating the transfer of nutrients to plants (Garg et al., 2006).

Vermiculture is practiced for the mass production of earthworms with the multiple objectives of waste management, soil fertility and detoxification and vermicompost production for sustainable agriculture. The practice was started in the middle of 20th century and the first serious experiments were done in The Netherlands in 1970, and later in England, and Canada. Then vermiculture practices were followed in USA, Italy, Philippines, Thailand, China, Korea, Japan, Brazil, France, Australia and Israel (Sinha et al., 2002). The American Earthworm Technology Company started a 'vermicomposting farm' in 1978–1979 with 500 t $month^{-1}$ of vermicompost production. Japan imported 3000 tons of earthworms from the USA during the period 1985–1987 for cellulose waste degradation. The Aoka Sangyo Co. Ltd., has three 1000 t $month^{-1}$ plants processing waste from paper pulp and the food industry. This produces 10 tons of live earthworms and 400 tons of vermicompost per month. Another company inJapan is using municipal sludge, paper waste and rice straw, for vermicompostinginvolving 20 plants which in total produce 2–3 thousands tons $month^{-1}$ (Sinha et al., 2002). Vermicomposting is used to biodegradation of municipal and paper mill sludges in Italy. Aerobic and anaerobic sludges were mixed and then they were aerated for more than 15 days and in 5000 m^3 of sludge 5 kg of earthworms are added. In 8 months, it si observed that the sludge is converted into vermicompost (Sinha et al., 2002).

Generally earthworms are resistant to many pesticides and have been reported to concentrate the pesticides and heavy metals in their tissues. They also inhibit the soil borne pathogens and work as a detoxifying agent for polluted soil (Sinha et al., 2002).

Vermicompost be formed worms, microorganisms, bedding materials and organic wastes at variousstages of decomposition. Nutrients invermicompost are often much higher than other traditional garden composts (Dickerson, 2004)(Table 1.1). Last product of vermicomposting should have a rich, goodsmell if properly processed by worms.

The comparison of compost with vermicompost is given in Table 1.2. In comparison with compost produced from the same parent material, vermicompost is richer in content of available nutrients after the feeding substrate passes through the worm gut. Furthermore, vermicompost is more effective for soil improvement than compost after the same period of maturity.

Table 1.1 Chemical characteristics of garden compost and vermicompost (Dickerson, 2004).

Parameter*	**Garden compost[1]**	**Vermicompost[2]**
pH	7.80	6.80
EC(mmhos/cm)**	3.60	11.70
Total Kjeldahl nitrogen(%)***	0.80	1.94
Nitrate nitrogen(ppm)****	156.50	902.20
Phpspsorous (%)	0.35	0.47
Potassium (%)	0.48	0.70
Calcium (%)	2.27	4.40
Sodium (%)	< .01	0.02
Magnesium (%)	0.57	0.46
Iron (ppm)	11690.00	7563.00
Zinc (ppm)	128.00	278.00
Manganese (ppm)	414.00	475.00
Copper (ppm)	17.00	27.00
Boron (ppm)	25.00	34.00
Aluminum (ppm)	7380.00	7012.00

Table 1.2 Comparison of composting and vermicomposting with some advantages(Phương-Nam Nguyễn, 2012).

Parameter	Composting	Vermicomposting
Heating requirement	Remaşned & controlled	Avoidingexcess heat
By-product	No by-product	Worm biomass
Decomposition time	Long (≥6 months)	Less
Decomposition degreeof OSW material	Incomplete in large & heterogeneous particles	Complete, resulting in small & uniform particles
Smell of product	Still bad odour	Less bad odour
Avaliable salt content in product	Increased	More increased
pH level of product	Less reduced, pH>7	Reduced, near neutral
C/N ratio of product	Decreased	More decreased
Avaliable N-P-K in product	Increased	More increased
Humification & stabilisation of product	Increased	More increased
Pathogenic microbes in product	Reduced	More reduced
Texture & structure of soil with product	Improved	More improved
Yield of plant crops with product amendment	Increased	More increased

1.1.1 Advantages of Vermicomposting

Degredation of organic matter can be relized by earthworms very rapidly, at the end of the process vermicompost occur with a better microbial content, and nutrient rich content than other composts. And also it is nontoxic and stable.This vermicompost have a high economic value as soil conditioners or environment for plant growth. Although the best products and the shortest retention times are obtained by high-technology systems, the low-technology ones can be easily adapted and worked in small farms. Vermicompost has low C/N ratio, excellent structure, porosity, aeration, drainage, and moisture-holding capacity, and it supplies a suitable mineral balance, improves plant nutrient availability, and could act as complex-nutrient-source granules (Dominguez &Edwards, 2011).

As an aerobic process, both composting and vermicomposting lead to nitrogen mineralization, but the presence of earthworms in vermicomposting increases and accelerates the nitrogen mineralization rate. Also, the humification rates that take place during the maturation stage are higher and faster during vermicomposting, resulting in a greater decrease of bio-available heavy metals (Dominguez et al., 2011).

1.1.2 Anatomy of Earthworms

Earthworms are cylindrical, long and narrow animals without bones. Rings that surround the moist, soft body allow the earthworm to twist and turn, especially since it has no backbone. With no true legs, bristles (setae) on thebody move back and forth, allowing the earthwormto crawl (Dickerson, 2004).The body is dark brown, glistening and covered with delicate cuticle. They weigh about 700–1400 mg after 10 weeks. They have a muscular gizzard which finely grinds the food (fresh and decaying plant debris, living or dead larvae and small animals, and bacteria and protozoa mixedwith earth) to a size of 2–4 microns. The gut of the earthworm is inhabited by millions of decomposer micro-organisms (Sinha et al., 2002).

The earthworm breathes through its skin. After food is taken through the mouth into a stomach, it passes throughout the gizzard, where it is ground up by ingested stones. After passing through the intestine for digestion, whats left is eliminated (Dickerson, 2004).

Earthworms have both male and female sex organs, in other words they arehermaphrodites. But they need another earthworm to breed. The wide band (clitellum) that surrounds a mature breeding earthwormsecretes mucus (albumin) after mating. Sperm from another worm is stored in sacs. As the mucus slidesover the worm, it encases the sperm and eggs inside (Dickerson, 2004). Copulation may last for about an hour, the worms then separate. Later the clitellum of each worm eject cocoon where sperms enter to fertilize the eggs. Up to 3 cocoons per worm per week are produced. From each cocoon about 10–12 tiny worms emerge (Sinha et al., 2002). Cocoons look like lemon-shape. Redworms take 4 to 6 weeks to become sexually mature (Dickerson, 2004).

Earthworms grow continuously during their life and the number of segments continuously prolife rate from a ‘growing zone’ just in front of the anus. Earthworms contain 70–80 percent high quality lysine rich protein on a dry weight basis. They can be useful as animal feed. Usually length of the life of an earthworm is about 3 to 7 years depending upon the type of species and the ecological conditions (Sinha et al., 2002).

Earthworms can eat their own weight in food residue and bed material in one day,under optimum conditions (Dickerson, 2004).

1.1.3 Ecology of Earthworms

Earthworms burrow through the soil. In other words they creat tunnels by eating their way through the soil. The distribution of earthworms in soil depends on parameters like pH, availability of organic matter and soil moisture of the soil. They occur in different habitats specially that are dark and moist.Organic materials like humus, cattle dung and kitchen wastes are highly attractive sites for some species (Sinha et al., 2002).

In soil with a coarse texture, and high clay content, or soil with $pH < 4$, earthworms are absent or rare. Earthworms are very sensitive to light, dryness and touch. Excess water in the soil can cause earthworms to come to the surface. Worms can tolerate a temperature range between 5^oC to 29^oC. A temperature of 20^oC to 25^oC and a moisture of 50–60 percent is optimum for earthworm function (Sinha et al., 2002).

1.1.4 Types of Earthworm

Different earthworm species exist in almost all regions of the world except those with extreme climates, such as deserts and glaciers. These species have quite different life cycles, behaviours and environmental requirements. They are classified into three major ecological categories based primarily on their feeding and burrowing strategies: epigeic, endogeic and anecic. Only epigeic earthworms seem to be relevant for vermicomposting (Nguyễn, 2012).

About 8000 species of earthworm worldwide have been described as epigeic from ~800 genera belonging to the order Oligochaetae. Of those, seven earthworm species are used in vermicomposting, namely *Eisenia foetida*, *Dendrobaena veneta*, *Dendrobaena rubida*, *Lumbricus rubellus*, *Perionyx excavates*, *Eudrillus eugeniae* and *Pheretima elongata*. These species show good growth on organic wastes compared with other species (Nguyễn, 2012). Edwards (2007) provides a comparative summary of the life cycle and cocoon production of the seven earthworm species suitable for vermicomposting (Figure 1.1, Figure 1.2)

But *E. foetida* remains the favoured earthworm species for laboratory experiments on vermicomposting due to its wide tolerance of environmental variables (temperature, moisture content,pH, etc.) (Suthar, 2009). Therefore, composting biology and waste recycling efficiency of this species is well documented by various authors (Table 1.3).

Table 1.3 Summary of some vermicomposting experiments using different eartworm species and bed materials (Suthar, 2009).

Organic wastes	Amendment or bulking material	Earthworm species	Reference
Crop residues	Cattle dung and cattle manure	*E. fetida*, *P. excavatus*	Bansal and Kapoor (2000); Suthar (2007b)
Household waste	Cow dung	*P. excavatus*, *P. sansibaricus*	Suthar and Singh (2008c)
Forest litter	Cow dung	*E. fetida*, *P. excavatus*, *Dichogaster bolaui*	Manna et al. (2003)
Kitchen waste	Grass clippings, shredded paper, cow dung	*Lumbricus rubellus*, *E. fetida*, *L. mauritii*	Tripathi and Bhardwaj (2004), Nair et al. (2006)
Textile mill sludge	Poultry droppings	*E. fetida*	Garg and Kaushik (2005)
Guargum industrial waste	Cow dung and saw dust	*E. eugeniae*	Suthar (2006)
Sewage sludge	Cow dung, sugarcane trash	*E. fetida*	Suthar (2008c), Gupta and Garg (2008)
Distillery sludge	Cow dung	*E. fetida*, *P. excavatus*	Suthar (2008e), Suthar and Singh (2008b)
Paper mill sludge	Cow dung	*E. andrei*	Elvira et al. (1996)

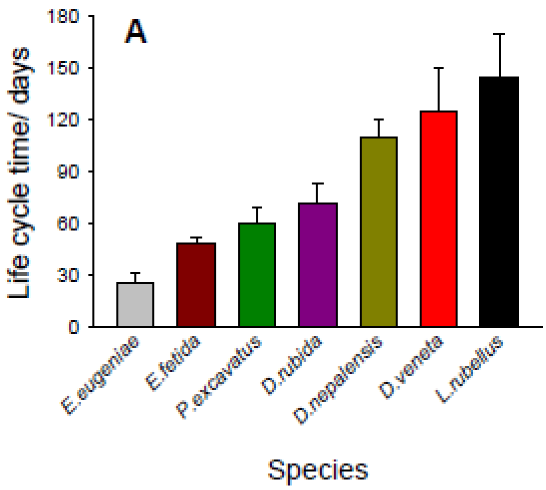

Figure 1.1 Life cycle of earthworm species (Edwards, 2007).

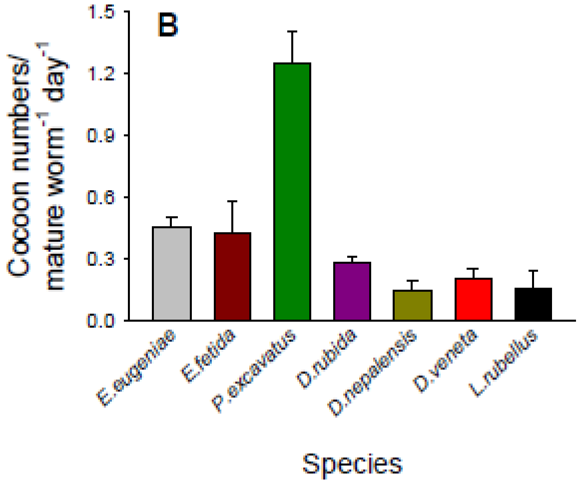

Figure 1.2 Cocoon numbers of earthworm species (Edwards, 2007).

1.1.4.1 Eisenia Foetida

Most studies of vermicomposting focus on the species *Eisenia foetida*. *E. foetida* has a rapid growth rate, good temperature tolerance (up to 35^{o}C) and accepts a large range of moisture (60-90%) (Reinecke *et al.*, 1992). Moreover, *E. foetida* can be handled easily and it is tolerant to other species (Nguyễn, 2012).

Under optimal conditions, the life cycle of *Esenia foetida* ranges from 45 to 51 days (Domínguez & Edwards, 2004). *E. foetida* cocoon depending on temperature. The maximum life time of *E. foetida* is 4.5-5.0 years (Domínguez & Edwards, 2004), but the average survival rate is ~20 months at 18-28^{o}C (Aira *et al.*, 2007b).

1.1.4.2 Dendrobaena Veneta

D. veneta does not grow rapidly, the worms have been used widely in vermicomposting and vermiculture systems, especially in industrial-scale processes. Because of their large size, they can be handled easily (Fayolle *et al.*, 1997).

D. veneta prefers temperatures in the range 9-30^{o} C for vermicomposting (Fayolle *et al.*, 1997; Munima *et al.*, 1994). Munima *et al.* (1994) also reported that *D. veneta* performs better in high moisture conditions (65-85%) than other species. The life cycle of the species is quite long (100-250 days). About 65 days is the average time for this worm to reach sexual maturity (Fayolle *et al.*, 1997).

1.1.4.3 Dendrobaena Rubida

The life cycle of the species is completed in 75 days and its rapid maturation and high reproductive rate could make the worm suitable for vermicompost processing (Edwards& Bohlen, 1996). *D. rubida* grows well in the range of 15-25^{o}C and needs 54 days to reach sexual maturity after hatching (Domínguez & Edwards, 2004).

1.1.4.4 Lumbricus Rubellus

L. rubellus has a long maturation time and a low reproductive rate, which suggests that the species is not a proper earthworm for vermicomposting, although its size, vigour and ability to survive in soils could make it interesting as fish bait or for soil improvement (Adi & Noor, 2009).

L. rubellus has a long life cycle in the range of 120-170 days, with a slow growth rate and a long maturation time of 74-91 days (Edwards, 2007). The optimum temperature of this worm is 15-18^{o}C and the optimum moisture is 80-85% (Edwards, 2007).

1.1.4.5 Perionyx Excavates

Under optimal conditions, the life time of *P. excavatus* tis 40-71 days from hatching to maturity (Maboeta *et al.*, 1999). This species prefers high temperatures (20-30^{o} C) with an optimum of 25^{o} C and may die at temperatures <9^{o}C (Loehr *et al.*, 1985). With about 90% hatching rate and 1.1 worms cocoon^{-1}, the species has a high net reproductive rate from nearly 6.7 cocoons day^{-1} (Reinecke *et al.*, 1992).

1.1.4.6 Eudrilus Eugeniae

E. eugeniae could be considered an ideal species for vermicomposting because it is handled and harvested more easily than the others (Domínguez et al., 2001). However, its disadvantage is that it has a relatively narrow temperature tolerance, from only 20 to 28^{o}C (Domínguez et al., 2001).

E. eugeniae has a life cycle of 43-122 days depending mainly on temperature. The maximum life expectancy of the worm is up to three years (Edwards, 2007).The time requirement of *E. eugeniae* for maturity is 40 days and for cocoon incubation is ~16 days. The average number of hatchlings is 2.7 earthworms cocoon-1 (Domínguez & Edwards, 2004).

1.1.4.7 Pheretima Elongata

*P. elongata*survives by dwelling deeply in low organic matter and tolerates in a temperature range from 19 to 30^{o}C (Somani, 2008). The duration of its reproductive life is longer than 200-400 days and the period of maturity from hatchling to adult seems to be in the range of 120-150 days (Domínguez & Edwards, 2004). The average time for cocoon production of P. elongate is ~20-24 weeks and its cocoon incubation period is 28-31 days.

1.1.5 Vermitechnology

Vermitechnology is using surface and subsurface varieties of earthworms in soil management and composting. Regular inputs of feed matter for the earthworms can be from kitchen wastes, agro wastes, and nitrogen rich materials like goat manure, pig manure and cattle dung. However, poultrymanure should be handled carefully dueto toxic impacts.We can get rid of the organic solid wastesby using these wastes into organicfertilizers.Vermicomposting therefore is also solid wastemanagement, where organic wastes are considered as resources (Aalok et al., 2008).

According to Aaloket al. (2008) vermitechnology includes three main processes:

1. Vermiculture - growth of earthworms.
2. Vermicomposting - biodegradation of wastes with earthworms.
3. Vermiconvertion - mass maintenance of sustainability of waste lands through earthworms.

Also according to Ndegwa & Thomson (2000), vermicomposting can be divided into two main processes:

Physical and mechanical processes: Organic matter is aerated, mixed and homogenised by earthworms. In the case of composting, the process usually requires large tools/units that are associated with high cost. Vermicomposting avoids these operation costs (Nguyễn, 2012).

Ecological and biochemical processes: Vermicomposting is a process with several interactions between microorganisms and earthworms. In the worm intestine (or gut), there are many biochemical processes among bacteria, protozoa, actinomycetes and fungi. In addition, some digestive enzymes which are known as catalytic reagents for biochemical reactions exist in the worm gut (Nguyễn, 2012).

1.1.5.1 Interaction Between Microorganisms and Earthworms

The interactions between earthworms and microorganisms in soil and organic solid waste materials seem to be of major importance in the degradation of organic matter and the release of mineralised nutrients (Nguyễn, 2012).

Vermicomposting is a mesophilic bio-oxidative process in which detritivorous earthworms interact intensively with microorganisms and soil invertebrates within the decomposer community, strongly affecting decomposition processes, accelerating the stabilization of organic matter, and greatly modifying its physical and biochemical properties (Dominguez, 2011).

The enzymes provides the biochemical decomposition of organic material. These enzymes produced by microorganisms. But earthworms are important for the process. Earthworms are involved in the stimulation of microbial populations during fragmentation and ingestion of fresh organic material, which results in a greater surface area available for microbial colonization, thus dramatically increasing microbiological activity. Earthworms also modify microbial biomass and activity through stimulation, digestion, and dispersion in the casts (Figure 1.3) and interact closely with other biological components of the vermicomposting system, thereby affecting the structure of the microflora and microfauna communities (Dominguez, 2011).

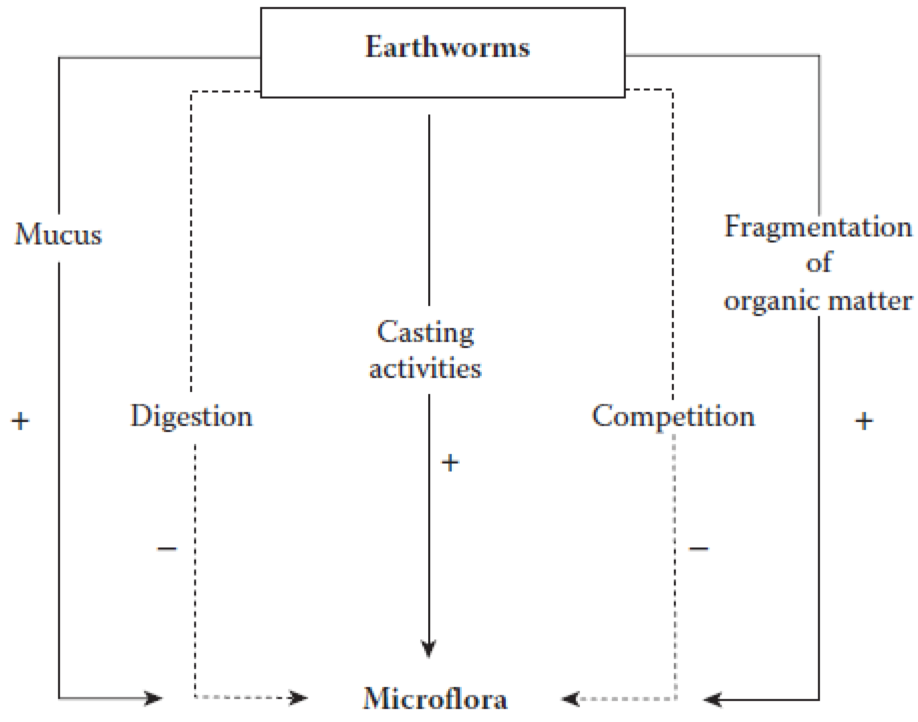

Figure 1.3 Positive and negative effects of earthworms on microbial activity (Dominguez, 2011).

The vermicomposting process occurs two different phases. They involve the activity of earthworms, an active phase during which earthworms process wastes, thereby modifying their physical state and microbial composition (Lores et al. 2006) and a maturation-like phase marked by the displacement of the earthworms toward fresher layers of undigested waste, during which the microbes take over the decomposition of the earthworm-processed waste (Domínguez 2004; Lazcano et al. 2008).

The impact of earthworms on the decomposition of organic waste during the vermicomposting process is, in the first instance, due to gut-associated processes (GAPs). These processes include all the modifications that the decaying organic matter and the microorganisms undergo during transit through the earthworms' intestines (Dominguez, 2011). Decomposition is also enhanced through the action of endosymbiotic microbes that reside in the earthworm gut. These microbes produce extracellular enzymes that can degrade cellulose and phenolic compounds, thereby further enhancing the degradation of ingested material (Dominguez, 2011).

1.2 Literature Review

Vermicomposting techniques are easily applicable and low cost. A high valuable product which is effective as bio-pesticide and bio-fertilizer could be obtained in the end of the vermicomposting process which was applied correctly. In recent years, liquid spray formulations named as vermicest tea produced from vermicomposting are applied to plants to control the diseases and stimulate the plant growth (Erşahin, 2007).

Earthworms provides aeration of the soil while making their ways by eating the soil. Thus, aerobic conditions that are necessary for vermicomposting naturally be ensured. Earthworm distrubiton in the soil depends on pH, humidity and organic matter content of the soil (Singh et al., 2002).

In diffirent studies, in order to product vermicompost profited by industrial solid wastes (Sen &Chandra 2007, Subramanian et. al. 2010, Vivas et al. 2009),domestic organic solid wastes (Suthar 2009; Suthar &Singh 2008; Kaviraj 2003) and sewage sludge (Vig et al. 2011, Suthar & Singh 2008, Yadav& Garg 2009; Li et. al. 2009).

In this studies, the effects of mixture ratio, worm number, surface/volume ratio, bed material were researched. Vermicompost production was achieved effectively from domestic/industrial organic wastes and agricultural wastes. The system need to enhance about sewage sludgesdue to their toxic content. Vermicomposting of food industry sludges was not achieved without admixture. Yadav & Garg (2009), were determine that minimum manure addition could be 30%.

pH of wastes which stabilizes by vermicomposting decreases to neutral levels. (Mitchell, 1996; Padmavathiamma et al., 2008, Mainoo et al., 2009; Subramanian et al., 2010) decrease in pH arise from the formation of organic acids such as humic acid, fulvic acid. Up to 60% reduction in total organic carbon content can be provided (Gupta&Garg, 2009; Sen et al., 2007). The C/N ratio in vermicompost should be 20/1 or less in order that plants take the nitrogen in soil by their roots (Singh et al., 2011).

0.5-2 fold increase can be provided in N, P, K, Ca etc. Micro nutrient contents that required by plants (Ndegwa & Thompson, 2001, Gupta & Garg, 2009, Suthar, 2009). Also the electrical conductivity increases due to release of inorganic content as a result of decay of the organic substance (Yadav & Garg, 2009).

Worms can also store heavy metals in the body and can contribute to the removal of heavy metals (Singh et al., 2011). However, an increase in heavy metals in the final product of vermicomposting can be observed (Gupta & Garg, 2008; Suthar et al., 2008).

Vermicompost contains useful hormons and vitamins that promote plant growth. Enzymes such as dehydrogenase, β-glukosidase, urease, amylase, protease, lipase ve cellulose enhance the quality of vermicompost and provide degradation of the organic fraction such as lignin, cellulose and hemicellulose which is difficult to hydrolyze (Sen et al., 2007, Kumar, 2010). Harmful microorganisms such as *E.coli*, *Salmonella* and *Aspergillus* in vermicompost decrease up to 70%. Also while earthworms mineralise the nitrogen they convert the nitrate which taken by plants easily (Atiyeh et al., 2000).

In a study, vermicompost was producted by leather industry sludge, however increasing in worm number and worm weight was observed. According to Vig et al. (2011),in waste mixtures, increasing in manure amount effected the vermicomposting positively, and also manure ratio must be 75%. Production rate related to worm number and also when worm number is 250 number / kg waste high speed vermicomposting occurs **(**Gajalakshmi et al. 2002)**.**

At the end of the vermicomposting, there was a decrease the variation of pH, organic carbon content and C/N ratio, and there was an increase the variation of ash content, electrical conductivity, nitrogen, phosphorus and potassium content. The decreasing in pH was related to production of organic (Singh et al. 2011). A decrease in C/N ratio was observedduring the vermicomposting and this could be attributed to the lossof carbon as carbon dioxide through microbial respiration andsimultaneous addition of nitrogen by worms in the form of mucusand nitrogenous excretory (Vig et al., 2011).

By considering the advantages of vermicomposting and adverse effect of wastewater treatment sludges on environment, the thesis designed to investigate the effect of substrate composition on vermicomposting waste sludge. For this purpose, yeast industry wastewater treatment sludge was mixed with cow dung for vermicomposting by *E. foetida*.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Earthworms

Earthworms were purchased from Ekosol Agriculture and Stockbreeding and reveived from Ege University, Agricultural Faculty. *E. foetida* was used as composting earthworm because of its wide spread use in vermicomposting of relatively moist organic material. The age of the eathworms varied during experiments. Stock vermicompost was cultuvated in a (40cmX50cmX40cm) vermireactor with dried cow dung substrate.

2.2 Collection of Organic Wastes

The substrate or bed materials were mixture of dried cow dung and aerobic wastewater treatment plant sludge (Table 2.1). The sludge was obtained from wastewater treatment plant of PAK-MAYA Baker's Yeast Industry, İzmir. The other substrate was dried cowdung. The sludge and cow dung was mixed in different ratios.

Table 2.1 Properties of cow dung and sludge.

	TOC(%)	**Total N (%)**	**C/N**	**pH**
cow dung	30.872	1.521	20.297	8.62
sludge	18.627	4.275	4.357	7.22

2.3 Experimental Setup

The vermicomposting experiments were carried out in plastic boxes with 1.5 L capacity as vermireactors. The dimentions of vermirecator were diamaeter 15 cm and depth 15 cm. Small holes with approximateley 3 mm in diameter were opened to the bottom of the vermireactor for aeration (Figure 2.1). The boxes were covered with a jute mat to maintain the moisture. The moisture content of the substrate in each box was maintained at between 60% and 70% during the study period by periodic sprinkling of water.

The total substrate in the vermireactor was 500 g. Cow dung (CD) was mixed with sludge in ratios from CD=80%, 70%, 60%,50% and 0%. The study was started with low sludge content (20%) then gradually increased to 100% in order to determine the maximum tolerable sludge content.

The total number of worm in every bed was 30 from different age groups (Figure 2.2).The duration of experiment was 75 days. Variation of pH, TOC and TN content of bed were monitered every 15 days.

Figure 2.1 Eartworms in the experiment box.

Figure 2.2 Eartworms from different age groups.

2.4 Analytical Methods

The samples were analyzed for pH, total organic carbon (TOC), total nitrogen (N) and then the changes in C/N ratio throughout the operation were calculated.

2.4.1 Sampling

About 20 g samples were taken from the vermibed in every 15 days. All of the samples were dried at 40^{o}C for 24 hours. And then they pounded.

2.4.2 pH Analysis

pH of vermicompost was monitored by using a pH meter. It was determined using distilled water suspension of vermicomposting in the ratio of 1:10 (vermicompost : distilled water). This suspension was shaked in a gyratory shaker at 230 rpm for 2 hours and allowed to stand for an hour prior to pH mesaurement. pH measurements were conducted on supernatant.

2.4.3 Total Nitrogen (TN) Analysis

Total nitrogen (TN) content was determined by Advanced Kjeldhal digestion method for total soil quality (TS 8337 ISO 11261). TN was calculated as percentage.

2.4.4 Total Organic Carbon (TOC) Analysis

TOC content of samples was determined by using TRLInstruments for TOC determination in solid and sludge (TRL-TOC) TOC anlayser. TOC was calculated as percentage.

CHAPTER THREE
RESULTS AND DISCUSSIONS

The main aim of the thesis was to determine the operating condition of a vermireactor to obtain vermicompost from wastewater treatment plant sludge. Therefore, the study was designed to use waste sludge as main substrate and mixture of sludge and cow dung as co-substrate. The percentages of sludge in the total bed mass were varied between 100% and 20%. The corresponding cow dung ratio was CD=80%, 70%, 60%, 50% and 0%. Worms survived at sludge contents between 20% and 40 %. However, further increase in sludge content to 50% to 100 % resulted in death of worms after 15 days of operation. Therefore, no results for the latter sludge contents were presented. The total number of worm in the bed was 30. It was kept constant as much as possible. Variation of pH, TOC and TN content of bed were monitored every 15 days. The changes in C/N ratio throughout the operation were calculated.

3.1 Effect of Sludge Content on Vermicompost Quality

3.1.1 20% Sludge + 80% CD

Variation of pH, TOC and TN content of bed material with time at 20 % sludge content is given in Figures 3.1, 3.2 and 3.3, respectively. As from Figure 3.1, pH value was 8.48 in the beginning of operation. There was no change in pH value for the first 15 days. It remained around 8.48. This result indicates that the worms were not active enough to digest the sludge and cow dung together. It could be because of the environmental conditions. No organic acid production was achieved for the first two weeks. However, as light decrease in pH value was observed for further operations and it decreased to pH= 8.29. The results indicated that rate of substrate digestion was slow.

Figure 3.2 depicts the variation of TN in the vermicompost with time. The initial %TN was 1.36%, then a slight increase in TN content was observed for the first 15 days and it reached to 1.49%. However, TN content sharply increased from 1.73% to 2.30% for the operation days between 30 days and 45 days, respectively. The rate of increase in TN content slowed down for the rest of the operation days up to 75 days. The final content was obtained as 2.69%. Although it seems that processing waste by worms slowed downed for the last weeks, TN content almost doubled at the end of the operation period with regard to the initial TN% content.

Variation of TOC content throughout the vermicomposting process is given in Figure 3.3. The initial TOC was 26.17%. At the end of 15 days operation, the content decreased to 24%. The change in the TOC content was not substantial until the second sampling period. It almost remained constant. A slightly rapid carbon consumption period was observed between 30 days and 60 days and TOC content was obtained as 22.47%. For the final 15 days of operation, no consumption of carbon was obtained; even there was a slight increase.

C/N ratio was calculated throughout the operation period. As seen from the Figure 3.4 there was a substantial change in the C/N ratio for 75 days of operation. The initial ratio was C/N= 19.23. A sharp decrease from about C/N=20 to C/N=9 in the ratio was observed for the first 45 days. At the end of the experiment, the ratio was even better as C/N=8.64. The main factor that affects the C/N ratio in this process is enrichment of nitrogen content of the process through vermicomposting rather than carbon removal from the substrate. If Figures 3.2 and Figure 3.4 are evaluated together, it is obviously seen that the decrease in the ratio and increase in the TN correspond to the same days.

Earthworms enhanced the nitrogen mineralization in the substrate, so that the mineral nitrogen was retained in the nitrate form. Earthworms also increase nitrogen by adding their nitrogenous excretory products, mucus, growth stimulating hormones and enzymes during the fragmentation and digestion of organic matter (Vig et al, 2011, Yadav & Garg, 2009).

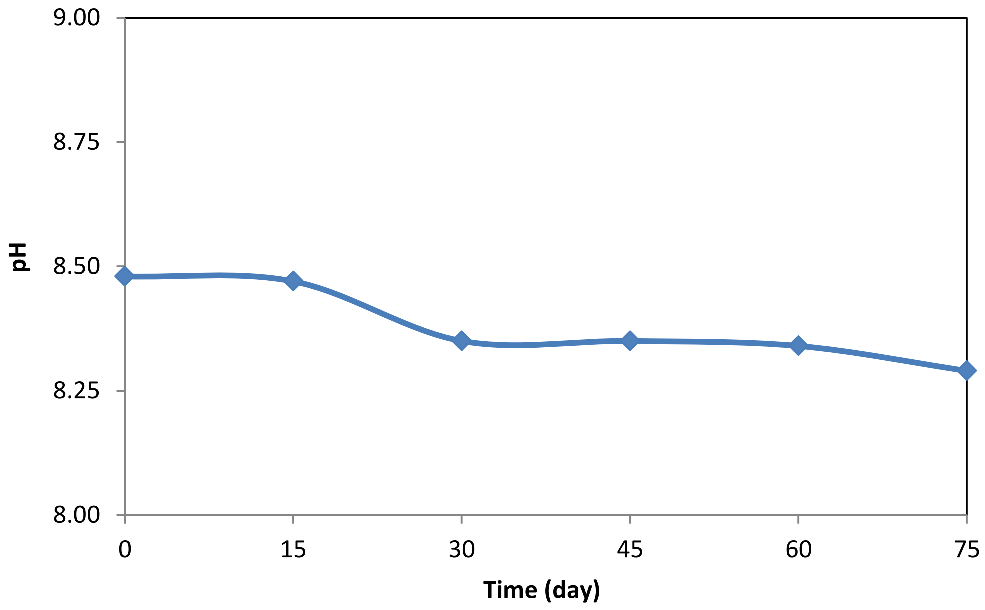

Figure 3.1 pH changes during vermicomposting of 20% sludge +80%cow dung mixture.

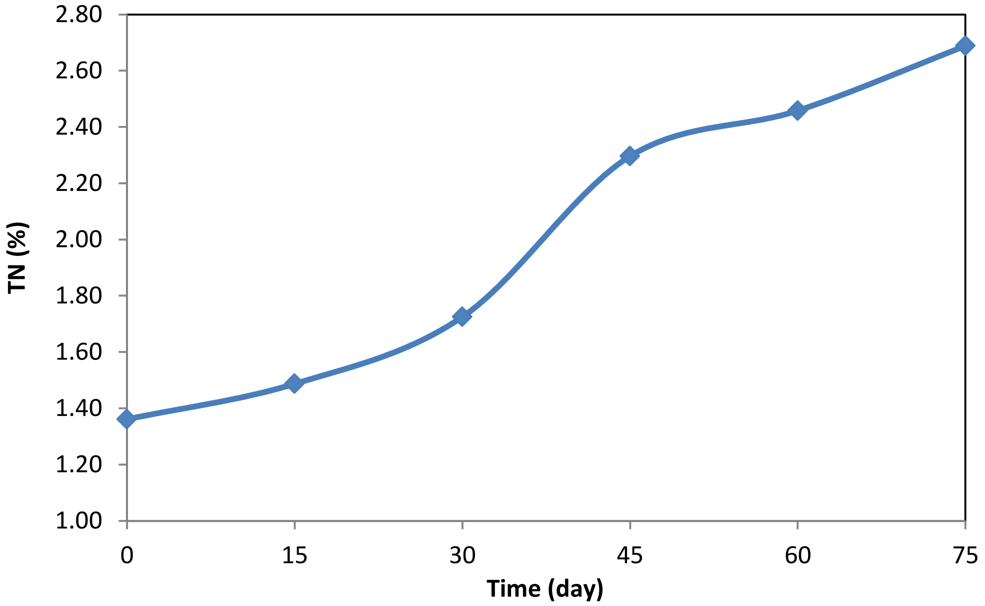

Figure 3.2 TN (%) changes during vermicomposting of 20% sludge +80%cow dung mixture.

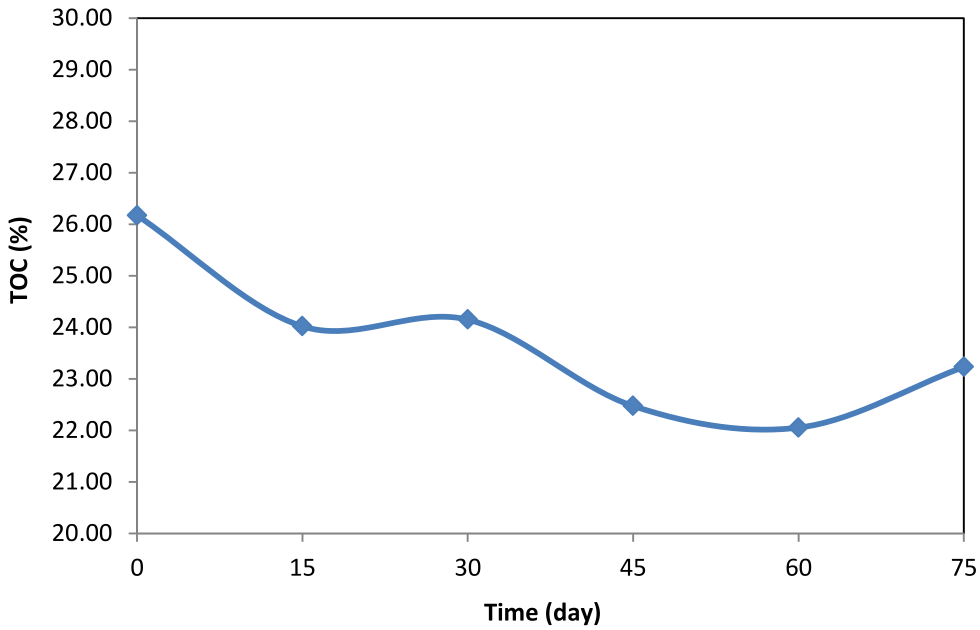

Figure 3.3 TOC (%) changes during vermicomposting of 20% sludge +80%cow dung mixture.

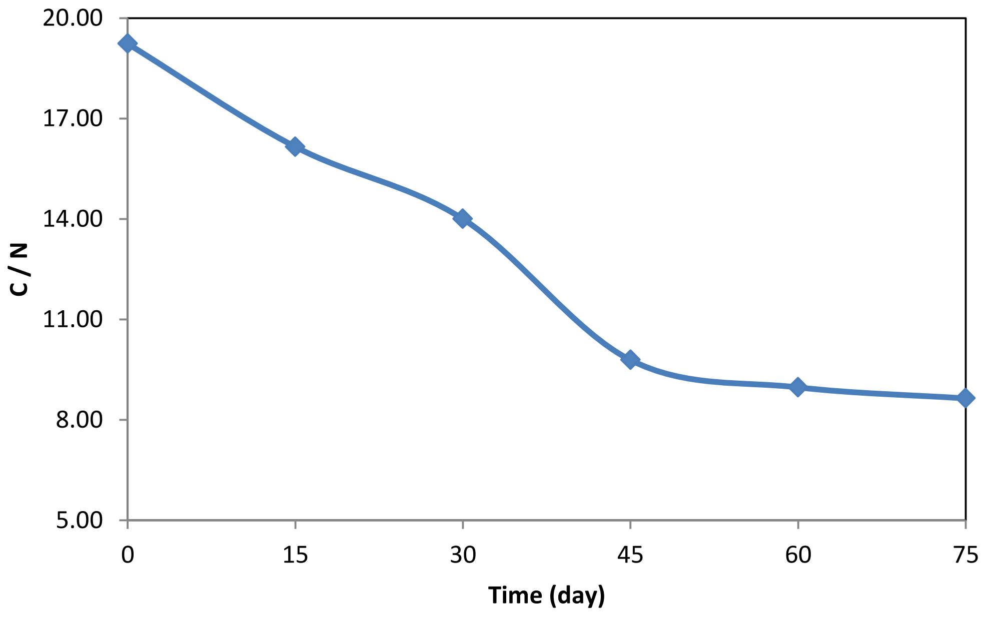

Figure 3.4 Changes in C/N ratio during vermicomposting of 20% sludge +80%cow dung mixture.

3.1.2 30% Sludge + 70% CD

Variation of pH, TOC and TN content during vermicomposting with time at 30 % sludge content is given in Figures 3.5, 3.6 and 3.7, respectively. The initial pH value was pH=8.07. No substantial change in pH was observed up to 45th day of operation. A slight decrease to around pH= 7.8 occurred for the days between t= 45 and 60 days. However, the final pH value was pH=7.4 which was desired pH value for a vermicompost. This slight decrease in pH can be evaluated that, worms need a time to adapt the new environmental conditions in terms of substrate composition.

The initial %TN at 30% sludge content was around TN=2.7% (Figure 3.6). Then it increased slowly for the first 15 days and reached to 2.95%. This gradual increase in the TN content was observed until the end of operation period. The resulting content was 4.21%. TN content almost doubled at the end of the operation with regard to the initial TN content. It seems that earthworms enhanced the nitrogen mineralization in the substrate throughout the processing.

At 30% sludge content, TOC was 25.63% in the beginning of the operation. As seen from the Figure 3.7, there was a slight decrease to 22.5% until the end of the 45^{th} day. These results can be evaluated as a good mineralization of organic matter. However, TOC showed an increasing trend up to 29% for the days between 45 and 75. It is not clearly understood the reason for this increase in the content. One of the possible reasons could be decay of the dead worms and release of their organic content to the wermibed.

At the end of the operation, C/N ratio calculated. As seen from the Figure 3.8 there was an important decrease in the C/N ratio. While the initial ratio was 9.44 then a slight decrease in C/N ratio was observed at the end of 60 days and it reached to 6.77. At last 15 days a little increase was observed in C/N ratio due to the increase in TOC.

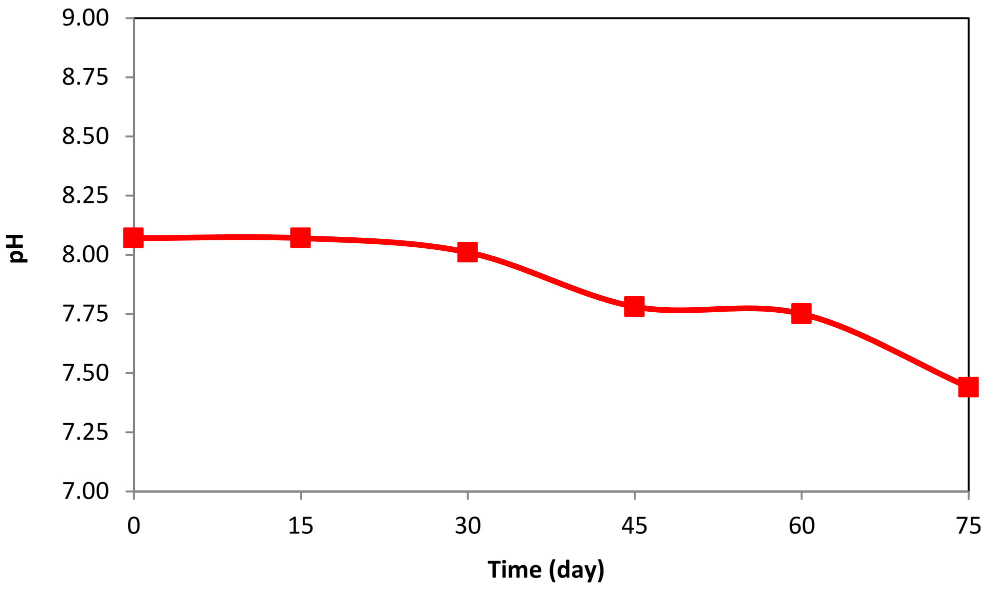

Figure 3.5 pH changes during vermicomposting of 30% sludge + 70% cow dung mixture.

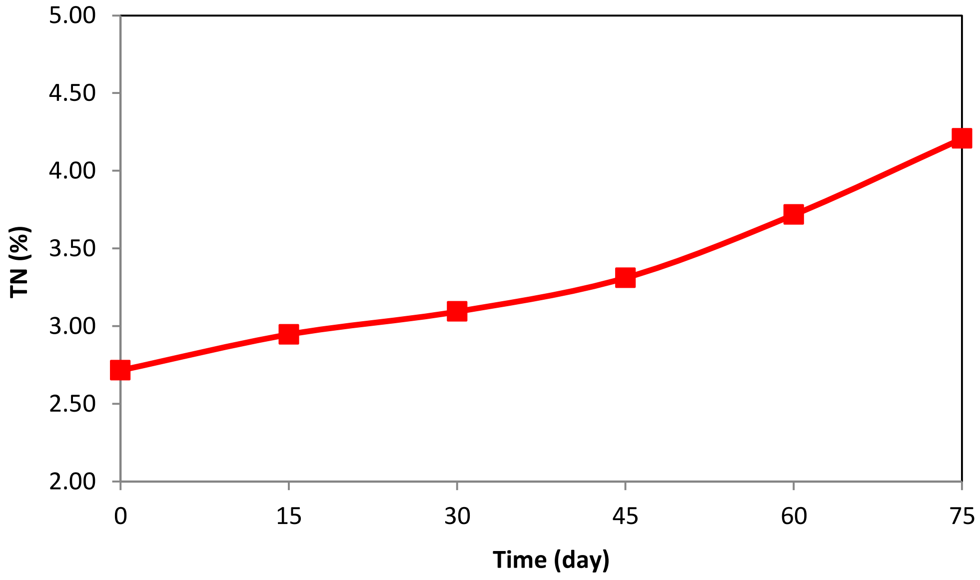

Figure 3.6 TN(%) changes during vermicomposting of 30% sludge + 70% cow dung mixture.

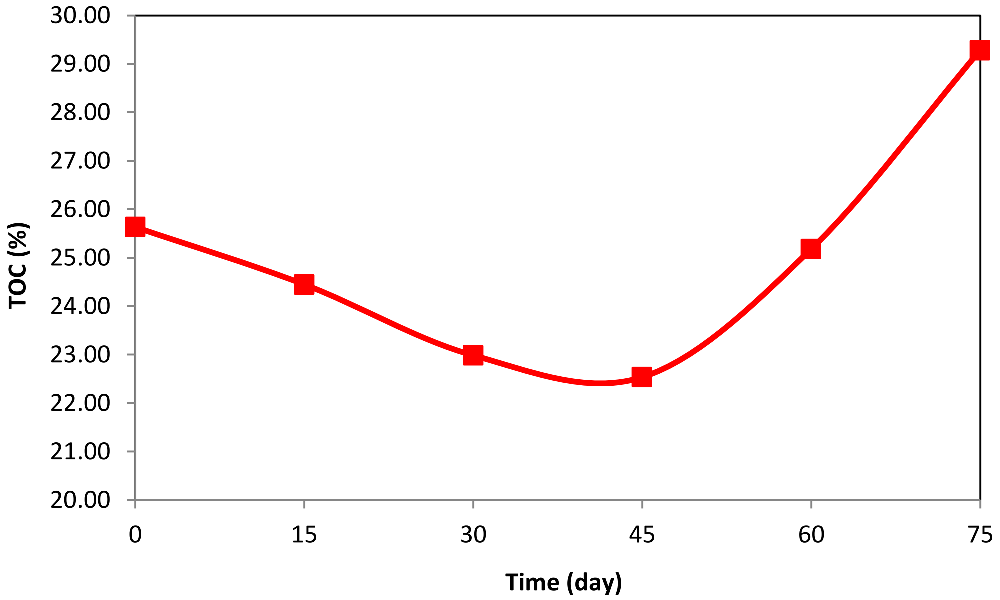

Figure 3.7 TOC (%) changes during vermicomposting of 30% sludge + 70% cow dung mixture.

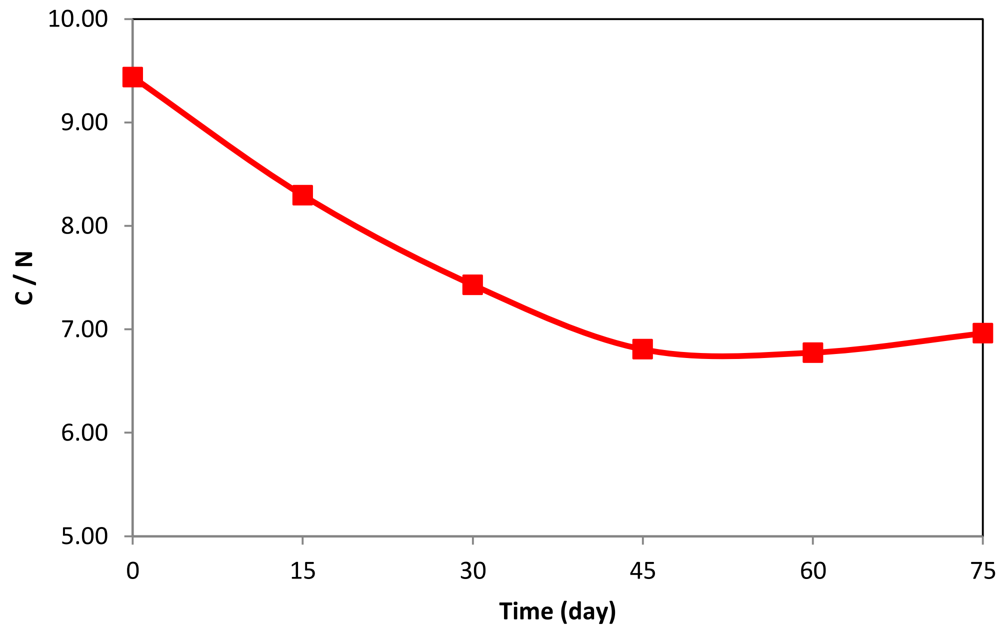

Figure 3.8 Changes in C/N ratio during vermicomposting of 30% sludge + 70% cow dung mixture.

3.1.3 40% Sludge + 60% CD

Variation of pH, TOC and TN content of bed material with time at 40 % sludge content is given in Figures 3.9, 3.10 and 3.11, respectively. pH value was 7.45 in the beginning of the operation. For the first 15 days, pH value decreased to 7.38 and it remained almost constant up to 45 days of operation. Then at the end of the operation it decreased top pH= 7.23. The decrease in pH indicates bioprocessing of organic content with the end product of organic acids. A long term adaptation to the new environmental condition due to media composition was observed, as well; in this case as expected. In fact, main organic substance removal started after 45 days of operation.

The initial TN% was 4.27% for 40% sludge content (Figure 3.10). There was steady increase in TN content to 5.14% throughout the vermicomposting process. However, about 1% increase in the content was substantially lower than the rise in TN content in other studied media compositions. One of the reasons for this result is the long adaptation period to media composition. If operation period was extended to over 75 days, the increase in TN content could have been higher.

The effect of long adaptation period was also observed in the case of TOC removal. TOC was 28% in the beginning of the operation. There was no significant change in TOC and it remained about 27%until the end of 45 days. But at the end of operation, the content was 22.528%. Decreasing in TOC was parallel to the decrease in pH. The main TOC removal was achieved after 45th days.

The initial C/N ratio for 40% sludge containing wermibed was C/N=6.56. The variation of C/N was parallel to variation of TN content up to 45 days then excess removal of TOC slowed down the decreasing in C/N ratio. The final ratio was C/N=4.38 which is lower than desired wermicompost C/N ratio.

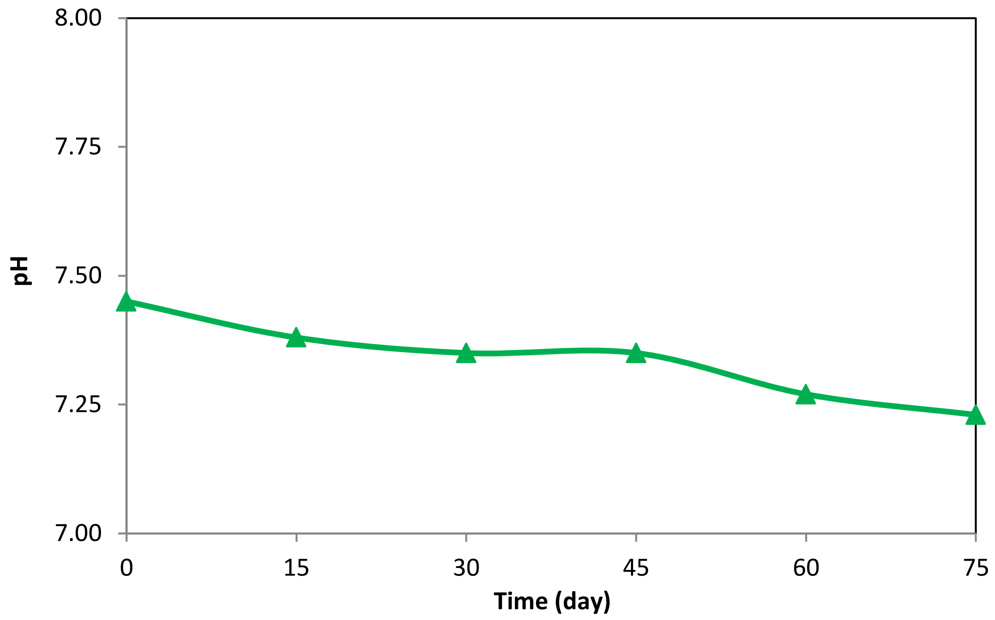

Figure 3.9 pH changes during vermicomposting of 40% sludge+ 60% cow dung mixture.

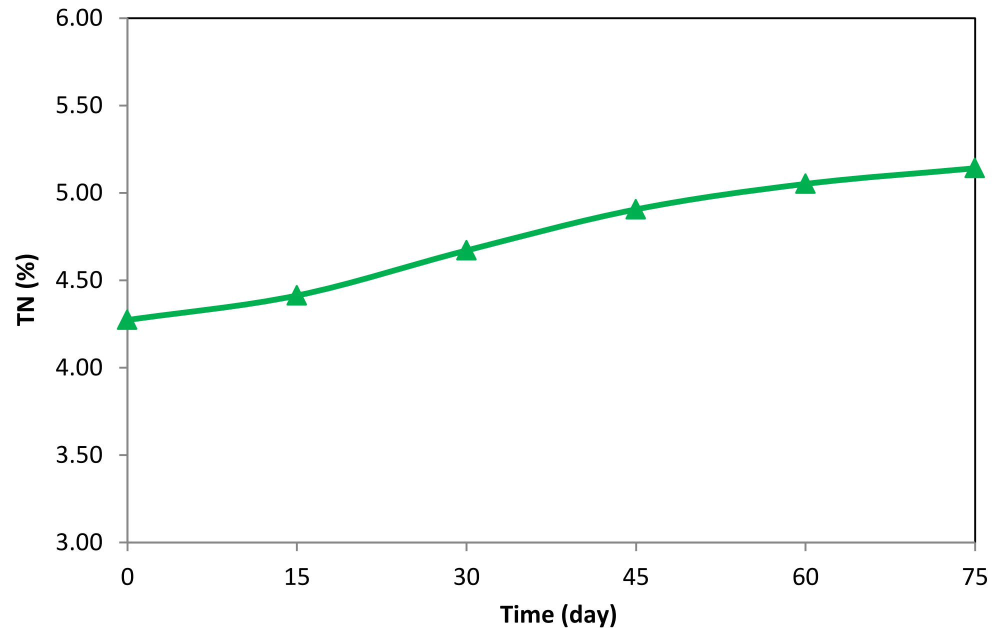

Figure 3.10 TN (%) changes during vermicomposting of 40% sludge+ 60% cow dung mixture.

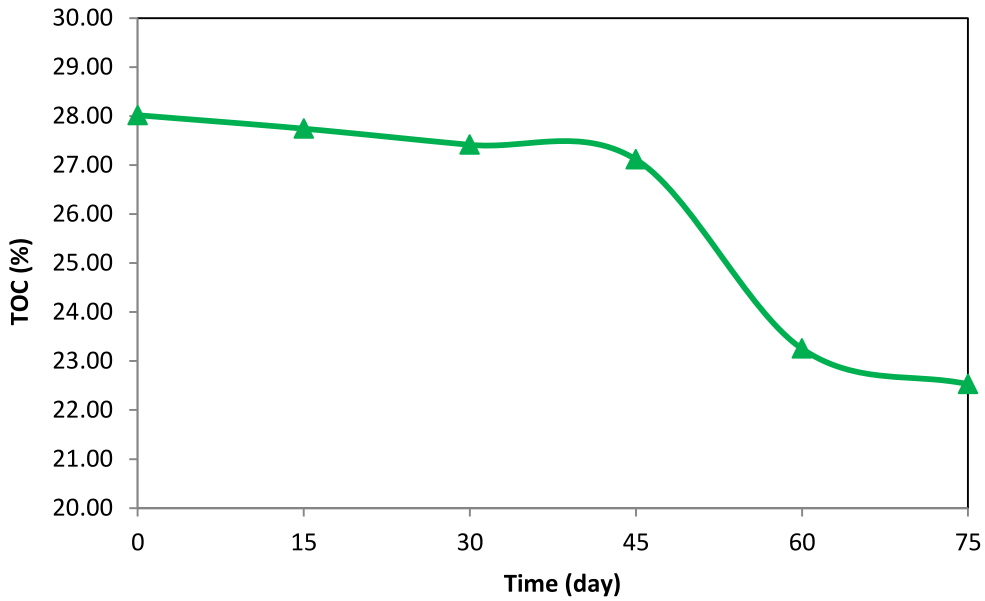

Figure 3.11 TOC (%) changes during vermicomposting of 40% sludge+ 60% cow dung mixture.

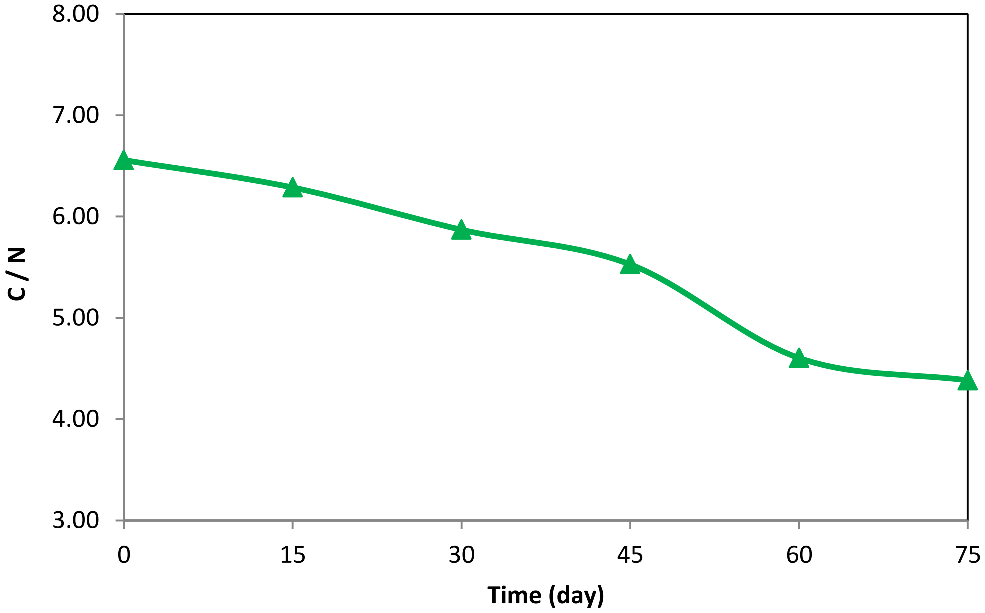

Figure 3.12 Changes in C/N ratio during vermicomposting of 40% sludge+ 60% cow dung mixture.

3.2 Evaluation of Process Performance

Vermicomposting process performance at different sludge ratios was evaluated in this section. Variation of pH, TN, TOC content and C/N ratio of bed material with time at three sludge contents were given in Figures 3.13, 3.14, 3.15 and 3.16, respectively.

The physico-chemical parameters showed significant changes in different feed mixtures. Decreasing pH values with respect to initial condition during vermicomposting were explained as formation of CO_2, organic acids and the mineralization of the nitrogen into nitrites/nitrates besides bioconversion of the organic material into intermediate compounds (Khwairakpam & Bhargava, 2009). There were slight changes in the pH throughout the vermicomposting for different sludge ratios with respect to initial pH values (Figure 3.13). Initial pH values decreased from 8.48 to 7.45 with the decrease in cow dung content. Fortunately, vermicomposting tried to shift the pH towards neutrality by processing the organic materials to organic acids. Worms prefer neutral pH values for the growth but can tolerate high pHs. At 20 % sludge content, pH was high enough to slow down the activity of worms. Rate of organic acid production was slowed down in parallel to metabolic activity of worms which resulted in final pH value around pH=8. Extending vermicomposting process could have provided neutral pH. In addition, microbial activity that helps mineralization of organic matters to CO_2 would be lower at this pH level. The maximum decrease in pH was observed in 30% sludge content (approximately 8%). The initial pH value was slightly better for the activity of worms compared to 20% sludge content. Therefore, organic acid production was enhanced and pH value decreased to around neutral level. In the case of 40% sludge content, pH was already around pH=7.4 which is the most suitable pH value for the growth of worms. This pH was maintained by the activities of both worms and microorganism.

The main aim of vermicomosting is to enrich the nitrogen concentration in the product. Nitrogen processing mechanisms in vermibed has been explained as earthworms mineralize the nitrogen in the substrate, so that the mineral nitrogen is retained in the nitrate form (Garg et al, 2006, Vig et al, 2011). Decrease in pH may be an important factor in nitrogen retention as this element is lost as volatile ammonia at higher pH (Garg et al, 2006). According to Viel et al. (1987) loss in organic carbon might be the main mechanism for nitrogen enhancement in vermicomposting. The results of this study were in parallel to the results in the previous studies. TN content was increased significantly with the time during the vermicomposting processes (Figure 3.14). The rate of increase in TN content during operation in different sludge contents was dependent upon the initial nitrogen present in the feed material and the degree of decomposition. The initial TN content was around TN=1.7% at 80% cow dung (CD)+20 % sludge (S) mixture and raised to 4.3% for 60%CD+40%S. Cow dung is a carbon rich but nitrogen deficient substrate. Therefore decreasing cow dung content in vermibed resulted in higher initial TN concentration. The percent increase in TN was in the order of 20% > 30% > 40% sludge content. In other words, $TN_{final}/TN_{initial} = 2$ for 20% sludge and $TN_{final}/TN_{initial}=1.2$ for 40% sludge containing vermibed. It was expected to have lower enrichment of nitrogen for 20% sludge. Since, pH was suitable for volatilization of nitrogen in the form of ammonia. This result indicated that there was no ammonia volatilization under this operation conditions.

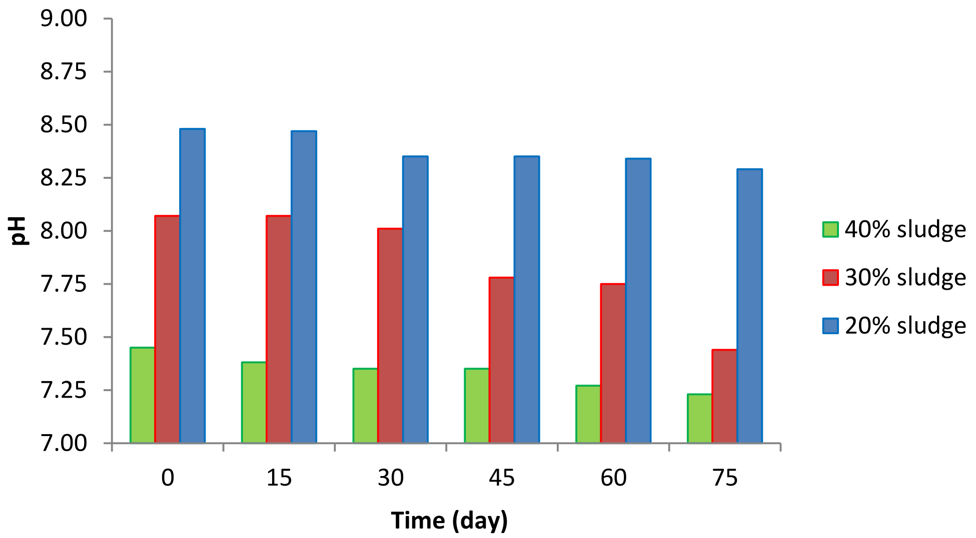

Figure 3.13 Effect of sludge content in vermibed on pH of vermicompost.

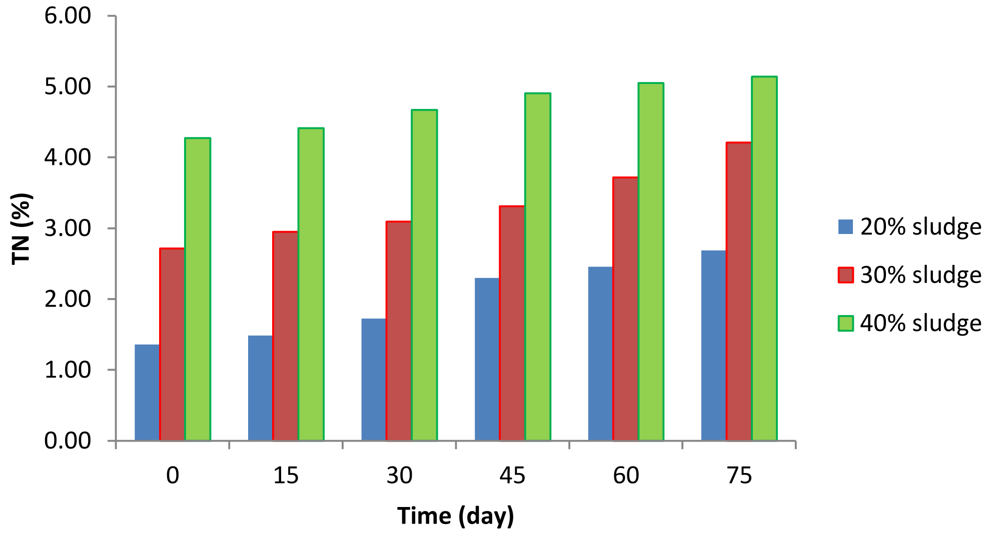

Figure 3.14 Effect of sludge content in vermibed on %TN of vermicompost.

A large fraction of TOC is lost as CO_2 as well as due to the consumption of the available carbon as a source of energy by the earthworms and the microorganisms (Khwairakpam et al., 2009). The decrease in TOC after vermicomposting indicates organic matter stabilization in the substrate due to combined action of earthworms and microorganisms. It has been reported that earthworms modify the substrate conditions, which subsequently enhance the carbon losses from the substrates through microbial respiration in the form of CO_2 (Vig et al, 2011). A significant decrease was observed in TOC content (Figure 3.15). Maximum reduction in TOC content was observed in 40% sludge content. The percent decrease in TOC content was in the order of 40% > 20% > 30%.

The C/N ratios of substrate material reflected the organic waste mineralization and stabilization during the process of decomposition. A decrease in C/N ratio was observed during the vermicomposting and this could be attributed to the loss of carbon as carbon dioxide through microbial respiration and simultaneous addition of nitrogen by worms in the form of mucus and nitrogenous excretory material (Vig et al, 2011). The initial C/N ratio was around C/N=20 and C/N= 7 for 20% and 40% sludge containing mixtures (Figure 3.16). Decreasing cow dung content in the mixture shifted the condition from nitrogen deficient to carbon deficient. At the end of the operations final product had lower C/N ratio, as compared to the initial value. The reduction in the C/N ratio was mainly due to TN enrichment. Maximum decrease in C/N ratio was observed in 20% sludge content at which the highest enrichment of nitrogen was observed. The percent decrease in C/N ratio was in the order of 20% > 30% > 40% which was the same as enrichment of TN.

An adaptation period to new media composition was observed during experiments. The time required for adaptation increased as sludge content was increased. It was about 15 days for 20 % sludge and rose to 45 days for 40% sludge content. Depending on this adaptation period, the total composition time necessary to obtain desired C/N ratios may need to be extended. The resulting C/N ratios were already in the range of desired condition in this study. Therefore, the process was ended at 75 days.

The experiments at 50% and 100% sludge content were not successful. The worms couldn't survive at these sludge percentages in vermibeds. One of the reasons for this result could be adverse effect of high sludge content on worms. The age of the worms could be another factor. It was not possible to monitor the life cycle of worms during experiments. Another one is the microflora necessary to help composting process. Cow dung is the main substrate to provide microbial flora for this purpose. Accumulation of worm end products at high sludge contents may not be further processed due to lack of microflora at low cow dung contents.

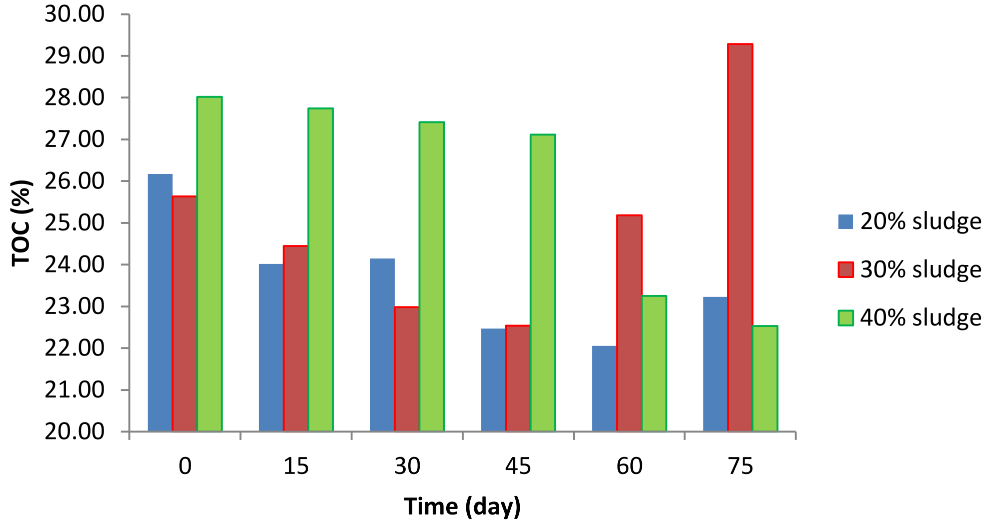

Figure 3.15 Effect of sludge content in vermibed on %TOC of vermicompost.

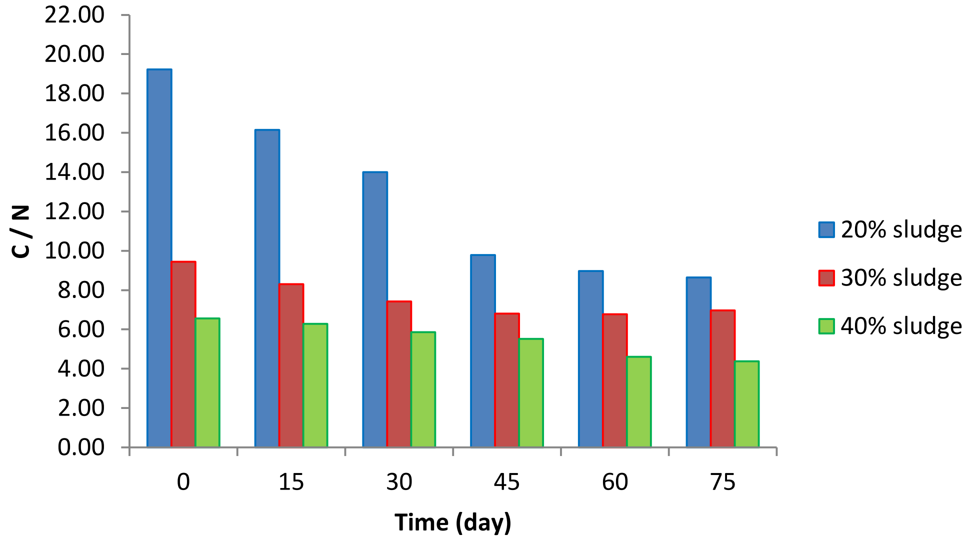

Figure 3.16 Effect of sludge in vermibed on C/N ratio of vermicompost.

CHAPTER FOUR
CONCLUSIONS AND RECOMMENDATIONS

Vermicomposting is a challenging process for conversion of waste materials to valuable products. It is a natural waste treatment process. There are limited control parameters like keeping moisture content of bed around 75% just by sprinkling waster, no aeration as required in conventional composting. However, keeping worms alive and reproduction of worms are the main problems encountered in this study.

Wastewater treatment sludges are main problems of treatment plants. Low to high cost processes are applied for stabilization of this waste. Sludge can be used as substrate in vermicomposting but it needs a co-substrate as cow dung to provide microbial flora for further mineralization of worms' end products. The best condition for vermicomposting of sludge can be suggested as 30% sludge and 70% cow dung and 60 days of operation to reach steady state condition. But it is possible to run the system at 40% sludge and 60% cow dung mixture to be able process higher sludge masses. Although the time required for this condition is 75 days, it will be shorter when system was reloaded with fresh media at the same ratios due to elimination of adaptation period.

The product obtained at the end of process was in good quality in terms of C/N ratio and can be used as natural fertilizer. Although initial C/N ratio was already low in this study, further processing or further decreasing in C/N ratio made the sludge more suitable to be used as fertilizer. Moreover, it was observed that sludge can be processed by the worms as substrate.

Vermicomposting of sludge can be improved by conducting further studies as suggested below;

1. Different co-substrates as domestic organic waste, agricultural organic wastes, can be used as co-substrate instead of cow-dung in vermicomposting of sludge.

2. Worm number is an important to increase the rate of vermicomposting. However, high worm numbers can adversely affect the process due to stress on worms at high individual number. Therefore, optimal number of worms per mass of vermibed can be determined.

3. Most of vermicpomposting process is operated batch wise. The process performance can be evaluated for fed-batch operation.

4. The quality of vermicompost can be evaluated by considering other factors like humic acids or organic acids, metal concentrations, forms of nitrogen as ammonia, nitrate or nitrite pathogens etc.

5. The fertilizer quality of product can also be evaluated by applying to the plants.

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