# Effects and promotion of Earthworm on municipal sewage sludge degradation.

Nasasira Boeres, Fu Xiao yong,

**Abstract:**
There are several techniques available for dealing with the municipal sewage sludge received during waste water treatment. Since municipal sewage sludge is a waste with high organic load generated in large quantities, a biological approach such as vermicomposting that uses Earthworms to degrade and partially digest the waste sludge was studied. To determine the effects and promotion of earthworms on degradation of municipal sewage sludge, obtaining a stable product with a high agronomic value by mineralize the essential nutrients like nitrogen, phosphorus and potassium from the sludge and reduce its risk to the environment. It is well established that many organic wastes can be ingested by earthworms and egested as peat like material termed as vermicompost, reduce the pathogens to safe levels (by devouring them and by discharge of antibacterial coelomic fluid) and toxic bioaccumulation by ingestion of the heavy metals through their intestine as well as through their skin.
This study evidences that earthworms can facilitate the stabilization of PDS through modifying microbial activity and number and
community during vermicomposting.

**Keywords:** Earthworms, vermicomposting, Pelletized dewatered sludge, and sludge stabilization.

## Introduction

Sludge is an inevitable, hazardous and odorous by-product from conventional water and waste-water treatment plants which eventually requires safe disposal either in landfills or by incineration incurring heavy cost. When sludge is dewatered and dried, the residue is termed 'biosolids'. Management of biosolids remains problematic due to the high cost of installing sewage sludge stabilization reactors and dehydration systems. Sewage sludge is a potential health hazard as it contains high numbers of cysts of protozoa, parasitic ova, fecal pathogens like Salmonella spp., Shigella spp. and Escherichia coli and heavy metals such as zinc, cadmium, mercury and copper. In addition, sludge contains organic molecules and essential plant nutrients like nitrogen, phosphorus, potassium and various trace elements.
Where in such setting of excess sludge as the byproduct of biological wastewater treatment process which can be costly and slow upon handling such residual byproduct. With the new wave of industrialization and energy based intensive agriculture, chemical pathways for raw materials conversion became predominant with extensive use of agrochemicals. The damaging long-term environmental impacts and resource depletion indicate un-sustainability of the current methods.

There is an impetus to develop a low-cost approach to promote municipal sludge degradation and to fasten the rated its stabilization. It is an increasing knowledge that vermicomposting is a promising technology for the safe, effective, and sustainable treatment of organic wastes. Its final product – vermicompost is regarded as a gold bio-organic fertilizer because it contains a higher content of water-soluble nutrients and a wider richness of microbial community diversity relative to composting product ((Domínguez, Edwards et al. 2000, Khwairakpam and Bhargava 2009);

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(Fernández-Gómez, Nogales et al. 2010, Fu, Cui et al. 2016). Earthworms feed readily upon the sludge components, rapidly converting them into vermicompost, reduce the pathogens to safe levels and ingest the heavy metals. Volume is significantly reduced from 1 m3 of wet sludge (80% moisture) to 0.5 m3 of vermicompost (30% moisture; (Sinha, Herat et al. 2010).

**Vermicomposting process:**
It is the biochemical degradation process of organic materials, which implicates the complex relationships between earthworms and microorganisms. During this process, organic wastes can be effectively converted into valuable products with higher available nutrients for plant or soil, meanwhile massive earthworms are also harvested. The use of earthworms for the management of sewage sludge is termed as vermistabilization. Until now, vermicomposting trials have been successfully applied for treating several types of sludge like industrial and municipal sludge (Yadav and Garg 2011, Fu, Huang et al. 2015). However, for this technology, an important point should be noted that fresh sludge cannot be directly used as the substrate for earthworms, because it easily generates the leachate with toxicants and gives rise to an anaerobic environment where earthworms cannot survive (Gupta and Garg 2008); (Suthar 2010, Hait and Tare 2011, Yadav and Garg 2011, Fu, Huang et al. 2015).

Therefore, a suitable approach for the pretreatment of fresh sludge before vermicomposting is essential for earthworm's survival. To push on the realization of vermicomposting industrialization, the effectively simplified pretreatment methods are desired to be explored. The palletization of sewage sludge combined with thermal dry is perceived as a rapid and effective technique for dealing with the dewatered sludge (Fu, Huang et al. 2015). This technique provides a new dimension for vermicomposting pretreatment of dewatered sludge. Because, the pelletized method can increase the specific surface area of dewatered sludge and enhance its aerobic qualities, which provides a suitable condition for the growth of earthworms. Hence, the cumbersome blending bulking materials may be omitted prior to vermicomposting. Based on above mentioned, it is reasonable to hypothesize that the pelletized dewatered sludge (PDS) could be vermicomposted directly by earthworms without other pretreatments or bulking material (Fu, Huang et al. 2015). The primary consumers of the vermicomposting food web are the microbes (mainly bacteria, fungi and ciliates) that break down and mineralize organic residues. Microbes are the most numerically abundant and diverse members of the vermicomposting food web and include thousands of organisms. Secondary and higher-level consumers, i.e., the soil fauna including the earthworms, exist alongside microbes, feeding on and dispersing them throughout the organic matter (Domínguez, Gómez-Brandón et al. 2010)

Although earthworms guide the fauna and microorganisms in vermicomposting system, microorganisms may play a more important role in degrading organic material, as reported by (Sen and Chandra 2009) and (Fu, Cui et al. 2016). Hence, in vermicomposting system, the examination with respect to microbial activity, number, and community is of high significance, because this can well clarify the underlying mechanism regarding the decomposition and stabilization of organic substance during vermicomposting process.
Vermicomposting was based on the view of introducing earthworm into the traditional sewage treatment, which prolongs and extends the food chain in the reactor. The microorganism in the system feeds on the organic pollutants of the sewage. Earthworm grows between 5-20cm of

surface of the filter mediums, Earthworm feeds on suspended substance and biological sludge of the sewage.
The feeding ability of earthworm has powerful enzyme system, by which the devoured material can be broken down and digested, which finally can be transformed into own proliferation and the high stability of organic and inorganic compounds-worm cast, and then to achieve stabilization of sludge.

Vermicompost requires a good balance of C and N that is beneficial for the worm (in biomass, reproduction and reduction of mortality rates). A C/N ratio of 25 is considered good for vermicomposting and stabilization of sewage sludge and reduction of volatile solids. Sewage sludge may have low C/N ratio due to higher nitrogen content(Sinha, Herat et al. 2010).
During the process, cooperating of microbes with earthworms could increase the degradation of organics, circulation of carbon, nitrogen and phosphorus, and enhance the sludge fertility (Yang, Zhao et al. 2013).
The quality of vermicompost upon stabilization is significantly better, rich in key minerals and beneficial soil microbes as compared to the conventional composting which is thermophilic (temperature rising to 55°C) in which many beneficial microbes are killed and nutrient especially nitrogen is lost (due to gassing-off of nitrogen). Worms also keep the system fully aerated and aerobic processes are about 10 times faster than anaerobic processes(Sinha, Herat et al. 2010).
The earthworms release coelomic fluids that have antibacterial properties and destroy all pathogens in the waste biomass (Sinha, Herat et al. 2010). They also devour protozoa, bacteria and fungi as food. They seem to realize instinctively that anaerobic bacteria and fungi are undesirable and so feed upon them preferentially, thus arresting their proliferation.
In the intestine of earthworms, some bacteria and fungi (Penicillium spp. and Aspergillus spp.) have also been found (Singleton, Hendrix et al. 2003, Sinha, Herat et al. 2010). They produce antibiotics which kill the pathogenic organisms in the sewage sludge making it virtually sterile. The removal of pathogens, fecal coliforms (E. coli), Salmonella spp., enteric viruses and helminth ova from sewage and sludge appears to be much more rapid when they are processed by E. foetida. Of all, E. coli and Salmonella are greatly reduced (Bajsa, Nair et al. 2003, Sinha, Herat et al. 2010).
Vermicomposting lead into the increase in ash concentration during vermicomposting suggests that vermicomposting accelerates the rate of mineralization (Albanell et al. 1988). Mineralization is the process in which the chemical compounds in the organic matter decompose or oxidize into forms that could be easily assimilated by the plants. Ash is an alkaline substance which hinders the formation of $H_2S$ as well as improves the availability of O2 and thereby renders composts odorless. Thus, vermicomposting increases the ash content and accelerates the rate of mineralization which is essential to make nutrients available to plants.

The present paper stresses on the impact of earthworms on the stability of municipal sludge to provide theoretical basis for vermicomposting process, design comprehensive and systematic research on how earthworms accelerate the degradation of municipal sludge. To obtain the prospected purpose, this paper analyzes the internal and external factors and the role of earthworms in accelerating the degradation of municipal sludge by analyzing the physical and chemical indexes change during vermicomposting.
Therefore, aim of this study was to investigate the effects and promotion of Earthworm on municipal sewage sludge degradation of fresh pelletized dewatered sludges (PDS) without any

bulking materials using earthworms Eisenia fetida.

## 2.1 Materials and Methods

### 2.1.1 Earthworm and sludge collection

The earthworm *Eisenia foetida*, a common vermicomposting species, were cultured under laboratory conditions by dewatered sludge in a specific reactor. The Eisenia foetida is the most suitable species for vermicomposting due to their short life cycle with reproduction and regeneration rate it can tolerate with wide range of temperature and humidity(Hartenstein, Neuhauser et al. 1979, Ismail and Thampan 1995). The clitellated adult earthworms with the weight of approximate 1 g/worm were randomly chosen for vermicomposting experiment. Fresh dewatered sludge was collected from the Qilihe Anning Wastewater Treatment Plant, Lanzhou, China. Subsequently, the fresh dewatered sludge was placed on wire meshes with sizes of 4.5 mm x 4.5 mm and then gently squeezed by hand in laboratory to obtain ellipsoidal fresh PDS with the sizes of the 4.5 mm were obtained for this study. A steel pot, without a hole on the bottom, having a size of 36 cm x 12 cm (diameter x depth), was used as vermireactor. The physicochemical properties of dewatered sewage sludge are given in in Table 1 below.

### 2.1.2 Experimental design

In this experiment two degrading treatments with and without earthworms were set up in parallel. Each treatment was repeated in triplication to minimize the experimental errors. Therefore, a mesocosm was established using six metal pots with each size of 36 cm×12 cm (diameter × height). For each reactor, a total of 4 kg fresh 4.5 mm PDS was gently added as substrate and as vermi-bedding material of earthworms. Then, 100 *Eisenia foetida* earthworms were inoculated in three reactors as a vermicomposting system. Meanwhile, another three reactors were designed as a control system without earthworms.

Thereafter, a plastic film without any piercings was covered on each reactor to maintain the constant moisture. Subsequently, black braided fabric was placed on the plastic film to keep the dark environment. To supply the oxygen for earthworms, all reactors could be turn over once a day. The PDS could be stabilized from the 50 days of vermicomposting. As a result, this experiment was performed at 25±1 °C for 60 days. The earthworms were picked out by hands, and then their body weight and numbers were counted. The samples were collected for physicochemical and biological properites analysis. On the basis of the previous study of (Fu, Cui et al. 2016).

### *Statistical analysis*

All parameters were assayed in triplicate. All data were normalized before ANOVA treatment using the software of STATISTIC 8.0. One-way analysis of variance (ANOVA) with mean separation by Tukey's significant difference (HSD) test at 95% confidence level was performed to assess the differences of two treatments. Repeated measured ANOVA was used for the assessment of integral difference of samples with the vermicomposting time as stated well by (Fu, Huang et al. 2015)

### Analysis of physicochemical and biochemical properties

In this study pH and electrical conductivity (EC) were measured with a mixture of composting sample and water at the ratio of 1:50 (dry weight: volume basis). The suspension of the fresh sample and water (fresh sample: water = 1:5, wet weight basis) was filtered through 0.45 µm membrane filter for DOC determination by titration with ferrous sulfate after oxidation of sulfuric acid potassium dichromate at 200-220 °C. In this study, DOC means the organic carbon that could

be released from the composting sample to water. The determinations of water content and organic matter were conducted by oven for 12 h at 105 °C and 5 h at 550 °C, respectively.
Total nitrogen was analyzed by ultraviolet spectrophotometry after digestion by alkaline potassium persulfate by the mixture of $H_2SO_4$ and $H_2O_2$. The extracted samples with potassium chloride solution were used for the analysis of ammonia-nitrogen and nitrate-nitrogen by the spectrophotometric method using the standard published by Ministry of Environmental Protection of the People's Republic of China(Fu, Huang et al. 2015) to gain homogenized samples were dissolved into deionized water in the form of powders. After shaking, one of the suspensions was extracted by potassium sulfate solution and then filtered by quantitative filter paper with the pore of 30-50 µm and subsequently used for determination of microbial biomass carbon (MBC) by fumigating the sludge with chloroform, based on the method of Wu (2016).

**Table 1 Physicochemical properties of initial PDS, final products of control, and vermicomposting system.**

| Parameters | Initial PDS | Control | Vermicompost |
|---|---|---|---|
| **Water content (%)** | 80.6±0.01a | 72.2±0.03b | 76.4±0.03c |
| **Organic matter (%)** | 68.00±0.01a | 51.8±0.02b | 49.8±0.02c |
| **pH** | 6.8±0.00a | 7.1±0.00b | 7.00±0.00b |
| **Electrical conductivity (µS/cm)** | 569.00±8.50a | 1588±5.70b | 2107.00±28.20c |
| **Dissolved organic carbon (g/kg)** | 16.70±0.20a | 7.60±0.20b | 9.2±0.20c |
| **Ammonium (mg/kg)** | 7.40±0.10a | 289.10±5.40b | 240.90±5.00c |
| **Nitrate (mg/kg)** | 10.30±2.70a | 573.40±77.60b | 1390.50±81.50c |
| **Ammonium/ nitrate** | 0.74±0.19a | 0.51±0.06b | 0.17±0.01c |
| **Total nitrogen (g/kg)** | 51.70±0.30a | 35.30±0.40b | 36.00±0.40b |
| **Total phosphorous (g/kg)** | 18.20±1.40a | 26.50±1.10b | 28.00±0.80b |
| | | | |

***Value are means ± SE (n=3). Different lowercase letters in a given row mean significant difference between groups. (P<0.05, based on least significant different (LSD) test).***

# Results and discussion

**Changes of physiochemical properties-**

**The changes in some typical parameters like pH, EC, OM, DOC, C: N, and WHC of mixture material for successive mixing cycle during AAC are presented in table**

### 1. pH

The changes of the basic physicochemical properties during vermicomposting of Municipal Sewage Sludge under different temperature are shown in the Table 1. For 60 days, pH values showed a slight fluctuation at the range of 6.3-7.1 in the reactor as shown in the figure- 2 below. At the end, the significant pH fluctuation values were recorded from 0-60 days at incrementing temperatures of 5 $^0$C values between 15- 25 $^0$C were recorded in vermicomposting products, showing the highest and lowest values in treatments under 25 $^o$ C and 15 $^o$ C, respectively.

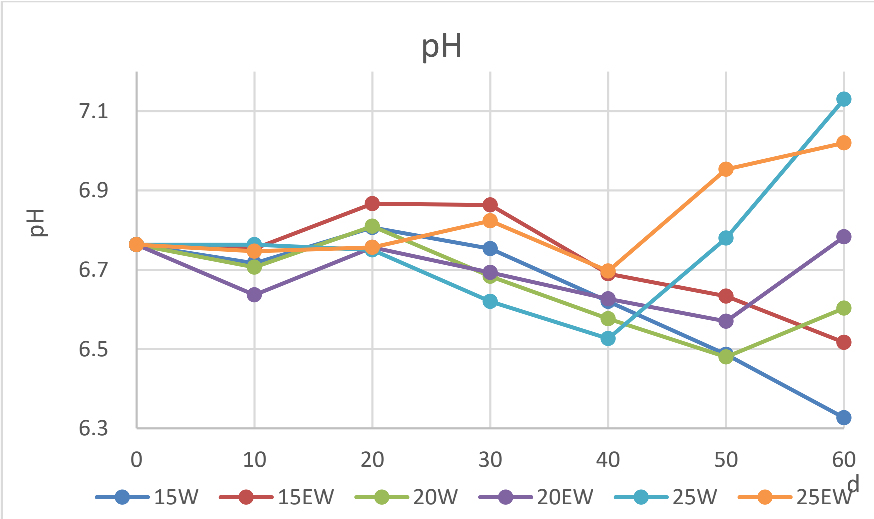


***Figure 1 showing the change of pH in vermicomposting system at different temperature.***

The change in pH showed indefinite trend in vermicomposting systems, involving in inorganic acids reactions and the decomposition of organic acids. The slight variation in pH identified in this study may be probably because earthworms can have a capacity of alter the buffering capacity of acids and alkaline and thus maintaining the pH values at a stable level. A similar trend between the pH and $NH_{4+}$ at the end stage of vermicomposting can indirectly intimate that $NH_{4+}$ played a critical role in regulating pH.

During vermicomposting, the final pH of all the vermibed reactors was slightly decreased in first few days of treatment with respect to the initial feed mixture (fig.1) above which notably increased later after 40 days of the experiment in all vermi reactors. The significant lowest pH was observed as 6.5 ± 0.03 with earthworm which is suitable for earthworm development. As it was documented on earlier that worms are adapted to survive in harsh environments of moderately acidic-to-alkaline conditions with pH values ranging from 4.5 to 9. These changes in pH may be due to the degradation of organic solid wastes in sludge, and during this process, formation of humic acid and ammonium ions occurred; similar results were supported by (Short, Frederickson et al. 1999). The low pH in vermicompost may also be due to the production of fluvic acid and humic acid，(Chauhan and Singh 2012, Fu, Huang et al. 2015) concluded that production of CO2 and organic acids by microbial decomposition during vermicomposting lowers the pH of substrate. Similarly, (Ndegwa, Thompson et al. 2000) pointed out that an alternation in pH might be related to the mineralization of the nitrogen and phosphorus into nitrites/nitrates and orthophosphates and biomodification of the organic material into intervening species of the organic acids. This finding had a direct correlation with other researchers (Mitchell and Edwards 1997, Atiyeh, Domínguez et al. 2000, Ndegwa, Thompson et al. 2000, Khwairakpam and Bhargava 2009).

There is a significant difference in pH value between the earthworm treatment and the control from the 20$^{th}$ day. pH in the control display a decreasing and then increasing trend. While, the pH in vermicomposting showed a fluctuating increasing trend. The end values between the treatment with earthworms and the treatment of control did not remarkably differ. pH acid buffer capacity (pHBC) (result not shown here) of the earthworm treatment and control generally displayed a trend of initial decreasing and then subsequent increasing. The 40$^{th}$ day was dividing point pHBC slightly decreased in the first 40 days and then increased in the later 40 days (Fu, Huang et al. 2015).

At the end, the increment of pH in the earthworm treatment was slightly higher than that of the control. The pH within the parity range can as well reflect the effect of acid buffer capacity (ABC) during vermicomposting. Even though it was not experimented here, (Fu, Huang et al. 2015) noted that ABC decreased, in the first 40days and then sharply increased until the end and such findings are close similar with the pH trend seen in figure above. Compared to control, significantly higher pH and its buffering capacity were found in the vermicomposting treatment, indicating that earthworms could pronouncedly enhance the pH buffering capacity.

## 2. Electrical conductivity (μS/cm)

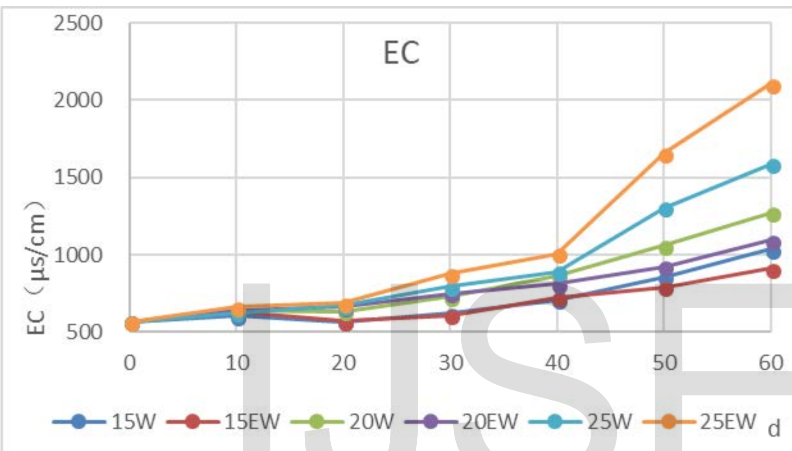


*Figure 2 showing the change of EC in vermicomposting system at different temperature.*

The electrical conductivity (EC) reflects the salinity of an organic amendment. High salt concentration may cause phytotoxicity problems and therefore EC is a good indicator of the suitability and safety of a compost or vermicompost for agricultural purposes. EC was affected in different ways by the application of the different treatments. The value of this parameter increased after the active process of composting relative to the control (Lazcano, Gómezbrandón et al. 2008). A sharp increase due to the release of soluble salts like ammonium and phosphate after the degradation of the most labile compounds in the thermophilic stage of composting has also been reported by several authors (Villar, Beloso et al. 1993)

The electrical conductivity displayed a gradual increment trend in all treatments and the highest value of electric conductivity appeared in 25$^0$C treatment, followed by 20$^o$C and 15$^o$C. The enhanced electrical conductivity may be attributed to mineralization of organic substances in the dewatered sludge (Suthar and Singh 2008).

A significant increase in EC in vermicompost was observed in all the reactors as compared to the initial material (Hait and Tare 2011). Some researchers like (Kaviraj and Sharma 2003) postulated that the increase in EC might have been due to loss of organic matter and release of different mineral salts in available forms such as phosphate, ammonium, potassium, etc. The augment of electrical conductivity suggests that vermicomposting can lead to the releases of inorganic ions and mineral salts from the original substrate. It has been demonstrated that earthworms could accelerate the shift of insoluble organic matter into a soluble form. (Fu, Huang et al. 2015)

### 3. Organic matter (%)

The content of organic matter along with the microbial number and activity are often chosen for assessment of stability in the vermicomposting process.

As presented in Fig 3 below, a continuous reduction of organic matter content was observed in both control and vermicomposting treatments. In contrast to the initial PDS, the control and vermicomposting significantly decreased by 23.8 and 26.7 %, respectively. The finding that earthworms can accelerate the decomposition of sewage sludge coincided with others(Lazcano, Gómezbrandón et al. 2008, Liu, Lu et al. 2012) in addition, this acceleration effect was associated with earthworm's density. Because the nutrients of sludge could not satisfy the requirement of earthworms' growth, where the higher the density of earthworms led to rapid loss of organic matter in the sludge.

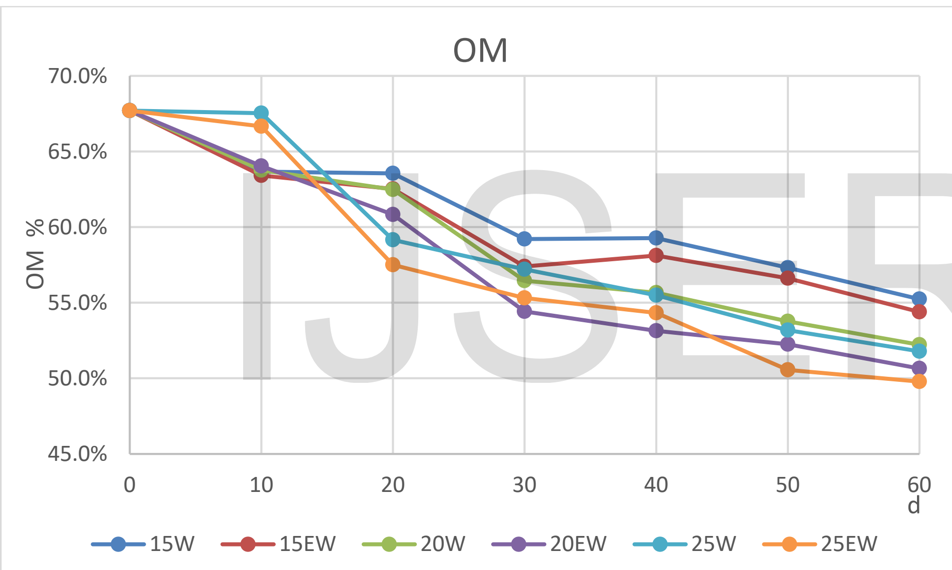


***Figure 3 showing the change of OM in vermicomposting system at different temperature.***

The inoculation of earthworms can accelerate the decomposition of protein-like substance in sludge. Moreover, earthworms, to some extent, have a promotion effect on the transformation of organic matter in all treatments, thus it declined with the vermicomposting time. Among these, the largest drop was found in the treatment at 25°C. At the end, the content of organic matter dropped in the reactors at 15°C, 20°C and 25°C condition, respectively.
In vermicomposting systems, earthworms degrade organic materials together with microorganisms. It is affirmed in this study that the temperature could strongly affect the activity of earthworms' growth and reproduction as it was also asserted by (Frederickson and Howell 2003); Meanwhile, the fact that the microorganisms were strongly affected by temperatures is also well understood in such environment systems as composting and anaerobic digestion.
Vermicomposting process could promote the decomposition and transformation of liable water soluble organic matter in sewage sludge, thus leading to the stabilized products were achieved in end products(Xing, Li et al. 2012). DOC as the most easily degradation carbon fraction of the

substrate, involving several biochemical reactions, is considered as a critical index for assessing the stabilization of organics during composting/vermicomposting process.
After 30 days, the DOC content in all treatments declined markedly and then kept stable values. Given that a threshold DOC value less than 4.0 g/kg was suggested as an indicator for assessing the maturity of vermicomposts (Xing, Li et al. 2012), the matured products were harvested in this study. This finding thus evinces that the pelletized pretreatment of dewatered sludge for vermicomposting appears to be an effective method for its stabilization.

On another hand, Municipal Sewage Sludge (MSS) is mainly comprised of microbial organics, in which the protein content is higher than other materials such as saccharides, lipid and nucleic acid (Neyens and Baeyens 2003). Therefore, the degradation speed of nitrogenous substances is closely associated with stabilization process of sludge. Generally, NH4+ and NO3 are considered as the important indices for affecting the decomposition process of organic nitrogen. The highest ammonification speed was recorded in the reactor operated under $25^0$C, being significant higher than other two treatments. The suitable growth temperature of ammonification bacteria was reported in the range of 25-35 $^0$C (Xu, Sung et al. 1986). The population of ammonification bacteria and its corresponding ammonification efficiency were enhanced with the increment of temperature at a certain range (Kuang et al., 2013 and Zhang, 2012) also $25^0$C was proven to be the optimal temperature condition for the growth ammonification bacteria (Guo et al., 2013) of solvable microbial byproducts-like substance.

***The change of organic matter and earthworms' density during vermicomposting of municipal sludge.***

Earthworms promote the growth of 'beneficial decomposer bacteria' in solid wastewater material and acts as aerators, grinders, crushers, chemical degraders, and biological stimulators (Sinha, Herat et al. 2002) Earthworms also granulate the sludge particles and increase the hydraulic conductivity and increase the total specific surface area and thereby enhance adsorption of the organic and inorganic matter from the solid wastewater. Earthworms are the crucial drivers as they stimulate and increase biological activity by fragmentation and ingestion of organic matter and this will increase the surface area to be exposed to microorganism

Earthworms' population is considered as an important parameter that can reflect the affecting intensity of earthworms on the substrate during vermicomposting.

## 4. Dissolved organic carbon (g/kg)

There was constant drop of Organic C in final vermicomposting product, when compared to the initial level in the substrate as demonstrated in figure 4 below.

Comparatively, substrate with earthworm (vermicompost) showed the greater carbon loss as compared to control containers (all significant: t-test), which suggests earthworm-mediated rapid organic matter mineralization rate in vermi-beds. According to (Domínguez 2004) vermicomposting is a combined operation of earthworm and microorganisms in which earthworm fragments and homogenizes the ingested material through muscular action of their foregut and also adds mucus and enzymes to ingested material and thereby increases the surface area for microbial action while, microorganisms perform the biochemical degradation of waste material providing some extracellular enzymes required for organic waste decomposition within the worm's gut.

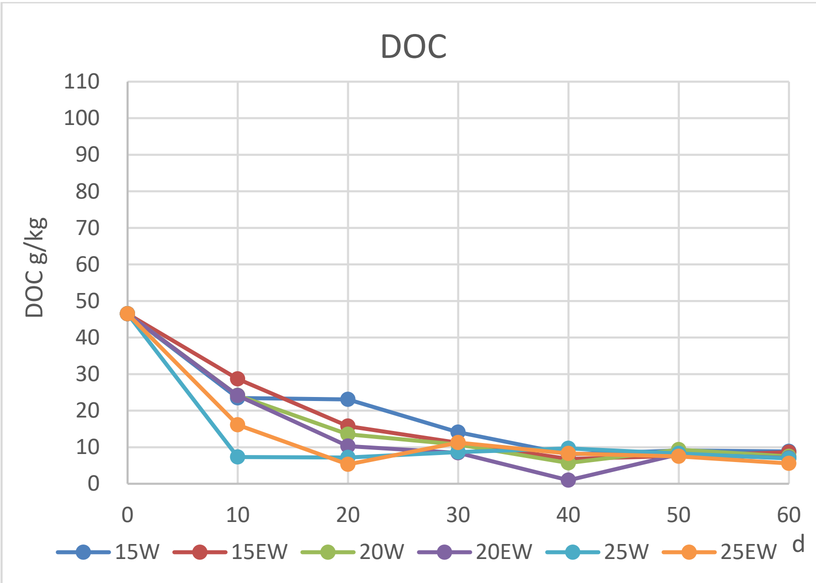


***Figure 4 showing the change of DOC in vermicomposting system at different temperature.***



Moreover, this biological mutuality caused C loss in the form of CO2 from the substrates during the decomposition and mineralization of organic waste. The conversion of some part of organic fractions of waste into worm biomass can also reduce the C from the substrate.
Benitez et al, concluded that glucosidase and BBA-hydrolyzing protease, which are hydrolytic enzymes involved in the C and N cycles, respectively, showed sharp decrease because of the decrease in available organic substances.

## 5. The carbon: nitrogen ratio

In this study, Organic C was lower in final vermicomposting product, and Nitrogen was higher when compared to the initial level in the substrate. (Suthar 2009) postulated that the organic C loss was in the ranges of 17.5 % to 67.0%. Comparatively, substrate with earthworm (vermicompost) showed the greater carbon loss as compared to control containers which suggests earthworm-mediated rapid organic matter mineralization rate in vermibeds.
Moreover, this biological mutuality caused C loss in the form of CO2 from the substrates during the decomposition and mineralization of organic waste (Suthar 2007, Suthar 2007). It indicates that the amount of organic matter in substrate affects the rate of microbial enzymes production and their activities. (Benitez, Nogales et al. 1999) concluded that β-glucosidase and BBA-hydrolysing protease, which are hydrolytic enzymes involved in the C and N cycles, respectively, showed sharp decrease because of the decrease in available organic substances. Present result extends and confirms above hypothesis. It is also suggested that in addition to releasing N from compost material, earth worms also enhance nitrogen levels by adding their excretory products, mucus, body fluid, enzymes, etc. to the substrate. Decaying tissues of dead worms also add a significant

amount of N to vermicomposting sub-system. According to (Benitez, Nogales et al. 1999) the hydrolytic enzyme production, which plays an important role in C and N cycle in waste decomposition system, is drastically influenced by the availability of easily degradable organic compounds in the substrates. However, the greater concentration of total N was not related to decomposition or nitrification activity. It was related more likely to the higher earthworm mortality that enriched the bedding with N content due to decaying earthworm tissue(Suthar and Singh 2008). According to (Suthar 2009) in general, earthworm contains about 60–70% (of dry mass) proteins in their body tissue, and this pool of N returned to the soil upon mineralization.

The C/N ratio is an important factor to determine the growth and reproduction of the vermicomposting earthworm (Suthar 2008, Chauhan and Singh 2012). The significant lowest C/N ratio may be due to the decrease in organic carbon and increase in nitrogen in the final vermicompost. Similar results were reported during the experiment of vermicomposting by Garg et al. (Garg, Yadav et al. 2006).
(Suthar 2010) has reported that loss of carbon during vermicomposting is due to the digestion of carbohydrate and another polysaccharides from initial substrate material. Also, some part of organic carbon may be assimilated by the earthworm as the biomass increases. Increase in nitrogen is responsible for the reduced C/N ratio by the change of ammonium nitrogen to nitrate.

The change in C/N ratio reflects the degree of organic waste mineralization and stabilization rate during the process of composting and vermicomposting. The C/N ratio below 20 is indicative of an advanced degree of stabilization and acceptable maturity, while a ratio of 15 or less being preferable for agronomic use of composts as plants cannot assimilate nitrogen unless the ratio is in the order of 20 or less. The C/N ratio of the final vermicompost was decreased considerably as compared to the initial compost material under all the experimental conditions. The C/N ratio of the vermicompost was in the range of 5.97–9.36. The decreasing trend of C/N ratio during vermicomposting is in good agreement with other authors (Gupta and Garg 2008). Therefore, in the present study, a high degree of organic matter stabilization was achieved in all the reactors and certainly indicative of the agronomic potential of vermicompost. The loss of organic carbon as $CO_2$ due to microbial respiration and addition of earthworm N excrements increase the levels of nitrogen and thereby lowers the C/N ratio during vermicomposting

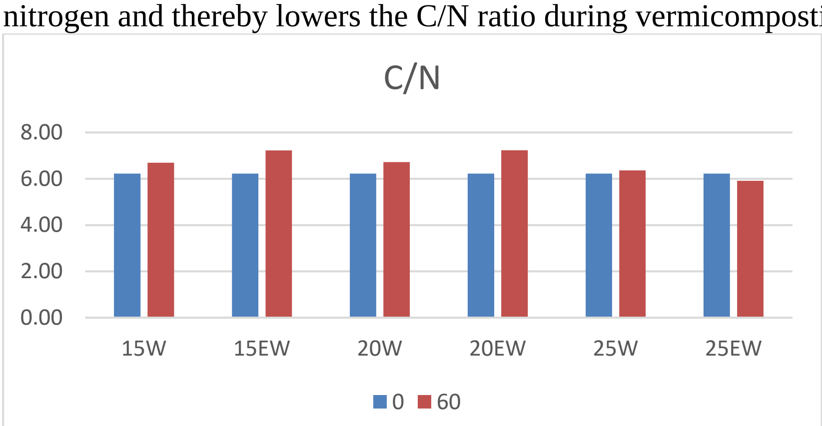


***Figure 5 showing the change of C/N in vermicomposting system at different temperature.***

### 6. Total nitrogen (g/ kg)

Total N was significantly higher in the product (Table 4) than initial substrate material (Table 3).). Comparatively the vermicomposted material showed nearly 4.87–17.11% more nitrogen content than control treatments (all, $P < 0.05$: t-test)

A significant increase in TN content in vermicompost was observed in all the reactors as compared to the initial composted material. The TN content of the vermicompost varied from 2.92– 4.15% (Fig. 2). The increase in TN content during vermicomposting was in the range of 31.3–90.0%. The maximum increase was observed in experimental reactors C4 and SD3.0 (90.0%), whereas C1 and SD0.5 (31.3%) showed the minimum increase for TN content. The increase in TN content for control was in the range of 15.2– 49.5%. The increasing trend in TN content during vermicomposting corroborates with the findings of other researchers (Kaushik and Garg 2004, Suthar and Singh 2008, Khwairakpam and Bhargava 2009) The reduction in dry mass i.e. organic carbon in terms of CO2 due to substrate utilization by earthworms and microorganisms and their metabolic activities as well as addition of earthworm N-excrements and evaporative moisture loss might have led to relative increase in TN content (Suthar and Singh 2008). Therefore, it is suggested that TN content in vermicompost depends upon the nitrogen content and C/N ratio in the parent substrate, extent of decomposition and evaporative moisture loss due to prevailing environmental condition. Therefore, in the present study, a high degree of organic matter stabilization was achieved in all the reactors and certainly indicative of the agronomic potential of vermicompost. The loss of organic carbon as CO2 due to microbial respiration and addition of earthworm N excrements increase the levels of nitrogen and thereby lowers the C/N ratio during vermicomposting (Senapati et al., 1980). The decrease in C/N ratio over time might also be attributed to rapid reduction in organic carbon due to increase in earthworm population.

It is suggested that in addition to releasing N from compost material, earthworms also enhance nitrogen levels by adding their excretory products, mucus, body fluid, enzymes, etc. to the substrate. Decaying tissues of dead worms also add a significant amount of N to vermicomposting sub-system.

### 7. Total phosphorous (g/ kg)

Total phosphorous (TP) content of the final vermicompost was significantly increased as compared to the initial composted material under all the experimental conditions.

The increasing trend in TP content during vermicomposting is consistent with the findings of other researchers(Gupta and Garg 2008, Suthar and Singh 2008, Khwairakpam and Bhargava 2009). It has been postulated that the increase in TP content during vermicomposting is probably through mineralization, the release and mobilization of available P content from organic waste performed partly by earthworm gut phosphates, and further release of P might be due to the P-solubilizing microorganisms present in worm casts(Lee 1992, Ghosh, Chattopadhyay et al. 1999)

After vermicomposting, all treatments showed higher concentrations of available P in vermicomposted material than initial material. As compared to control bedding vermicomposted material showed the great concentration of available P, after 90 days of experimentation. When organic matter passes through the gut of earthworm, results in some amount of phosphorus is converted to more available form. The release of phosphorus in available form is performed partly by earthworm gut phosphatases, and further release of P might be attributed to the P-solubilizing microorganisms present in worm casts.

Recently (Suthar 2006) and (Gupta and Garg 2008) claimed a higher concentration of available P in earthworm processed organic waste. In present study, however, there was a consistent trend of decreasing P mineralization rate with increasing proportion of sewage sludge. This difference could be attributed to the increased level of heavy metals and other toxic compounds in substrates. Exchangeable K content in ready vermicompost was lower (Table 4) than initial substrate material. The loss of available K in the presence of earthworm is evidenced in some previous studies of (Orozco, Cegarra et al. 1996, Suthar 2006). (Bettiol 2007) correlated the K loss with leaching process, during vermicomposting. (Garg and Kaushik 2005) reported a similar trend of potassium loss during vermicomposting process. Dramatically the control treatments showed the higher content of exchangeable K in end products. Therefore, in this study the loss of exchangeable K in vermibeds was not the result of leaching process. There might be possibility of potassium absorption or assimilation by inhabiting earthworms as potassium is important physiological supplement

The changes of available phosphate content during the decomposition of PDS are given in Fig. 2. Gradual increasing trends of available phosphate contents were found in both treatments of earthworm addition and earthworm-free systems earthworm system showed a significantly higher content of available phosphate than the control system, with final content at the values of 4.57 and 4.15 g/kg, respectively. This result could be explained by the reason that the phosphatase in earthworms gut could accelerate the transformation of organic phosphorous compound into the available phosphate of plant, as reported by (Yadav and Garg 2009). Overall, the inoculation of earthworms could increase the available phosphate and nitrate contents of PDS, suggesting that its final product was more suitable for agriculture field.
Available Phosphorus: After vermicomposting, all treatments showed higher concentrations of available P in vermicomposted material than initial material.
When organic matter passes through the gut of earthworm, results in some amount of phosphorus is converted to more available form. The release of phosphorus in available form is performed partly by earthworm gut phosphatases, and further release of P might be attributed to the P-solubilizing microorganisms present in worm casts.
In present study, however, there was a consistent trend of decreasing P mineralization rate with increasing proportion of sewage sludge. This difference could be attributed to the increased level of heavy metals and other toxic compounds in substrates.

## Conclusions

The results proved that the earth worm has both direct and indirect effect on vermistabilization of municipal sewage sludge. Where palletization of fresh dewatered sludge was considered as suitable and alternative to pretreatment approach or vermistabilization of sludge.
The final vermicomposted materials contained the higher values of electrical conductivity and indefinite trend of pH change, lower C: N content and higher available phosphorus and the lower values of DOC, lower content of organic matter. The observed increase of total phosphorous (TP) in vermicompost is probably due to mineralization and mobilization of phosphorus resulting from the enhanced phosphatase activity by microorganisms in the gut epithelium of the earthworms. Vermicomposts showed a significant increase in exchangeable $Ca^{2+}$, $Mg^{2+}$ and $K^{+}$ compared to fresh sludge indicating the conversion of nutrients to plant-available forms during passage through the earthworm gut. Vermicomposts contain higher nutrient concentrations, but less likely to produce salinity, than composts. All these are achieved in vermicomposting process by earthworms

in favorable temperature of about 20-25 $^{0}$C to interact with microorganism within decomposer community to both increase the rate of aerobic decomposition and decomposition of organic matter in the sewage sludge, thereby stabilizing organic residues by removing harmful pathogens and greatly modifying its physical and biochemical properties.

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