COMPOSTING OF VEGETABLE WASTE THROUGH DIFFERENT COMPOSTING TECHNIQUES

A Thesis submitted

in partial fulfillment of the requirement for the degree of

Doctor of Philosophy

By

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Candidate's Declaration

I hereby declare that the work presented in this thesis is to the best of my knowledge, original, except as acknowledged in the text. This material has not been submitted, either in whole or in part, for degree at any University.

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Certificate

This is to certify that the thesis entitled **"Composting of Vegetable Waste through Different Composting Techniques"** submitted by **V. Sudharsan Varma** (Registration No. 126104034) to the Indian Institute of Technology Guwahati for the degree of Doctor of Philosophy is a record of bonafide research work carried out by him under my supervision and guidance. The thesis work, in my opinion has reached the requisite standard fulfilling the requirement for award of the degree of Doctor of Philosophy. This work has not been submitted earlier for the award of any degree or diploma to the best of my knowledge and belief.

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ABSTRACT

Vegetable waste with high moisture content and readily biodegradable nature is causing major environmental problems due to improper waste management practices in India. So, composting and vermicomposting could be considered the best alternative for the treatment of these organic fractions. Therefore, studies were carried out on the degradation of vegetable waste by adding cow dung, saw dust and dry leaves during the 20 days of drum composting, 30 days of pile composting and 45 days of vermicomposting. Initially, drum composting of vegetable waste was carried out by adding cow dung and sawdust based on C/N ratio 24 and 30, respectively. Eventhough C/N ratio was optimum, leachate production was observed due to insufficient bulking agents. Therefore, the further experiments were carried out by adding dry leaves as bulking agent to control leachate production and observed a prolonged thermophilic phase. Appropriate combination of waste materials i.e. vegetable waste, cow dung and saw dust in (5:4:1) ratio along with 10 kg of dried leaves to make a final mass of 100 kg wet weight was found successful. A maximum of 66.5^{o}C was observed with temperature in the range of 55 to 64^{o}C for 5-7 days resulting in higher organic matter degradation and destruction of pathogens. Further, the best (5:4:1) ratio of waste combinations was tested for pile composting under three different operating conditions i.e. agitated pile (AP), passive pile (PP) and forced aerated pile (FAP). The rate of aeration and mixing of the materials was found crucial to maintain the thermophilic temperature inside the composting mass during pile operation. Hence, piles operated in passive and forced aeration mode were observed with higher degradation of VS (19.6 and 22.9% respectively). However, passive operated pile was observed with highest temperature and considerable degradation as compared to other piles. Further, *Eisenia fetida* and *Eudrilus euginea* were employed for the degradation of vegetable waste during vermicomposting. Trial 1 and 2 were performed for vermicomposting using *E. fetida* and *E. eugenia* for 45 days; trial 3 was performed for drum composting for 8 days and followed by vermicomposting using *E. fetida* for another 20 days. Trial 1 and 3 employed with *E. fetida* was observed with higher volatile solids (VS) reduction and biomass production at the end of composting period. However, pretreating the vegetable waste in drum composter for 8 days was found to be optimum for degradation during vermicomposting, as the overall composting period was shortened to 28 days.

However, considering the VS degradation with respect to 60-70% volume reduction during drum composting, the VS loss was observed low. Hence, the treatment efficiency was improved by adding waste carbide sludge (WCS) and inoculating white rot fungi (*P. Chrysosporium*). WCS was added in the concentration of 1, 2 and 3% during the drum composting and observed that 1% addition was found optimum to achieve 22.4% of VS reduction and higher temperature. However, higher additions of WCS lead to rise in pH of the composting and thereby volatilizing the nitrogen as ammonia. Furthermore, addition of WCS during vermicomposting was also found efficient in terms of higher biomass growth and VS reduction. However addition of WCS during vermicomposting had no negative effects on biomass growth and degradation pattern during vermicomposting, while 1.5 to 2% of WCS addition was observed optimum. The mycelia of *P. Chrysosporium* were inoculated in the order of 10^6-10^8 CFU/g of compost in three different trials i.e. Trial 1 (non-inoculated), Trial 2 (0th day) and trial 3 during the 8th day after thermophilic phase. Finally, a maximum of 19.4% of VS reduction was observed in trial 3, followed by 15.4% in trial 2. Inoculation of fungus after thermophilic phase was observed optimum as compared to the non-inoculated and 0th day inoculated fungal treatment. Finally, the best combinations of waste materials (5:4:1) ratio along with 10 kg of dry leaves from drum composting was performed for 16S Metagenome sequencing and showed that 89.5% sequences belonged to bacteria, 9% to Eukaryota followed by 1.4% Archaea. Taxonomic hit distribution at family level showed that compost was enriched with *Thermomonosporaceae*. *Thermomonospora curvata* (strain ATCC 19995/DSM 43183/JCM 3096/NCIMB 10081) is an aerobic, cellulolytic, thermophilic gram-positive bacterium which produces a number of enzymes like cellulase, alpha-amylase and polygalacturonate lyase. In addition, the lowest common ancestor (LCA) classification plot showed the high abundance of the phylum proteobacteria followed by actinomycetes in the compost. The ability of actinomycetes to degrade lignocelluloses implies that this group of bacteria has potential to be useful indicators for compost maturity. Finally, it can be concluded that drum composting of vegetable waste with the combinations of cow dung and sawdust in (5:4:1) ratio along with 10 kg of dry leaves was successful. Further, pre-stabilization of vegetable waste in drum composter followed by vermicomposting can also be recommended.

Keywords: Vegetable waste, Rotary drum composting, Pile composting, Vermicomposting, Bulking agents, Volatile solids, Lignocellulosic fractions, Microbial diversity, Waste carbide sludge (WCS), *P. Chrysosporium*, 16S Metagenome sequencing.

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NOTATIONS

MSW	Municipal Solid Waste
L	Litre
mg	Milligram
g	Gram
Kg	Kilogram
EC	Electrical Conductivity
TN	Total Nitrogen
TP	Total Phosphorous
AP	Available Phosphorous
C/N	Carbon/Nitrogen
TOC	Total Organic Carbon
NH_4-N	Ammoniacal Nitrogen
APHA	American Public Health Association
NH_3	Ammonia
US EPA	United State Environmental Protection Agency
ND	Not Detected
h	Hour
BIS	Bureau of Indian Standard
VS	Volatile Solids
AAS	Atomic Absorption Spectrometer
M	Molar
kcal	Kilo Calories
RPM	Round Per Minute
VS	Volatile Solids
NH_3	Ammonia
CFU/g	Colony Forming Unit/gram
MPN/g	Most Probable Number/gram
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
WCS	Waste Carbide Sludge
TC	Total Coliform
FC	Fecal Coliform

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WCS	Waste Carbide Sludge
EF	*Eisenia Fetida*

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Chapter 1

INTRODUCTION

This chapter consists of brief discussion about vegetable waste problems, treatment through different composting techniques and combination of waste materials during the composting process. The chapter also deals with the major objectives, need of the study, scope of the thesis and finally the thesis organization.

1.1 OVERVIEW

India is the second largest producer of fruits and vegetables in the world (next to China) with 221.431 million metric tonnes. The cumulative wastages are estimated to be 5.8 to 18% of the total produced fruits and vegetables (CIPHET, 2013). The total population in India is 1.27 billion representing almost 17.31% of the world's population. With the population growth rate of 1.58%, India is predicted to have more than 1.53 billion people by the end of 2030. Rapid industrialization and population growth in India has led to the migration of people from villages to cities thereby generating thousands of tons of municipal solid waste (MSW) everyday throughout the country. Urban India is reported to generate 68.8 million tonnes of MSW per year with a per capita waste generation rate of 500 g/person/day (Annepu, 2012). The total waste generated included the total tonnage of wastes from 366 cities representing almost 70% of India's urban population. The composition of MSW in India is completely different when compared to western countries. The MSW is composed of large amount of organic fraction (40–60%), ash, fine earth (30–40%), paper (3–6%) and plastic, glass and metals (each less than 1%). The moisture content of urban MSW is 47%, C/N ratio ranges between 20 and 30, and the average calorific value is 7.3 MJ/kg (1745 kcal/kg) (Annepu, 2012).

Generally, MSW is disposed off in low-lying areas, dumped openly and most of it is landfilled without any operational control and taking proper precautions (Sharholy et al., 2008). The management of MSW is associated with several activities such as generation, storage, collection, transfer and transport, processing and disposal (Fig. 1.1). But in India, the practices are compromised with waste generation, collection, transportation and disposal. Due to the poor management of MSW, improper infrastructure facilities and maintenance facilities, the practices are becoming more complex and expensive. Since there is no segregation for MSW before disposal, it is leading to more emission of

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greenhouse gases and leachate production due to large fractions of organic matter (fruit and vegetable peels, food waste) (Suthar et al., 2005). The leachate from these wastes majorly contaminates the groundwater (Pokhrel and Viraraghavan, 2005). In addition, these illegally dumped wastes have adverse effects on human health and the environment (Achankeng, 2003). Moreover, these emissions are mainly due to the result of landfilling and other life cycle activities. With its high biodegradability nature, the organic waste of the vegetable market is causing much nuisance after reaching the landfill (Bouallagui et al., 2004). However, incineration of solid waste generally results in the production of more polluting gases and other toxic solid residues in the land.

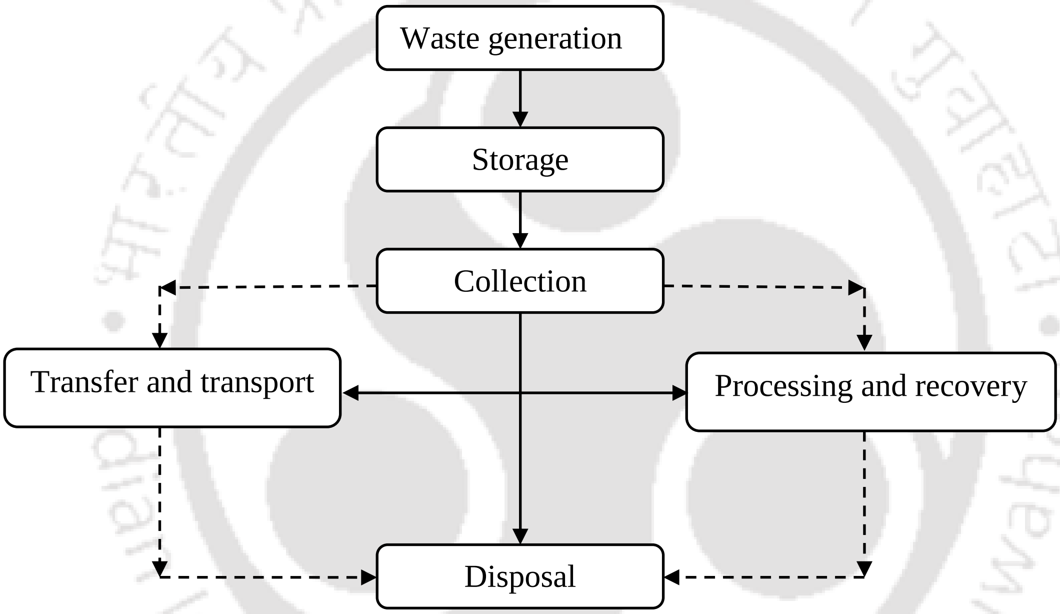

Fig. 1.1. Life cycle of municipal solid waste management system (MWSR, 2000)

Kulcu and Yaldiz (2004) have reported that biogas processes and composting can be preferred as sustainable alternative over landfilling, incineration and pyrolysis for disposing these organic wastes. The MWSR (2000) have recommended composting for the stabilization and processing of biodegradable wastes. Hence, management of these organic fractions is very crucial for the preservation of the environment and valorisation of the by-products formed during the process. Composting of organic wastes produces a stabilized final product, free of phytotoxicity and pathogens and with certain humic properties (Zucconi and de Bertoldi, 1987).

Over 50 percent of an average city's MSW stream in a developing country could be readily composted (Hoornweg et al., 2000). Composting is a relatively simple process, optimization efforts increase the rate of decomposition (thereby reducing costs), minimize nuisance potential, and promote a clean and readily marketable finished product. Composting is highly compatible with other types of recycling. Diverting organic material helps to increase the recovery rate of recyclable materials and also improves the quality of the finished compost. Household source separation of recyclable paper, metal and glass is already common in many developing countries. Many cities in developing countries are plagued with poor waste collection. While a few, more influential residents may get daily waste collection, others may never have such services. Daily waste collection in wealthy neighbourhoods is usually too frequent and contributes to the lack of collection in poorer areas (Hoornweg et al., 2000). In more affluent areas of a city, the use of containers and diversion of organic waste for composting is a good way to quickly improve the cities overall waste collection service. Many cities have switched from unreliable daily collection to bi-weekly organic waste collection and weekly non-organic waste collection. Variations of this schedule are easily tailored to each area’s individual characteristics. Introducing waste diversion for composting program provides a city with a unique opportunity to improve its overall waste collection service (Diaz et al., 2007).

In developing countries like India, the high animal and vegetable waste content of the waste stream combined with existing materials recovery systems (mixed waste stream) is sufficiently compostable to produce good compost at a small or medium scale. The compostability can be accomplished by facilitating the recovery of non-compostables and reducing the introduction of new packaging into the waste stream (bearing in mind that the public health benefits of good packaging are significant) (UNEP, 2014). In India, composting and vermicomposting have been recommended for the management of organic waste separately from MSW under the Jawaharlal Nehru National Urban Renewal Mission (JnNURM, 2006). In the first attempt at developing a regional facility in India was by Ahmedabad Urban Development Authority (AUDA) in 2007 to address the SWM requirements of 11 towns in its (then) jurisdiction. The project facility integrated composting facilities for approximately 150 tonnes per day (TPD) and a scientific landfill site of 50 TPD capacities. The overall strategy included the development of three transfer stations. Another project developed in Asansol Urban Agglomeration (AUA) area includes 5 urban local bodies. The project developed through JnNURM program has three treatment plants using composting technology (500, 300, and 200 TPD capacity) and a regional landfill at Mangalpur to accommodate 400,000 metric tonnes of waste (JNNRUM, 2012).

UNDP has recommended that instead of setting up single large mechanical compost plants, it would be beneficial and more effective to set up several small composting plants (UNDP/WB RWSG-SA, 1991). Decentralized composting in institutions, neighbourhood or community scale provides small groups to pursue it at a relatively low cost. Decentralized composting allows reuse of organic waste where it is generated, thereby reducing waste quantities to be transported as well as transport costs. This has a positive effect on the overall municipal waste management costs. An efficient and promising technique in decentralized composting is the rotary drum composter. Rotary drum provides agitation, aeration and mixing of the compost, to produce a consistent and uniform end product without any odour or leachate related problems. In warm and moist environment, ample amount of oxygen and organic material are available and aerobic microbes flourish and decompose the waste at a quicker pace. The composting time is drastically reduced to 2-3 weeks (Kalamdhad et al., 2009; Singh et al., 2012; Singh and Kalamdhad, 2014).

Several successful studies were conducted on the application of cattle manure, swine manure, municipal bio-solids, brewery sludge, chicken litter, sewage sludge, water hyacinth, animal mortalities and food residuals using rotary drum composter (Mohee and Mudhoo, 2005; Tolvanen et al., 2005; Smith et al., 2006; Kalamdhad et al., 2008; Kalamdhad et al., 2009; Singh and Kalamdhad, 2013; Nayak and Kalamdhad, 2014). The optimum C/N ratio for rotary drum composting of vegetable waste mixture is in the range of 20 to 25 (Kalamdhad et al., 2008). Even though low C/N ratio is recommended for drum composting (in-vessel), it has been reported that the production of leachate from organic waste with high moisture content during decentralized composting (Tchobanoglous et al., 2000). Compared to many composting process such as pile/windrow composting process operated in agitated, passive and aeration mode, in-vessel system composting is proven economical and very fast process (Kalamdhad et al., 2009). Moreover, pile composting methods are reported to produce the same quality of compost operated in passive and active mode (Solano et al., 2001).

Since composting is an exothermal process, biological oxidation of organic matter is carried out by a dynamic and quick succession of populations of aerobic microorganisms. The transformation and mineralization of organic matter during composting is carried out by many microbial communities such as bacteria, fungi and actinomycetes (Zucconi et al., 1987; Davis et al., 1992, Bhatia et al., 2013). However these microbial communities were greatly affected by the varying temperature during the process and the physical properties of the waste material. Ruggieri et al. (2008) composted organic fractions of municipal solid waste and studied this extensively at the industrial level. Chanakya et al. (2000) has reported the production of large amounts of leachate fraction during the composting of food waste. It has been found that food waste decomposes rapidly to produce organic acid, thereby leading to leachate. In contrast to food waste (cooked), vegetable waste (uncooked) also contains high amount of moisture and organic content.

The successful operation of composting is always followed out by adding several bulking agents such as saw dust, rice straw, dry leaves and cattle manure to increase the efficiency of process for producing high quality compost (Chang and Chen, 2010; Kalamdhad et al., 2009; Kulcu and Yaldiz, 2014). Adding these bulking agents in appropriate combinations are reported to provide optimum moisture content, C/N ratio and pH for the survival of microbes during composting. However, these materials are rich in lignocellulose content contributing to the total organic matter, which is normally resistant for microbial degradation as compared to the readily biodegradable content of organic waste. Amendments of alkaline materials as bauxite residue, clay, coal fly ash and lime during co-composting of solid waste has been reported to increase the microbial metabolism and also reduces the availability of heavy metals in compost (Qiao and Ho, 1997; Wong et al., 1997; Fang and Wong, 1999, Singh and Kalamdhad, 2013; Singh and Kalamdhad, 2014). However, not much research has been carried out on the application of waste lime sludge on different organic waste combinations for improving the treatment efficiency during composting process. In India, about 0.75 million tones of lime sludge is being generated per year during acetylene gas production and expected to increase annually due to very limited utilization of this carbide sludge (CPCB, 2006).

Inoculation of white rot fungi *Phanerochaete Chrysosporium* during composting has been reported to increase the lignocellulose degradation. These basidiomycetes belong to the white rot fungi which are well known for lignocellulose degradation by producing a non-specific extracellular enzyme system consisting of manganese peroxidase, lignin peroxidase and laccases (Toumela et al., 2000; Taccari et al., 2009). In addition, there are many literatures available on the utilization of vegetable waste along with cattle manure, saw dust and dry leaves for producing nutrient rich end product during vermicomposting (Suthar, 2009; Garg and Gupta, 2011; Huang et al., 2013). The earthworms ingest the organic waste and convert them into humus like material termed vermicast containing N, P and K in such forms that they are more available to plants than those in the initial raw substrate (Ndegwa and Thompson, 2001). These reports are majorly concentrated on the organic matter transformation and stabilization of vegetable waste from 45 days to a maximum of 105 days for vermicomposting (Garg and Gupta, 2011; Khwairakpam and Kalamdhad, 2011).

Therefore, from the above literatures it is well established that composting and vermicomposting can be efficiently carried out for vegetable waste processing, however the quality of final compost and time duration for the process is of major concern to look upon. Most of these reports were experimented on the organic matter transformation, stability analysis and microbial dynamics during the process. But, there are not many literatures available on the effect of leachate on compost parameters and control methods during drum composting of vegetable waste. The best combination of waste materials such as vegetable waste, cow dung, saw dust and dry leaves for producing stabilized composting is still unproven. In addition, there are limited reports available on the application of waste carbide sludge addition and white-rot fungi i.e. *P. Chrysosporium* to increase the volatile solids reduction and lignocellulose degradation during drum composting of mixed organic waste. Also, there are only few literatures available on the application of rotary drum composting followed by vermicomposting of vegetable waste for improving the quality of compost and shortening the time duration.

Hence the present study was focused on the best combination of waste materials for producing the stabilized compost within shorter time period. Different composting methodologies were compared with the best combinations for higher degradation of organic matter. Effect of waste carbide sludge addition and inoculation of white rot fungi was also experimented during drum composting. Finally, the best trial was experimented for microbial succession through 16S Metagenome sequencing method.

1.2 OBJECTIVES

The main objective of the study was to find out the best combination of waste materials for producing stabilized compost within shorter time period. The purpose was also to find the best strategy for improved treatment efficiency by performing different composting methodologies. The scope of the present study was limited to:

- Performance and evaluation of vegetable waste composting using a batch scale rotary drum composter on optimum weight, ratio and the best combinations.
- Comparison of pile composting methods operated in agitated, passive and active mode condition.
- Efficacy of vermicomposting and rotary drum composting followed by vermicomposting technology for stabilizing vegetable waste.
- Effects of waste carbide sludge addition and white rot fungi inoculation during drum composting of vegetable waste for improved treatment efficiency.
- Enumeration of microbial communities (DNA extraction method) from the best ratio.

1.3 NEED OF THE STUDY

Composting is a potential recycling process in which the resources are conserved in a more available form so that they can be most efficiently used. Unlike other chemical and physical disposal process such as burning and landfill, this biological means of disposing can add much advantage to the ecosystem by conserving the plant nutrients. Installation of batch drum composters at community level and source separation of organic waste will reduce much burden of collection and transportation costs. Composting of organic waste will result in nutrient rich and stabilized end product. It also reduces the greenhouse gases and leachate production in landfill and open (illegal) dumping, if they are source segregated and composted using rotary drum composter.

Agriculture is receiving huge attention worldwide, as government and non-government authorities recognize that there is a need to increase productivity in a more accelerated way in order to ensure food security and improved nutrition to a growing population. Farmers will need to produce around 1.5% more grain every year, representing an increase of 35% by 2030 and greater than 70% by 2050 (USDA, 2011). Since the compost is primarily focused on nitrogen, phosphorous, potassium and other micronutrients, that can be well used as a soil conditioner. Application of compost as a fertilizer has improved the physical structure of the soil that includes potting soil mixtures. In addition there was an increased suppression of plant diseases caused by soil-borne nematodes, fungi and bacteria due to the addition of compost to the soil in various cropping systems (Schonfeld et al., 2003). Kostov et al. (1996) conducted a test by treating the soil with compost, mineral fertilizers and manure to study the yield efficiency and quality of vegetables and fruits. Study also reported that the use of

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compost derived from vine branch, rice husks, and flax from in soil significantly increased the yield of tomatoes and quality of fruits when compared to other two materials.

1.4 SCOPE OF THE THESIS

To achieve the above mentioned objectives, laboratory experiments as well as actual on-site plant studies were performed alternatively under different conditions. In the first attempt, the experimental set-up for the physico-chemical analysis, biological analysis, microbial analysis and learning the protocols and operation of instruments were performed. Secondly, the performance of the batch rotary drum composter and pile composting was performed at different aeration conditions on the basis of weight and different combinations of vegetable waste, cow dung, sawdust and dry leaves. This was followed by the addition of waste carbide sludge (WCS) and fungal inoculation for the improvement of treatment efficiency. Finally, the best combinations were studied for the microbial succession using DNA extraction method. A large part of the work was on the collection of vegetable waste, cow dung and saw dust from different places; and cutting of vegetable waste in proper size, handling and analysis of data including daily visual observations of the process during different composting methodologies.

1.5 THESIS ORGANIZATION

The thesis has been organized in following chapters:

- Chapter 1 gives brief introduction of solid waste management in India, vegetable waste problems, composting and vermicomposting, objectives, need of the study and scope of the thesis.
- Chapter 2 gives detail literature review of vegetable waste problem, utilization of vegetable waste, different types of composting process.
- Chapter 3 deals with collection and initial characterization of raw materials such as vegetable waste, cow dung, sawdust, dry leaves, white rot fungi, and waste carbide sludge. Experimental design of phase 1, 2, 3 and 4 is given in flow chart. The detail procedures for physico-chemical, biological, microbiological and biochemical analysis are provided.

- Chapter 4 gives the detailed results and discussion of physico-chemical parameters, biological, microbiological and biochemical parameters during composting and vermicomposting.
- Chapter 5 gives the results and discussion during the addition of waste carbide sludge and inoculation of white rot fungi during composting and vermicomposting.
- Chapter 6 gives the microbial succession during composting through 16srRNA gene sequence method.
- Chapter 7 lists the conclusions and recommendations of the thesis.

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CHAPTER 2

LITERATURE REVIEW

This chapter covers detail literature on vegetable waste and its problems during disposal, management of vegetable waste through different composting methods. Chapter also deals with detailed composting and vermicomposting process, effect of leachate and bulking agent addition, organic matter transformation, stability and microbial diversity during different composting methodologies.

2.1 SOLID WASTE PRACTICES IN INDIA

In India, the term municipal solid waste refers to solid waste from houses, streets and public places, shops, offices and hospitals (Asnani and Zurbrugg, 2007). Management of these types of waste is most often the responsibility of corporates or urban local bodies. Except in the metropolitan cities, solid waste management (SWM) is the responsibility of a health officer who is assisted by the engineering department in the transportation work. The activity is mostly labour intensive and 2-3 workers are provided per 1000 residents served (Asnani and Zurbrugg, 2007). The municipal agencies spend 5-25% of their budget on SWM. A typical waste management system in a low or middle-income country like India includes the following elements:

- Waste generation and storage
- Segregation, reuse, and recycling at the household level
- Primary waste collection and transport to a transfer station or community bin
- Street sweeping and cleansing of public places
- Management of the transfer station or community bin
- Secondary collection and transport to the waste disposal site
- Waste disposal in landfills
- Collections, transport and treatment of recyclables at all points on the solid waste pathway (Collection, storage, transport and disposal)

2.1.1 ADVERSE EFFECT OF OPEN DUMP

An open dumping is defined as a land disposal site at which solid wastes are disposed of in a manner that does not protect the environment, are susceptible to open burning, and are exposed to the elements, vectors and scavengers. Open dumping can include solid waste disposal facilities or practices that pose a reasonable probability of adverse effects on health or the environment. The health risks associated with illegal dumping are significant. Areas used for open dumping may be easily accessible to people, especially children, who are vulnerable to the physical (protruding nails or sharp edges) and chemical (harmful fluids or dust) hazards posed by wastes. Rodents, insects, and other vermin attracted to open dump sites may also pose health risks. Dump sites with scrap tires provide an ideal breeding ground for mosquitoes, which can multiply 100 times faster than normal in the warm stagnant water standing in scrap tire causing several illnesses (EPA, 1998).

Poisoning and chemical burns results from contact with small amounts of hazardous, chemical waste mixed with general waste during collection and transportation. Burns and other injuries can occur resulting from occupational accidents and methane gas exposure at waste disposal sites. Dust generation occurs from on-site vehicle movements, during placement of waste and materials. The waste in the dumping ground undergoes various anaerobic reactions and produces offensive greenhouse gases such as CO_2, CH_4 etc. These gases are contributing potentially to global warming and climate change phenomenon.

2.2 COMPOSTING

Composting is a microbiological conversion of organic residues of plant and animal origin to manure rich in humus and nutrients by various micro-organisms including bacteria, fungi and actinomycetes in the presence of oxygen (Fig. 2.1). During the process it releases by products such as carbon dioxide, water and heat (Bharadwaj, 1995; Abbasi and Ramasamy, 1999; Bhatia et al., 2012; 2013).

2.2.1 PHASES IN COMPOSTING PROCESS

The phases in the composting processes can be distinguished according to temperature patterns as shown in Fig. 2.2. In the mesophilic phase, the microorganisms acclimatize and colonize in the new environment in the compost heap. Growth phase is characterized by the rise of biologically produced temperature to mesophilic level. In thermophilic phase, the temperature rises to the highest level with stabilization of waste and pathogen destruction which are more effective. During maturation phase the temperature decreases to mesophilic and consequently ambient levels (Fig. 2.2).

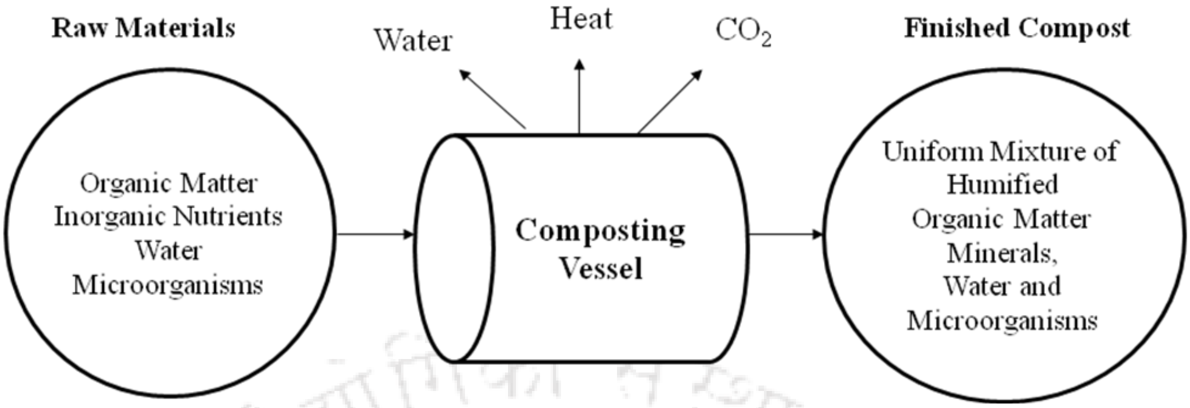

Fig. 2.1. Composting process (Haug, 1993)

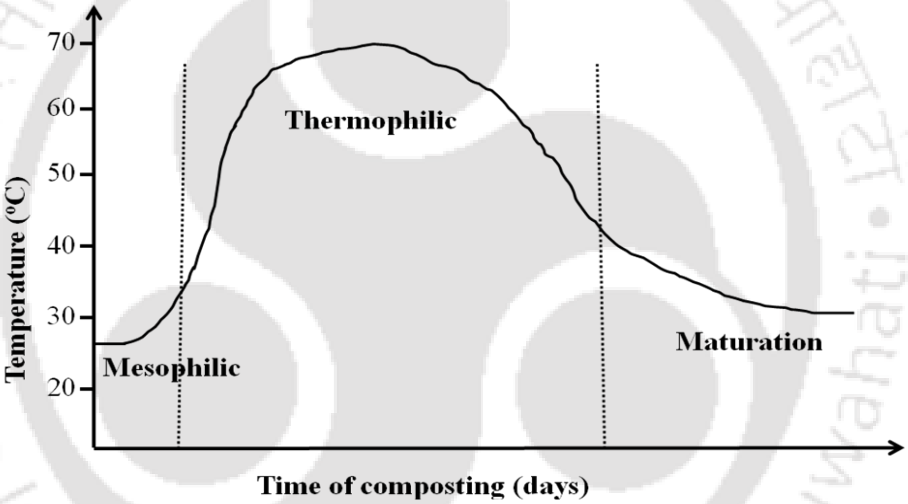

Fig. 2.2. Pattern of temperature during composting

In addition, humification takes place in which some of the complex organics are converted into humic colloids that are closely associated with minerals (iron, calcium, nitrogen, etc.) and finally to humus. Oxidation of ammonia to nitrite (NO_2^-) and finally nitrate (NO_3^-), also take place.

2.3 HISTORY OF COMPOSTING

Even though it is very difficult to attribute the birth of composting, the history of urban waste generation and its management begins with human civilization and urbanization. During the Neolithic period when human beings changed their habitat from essential hunters and gatherers to farmers, they started making pits out of stone for the storage of organic urban waste for the application of agricultural fields (Uhlig, 1976; Martin and Gershuny, 1992). However the most accurate and technical descriptions of composting has been conducted by the Knights Templar of thirteenth century. These Templar's were a military order during the time of the crusades.

There are references for the usage of manure in agriculture on clay tablets by the ancient Akkadian Empire in the Mesopotamian Valley, thousand years before Moses was born. There are evidences that Romans, Greeks and the Tribes of Israel knew about compost. Even in tenth and twelfth century Arab writings of both Bible and Talmud, have references for using rotted manure straw and organic materials to compost. Many New England farmers' composted 10 parts of muck to 1 part of boneless fish by periodically turning the compost heaps until the disintegration of fish was achieved (Martha et al., 2012).

Some of the advances made during the twentieth century include the work of Sir Albert Howard in the year 1933 in India. His work was one of the first documented efforts on the application of composting in the management of organic residues in India ever in the history of modern composting (Howard and Wad, 1935; 1938). Sir Howard in collaboration with few researchers developed the "Indore Process". Initially in Indore process only the animal manure was used for composting. But later readily biodegradable materials such as night soil, garbage, straw, leaves, municipal refuse and stable wastes were also composted on open ground. Indore process included two methods; the heap method and the pit method. In heap method the materials were piled up to height of 1.5 m and in pit method the materials were placed in trenches of 0.6-0.9 m deep. The leachate from the compost material was recirculated to maintain the moisture content and the composting process lasted for 6 months or longer.

Later in 1939, the Indian Council of Agricultural Research at Bangalore developed the "Bangalore Process" with some improvements of Indore method. This process overcame many of the disadvantages of Indore process such as heap protection from adverse weather; nutrient losses due to high winds/strong sun rays, frequent turning requirements and fly nuisance etc. An important modification to the Indore method was increasing the turning frequencies in order to maintain aerobic conditions, thus achieved more rapid degradation and shortened the composting period.

Later, a process that was used in a number of countries and heavily marketed throughout the world is the Dano Process. This is one of the widely known in-vessel systems which uses a large, slowly rotating drum with baffles incorporated inside it that carries the material during the digestion. This process was mainly concerned in the segregation and size reduction of the waste; however the output of this process can be composted by any of the procedures that were available at that time. This process was first developed in Denmark. The Dano Corporation later developed a mechanical silo-type digester known as the Bio-stabilizer (Golueke, 1992). The materials are fed to the stabilizer and maintained in thermophilic conditions for most of the time. The outputs are passed through a 1 mm mesh screen and further composted using windrows system if necessary. Later, Mr. T. van Maanen had started Vuilafvoer Maatschappij (VAM) company to compost city refuse in Netherlands. In the process, the refuse was placed in long and high piles. The piles were sprinkled periodically with the recirculated leachate to maintain the moisture content of the system (Diaz et al., 2007). Overhead cranes were used to turn the piles and the decomposed material was shredded, screened and sold as humus. Stovroff and his associates built an aerobic composting facility in Oakland, California, USA using the windrow method, which is also a modified version of the basic Indore method. This composting methodology was designed to compost 300 tons of mixed waste in an 8 h shift per day or 600 tons on a two shift i.e. 16 h/day basis (Stovroff, 1954a; b). Usually the piles were made in the range of 2 to 3 m in length and it was dependent on the site characteristics.

2.4 TYPES OF COMPOSTING

Generally, composting systems are of two types: the open process and reactor process. Open composting process are the first types systems originated and practiced from the evolution of composting times, which also includes windrow systems, static and household systems. Reactor systems include tunnel systems, the rotary drum and the reactor systems of various designs (Gajalakshmi and Abbasi, 2008; Haug, 1993). Furthermore, based on the supply of aeration to the composting system they are classified into two; the agitated and the static system. Normally in agitated system the compost materials are mechanically turned using large machines to supply air and to release inner temperature, which also includes mixing of the materials. Whereas in static systems, the compost heaps are made on a series of perforated tubes connected to a blower which is controlled manually or in timer basis to supply air into the system so the temperature is maintained within the system (Tchobanoglous et al., 1993). In the case of reactor operation, there are three major classifications as mentioned in Fig. 2.3. However, In-vessel (rotary drum) system of organic waste and municipal solid waste composting is the most successful process. An overview of major composting systems is discussed below.

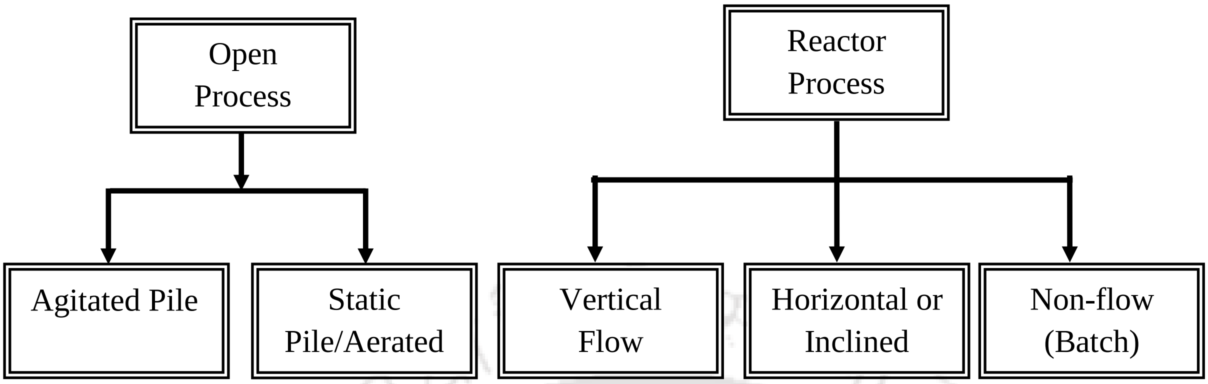

Fig. 2.3. Types of composting methods (Haug, 1993)

2.4.1 PILE/WINDROW COMPOSTING

Windrow composting involves aerobic bioconversion of organic matter to stable compost with release of heat, water vapor and CO_2, in which the pile composting can be used only for small quantities of input materials (Fig. 2.4). However the windrow composting allows large quantities of materials to be composted, having a geometrical shape ranging from 2 to 4 m wide and 2 to 3 m high at the starting of composting process (Martin, 2005). These types of systems usually acquire a trapezoidal shape, depending on the nature of raw material used for composting. Before forming the windrow, the material is shredded and screened to 3 to 9 cm, with moisture content adjusted to 50-60%. Usually, the windrows are turned twice in a week so that temperature is maintained at 55^{o}C and the process is accomplished in 3-4 weeks. Furthermore for curing, the compost is allowed without turning for another 3-4 weeks for the degradation of residual organics. The aerobic bioconversion reaction of organic waste has been expressed by the simplified chemical formula (Eq. 1).

$$(C_6H_{10}O_4)_a + 6.5O_2 = (C_6H_{10}O_4)_{a-1} + 6CO_2 + 5H_2O \qquad (1)$$

The reaction (1) is strongly exothermic with an assumed heat of formation of the organic matter of 230 kcal/mol and it generates 616 kcal of heat per mole of organic matters reacted; with comparison the heat of biodegradation of glucose is - 673 kcal/mol to produce carbon dioxide and water (Themelis and Kim, 2002). Addition of sawdust and sewage sludge along with the input material during composting process may improve the biodegradation process (Dumitrescu et al., 2009).

Fig. 2.4. Windrow/pile composting method (BPF, 2012)

Sawdust which is rich in sugar and lignin components; and sewage sludge with high lipid content and enzyme forming microorganisms helps in the conversion process. In addition compost quality can also be increased by adding some of the inoculating agents such as cow manure, poultry manure and yard waste to the organic matter (Gautam et al., 2010). Windrow composting of vegetable waste with 1.5 m high and three metric ton capacity has been studied for producing high quality green compost. In which a temperature controller has been placed at the core of the pile to monitor the temperature variance with a blower connected to it to maintain the temperature. From the work carried out, it has been suggested to perfect mechanical conditioning of vegetable waste, with the use of some slicer machinery that can be useful to prevent an excessive pulping of residues (Gautam et al., 2010). This windrow composting not only works on the volume reduction of the input waste material, but also makes good compost with quality parameters lying within the acceptable range set international standard. The compost had the appreciated amount of plant nutrients such as C (35%), N (0.05%), P (0.002%), Na (4.8%), K (0.35%) and pH ranging from 7.8 to 8.1 with organic matter of 45%, which can be used to maintain the soil fertility (Gautam et al., 2010).

Turning frequencies in composting plays a major role in deciding the quality of the compost in which the proper mixing of the waste ensures that organic matter receives equal exposure to the air at the surface and readily available to the microorganisms to feed on it for bioconversion. Most importantly it releases heat, water vapor and gases; and restores the gap eliminated by decomposition (Haug, 1993). In a study by Mayabi (2005) on turning frequencies in composting, he had stated that higher the organic matter in the process makes the turning cycle critical. Three days turning cycle will be suitable for organic matter greater than 70% and also organic matter of 50% in the process is not generally suitable. Hence, therefore pilot studies for the turning frequencies must be carried out for the given waste for the betterment of the process. Therefore it has been suggested to minimize the width of the compost for better aeration and for frequent turnings to enable air intrusion (Andersen, 2010). Since the MSW has more than 60% of organic waste with 2.8% of nitrogen content, composting can be successfully used for making the compost from yard waste (Maso and Blasi, 2008).

2.4.2 STATIC (ACTIVE/PASSIVE) COMPOSTING

Static composting is similar to windrow system in which the raw material is laid in parallel rows, with no restrictions to the size and shape of the compost (Fig. 2.5). But reports state that windrow and static composting are two different processes, in which the material to be composted is agitated manually in the windrow system to introduce oxygen and regulate temperature, whereas in static piles, air is blown through the mass (Tchobanoglous et al., 1993). Active aerated systems are the improved form of windrow composting, which has accelerated degradation and better control over the entire process (Nema, 2010). Hence aeration plays a critical role in composting as it supplies oxygen for the system and it removes CO_2, moisture and excess heat as a result of microbial degradation (Haug, 1993). Static pile composting (active and passive) is operated by placing them over a network of pipes connected to air blowers that delivers air in and out of the system. The waste material used for the system is mixed all together in one large pile and these piles are suitable only for high organics such as yard trimming and biodegradable MSW (USEPA, 1992). These types of system are effective in substantially reducing the mass and volume of the material being transported to the landfills (Larney and Hao, 2007). The above reports supported the application of aeration system in composting methodologies as they affect the greenhouse gas emissions. This is because during aeration, most of the carbon produced is released as CO_2. Furthermore, it was also reported that the turning frequency in active aeration treatment released higher greenhouse gas emission than passive aeration treatment due to the nitrogen transformation. Passive and active aeration systems produce the same compost quality with similar results (Solano et al., 2001), while the maintenance and operation costs differs with the methodology adopted (Haug, 1993) (Fig. 2.6). Contained pile is a modified variant of static pile in which the materials to be composted are placed between the walls over a perforated floor through which the air is blown. Due to the presence of walls the pumped air is forced to move upward preventing any sideway diffusion, thereby ensuring efficient aeration in the system. Furthermore, the management of leachate from the system is also easier.

Fig. 2.5. Windrow/pile composting in passive mode operation (MAF, 2012)

Fig. 2.6. Windrow/pile composting in active aeration mode (PKT, 2012)

One more advantage of the contained pile over static pile is that the material width size reducing with height due to sliding of material along the angle of repose. As the materials are loaded between the walls, with the decomposition the materials will be gradually slided down. Covering the roof of the contained pile system could also be beneficial in odor contamination (Gajalakshmi and Abbasi, 2008).

2.4.3 IN-VESSEL COMPOSTING

Rotary drum composters are one of the first types of In-vessel composting system design with engineering systems that are completely different from other conventional methods practiced earlier (Fig. 2.7). A common feature of these types of system is that large amount of waste material can be decomposed within an enclosed space in shorter time under controlled process. Therefore, drum composting of vegetable waste is an efficient and promising technique with its decentralized processing of the material, as it provides agitation, aeration and uniform mixing of the compost material to produce a stabilized end product with high quality (Kalamdhad and Kazmi, 2009a). In such systems aeration is optimized by various forced aeration and mechanical turning devices. Since the time of the composting is drastically reduced when compared with other composting methods, this methodology can be successfully used. There are many reports on the application of drum composting on vegetable waste in combination with many wastes such as cattle manure, tree leaves and saw dust (Tolvanen et al., 2005; Smith et al., 2006; Aboulam et al., 2006; Kalamdhad et al., 2008; Kalamdhad et al., 2009). A maximum of 70% reduction in the volume of the input can be achieved in vessel composting process of household wastes with high quality when compared to other reactors tested. The final compost can also be used as a soil conditioner by improving the quality of the soil and supplying basic nutrients to the plants (Iyengar and Bhave, 2006). Drum provides complete mixing of the material for better degradation organic matter as reported by many authors. There is always a major concern on C/N ratio of the material being added to the compost and this C/N ratio plays crucial part in the compost. Since the composting is fully dependent on microbial activity, if there is any improper supply of carbon and nitrogen sources it will greatly affect the quality of the end product.

In a study on stability evaluation of compost by Kalamdhad et al. (2008) on various C/N ratios of 16, 22, 30 and 38 of the vegetable waste by respiration techniques; it had been reported that C/N ratio of 22 was able to produce a stable compost and higher degradation of VS within 20 day period with final oxygen uptake rate (OUR) of 0.808 mg/g VS/day. The end product was considered as the very mature compost with a Solvita maturity index of 8 indicating that the compost was suitable as a soil conditioner, while the other compost had a value of 7.

Fig. 2.7. In-vessel composting method (Cadman, 2013)

Moreover drum composting seems to be more effective in degradation, as it reaches the thermophilic phase of 50 to 70°C within few days of the composting period. However this thermophilic phase is maintained continuously in the drum with the remaining matter, so that the leftover material serves as the inoculum to the newly added material, hence the degradation process is started as soon after adding the input material by directly entering the thermophilic phase (Kalamdhad et al., 2009b). Furthermore, it was reported that high temperature has greater influence in degrading the material faster. In addition, the metabolic activity of microbes is said to be dependent on the temperature of the compost under aerobic conditions (Amner et al., 1988).

There are also reports on the turning frequencies of the rotary drum which affects the quality of compost in terms of nitrogen and phosphorous values. There is a close connection between the turning frequencies of the drum with temperature variance which affects microbial activity (Kalamdhad and Kazmi, 2009a). When there is frequent turning of the compost, the time available for the microbes to act on the organic matter has been reduced and hence the utilization of carbon has been reduced to 8 to 13%. So it has been suggested that delayed turning, once a day in the early composting period will be favorable for the microbial action, such methods had a carbon uptake of 23% as reported. These delayed turnings also favor the nitrifying bacteria in nitrogen fixation, by preventing the loss of nitrogen in gaseous form and also extend the thermophilic phase for active microbial degradation.

Focusing the temperature influence in composting process as a major factor, a study by Yadav and Ganvit (2011) on temperature variation in co-composting of vegetable waste was reported to achieve 9-15% reduction of volatile solids within four weeks of the composting period. Therefore temperature seems to be an important factor and it is an indicator of active biological activity. Moreover it has also been suggested to co-compost vegetable waste along with cooked food and dry leaves together to speed up the compost process because of the high moisture content of vegetable waste. Furthermore, rotary drum composting can also be used as pretreatment step for the breakdown of organic material before adding it to the anaerobic digester (Ghosh, 1987, Cho and Park, 1995, Ghosh et al., 2000; and Xu et al., 2002). It was also reported that drum treated organic material is highly desirable as feedstock for the anaerobic digester with higher bioconversion efficiency of solid waste and high biogas yield. Table 2.1 provides the comparison of composting methods (Kalamdhad, 2010).

2.5 VERMICOMPOSTING

Vermicomposting is a modified and specialized bio-oxidative process of composting which uses earthworms to convert organic wastes into high quality compost (Fig. 2.8). Unlike other composting process vermicomposting is not an exothermic process. In this process, the organic matter containing major fraction of nutrients are converted into more available forms known as vermicast. Initially the substrate is broken into small fragments for ingestion, thereby it enters into gizzard of earthworms where mincing of the substrate occurs. This mincing helps in increasing the surface area of the substrate and facilitate microbial action (Chan and Griffiths, 1988). During vermicomposting, the earthworm body is reported to act as a bio-filter that can purify and also disinfect and detoxify solid wastes (Rahul and swetha, 2011). Apart from production of compost of high nutrient rich, vermicomposting has also proved to more efficient in removing pathogens, as they are eliminated as soon entering the food chain of earthworms (Canche et al., 2010; Arunugam et al., 2004; Ramos et al., 2005).

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Table 2.1. Comparison of composting methods (Kalamdhad, 2010)

Aerated Static Pile	**Windrow**	**In-Vessel**
Highly affected by weather (can be lessened by covering, but at increased cost)	Highly affected by weather (can be lessened by covering, but at increased cost)	Only slightly affected by weather
Extensive operating history both small and large scale	Proven technology on small scale	Relatively short operating history compared to other methods
Large volume of bulking agent required, leading to large volume of material to handle at each stage (including final distribution)	Large volume of bulking agent required, leading to large volume of material to handle at each stage (including final distribution)	High biosolids to bulking agent ratio so less volume of material to handle at each stage
Adaptable to changes in biosolids and bulking agent characteristics	Adaptable to changes in biosolids and bulking agent characteristics	Sensitive to changes in characteristics of biosolids and bulking agents
Wide-ranging capital cost	Low capital costs	High capital costs
Moderate labor requirements	Labor intensive	Not labor intensive
Large land area required	Large land area required	Small land area adequate
Large volumes of air to be treated for odor control	High potential for odor generation during turning; difficult to capture/contain air for treatment	Small volume of process air that is more easily captured for treatment
Moderately dependent on mechanical equipment	Minimally dependent on mechanical equipment	Highly dependent on mechanical equipment
Moderate energy requirement	Low energy requirements	Moderate energy requirement

Epigeic species including *Eisenia fetida*, *Eudrilus eugeniae* and *Perionyx excavates* have greater potential in decomposing organic waste and animal manure due to its humus consuming and surface dwelling nature (Hartenstein et al., 1979; Kale et al., 1982; Abbasi and Ramasamy, 1999). The elimination of pathogens by these earthworms is due to the release of coelomic fluids which have antibacterial properties. Even though there is no complete removal of pathogens in vermicomposting, the microorganisms microbial make up the compost harmless and nutrient rich to the soil (Rahul and Swetha, 2011).

Fig. 2.8. Earthworms during vermicomposting (Agrotech, 2012)

Vermicomposting is majorly influenced by moisture content, pH and temperature, as they have direct effect on cocoon production and growth of earthworms eventually affecting the composting stability. It has been well demonstrated by most researchers that earthworms have well defined limits of tolerance to the above mentioned parameters. Therefore, if there is much divergence in these limits the composting process may be slow down and in worse case fatal death of earthworms can occur. An average moisture content of 50 to 90% has been proposed to make a suitable environment for the earthworms to act effectively on the organic matter transformation (Dominguez and Edwards, 2004). Moreover, the optimum range of these parameters varies from species to species that is being used in the process. Earthworms are relatively sensitive to pH. They are reported to survive at slight acidic to alkaline conditions, but not below than 4.5. Bhawalkar (1995) has proposed neutral substrate pH to be used in vermicomposting and Edwards (1995) suggested a wide pH range of 5.0 to 9.0 for maximal growth of earthworms during the processes. In addition, Satchell (1955) had reported on the species classification based on their tolerance towards acidity. It also reported that from many species classified during his study that *Lumbricus terrestris* was not very sensitive to pH. C/N ratio also plays a major role in the degradation process during vermicomposting (Nayak et al., 2013).

Temperature range of 15 to 20^{o}C was found to be optimum for *Eisenia fetida* growth

(Dominguez and Edwards, 2004). This temperature variance has a major effect in reproduction and activity of earthworms. In a study by Frederickson and Howell (2003) on the influence of temperature on earthworms, it was reported that low ambient temperatures have very low rate of reproduction than that of moderate temperatures during processing. Hence temperature has a greater influence on earthworms during composting process as stated by Neuhauser et al. (1988). Moreover this thermophilic condition during the process favors the inactivation of many harmful pathogens in the bedding materials as reported by Jadia and Fulekar (2008) in a study on vermicomposting of vegetable waste in hydro-operating bioreactor.

Earthworms are very sensitive to anaerobic conditions. Eventhough they do not have any specialized respiratory organs, they use their body wall to suck oxygen in and diffuse carbon-di-oxide out. Edwards and bohlem (1996) have reported that respiration rate of 55 to 65% has been decreased during anaerobic conditions consequently resulting in reduction of their feeding rates. Dominguez and Edwards (2004) reported that the individuals of *E. Fetida* migrate from oxygen depleted water saturated substrate in large numbers. Trickling filters in which the aeration is high may be used for vermicomposting, which can increase the life span of earthworms. Organic waste containing high inorganic salts and other cations make earthworms to struggle in the compost. Finally, the vermicompost has proved to be rich in nutrients and source of beneficial microorganisms and can be used as a soil conditioner or fertilizer to improve its quality (Hattenschwile and Gaser, 2005; Rock and Martnes, 1995). Almost any type of organic material can be vermicomposted including agricultural, urban or industrial organic material, but sometimes they need preprocessing such as washing, precomposting and macerating to facilitate vermicomposting (Gajalakshmi et al., 2005).

2.6 MICROBIAL DIVERSITY AND ENZYME ACTIVITY

Microbes and enzymes play a major role in converting the organic matter to simpler units of organic carbon and nitrogen in compost. The active microbial population in the compost decides the quality and stability of the compost. The optimized environmental conditions such as pH, moisture content, C/N ratio and temperature etc., for the microbial survival and it varies irrespective of the composting methodologies used. There are many reports on the isolation and microbial diversity in composting methodologies (Raut et al., 2008; Bhatia et al., 2012; 2013).

2.6.1 BACTERIA

In composting, bacteria play a major role as they make up 80-90% of the billions of microorganisms found per gram of the compost (Trautmann and Olynciw, 2012). They can easily grow on soluble proteins and other soluble substrates. They also have the capability to attack more complex material by releasing extracellular enzymes (Golueke, 1992; Epstein, 1997). They contribute to a major proportion of 80% of the total microbial count in the compost and are responsible for the degradation of variety of organic materials by releasing a wide range of enzymes. They are also responsible for the initial decomposition and rise in temperature. In aerobic decomposition systems the commonly found bacterial species include *Bacillus, Cellulomonas, Pseudomonas, Klebsiella and Azomonas* (Nakasaki et al., 1985a; Strom, 1985; Bhatia et al., 2013). At the early stages of composting with temperature less than 40°C mesophilic bacteria of *Bacillus* spp. and *Azotobacter* spp. are responsible for the evolution of CO_2 from the composting heaps. Once the thermophilic stage reaches these microorganisms are partly killed or they will remain inactive at this period (Nakasaki et al., 1985b; Beffa et al., 1996).

Since temperature of the compost reaches a maximum of 65°C during thermophilic stage, at this stage bacillus species of *Bacillus sphaericus*, *B. subtilis*, *B. licheniformis and B. circulans* dominate at these temperatures (Ryckeboer et al., 2003). There are reports stating that more than 87% of the randomly selected isolates during thermophilic composting belong to *bacillus* species. Furthermore, Nakasaki et al. (1992) reported CO_2 evolution during thermophilic composting has contributed to thermophilic bacteria at initial stage of 60°C and to thermophilic actinomycetes at the later stages of temperature 60°C. As the thermophilic stage completes all the readily available carbon sources will be depleted by mesophilic organisms, only the complex material of lignin and cellulose content will be left out. In such stages, majorily thermophilic cellulose degrading bacteria plays a major role in degrading cellulose; bacterial species of *Geobacillus debilis* has been identified and characterized by Razali et al. (2012) during composting.

During composting, organic matter is mineralized by several microorganisms and finally undergoes humification. During such process, nitrogenous compounds undergo various biochemical transformations. In case of denitrification process, substances such as proteins and amino acids in the compost will produce ammonium ions. And these ammonium ions will be aerobically converted into nitrate (Hansen et al., 1990;

Gajalakshmi and Abbasi, 2008). Such transformations are due to ammonium-oxidizing and nitrite-oxidizing bacteria of *Nitrosomonas* spp. and *Nitrobacter* spp. from the compost (Focht and Verstraete, 1977). Bhatia et al. (2012) has observed gram positive rod bacteria and the dominance of gram negative bacilli shaped bacteria during drum composting. Transformation of organic compounds during the biodegradation of organic waste and difference in the utilization of nutrients (organic matter) was due to different group of microbes and higher temperature.

2.6.2 FUNGI

Fungi are majorly dominated during the degradation of lignin at the time of curing process, which is after the thermophilic phase once all the sugar and protein substances are utilized by bacteria. Temperature has adverse effect on the growth of fungi, as they are eliminated by higher temperature and recover during the maturation phase when the temperature is at moderate conditions. Most of the fungi are mesophilic with an optimum temperature of 25-45°C. At temperature range of 55°C and during the early stages of thermophilic composting, fungal growth will be significantly limited (Tiquia et al., 2001; Nakasaki et al., 1985b).

There are several other factors which influence the activity of fungi apart from temperature that include pH and sources of carbon and nitrogen. Most of the fungi tolerate wide range of pH, but they prefer much acidic conditions. Usually fungi require high level of nitrogen content for their growth except few wood rot fungi that prefer low nitrogen level. However even low nitrogen level is also a rate limiting factor in terms of degrading cellulose (Dix and Webster, 1995).

Thermophilic fungi are reported to withstand a temperature range from 40°C to a maximum of 55°C (Cooney and Emerson, 1964). However these fungi are reported to have cellulolytic/lignolytic activity. The optimum temperature of thermophilic microfungi is 40-50°C; therefore beyond this temperature they cannot survive, resulting in gradual reduction of cellulose degradation (Hellmann et al., 1997). Some of the thermophilic lignocellulolytic fungi include *Taloromyces emersonii*, *T. thermophilus*, *Thermoascus auranticus* and *Thermomyces lanuginosus*.

Since the vegetable waste is rich in lignin content which makes the composting process a little challenge to the microbes for the degradation, because of its complexity to degrade as well as it reduces the bioavailability of other cell wall constituents. In case of fungi, white rot fungal species *Phanerochaete chrysosporium and Coriolus versicolor*

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are the best known lignolytic microorganisms. Muthukumar and Mahadevan (1983) have reported a lot of fungi belonging to basidomycetes group are good in degrading lignin. The lignin degradation is usually carried out by the extracellular enzymes. In the case of white rot fungi lignin peroxidases (LiPs), manganese peroxidases (MnPs) and laccase were the best studied enzymes in the degradation of lignin (Hatakka, 1994).

2.6.3 ACTINOMYCETES

Actinomycetes possess the characteristics of both bacteria and fungi. They are classified to higher forms of bacteria, but produce a mycelium and their cell wall is without chitin and cellulose which resembles fungi also. Similar to some cellulolytic bacteria and fungi, actinomycetes are also capable of utilizing complex organic material and cellulose (Fig. 2.9). They are found to grow in more numbers during the later stages of composting by attacking lignin and cellulose (Epstein, 1997).

Fig. 2.9. Actinomycetes growth onto the compost material

Moreover cellulose is not the only carbon source left out to fungi and actinomycetes. It is believed that before cellulose degradation, the readily available carbon sources and metabolizable substances has shown to accelerate cellulose degradation. Once all the carbon sources has depleted, these microorganisms starts utilizing cellulose as the sole carbon source thereby resulting in increase of cellulose hydrolysis (Alexander, 1961). Hence most of the cellulose and lignin degradation is attributed to fungi and actinomycetes; frequent turning of the compost will result in breaking of the hyphae subsequently reducing the activity of cellulose degrading microorganisms.

Most frequently occurring actinomycetes during the later stages of composting include *Micromonospora*, *Streptomyces*, *Nocardia* and *Thermoactinomyces*. Some of the thermophilic actinomycetes isolated from compost include *Nocardia*, *Streptomyces*, *Thermoactinomyces* and *Micromonospora*.

They appear not only during the thermophilic phase but also during cooling and maturing phase. A white film over the compost material during the final stages of composting is due to over population of these species (Waksman et al., 1939; Strom, 1985; Gajalakshmi and Abassi, 2008). The metabolic activity of thermophilic bacteria and thermophilic actinomycetes was estimated by a nonlinear equation with respect to the evolution of CO_2 during thermophilic stages of composting. These CO_2 evolutions rate are considered to be specific to different volatile organic material degradation.

$$n_a R_b + n_b R_b = rCO_2 \qquad (2)$$

Where:

n_a - the amount of thermophilic actinomycetes (cfu/g dry weight)
n_b - the amount of thermophilic bacteria (cfu/g dry weight)
R_a - CO_2 yield of each thermophilic actinomycete (mol CO_2)
R_b - CO_2 yield of each thermophilic bacterium (mol CO_2/CFU)
rCO_2 - CO_2 evolution rate (mol CO_2/g dw)

Thermophilic bacteria were more active during the early stages of thermophilic temperature 56-59^{o}C, while at the latter stages thermophilic actinomycetes started increasing and played a major role in degradation of organic compounds. The optimum growth temperature of the actinomycetes falls within the range of 25-30^{o}C, whereas pH at the range of 5-9. Even higher level of nutrition in the compost will slow down the activity of the actinomycetes when compared to bacteria and fungi; while at low level they become more competitive. Certain species of actinomycetes are very resistant at higher temperatures even at 60^{o}C (Nakasaki et al., 1985a).

2.6.4 ENZYMES

The overall efficiency of the organic material break down in the compost by microorganisms is through the release of different kind of substrate based hydrolytic enzymes that favor the degradation process (Benitez et al., 1999). The enzymes in the compost can be majorly classified into intracellular enzymes and extracellular enzymes.

Intracellular enzymes catalyze the biochemical reactions occurring within the cell system, while the extracellular enzymes are those released into the external compost system for the degradation of complex materials into simpler units (Gajalakshmi and Abbasi, 2008; Vuorinen, 2000). To be more specific the released extracellular enzymes will breakdown the polymer structures into monomers. The released water soluble components will dissolve in water and enter the microbial cell system through cytoplasmic membrane, in which the intracellular enzymes will act upon them (Tabatabai, 1994; Ginkel, 1996).

Some of the important enzymes that control the rate of substrate degradation include cellulases, B-galactosidases, urease, phosphatases and arylsulphatase. Each of these enzymes is substrate specific in which cellulases are involved in depolymerizing cellulose, while B-galactosidases is involved in hydrolyzing glucosides. However, urease is involved in N-mineralization; phosphatases and arylsulphatase are involved in removing phosphate and sulphate groups from organic compounds (Mondini et al., 2002). Hydrolysis of cellulose, hemicellulose and proteins are majorly favored by the enzymes cellulase, xylanase and protease (Gajalakshmi and Abassi, 2008). Hence enzymes are considered to be the main mediators in degradation processes (Tiquia et al., 1996).

The dehydrogenase activity occurs by the following major hydrolytic enzymes such as B-galactosidases, acid-phosphatases and urease activity and these enzymes are substrate specific and act accordingly by releasing the enzymes for the degradation processes, from which the enzyme activity can be correlated to the microbial process. The composting process has been stated to follow three major processes of mineralization, conversion and finally degradation to simpler units with the release of ammonium that would alter the pH in the compost. The first step would be the mineralization of nitrogen to nitrates and nitrites, followed by the microbial action on organic matter to release CO_2, humic substances and rise in temperature; finally the degradation of simpler units. Furthermore, the higher metabolic activity will be evident by the release of CO_2 and temperature (Hellmann et al., 1997).

Characterization and quantification of extracellular enzymes during composting reflect the dynamics of organic matter decomposition and nitrogen transformations in the composting process. Once there occurs a well demonstrated relationship between enzyme activity, quantity and quality of organic matter, then compost quality could be well understood which is defined as the degree of decomposition of the readily biodegradable organic matter (Tiquia et al., 2001; Garcia et al., 1992; Lasaridi and Stentiford, 1998).

2.6.5 EFFECT OF INOCULUM AND ENZYME ADDITION

The effectiveness of the inoculum addition to the compost has been studied by adding ligno-cellulolytic bacteria at mesophilic stage followed by ligno-cellulolytic fungi at thermophilic stage and vice versa. From the studies it was reported that addition of bacteria followed by fungal species had a greater effect in increasing the efficiency of the compost material (Feng et al., 2011). It was stated that the bacteria added at the initial stage were thermophilic and they significantly survived at the thermophilic stage with increased population. Furthermore they also showed increased retention time of thermophilic stage leading to a maximum sanitation and biodegradation rate.

Addition of ligninolytic enzymes as the pretreatment for lignin degradation had an enhanced improvement in the process and also the carbon utilization ability of the microbes (Feng et al., 2011). However, the addition of ligno-cellulolytic fungal or bacterial inoculation instead of enzymes in thermo composting had no effect in small scale composting process. In addition, the composting performed equally as the mature compost when it is used in the premix even with the addition of inoculum. Moreover, it was also suggested to know the suitability of different inoculums to the specific substrates in the compost process for its efficient degradation (Nair and Okamitsu, 2010).

Finally, Zhang et al. (2011) concluded that physico-chemical parameters of the compost are directly influenced by the bacterial and fungal communities. It was reported that water soluble carbon (WSC), ammonium and nitrate of the compost influence the temporal variation of bacterial community and fugal communities. Further these communities are influenced by the pile temperature, WSC and moisture content. Inoculation of *Trichoderma viride, Aspergillus niger* and *Aspergillus flavus* spores were studied on the degradation of organic fraction of municipal solid waste (OFMSW) with different with turning frequencies (Awasthi et al., 2014). The authors concluded that the results demonstrated that inoculation of fungal consortium with weekly turning frequency effectively degraded OFMSW, as indicated by increased C and N mineralization, achieved faster compost maturity and limited or eliminated the phytotoxicity of the compost products (Awasthi et al., 2014).

2.6.6 EFFECT OF LIME ADDITION DURING COMPOSTING

In India, about 0.75 million tons of lime sludge is being generated per year during acetylene gas production and expected to increase annually due to very limited utilization (CPCB, 2006). The lime amendment was significant in reducing the exchangeable and acid extractable Cu, Mn, Pb and Zn of compost and mainly acts as buffering system. Addition of lime during composting resulted in lower diethylenetriamine pentaacetate (DTPA) extractable metal contents. However, >1% lime addition in a lime-sewage sludge compost was reported to affect the seed germination of cress (*Lepidium sativum L.*) (Fang and Wong, 1999). Therefore, lime can be used as a co-compost material for sewage sludge composting at the rate of not more than 1% (Wong and Selvam, 2006).

The lime amendment during drum composting of water hyacinth significantly reduced the water soluble metals (Zn, Cu, Fe and Cr), DTPA extractable metals (Zn, Cu, Fe, Ni and Cr) and leachable metals (Zn, Fe, Ni, Cr and Cd). Toxicity characteristic leaching procedure test confirmed that the heavy metals concentrations in control and lime amended compost were under the threshold limits. The maximum reduction of water soluble, plant available and leachable metals were observed in 2% treatment, which indicated optimum percentage of lime can enhance organic matter degradation and humification process; consequently it reduced the toxicity of the metals during water hyacinth composting (Singh and Kalamdhad, 2013). Amendments of alkaline materials as bauxite residue, clay, coal fly ash and lime during co-composting of solid waste has been reported to increase the microbial metabolism and also reduces the availability of heavy metals in compost (Singh and Kalamdhad, 2013). The higher calcium content in lime and waste carbide sludge is reported to increase the microbial activity during the composting process (Qiao and Ho, 1997; Wong et al., 1997; Fang and Wong, 1999; and Singh and Kalamdhad, 2012).

2.7 COMPOST STABILITY/MATURITY

The stability of compost can be defined as the degree to which the organic fractions in compost have been stabilized during the process. Compost is considered unstable if it contains a high proportion of biodegradable matter that may sustain high microbial activity. If the material contains mainly recalcitrant or humus-like matter, it is not able to sustain microbial activity and therefore, it is considered stable. Stability is an important aspect of composting in relation to its field application, potential of odor generation and pathogen regrowth (Zucconi et al., 1985). Stability prevents nutrient mineralization in rapid microbial growth, allowing them to be available for plant needs. Unstable compost can show phytotoxic behavior and therefore affect crops. This is due to the occurrence of toxic substances produced due to an insufficient biodegradation of organic compounds. Additionally, the degree of stability attained within a certain time can be used for process performance monitoring and comparative evaluation of different composting systems (Lasaridi and Stentiford, 1998; Gomez et al., 2006). Therefore, it is essential to prove the stability of compost to ensure about the technology and operational performance.

Different methods for measuring stability based on physical (temperature, aeration demand, odor and color, optical density of water extracts), chemical (volatile solids, C/N ratio, COD, polysaccharides, humic substances, etc.) and biological (respiration measured either as O_2 consumption, CO_2 production or heat generation, enzyme activities, ATP content, seed germination and plant growth, etc.) characteristics of composts have been proposed, but none has found universal acceptance (Lasaridi and Stentiford, 1998). Respirometric techniques are well suited for compost stability measurement. Respiration tests include both CO_2 production (Naganawa et al., 1990) and OUR (Palestki and Young, 1995). These are the most accepted methods for the determination of the biological activity of a material (Adani et al., 2001; and Iannotti et al., 1993). Respirometric techniques provide accurate information about the activity of a compost sample. Different commercial equipments are currently available (Costech, OxiTop, Micro-Oxymax, Sapromat, Comput-OX, N-Con systems, Colombus instruments, Arthur respirometers, etc.), but these respirometers are expensive and often cumbersome to use. A large number of methods are also available for determination of CO_2 evolution (Fibre-Optic Fluoro-Sensors, Amperometric, Conductometric, Potentiometric sensors, NaOH and KOH absorption). Their main disadvantage is that they need more specific instrumentation and more skilled labor. Furthermore, the equipment needs constant maintenance and frequent calibration.

Generally, a composted product should contain a low organic content that will not undergo further fermentation when discharge on land and the pathogens should be inactivated. Some of the approaches to measure the degree of compost stabilization are:

- Temperature decline at the end of the composting.
- Decrease in organic content of the compost as measured by the volatile solids content, chemical oxygen demand, percentage carbon or ash content and C/N

ratio.

- Presence of particular constituents i.e. nitrate and the absence of ammonia.
- Lack of attraction of insects or development of insects larvae in the final product.
- Absence of obnoxious odor.
- Presence of white or grey color due to the growth of actinomycetes.
- In cases where the composted products are to be applied to crops and where public health aspects are of concern, the time required for pathogen die-offs during composting is another important criterion to be considered.

2.8 ORGANIC MATTER TRANSFORMATION DURING COMPOSTING

The use of compost in agriculture as soil amendment is one of the practices for the sustainable management of soils and it also contributes to recycling organic residues. Composting of biological material generally means a full or a partial mineralization of organic compounds by producing CO_2, H_2O, NH_3, or NO_3, sulphates and carbonates of Ca, Mg and K, oxides of Fe and Mn, and phosphates. Some of these mineralization products get lost from the composting biomass as gaseous compounds (CO_2, H_2O, NH_3), some as solutes with the drainage water and some remain as precipitated or adsorbed compounds in the final compost product (Saad, 2001). A small metabolic sideway of all composting processes, even under strongly oxidative conditions allow the decaying of biological masses to the formation of fulvic and humic substances. These are able either to mummify decaying organic tissues or to become strongly precipitated as humates on the surface of clay particles (mull-formation). In both cases these relatively stable or even inert byproducts create the dark, blackish grey color of all composts.

Approximately 50% of the added organic matter becomes fully mineralized, mostly due to the degradation of easily degradable compounds such as proteins, cellulose and hemi-cellulose, which are utilized by microorganisms as C and N sources. The residual organic matter contains newly formed macromolecules along with non-degradable organic matter jointly forming the humic-like substance, the most stable fraction of the mature compost (Chefetz et al., 1996). Organic matter is decomposed for the most part of the soil micro-flora, although slight decomposition occurs even under biotic or photochemical conditions (Saad, 2001). In aerobic condition, there is a great diversity of decomposers, consisting of fungi, actinomycetes and a wide range of bacteria, which degrade the readily available organic components or transform them into stable humic components (Diaz-Burgos et al., 1994; Amir at al., 2004). It is reasonable to expect that the humification and transformation process might differ depending upon raw materials used for composting. Humic substances constitute the most important fraction of organic matter because of their effect on soil ecology, structure, fertility and plant growth. Many tests have been proposed to assess the biodegradation and humification of organic matter resulting in compost maturity and stability.

Changes in compost stability or the degree to which the composts have been decomposed can be predicted with C/N ratio in the solid phase (Jimenez and Garcia, 1992), soluble organic carbon content in water extract (Inbar et al., 1993), humification indices (Jimenez and Garcia, 1992; Chefetz et al., 1996), oxygen and CO_2 respirometry, plant growth bioassay, NMR and IR spectroscopy (Chen and Inbar, 1993). Several studies have investigated organic matter transformation during composting of municipal solid waste, municipal sewage sludge and separated cattle manure using chemical, spectroscopic and microbiological methods (Chen and Inbar, 1993; Adani et al., 2001; Jouraiphy et al., 2005).

Lignocellulose degradation during composting of agricultural waste (Vegetable waste, cow dung, saw dust and dry leaves) plays an important role during the process as it contributes to the major organic matter (Tuomela et al., 2000; Zeng et al., 2010; Feng et al., 2011). Lignin is considered as the most abundant renewable source on earth and it is very difficult to degrade, as it slows down the degradation of cellulose and hemicellulose (Huang et al., 2010). Huang et al. (2010) had reported that lignin as the most abundant renewable source on earth and its difficulties during degradation process. It has been estimated that there is $2.5\text{-}4\times10^{11}$ tonnes of cellulose and $2\text{-}3\times10^{11}$ tonnes of lignin in the earth, representing 40 and 30% of organic matter carbon respectively (Fengel and Wegener, 1989; Argyropoulos and Menachem, 1997). The balance of the global carbon cycle is maintained by the photosynthesis and degradation of these lignocellulosic fractions (Brown, 1985; Colberg, 1988). Temperature, moisture content and type of lignocellulose majorly govern the degradation rate (Rayner and Boddy, 1988). During composting, transformation and mineralization of organic matter is carried out by many microbial communities such as bacteria, fungi and actinomycetes (Zucconi et al., 1987). However, these microbial communities are greatly affected by the varying temperature during the process and physical properties of initial waste material.

2.9 THE BENEFITS OF COMPOST

Composting is a great recycling process in which the resources are conserved in a more available form so that they can be most efficiently used. Unlike other chemical and physical disposal process such as burning and landfill, this biological means of disposing that is composting can add much advantage to the ecosystem by conserving the plant nutrients. The application of compost can drastically reduce the usage of ammonia-type fertilizers, in which approximately 2% of the natural gas consumed in the United States is used up in the manufacture of these chemical fertilizers (Schonfeld et al., 2003). Since the compost is primarily focused on NPK and other micronutrients it can be well used as a fertilizer. Most of the nitrogen can be trapped into the compost if the loss of ammonia is reduced during the process. Application of compost as a fertilizer has improved the physical structure of the soil that includes potting soil mixtures. In addition there was an increased suppression of plant diseases caused by soil-borne nematodes, fungi and bacteria due to the addition of compost to the soil in various cropping systems (Schonfeld et al., 2003).

Gajalakshmi and Abbasi, (2002) studied the effect of compost/vermicompost obtained from a pernicious weed like water hyacinth on kitchen gardens with lady's finger (*Hibiscus esculentus*), brinjal (*Solanum melongena*), cluster bean (*Cyamopsis tetragonoloba*), chili (*Capsicum annum*) and tomato (*Lycopersicon esculentum*). They reported that there was total absence of any harmful effect by the use of such compost material and moreover the quality of vegetables was better than normal conditions. Authors have also studied the effects of same water hyacinth compost on the growth and yield of a flowering plant, *Crossandra undulaefoila*. The results stated that the plants in pots amended with water hyacinth compost showed significantly better height, larger number of leaves, more favorable shoot: root ratio, greater biomass per unit time and larger length of inflorescence.

2.10 CONCLUDING REMARKS

Due to huge production of MSW and improper management practices, the country is facing a lot of environmental effects as well spending huge amount of fund in the solid waste management. The major problem is the composition of MSW in India and the practices being followed. Since disposal and landfilling is the major practice being followed, it is having a huge impact on environment by greenhouse gas emission, leachate production and other air borne diseases. The primary reason is only due to the

40-60% composition of organic waste in the MSW. The best alternative for the issue is the source segregation of wet and dry waste at the generation point and opting suitable treatment process. From the above literatures, many researchers and government policies have recommended composting and vermicomposting for the processing of organic (vegetable) waste as the sustainable method. Composting of vegetable waste may reduce the environmental impact on climate change by 40–70% compared to landfilling and incineration. During composting, the organic matter is biologically degraded by several groups of microorganisms to form a final product containing stabilized carbon, nitrogen and other nutrients in the organic fraction. During composting, about 50% of added organic matter has been completely mineralized due to the degradation of easily degradable compounds such as proteins, cellulose and hemicellulose by microorganisms. The final residual organic matter consisted of humic-like substances which are highly non-biodegradable and also the most stable fraction of mature compost. In composting, the organic matter degradation is carried out by different diversity of microorganisms including mesophilic bacteria, spore-forming bacteria, fungi and actinomycetes to transform them into stable humic components. However, the degradation pattern and humification during composting is considered to follow different pattern depending on the raw materials used for composting.

In addition the quality of final compost and time duration for the process carried out is of major concern to look upon which is reported to change according to the type of composting methodology adopted and also by environmental factors. Most of the reports were experimented on the organic matter transformation, stability analysis and microbial dynamics during the process for 45 to 120 days. But, there are not many literatures available on the effects of leachate on compost parameters and control methods during drum composting of vegetable waste. The best combination of waste materials such as vegetable waste, cow dung, saw dust and dry leaves for producing stabilized compost within shorter time period is still unproven. In addition, there are limited reports available on the application of waste carbide sludge addition and white-rot fungi i.e. *P. Chrysosporium* to increase the volatile solids reduction and lignocellulose degradation during drum composting of mixed organic waste. There are only few literatures available on the application of rotary drum composting followed by vermicomposting of vegetable waste for improving the quality of compost and shortening the time duration.

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Chapter 3

MATERIALS AND METHODS

Different experimental approaches were used to accomplish the stipulated objectives. The research was carried out in different phases using various waste material combinations. The detailed methodology is given in this chapter.

3.1 EXPERIMENTAL DESIGN

In order to accomplish the objectives, the proposed research was carried out in four different phases (Fig. 3.1). In phase 1, experiments were conducted on the effects of leachate and effects of bulking agent addition during drum composting of vegetable waste. In phase 2, the best combination ratio were compared with different composting methodologies such as pile composting operated in agitated, passive and active aeration mode; and vermicomposting. In phase 3, the effects of waste carbide sludge addition and white rot fungi inoculation were performed for improved treatment efficiency. In final phase, the microbial diversity of the best combination was performed using the 16srRNA sequence method.

3.2 FEEDSTOCK MATERIALS

Vegetable waste, cow dung, saw dust and dry leaves were used for the preparation of different waste mixtures. Vegetable waste was collected from different hostel mess of Indian Institute of Technology Guwahati campus. Dust bin was kept in the mess of each hostel and the waste was collected for the experiments. Fresh cow dung was obtained from nearby Amingaon village, Guwahati city. Saw dust was purchased from a nearby saw mill. Dry leaves were collected from the Indian Institute of Technology Guwahati campus. All the materials were mixed together in different proportions for making the compost (Fig. 3.2). Table 3.1 provides initial characterization of waste materials used for preparing compost. The observed values were found very much optimum for the waste materials to produce compost.

Exotic earthworm species *Eisenia fetida* and *Eudrilus euginea* was collected from Central Plantation Crops Research Institute (CPCRI), Indian Council of Agricultural Research, Regional Station, Kahikuchi, Guwahati, India. For developing the cultures, Perspex bin sizes 450×300×450 mm were fabricated in the laboratory.

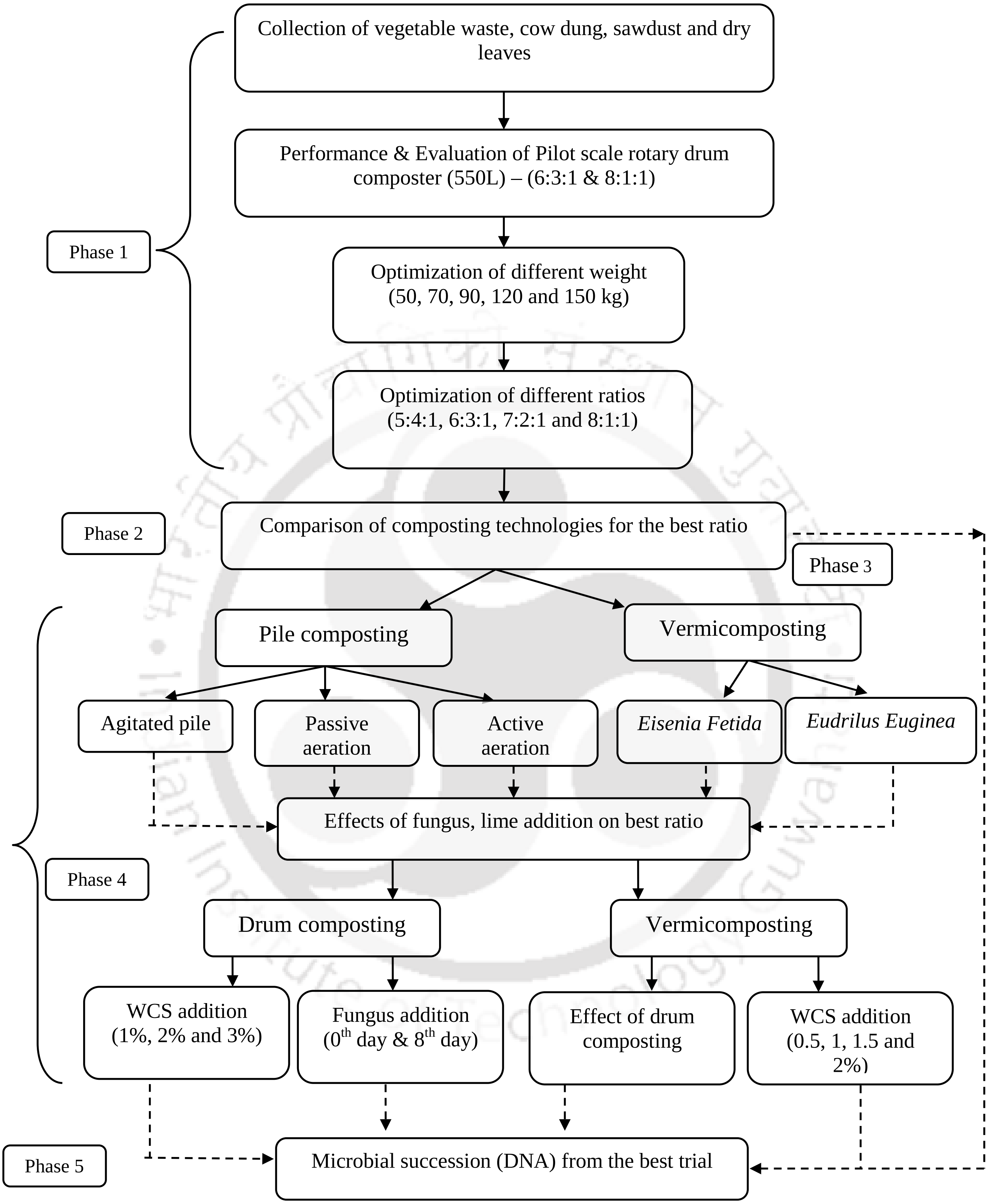

Fig. 3.1. Experimental design of the research work

Fig. 3.2. Raw materials for making compost

Fig. 3.8 shows pictorial view of Perspex bin and cultured earthworm. For aeration and drainage purpose 16 holes of 10 mm diameter were drilled along the length and at the bottom respectively. The earthworms, bedding was prepared using chopped hay (about 50 mm), cow dung, banana pulp (chopped about 50 mm), tree leaves all were partially degraded. The moisture level was maintained about 60-70% by periodic sprinkling of adequate quantity of tap water to keep it moist to enable the worms to breathe. The temperature of the experimentation room was maintained at 25^{o}C by placing hot and cool blowers.

3.3 COMPOSTING METHODS

3.3.1 PHASE I - ROTARY DRUM COMPOSTING

Vegetable waste collected from different hostel mess was chopped into 1 to 2 cm size and mixed along with cow dung and saw dust. After that, the different waste combinations were mixed together before feeding into the reactor.

Table 3.1. Initial characterization of waste materials

Parameters	**Waste materials**		
	Vegetable Waste	**Cow dung**	**Sawdust**
pH	5.23±0.02	7.92±0.01	6.86±0.02
Electrical conductivity (dS/m)	1.88±0.01	3.10±0.02	1.06±0.02
Moisture content (%)	91.20±2.22	75.14±0.52	41.02±0.32
Total organic carbon (TOC) (%)	49.84±2.22	32.22±1.24	53.44±1.22
Total nitrogen (%)	2.59±0.07	1.35±0.20	0.55±0.02
Ammoniacal Nitrogen (NH_4-N) (%)	0.65±0.04	0.36±0.04	0.05±0.02
Total phosphorous (mg/L)	6.6±0.25	7.8±0.41	1.22±0.05
Available phosphorus (mg/L)	1.10±0.14	1.15±0.04	0.61±0.06
C/N ratio	19±0.24	23.46±0.40	95±2.16
Sodium (Na) (g/kg dry matter)	2.6±0.8	1.25±0.12	0.62±0.08
Potassium (K) (g/kg dry matter)	2.82±0.6	8.25±0.65	1.95±0.15
Calcium (Ca) (g/kg dry matter)	3.82±0.65	1.7±0.25	0.95±0.20
Iron (Fe) (g/kg dry matter)	4.95±1.5	8.55±0.20	2.9±0.25
Nickel (Ni) (mg/kg dry matter)	42±1.25	22.2±3.8	245±2.65
Chromium (Cr) (mg/kg dry matter)	23.5±1.5	130±2.5	142±2.75
Manganese (Mn) (mg/kg dry matter)	164±3.5	160±2.0	135±3.25
Cadmium (Cd) (mg/kg dry matter)	68.2±0.08	45±2.5	72.15±2.10
Copper (Cu) (mg/kg dry matter)	45.6±2.7	36±1.5	42±2.77
Lead (Pb) (mg/kg dry matter)	32±0.95	86±3.5	146.35±3.0
Zinc (Zn) (mg/kg dry matter)	160.5±4.5	130±4	125±2.3
Chemical oxygen demand (COD) (mg/L)	4300±20	440±16	480±30
Biological oxygen demand (BOD) (mg/L)	1950±30	120±20	250±20
CO_2 evolution (mg/g VS/day)	26±2.83	17.2±0.2	13.2±0.6
Oxygen uptake rate (OUR) (mg/g VS/day)	29.4±0.8	18.9±0.7	10.9±0.54

Note: (mean ± SD, n=3) SD- standard deviation, ND- Not detected

Fig. 3.3 and 3.4 shows the schematic diagram of a pilot-scale rotary drum composter of 550 L capacity; operated by batch mode. The main unit of the composter, i.e. the drum is of 1.022 m in length and 0.76 m in diameter, fabricated by a 4 mm thick metal sheet.

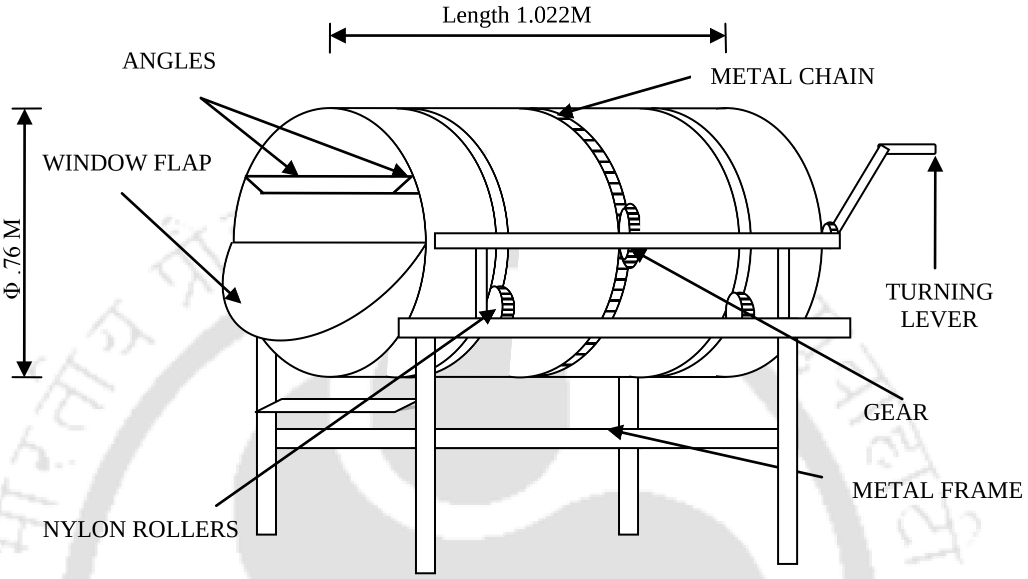

Fig. 3.3. Schematic diagram of rotary drum composter

Fig. 3.4. Pictorial representation of drum composting

The inner side of the drum is covered with an anti-corrosive coating. The drum is mounted on four rubber rollers attached to a metal stand and is rotated manually with its handle. In order to ensure appropriate mixing, agitation and aeration of the wastes during rotation, 40×40 mm angles were welded longitudinally inside the drum. In addition, two adjacent holes of 10 cm each are made on top of the drum to drain out the excess water. The capacity of the pilot scale rotary drum batch reactor was decided keeping in view of the capability of a single person who can handle around 150 kg of wastes by manual rotation. The composting period of 20 days was decided based on higher degradation and stabilization of waste material in In-vessel composting reactors (Kalamdhad et al., 2008; Singh and Kalamdhad, 2013). Manual turning was done at every 24 h through one complete rotation of the rotary drum to ensure that the material on the top portion moved to the central portion, where it was subjected to higher temperature. Table 3.2, 3.3 and 3.4 provide the waste combinations for the effect of leachate, effect of bulking agent addition and optimization of weight ratios during rotary drum composting of vegetable waste along with cow dung, saw dust and dry leaves.

Table 3.2. Waste combinations during leachate production

Experiment	**Vegetable waste (kg)**	**Cow dung (kg)**	**Sawdust (kg)**	**C/N**
Trial 1 (6:3:1)	90	45	15	24
Trial 2 (8:1:1)	120	15	15	30

Table 3.3. Waste combinations during effect of bulking agent addition

Feedstock material	**Trial 1 (6:3:1:2)**	**Trial 2 (6:3:1:1.4)**	**Trial 3 (6:3:1:1.1)**	**Trial 4 (6:3:1:0.8)**	**Trial 5 (6:3:1:0.6)**
Vegetable waste (kg)	30	42	54	72	90
Cow dung (kg)	15	21	27	36	45
Saw dust (kg)	5	7	9	12	15
Dry leaves (kg)	10	10	10	10	10
Total weight (kg - Initial) (Dry leaves excluded)	50	70	90	120	150
C/N ratio	19.37	17.79	22.15	23.27	23.41

Table 3.4. Waste combination during optimization of weight ratios

Experiment	Waste materials		
	Vegetable waste (kg)	Cow dung (kg)	Sawdust (kg)
Trial 1 (5:4:1) + 10 kg dry leaves	45	36	9
Trial 2 (6:3:1) + 10 kg dry leaves	54	27	9
Trial 3 (7:2:1) + 10 kg dry leaves	63	18	9
Trial 4 (8:1:1) + 10 kg dry leaves	72	9	9

3.3.2 PHASE II - PILE COMPOSING AND VERMICOMPOSTING

Fig. 3.5-3.7 shows the pictorial view of agitated and passive pile. The composting materials were mixed together and made to form a trapezoidal shape with length 2.13 m, width 0.61 m and height 0.55 m. The compost was prepared in 5:4:1 ratio with 10 kg dry leaves and the mass was turned manually on every third day for agitated pile and samples were collected from six different locations mainly from the mid and end portions of the piles to make up 500 g to form a homogenous sample. Passive operation was carried out by placing a polyvinyl chloride (PVC) pipe on a 10 cm layer bed of old compost. The PVC pipe was 7 cm diameter with holes of 2.5 cm diameter drilled at every 20 cm distance for the air flow at every side starting from the centre. After making the combinations in (5:4:1) ratio along with dry leaves, the mixed waste materials were discharged on the bed with the pipe in the center of the composting mass.

Fig. 3.5. Agitated pile composting

Fig. 3.6. Passive pile composting

Forced aerated pile was operated with the same PVC pipe on 10 cm layer bed of old compost connected to an air blower at one end while the other end was sealed. The air flow from the compressor was 0.34 L/min kg/VS for the total mass of 100 kg as suggested by Kulcu and Yaldiz (2004). Three different piles containing 100 kg of waste mixture was composted for 30 days and sampling was carried out on 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30th day. The composting mass was turned on every third day for trial 1, while there was no turning for trial 2 and 3.

Fig. 3.7. Aerated pile composting

However for trial 3, the forced aeration was done for first three days (10 mins per day) and further it was stopped due to drop in temperature. After turning and aeration, about 500 g of each grab samples were collected mostly at the mid span and end terminals of pile and samples were mixed together and considered as homogenized sample. Table 3.5 provides combination of waste materials used for experimenting pile composting.

Table 3.5. Waste combination during pile composting

Experiment	**Waste materials**			
	Vegetable Waste (kg)	**Cow dung (kg)**	**Sawdust (kg)**	**Dry leaves (kg)**
Trial 1 (Agitated pile)	45	36	9	10
Trial 2 (Passive pile)	45	36	9	10
Trial 3 (Aerated pile)	45	36	9	10

Vermicomposting experiments were conducted in duplicate in curved bamboo containers (reactors) (Radius; 120 mm, depth; 90 mm and volume; 904.70 cm^3). Temperature in the experimentation room was maintained at 25±1°C which is the optimum temperature for *E. fetida*. Fig. 3.8 and 3.9 shows the pictorial view of vermicompost reactors and vermicompost. The earthworm weight was calculated according to the weight of the feedstock added and the number of days for experimentation, based on the literature suggested; earthworms can consume materials half of their body weight per day under favorable conditions (Haimi and Huhta, 1986; Khwairakpam and Bhargava, 2009).

Fig. 3.8. Culturing of earthworms

Fig. 3.9. Vermicomposting of vegetable waste

The moisture level was maintained about 60-70% throughout the study period by periodic sprinkling of adequate quantity of tap (potable) water. To prevent moisture loss, the reactors were covered with gunny bags. The proper aeration was provided by perforated reactor design and periodic turning of waste mixture. The reactor was designed for a total weight of 2.5 kg for 45 days (based on worm mass added) composting period (Fig. 3.9). Table 3.6 provides the combination of waste materials used for the study.

Table 3.6. Waste combinations during vermicomposting

Experiment	**Waste materials**					
	Vegetable waste (kg)	**Cow dung (kg)**	**Sawdust (kg)**	**Dry leaves (kg)**	**Total Weight (kg)**	**Adult biomass (No's)**
Trial 1 (*Eisenia fetida)*	1.25	1	0.25	0.27	2.77	180
Trial 2 (*Eudrilus eugeniae)*	1.25	1	0.25	0.27	2.77	180
Trial 3 (*Eisenia fetida)*	Mix of prestabilized waste from drum composting after 8 days				2.77	180

Acclimatized 180 earthworms (adult and juvenile, average weight of 70 g) were randomly picked from the Perspex bin culture and used for the purpose of investigation. After turning and earthworm counting, about 80 g of each grab samples were collected on 0, 15, 30, and 45th day from three different locations of vermireactors and samples were mixed together and considered as homogenized sample.

3.3.3 PHASE III – EFFECT OF CHEMICAL ADDITION AND FUNGAL INOCULATION

Table 3.7 and 3.8 provides the waste combinations during Waste carbide sludge (WCS) addition and fungus inoculation. WCS was collected from Assam Air Products Pvt. Ltd., Guwahati, Assam, India. WCS is produced during acetylene gas production by calcium carbide in semisolid condition. The fungus *P. chrysosporium* (MTCC 787) was used in this study that was procured from IMTECH, Chandigarh, India. The culture was grown on potato dextrose agar (PDA) plates maintained at 25°C for 12 to 15 days. Fig. 3.10-3.12 provides WCS and fungal preparation.

Table 3.7. Waste combination during waste carbide sludge addition

Experiment	**Waste materials**					
	Vegetable waste (kg)	**Cow dung (kg)**	**Sawdust (kg)**	**Dry leaves (kg)**	**Total (kg)**	**WCS (%) added**
Trial 1 (WCS)	45	36	9	10	100	0
Trial 2 (WCS)	45	36	9	10	100	1
Trial 3 (WCS)	45	36	9	10	100	2
Trial 4 (WCS)	45	36	9	10	100	3

Table 3.8. Waste combination during fungal addition

Experiment	**Waste materials**					
	Vegetable waste (kg)	**Cow dung (kg)**	**Sawdust (kg)**	**Dry leaves (kg)**	**Total (kg)**	**Fungus inoculation time**
Trial 1 (-)	45	36	9	10	100	--
Trial 2 (Fungus)	45	36	9	10	100	0th
Trial 3 (Fungus)	45	36	9	10	100	8th

Fig. 3.10. Waste carbide sludge

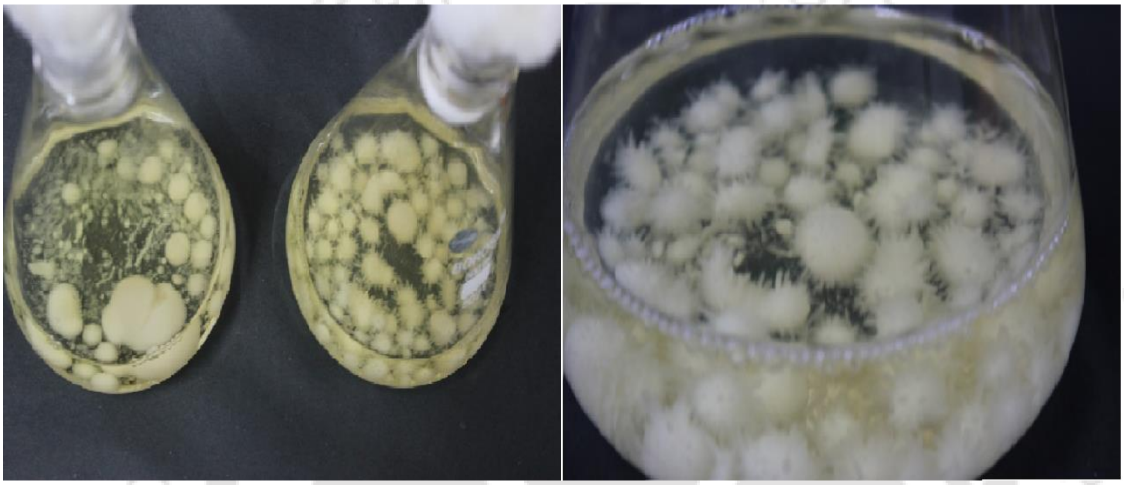

Fig. 3.11. *Phanerochaete chrysosporium* preparation in liquid broth

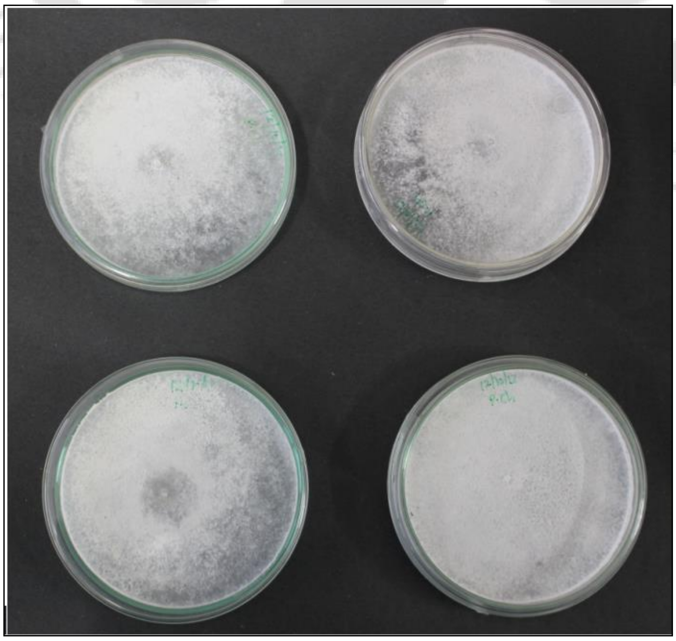

Fig. 3.12. *Phanerochaete chrysosporium* mycelia preparation in agar plate

3.4 SAMPLING AND ANALYSIS

A total of 500 g and 200 g sample was collected from composting and vermicomposting reactors for complete analysis. The sample was prepared by taking representative samples from 9 different points mainly from the mid span and end terminals of the pilot-scale rotary drum composter after drum rotation and pile so as to ensure homogenized sample. These homogenized samples was collected on every two day interval for drum composting, three day interval period for pile composting and 15 day interval period for vermicomposting. Finally the collected samples were mixed thoroughly to make a homogenized sample. Triplicate samples were collected and air dried immediately, ground to pass through 0.2 mm sieve and stored for physico-chemical analysis. The sub-samples were either used or stored at $4^{o}C$ for biological analysis of the wet sample within 2 days.

3.5 PARAMETERS ANALYZED

Different experimental methods were used in the study to accomplish the stipulated objectives. Physico-chemical and biological analysis of the solid waste samples including vegetable waste, cow dung and saw dust were carried out in Environmental engineering laboratory (Department of Civil Engineering, IIT Guwahati). Experimental procedures of the biological, microbiological and physico-chemical parameters were explained below.

3.5.1 BIOLOGICAL ANALYSIS

- *Soluble Biochemical Oxygen Demand (APHA, 2005)*

About 10 ± 0.1g of fresh compost was taken in a conical flask and dissolved in 100 ml of distilled water. The flask was kept in a horizontal shaker for 2 h. Then it was filtered using whattman filter paper (Standard filter paper). The supernatant of the samples were taken and analyzed for BOD test using BOD_3 test (Eq. 3).

Calculation:

$$BOD_3\text{, mg/L} = \frac{D_1 - D_2}{P} \times 1000 \qquad (3)$$

Where,

D1= initial DO of sample in mg/L, D2= final DO of sample after 3 day incubation in mg/L, P =sample volume (in mL) diluted to 300 mL with dilution water

- *Chemical Oxygen Demand (APHA, 2005)*

About 10 ± 0.1 g of fresh compost was taken in a conical flask and dissolved in 100 ml of distilled water. The flask was kept in a horizontal shaker for 2 h. Then it was filtered using whattman filter paper (Slandered filter paper). The supernatant of samples were taken and analyzed for COD using closed reflux method 1.5 mL of $K_2Cr_2O_7$ + 2.5 ml of sulfuric acid reagent were added to cod vial. It was digested for 2 h in COD digester at 150^{o}C and cooled down to room temperature. Then cooled and titrated against ferrous ammonium sulphate using ferroin indicator till color changes from green to wine red.

- *CO_2 evaluation by Soda-Lime method (Kalamdhad et al., 2008)*

About (25 ± 0.1g) of fresh compost sample was taken in 1 L PVC airtight container. About 10 g of oven dried (105°C) soda lime (1.5-2.0 mesh) was taken in a 100 mL beaker and was placed in the above mentioned container. The initial weight of the soda-lime was taken as (W_1)g (Eq. 4).

Calculation:

$$CO_2 \text{ evolution rate (mg/g VS/day)} = \frac{W_2 - W_1}{W \times T} \times 1000 \qquad (4)$$

Where, W_1 = Initial weight of the soda-lime (g), W_2 = Final weight of the soda-lime (g), W = Weight of compost sample taken (g), T = Time duration of incubation (h)

Then the container with soda-lime beaker was kept in an incubator at 25°C. After 20-24 hours the soda-lime was taken out and oven dried it again, then the final weight (W_2) g was noted. The difference in mass of soda lime will give the amount of CO_2 absorbed.

- *Oxygen uptake rate (OUR) (Kalamdhad et al., 2008)*

The oxygen uptake rate (OUR) was performed according to the method described by Lasaridi and Stentiford (1998). The OUR was measured in a liquid suspension of compost (5-8g of compost in 500 ml of distilled water added with $CaCl_2$, $MgSO_4$, $FeCl_3$ and phosphate buffer at pH 7.2) the solution was kept in suspension by placing it on the magnetic stirrer at constant temperature by keeping the whole assembly into the water bath held at 30°C . During this time, the dissolved O_2 of the suspension was continuously observed through the digital (DO) meter attached to it. The oxygen consumption rate was calculated by taking the difference of DO with respect to the time intervals and this value was quoted as the OUR in mg O_2/g VS/h.

3.5.2 MICROBIAL ANALYSIS

- *Sampling for microbial analysis*

Microbial count was done by adding 10 g of waste or compost into 90 mL of sterile distilled water containing 0.85% (w/v) sterile sodium chloride solution in 250 mL Erlenmeyer flasks. The solution is mixed mechanically at 150 rpm for 2 h at 25^{o}C. Finally, the waste suspensions were diluted serially and used for microbial counts on appropriate media.

- *Culture media and conditions*

For bacterial growth nutrient agar medium was used, total count of the prokaryotes was examined. Petri plates were poured with the nutrient agar medium and kept in the BOD incubator for 24-48 h at 25°C for mesophilic bacteria and for 24 h at 55°C for the spores of bacteria. Cycloheximide was added in concentration of 0.2 g/L in order to restrict the growth of fungi.

For actinomycetes growth, Actinomycete isolation agar was supplemented with 2 g sodium caesinate, 0.1 g/L-Asparagine, 4 g sodium propionate, 0.5 g K_2PO_4, 0.1 $MgSO_4$, 0.001 g $FeSO_4$ and 5 mL glycerol per liter and cycloheximide (0.2 g/L) (for fungal growth inhibition). Final petri plates poured with this medium were incubated in the BOD incubator at 25°C for 4-5 days.

Fungal count was done in the Sabouraud 4% dextrose agar with 5 g peptone from casein, 5 g peptone from meat, 40 g D(+)Glucose per liter. Prepared plates were incubated at 25°C for 3-4 days.

Streptomycetes were counted in the ISP medium No.4 supplemented with 10 g starch soluble, 1 g K_2HPO_4, 1 g $MgSO_4.7H_2O$, 1 g NaCl, 2 g $(NH_4)_2SO_4$, 2 g $CaCO_3$ per liter and 0.2 g/L of cycloheximide for fungal inhibition.

Lauryl tryptose broth and EC medium respectively were used for total coliforms (TC) and fecal coliforms (FC) analyses in the culture tube by using the most probable number (MPN) method (APHA, 1995).

3.5.3 TOTAL GENOMIC DNA EXTRACTION FROM COMPOST

DNA was isolated using modified Xcelgen Soil gDNA kit. Quality of gDNA was checked on 1% agarose gel (loaded 5 µL) for the single intact band. The gel was run at 110 V for 30 mins. 1 µL of each sample was loaded in Nanodrop 8000 for determining A260/280 ratio. The DNA was quantified using Qubit dsDNA BR Assay kit (Thermo

Fisher Scientific Inc.). 1 µL of each sample was used for determining concentration using Qubit® 2.0 Fluorometer.

3.5.4 PHYSICO-CHEMICAL ANALYSIS

Temperature was monitored using a digital thermometer throughout the composting period. The EC and pH was measured in filtered supernatant (BIS: 10158-1982). Volatile solid (VS) and ash content were also measured according to BIS, 10158-1982. Initial weight of the crucible was taken as W_1 g. Weighed (10 ± 0.1g) ground sample (screened through 0.22 mm sieve) in crucible and kept it in a muffle furnace operating at a temperature of 550-600^{o}C for 2 h. After 2 h crucible was taken out of the muffle furnace and kept in desiccator for ½ h for cooling and then final weight of crucible with sample was taken as W_2 g. Volatile solids content of the sample was calculated as (Eq. 5):

$$VS(\%) = \frac{(5-(W_2 - W_1))}{5} \times 1000 \qquad (5)$$

Total organic carbon (TOC) was calculated from VS with a factor of 1.8. Total nitrogen (TN) was analyzed using the Kjeldahl method and NH_4-N using KCl extraction (Tiquia and Tam, 2000). For TN analysis 0.2 g of sample (passed through 0.22 mm sieve) was taken and catalyst mixture (potassium sulphate and cupric sulphate, 5:1) of 3 g was added, and digested with 10 mL conc. H_2SO_4 using digestion equipments at 400^{o}C for 2 h (end color of digested sample was green). After digestion, make the digested sample 100 mL. 10 mL of diluted sample distillate using distillation unit (Pelican Equipments Chennai, India) with 40% NaOH and distilled water, distillate was collected in 25 mL boric acid with mixed indicator. Collected distillate (clear green color) and titrate with 0.02 N H_2SO_4 at end point purple colour.

The TN was calculated as follow (Eq. 6):

$$TN(\%) = \frac{14 \times (S - B) \times N}{Wt.} \qquad (6)$$

Where, S = mL of standard sulfuric acid used for sample, B = mL of standard sulfuric acid used for blank, N = Normality of standard sulfuric acid, Wt. = Weight of compost sample in g.

For the analysis of NH_4-N, 5 g sample (passed through 0.22 mm sieve) was taken in a reagent bottle to which 50 mL of 2M KCl was added and kept in a horizontal shaker for 2 h. After shaking sample was filtered and supernatant was taken for NH_4-N analysis using Phenate method of Standard methods (APHA, 2005).

3.5.5 HEAVY METAL ANALYSIS

The Flame photometer (Systronic 128) was used for analysis of Na, K and Ca concentration and Mg concentration was measured by atomic absorption spectrometer (AAS) (Varian Spectra 55B) after the digestion of 0.2 g sample with 10 mL of H_2SO_4 and $HClO_4$ (5:1) mixture in block digestion system (Pelican equipments, Chennai, India) for 2 h at 300^oC. The total concentration of Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr was measured using atomic absorption spectrometer (AAS) after the digestion of 0.2 g sample with 10 mL of H_2SO_4 and $HClO_4$ (5:1) mixture in block digestion system (Pelican equipments Chennai, India) for 2 h at 300^oC.

3.5.6 BIOCHEMICAL ANALYSIS

Lignin was determined in 0.3 g (dry weight) portions of each sample using National renewable energy laboratory (NREL) procedure (Templeton and Ehrman, 1995; Ehrman, 1996). Cellulose was obtained using method adopted by Updegraff (1969) and hemicellulose was determined from the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF) using method provided by Goering and Van (1975).

3.6 INSTRUMENTS USED

Table 3.9 shows the different instruments that were used during the investigation and experimental analysis.

Table 3.9. Different instrument used for experimentation

Parameter Analysis	Instrument	Company (Make/Model)
Sieving	Sieve	Unique Drawing & Survey emporium
Moisture content	Class- II High Accuracy	Satwik Scale Industries
pH	µ pH system 361	Systronics
Electrical conductivity	Conductivity meter VSI-04 Deluxe	VSI Electronics Pvt. Ltd.
Volatile solids	Muffle Furnace	International Commercial Traders

CO_2 evolution	BOD Incubator	International Commercial Traders
OUR	DO meter	Eutech Instrumets Pvt. Ltd.
COD	COD Digester	HACH
BOD	BOD Incubator	International Commercial Traders
Serial dilution	Vortex	SPINIX
Plating, MPN	Laminar flow chamber	CleanAir
Sterilization	Autoclave	Equitron
Centrifuge	Micro centrifuge	ThermiSci
Spectra analysis	FTIR	PerkinElmer's
Spectra analysis	Fluorescence Spectroscopy	PerkinElmer's
Heavy metals	AAS	Varian Spectra 55B

Indian Institute of Technology Guwahati

Chapter 4

COMPOSTING OF VEGETABLE WASTE

This chapter deals with the performance of rotary drum composter for the degradation of vegetable waste in combination with bulking agents such as cow dung, sawdust and dry leaves. In addition, pile composting of vegetable waste was experimented at agitated, passive and forced aeration condition. Vermicomposting of vegetable waste was also performed by employing *Eisenia fetida* and *Eudrilus eugeniae*. The overall study is conducted in three phases:

1. Phase 1: Performance and optimization of rotary drum composter
2. Phase 2: Performance of pile composting operated at agitated, passive and forced aerated conditions.
3. Phase 3: Performance of vermicomposting using *Eisenia fetida* and *Eudrilus eugeniae* and drum followed by vermicomposting.

4.1 PHASE I – PERFORMANCE AND OPTIMIZATION OF ROTARY DRUM COMPOSTER

Composting is an exothermal process and biological oxidation of organic matter carried out by a dynamic and quick succession of populations of aerobic micro-organisms. Bulk treatment of organic wastes can be done successfully with composting process. Among the major waste management strategies practiced, composting is gaining much interest for organic waste disposal with more economical and environmental profits leading to a stabilized end product. The final product can be used to improve and maintain soil quality and fertility (Larney and Hao, 2007). Application of vegetable waste compost consistently as a soil conditioner increases soil organic matter content and soil C/N ratio to greater levels than those of unamended soil (Montemurro et al., 2006; Xiao et al., 2011).

Rotary drum composting is an efficient and promising technique for the treatment of organic waste. The composting time can be drastically reduced to 2-3 weeks with pathogen free end product (Kalamdhad and Kazmi, 2008). However, there are limited literatures available on the composting of vegetable waste using cow dung, sawdust and dry leaves for the control of leachate and the role of bulking agent addition during drum composting. Due to higher proportions of vegetable waste, there are chances for leachate production because of its higher biodegradable nature. In order to study the composting process and the effects of moisture production, composting was carried out and reported about the effect of leachate on compost properties. Moreover the role of bulking agent during vegetable waste composting was discussed in detail. Compost properties such as physico-chemical, biological and microbial dynamics during different intervals of composting period has been extensively studied and reported.

In phase I, composting was carried out by mixing vegetable waste, cow dung and sawdust based on the initial C/N ratios of 24 and 30. Further, different combination of waste materials were studied by adding fixed amount of dried leaves. The composting period was carried out for 20 days with manual turning of drum once in every 24 h. The influence of different combination of waste materials and addition of dried leaves was studied on the changes in physico-chemical parameters, microbial diversity and lignocellulose degradation during rotary drum composting of vegetable waste.

4.1.1 EFFECTS OF LEACHATE DURING VEGETABLE WASTE COMPOSTING USING ROTARY DRUM COMPOSTER

The compost was prepared with two different proportions using vegetable waste, cattle manure and sawdust for two different ratios, trial 1 (6:3:1) of C/N 24 and trial 2 (8:1:1) of C/N 30 respectively with a total volume of 150 kg. The effect of leachate production during the process was studied on the physico-chemical and biological parameters. Prior to composting, the maximum particle size of the composting material was restricted to 1 cm in order to provide better aeration and moisture control. The combination of waste materials is explained in Table 3.2.

4.1.1.1 Physico-chemical parameters

- **Temperature**

Temperature is an important factor determined by the microbial diversity and the intensity of metabolic activities during the composting process. An increase in temperature was observed during early stages of composting i.e. 18 h in both the trials 1 and 2. Although the rise in temperature preceded in the same manner in both the trials, but it was observed maximum in trial 1 than trial 2. A maximum of 63.5^{o}C was observed in trial 1 and 61.2^{o}C in trial 2; maintained an average of 55^{o}C till the end of 5th day and thereafter started to decrease (Fig. 4.1). Temperature from 52 to 60^{o}C is considered to maintain the greatest thermophilic activity in composting systems (Mohee and Mudhoo, 2005). Since the organic content is very high in vegetable waste the microbial activity started at the early hours of the composting leading to rise in temperature. Furthermore it also resulted in gradual release of CO_2 and high amount of moisture. Sawdust was used as bulking agent to maintain moisture content and provide better aeration during the composting process as suggested by Tsai et al. (2007). But it was not found successful in the present study with the usage of vegetable waste mixture at 150 kg working volume. However it was successful in the same range for different substrate i.e. water hyacinth with the same proportions studied by Singh and Kalamdhad (2012).

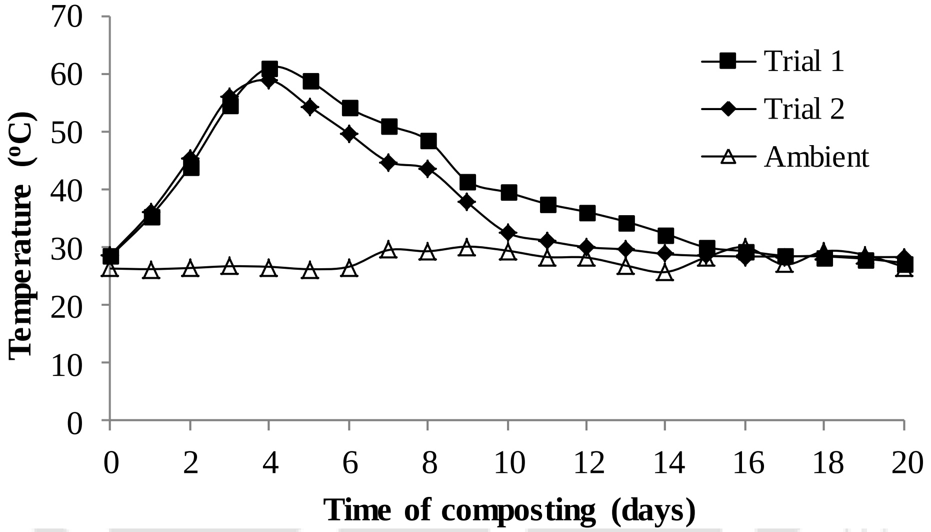

Fig. 4.1. Temperature profile during drum composting

Leachate production started soon after thermophilic phase in both the trials, but was found very high in trial 2 compared to trial 1, which might be due to insufficient bulking agent. Sawdust has been proposed as bulking agent by many researchers for moisture control and to provide better aeration during composting for various substrates (Smith et al., 2001; Smith et al., 2006; Tsai et al., 2007). But it was not found optimum during vegetable waste composting. Therefore, an alternate and optimized bulking material should be proposed for vegetable waste which provides more aeration and porosity for controlling the moisture, because when the thermophilic stage is at maximum due to active microbial action the byproducts formed mainly water vapor will completely saturate the bulking agent. In such cases proper bulking agent should be provided for maintaining the loss of moisture. In absence of such agents in the system, it will consequently lead to leachate production. Initial moisture content of the raw material was maintained around 77 and 84% in trial 1 and 2, respectively. Not much appreciable amount of moisture content reduction was observed at the end of 20 days compared to studies conducted in drum composting (Kuhlman, 1989; Bhatia et al., 2012). During leachate production, sudden drop of temperature was observed during the active thermophilic phase, thereby reducing the loss of moisture during the process. It observed a direct relation between the active thermophilic phase and leachate production during the composting was observed. During increase in temperature good reduction in moisture content was observed, however after the production of leachate the moisture content was found to be almost constant, finally it was in the range of 67% in trial 1 and 76% in trial 2. Moreover the leachate production had great influence on the compost parameters.

- **pH and EC**

Most compost had the pH value in the range of 6 to 8 and it generally increases during the composting process (Singh et al., 2009). Similar results were observed in the present study with initial pH 6.6 and 6.45 increased to 7.4 and 6.9 in trial 1 and 2. EC of trial 1 was observed to increase from 3.5 dS/m to 7.4 dS/m, which could be due to release of mineral salts and ammonium ions through the decomposition of organic matter as reported by Nair and Okamitsu (2010); whereas in trial 2 there was a gradual reduction from 6.2 dS/m to 4.7 dS/m, which might be due to large amount of leachate production. While the metabolic process is in progress, the volatilization of ammonia and precipitation of mineral salts occurs through which the EC of material will be increased and also leads to the stabilization of pH to alkaline conditions. Due to leachate production and inconsistent moisture content during composting, there was not much loss of organic carbon during the process (Table 4.1).

- **Total organic carbon (TOC) reduction**

Decrease in TOC from 26 and 44% to 22 and 42% in trial 1 and 2 respectively was observed as given in table 4.1. This minimal loss of carbon is contradictory as compared to traditional compost process that leads to great loss in carbon content. But, such reduction correlates with the reports by Andersen et al. (2010) in which he stated that carbon loss is greatly dependent on leachate production.

- **Nitrogen and phosphorous dynamics**

From the results it was observed that leachate production did not have much effect on the total nitrogen dynamics in both the trials, as the values increased from 1.05 and 1.45% to 1.75 and 1.85% finally in trial 1 and 2 respectively. However, ammoniacal nitrogen showed considerable decrease from 0.70 and 0.83% to 0.50 and 0.62% in trial 1 and 2, respectively. During mineralization of organic matter, phosphorous is released by the action of micro-organisms. It was observed with a good increase in total and available phosphorous in both the trials.

- **Micronutrients and heavy metals**

Table 4.2 illustrates the total concentration of micronutrients (K, Na and Ca) and heavy metals (Ni, Co, Cr, Cu, Fe, Mn, Mg, Pb and Zn) during the composting process. These nutrients are used as mineral fertilizers in the compost. Trial 1 showed considerable amount of increase in the total amount at the end of the composting period, whereas in trial 2 there was a drastic reduction in all the nutrients which is considered due to large amount of leachate production most of the nutrients might have been washed away.

4.1.1.2 Biological parameters

- **CO_2 evolution rate**

CO_2 evolution is used to evaluate the compost stability as it measures carbon derived directly from the compost being tested and it is directly correlated with aerobic respiration. The composting process is believed to continue as long the total amount of biodegradable organic material is stabilized and the percentage of readily available biochemical oxygen demand (BOD) is believed to be an important aspect of compost quality (Kalamdhad et al., 2009; Wang et al., 2004). Higher CO_2 evolution was observed at the initial stage of the composting period in both the trials 1 and 2; but the values were inconsistent in between the composting period as it correlates the minimal of carbon during the process, finally it was in the range of 2.1 mg/g VS/day and 2.8 mg/g VS/day in trial 1 and 2 respectively. The lower emission of CO_2 during the final stage of compost denotes that the material is in the maturation phase as suggested by Kalamdhad et al. (2009).

- **Oxygen uptake rate (OUR)**

OUR determines the biological activity of the compost and it is well accepted. It relates the compost stability to the amount of readily biodegradable organic matter present in the sample through its carbonaceous oxygen demand (Kulhman, 1989). At the end of 20 days it was observed in the range of 2.4 mg/g VS/day and 2.7 mg/g VS/day in trial 1 and 2 respectively. Soluble BOD and soluble chemical oxygen demand (COD) can be measured by the respiration of BOD through aerobic conditions maintained in the compost. Soluble BOD and soluble COD decreased as a result of destruction of biological organic matter, resulting in decreased emission of carbon dioxide. Similar observations were found in the present study indicating the reduction of soluble COD from 14 and 15.6 mg/kg to 5.5 and 6.1 mg/kg in trial 1 and 2. Consequently soluble BOD reduced from 7.18 and 9.40 to 2.95 and 3.70 mg/kg in trial 1 and 2 respectively.

4.1.1.3 Microbial dynamics

- **Heterotrophic bacteria**

Bacterial populations are the natural microflora colonizing the first substrate preferentially degrading the liable organic-matter. Fig. 4.2 compares the changes in the cell density of mesophilic bacteria over time during composting process in trial 1 and 2. During mesophilic stage, there is a predominance of mesophilic population in both the trials in the range of 6.5×10^9 CFU/g of compost and 6.8×10^{10} CFU/g of compost. These mesophilic bacteria are mainly involved in the degradation of simple organics. During this stage metabolic activities by mesophilic population will result in the release of CO_2 and temperature (Nair and Okamitsu, 2010); thereby heating up the material in the reactor. Due to high organic content in vegetable waste, the microbial activity started at the early stage of mesophilic phase resulting in the rise of temperature. The temperature rise had major effect on the growth of mesophilic bacteria which is evident during the fourth day of composting period in both the trials. Major drop in the population of mesophilic bacteria was observed in the range of 4.25×10^9 CFU/g of compost and 4.45×10^9 CFU/g of compost in trial 1 and 2 respectively. At the same time there was an enormous growth in spore forming bacteria from 3.9×10^6 CFU/g and 4.4×10^6 CFU/g to 5.6×10^8 CFU/g and 4.75×10^7 CFU/g in trial 1 and 2 respectively from initial day to the fourth day of the process. Most of these thermophilic bacteria are spore forming ones, which can survive at 10 to 70°C in the composting environment (Amner et al., 1988). The decline in mesophilic bacteria due to higher temperature in the compost correlates the report stated by Ryckeboer et al. (2003); furthermore it was reported that the bacterial and fungal populations are clearly influenced by temperature especially in the thermophilic stage. Soon after the decline in thermophilic stage rise in mesophilic bacteria was observed in the range of 3.0×10^7 CFU/g and 4.5×10^5 CFU/g in trial 1 and trial 2 respectively. Higher microbial population was observed at the end of 20 days which might be due to considerable amount of moisture content.

Table. 4.1. Physico-chemical and biological pattern during composting

Days	**Moisture content (%)**		**pH**		**EC (dS/m)**		**TOC (%)**	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
0	77.04±0.14	84.06±0.23	6.6±0.01	6.44±0.02	3.5±0.02	6.16±0.01	26.12±0.21	44.42±0.28
20	67.77±0.79	75.87±0.59	7.4 ± 0.01	6.9±0.01	7.4 ± 0.01	4.7±0.01	22.24±0.15	41.89±0.09
Days	**Total Nitrogen (%)**		**Ammonia (%)**		**Available phosphorus (%)**		**Total phosphorus (%)**	
0	1.05±0.14	1.45±0.07	0.7±0.014	0.825±0.02	0.22±0.02	0.42±0.01	0.35±0.02	0.57±0.01
20	1.75±0.22	1.85±0.22	0.5±0.03	0.62±0.09	0.34±0.01	0.53±0.02	0.48±0.01	0.78±0.02
Days	**Soluble COD (mg/kg)**		**Soluble BOD (mg/kg)**		**CO$_2$ (mg/g VS/day)**		**OUR (mg/g VS/day)**	
0	14±0.84	14.60±0.35	7.17±0.24	9.40±0.84	7.9±0.6	8.5±0.4	6.4±0.6	6.9±0.4
20	5.50±0.63	6.10±0.42	2.95±0.21	3.70±0.14	2.1±0.3	2.8±0.4	2.4±0.2	2.7±0.4
Days	**Total Coliform (MPN/g)**		**Fecal Coliform (MPN/g)**		**Total Streptococci (CFU/g)**		**Total Enterococci (CFU/g)**	
0	11×10^{8}	24×10^{8}	4.3×10^{5}	3.5×10^{5}	4.05×10^{5}	5.5×10^{5}	3.5×10^{5}	4.05×10^{5}
20	4.6×10^{3}	9.3×10^{3}	3.5×10^{2}	4.6×10^{2}	2.55×10^{2}	2.05×10^{2}	1.33×10^{2}	2.55×10^{2}

Note: (mean ± SD, n=3) SD - standard deviation

Table. 4.2 Variation of nutrients and heavy metals during composting

Days	**Na (g/kg)**		**K (g/kg)**		**Ca (mg/kg)**		**Mg (mg/kg)**	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
0	2.71±0.03	2.61±0.05	3.63±0.07	2.61±0.05	27.23±0.17	18.67±0.07	46.75±1.47	58.25±1.64
20	3.82±0.01	2.81±0.05	5.31±0.05	7.68±0.02	68.06±0.12	68.51±0.07	92.5±0.84	39.25±1.22
Days	**Mn (mg/kg)**		**Cr (mg/kg)**		**Cd (mg/kg)**		**Cu (mg/kg)**	
0	498.25±1.06	436±0.71	55.25±1.77	43.5±1.41	86.75±2.47	93.25±1.06	53.25±1.76	71.75±1.06
20	600.75±1.06	301.5±2.12	62±2.12	53.75±1.76	96.5±0.71	83.5±1.41	107±2.12	53±3.53
Days	**Pb (mg/kg)**		**Ni (mg/kg)**		**Zn (mg/kg)**		**Fe (g/kg)**	
0	1162.5±17.68	1385±14.14	234±2.12	257.75±2.47	336.75±1.76	553.5±2.12	9.92±1.21	6.79±0.47
20	1375±21.21	1240±14.14	261.5±1.41	226±0.71	391±1.42	309.05±0.21	10.48±1.61	3.75±1.21

Note: (mean ± SD, n=3) SD - standard deviation

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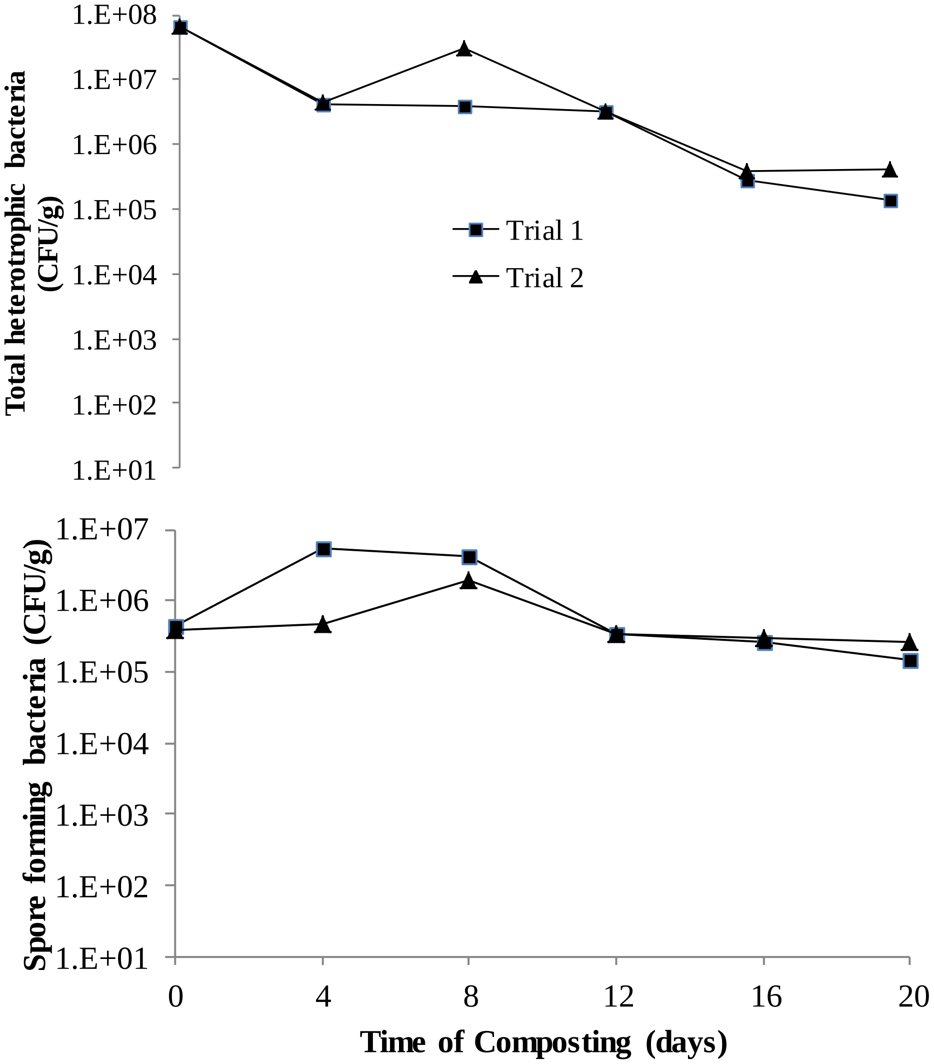

Fig. 4.2. Variation in heterotrophic and spore forming bacteria during composting

Whereas studies by Bhatia et al. (2012) with vegetable waste composting using Invessel system reported much reduction in the microbial population at the end of composting period but no leachate was reported. Hence, in the present study due to high moisture content the microbial population prevailed in large amount even at the end of composting period and it is correlated with the report by Neyla et al. (2009). Therefore leachate production adversely affects the microbial population during the compost process which in turn results in poor degradation process.

- **Fungi, actinomycetes and streptomycetes population**

Moreover fungi, actinomycetes can survive at high temperature as spores and started to increase soon after the decline of thermophilic phase. The results were in agreement with the report by Ryckeboer et al. (2003) for low population of these species during the initial stages of composting due to domination of bacteria and high proliferation soon after thermophilic phase. The reason for the rise in population may be due to the loss of readily available carbon source during the initial phase and hence the left out lingo-cellulolytic material had been degraded by these population. Xiao et al. (2011) also suggested that temperature higher than 50°C favors the growth of actinomycetes. There was a tremendous growth in fungi, actinomycetes and streptomycetes during late thermophilic stage as reported by Tchobanoglous et al. (1993). The fungi population decreased from 4.1×10^6 CFU/g and 3.2×10^5 CFU/g to 2.8×10^6 CFU/g and 3.0×10^5 CFU/g at the end of fourth day in trial 1 and 2 respectively (Fig. 4.3). Actinomycetes, which are considered for the degradation of cellulose and lignin was found in the range of 3.85×10^6 CFU/g and 2.8×10^4 CFU/g in trial 1 and 2 at the end of 20 days.

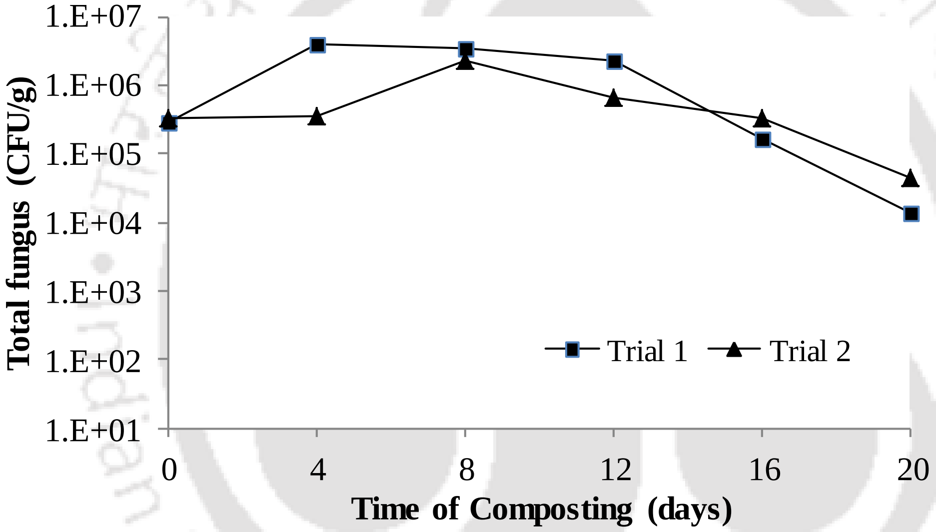

Fig. 4.3. Total fungus during drum composting

Streptomycetes population was observed in the range of 4.15×10^5 CFU/g and 3.6×10^4 CFU/g in trial 1 and 2 respectively (Fig.4.4). Ishii et al. (2000) has reported that by the end of composting period there was no fungi and fewer amounts of actinomycetes were left. However these reports were disagreed by Ryckeboer et al. (2003) and it was explained that the differences can be possible by high moisture content and low C/N ratio. Furthermore it might also be due to slow growth rate of fungi and actinomycetes. The present study results agree with the above statement for high moisture content and also for high populations of fungi, actinomycetes and streptomycetes at the end of composting period. Mustin (1987) indicated that the micro-organisms of compost can

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create the conditions for their own destruction at any moment that are optimal for the micro-organisms engaged in composting.

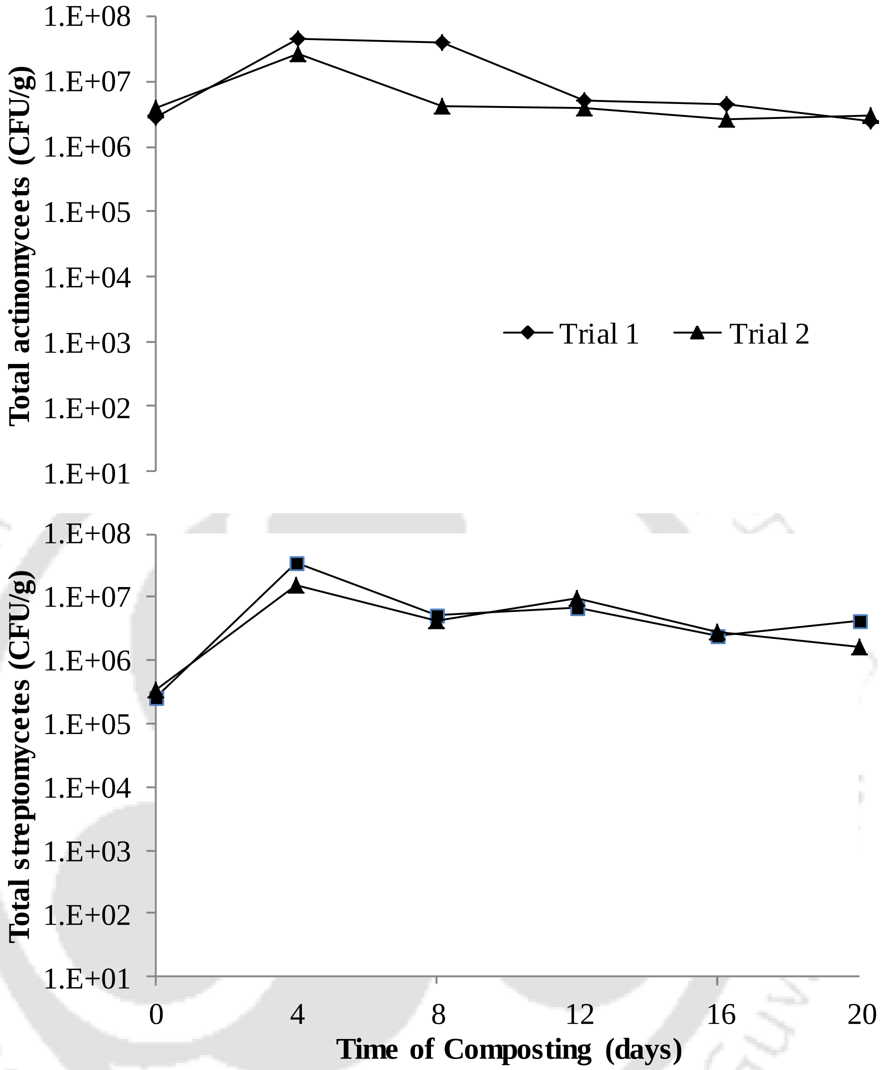

Fig. 4.4. Actinomycetes and streptomycetes during drum composting

- **Pathogens**

There is always a concern on the hygiene of the final compost. The recommended FC and fecal streptococci (FS) densities for compost hygienization are 5×10^2 MPN/g and 5×10^3 MPN/g, respectively. Moreover the presence of coliform bacteria is often used as an indicator of overall sanitary quality of the compost (Kulhman, 1989). Salmonella species were regarded as a problem of the hygienic quality of MSW compost and is destroyed when the temperature of the compost reaches 55°C. It is also reported that *Escherichia coli* and *Salmonella sp*. are killed in 20 min at 60°C. In addition the thermal death points of most disease causing organisms including *Salmonella typhosa*, *Mycobacterium tuberculosis*, *Mycobacterium diptheriae* and *Brucella abortus* or *sius* are well within the temperature range achieved in composting when exposed to these temperatures for at least a few minutes. Finally organisms are exposed to high temperatures for many hours and/or days during composting which is sufficient for eliminating pathogens (Kalamdhad and Kazmi, 2008; Nair and Okamitsu, 2010).

The present study also achieved a maximum temperature of around 61°C that was able to reduce the TC from 11×10^8 MPN/g and 24×10^8 MPN/g to 4.6×10^3 MPN/g and 9.3×10^3 MPN/g in trial 1 and 2 respectively. Similarly, average number of FC considerably decreased from 4.3×10^5 MPN/g and 3.5×10^5 MPN/g to 3.5×10^2 MPN/g and 4.6×10^2 MPN/g in trial 1 and 2 respectively. In addition streptococci and enterococci population were reduced to considerable amount at the end of 20 days in trial 1 as compared to trial 2 as given in Table 4.1.

4.1.2 EFFECTS OF BULKING AGENT IN COMPOSTING OF VEGETABLE WASTE AND LEACHATE CONTROL USING ROTARY DRUM COMPOSTER

The study involved the effects of bulking agent in vegetable waste composting using rotary drum with different waste combinations in (6:3:1) ratio for five different trials along with cow dung and sawdust (Table 3.3). 50 kg (Trial 1), 70 kg (Trial 2), 90 kg (Trial 3), 120 kg (Trial 4) and 150 kg (Trial 5) were carried out by adding 10 kg of dry leaves in each of the trials as bulking agent. Changes in physico-chemical and biological parameters have been studied during the 20 days of composting period.

4.1.2.1 Physico-chemical parameters

- **Temperature**

Due to higher indigenous microbial population in initial waste material, rise in temperature was observed in early stages of composting i.e. within 24-36 h of composting period. Fig. 4.5 shows the temperature variations in different trials during the composting process. Due to appropriate combination of waste materials and bulking agent, biodegradation of vegetable waste was carried out resulting in rise of temperature. A maximum of 61.4°C was achieved in trial 3, showing maximum organic matter reduction as compared to all other trials. In addition, an average of 54 to 56°C temperature was maintained for seven days in trial 3, which was considered as prolonged thermophilic stage leading to more organic matter degradation and removal of pathogen at such elevated temperature. Moreover, Mohee and Mudhoo (2005) have reported

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temperatures from 52 to 60°C are considered to maintain the greatest thermophilic activity in composting systems. Therefore, it can be considered that temperature variation and maintenance during composting process is directly dependent on the degradation process and active microbial action on the feed materials. Hence, the rise in temperature due to appropriate addition of waste materials has been supported by Singh and Kalamdhad (2012).

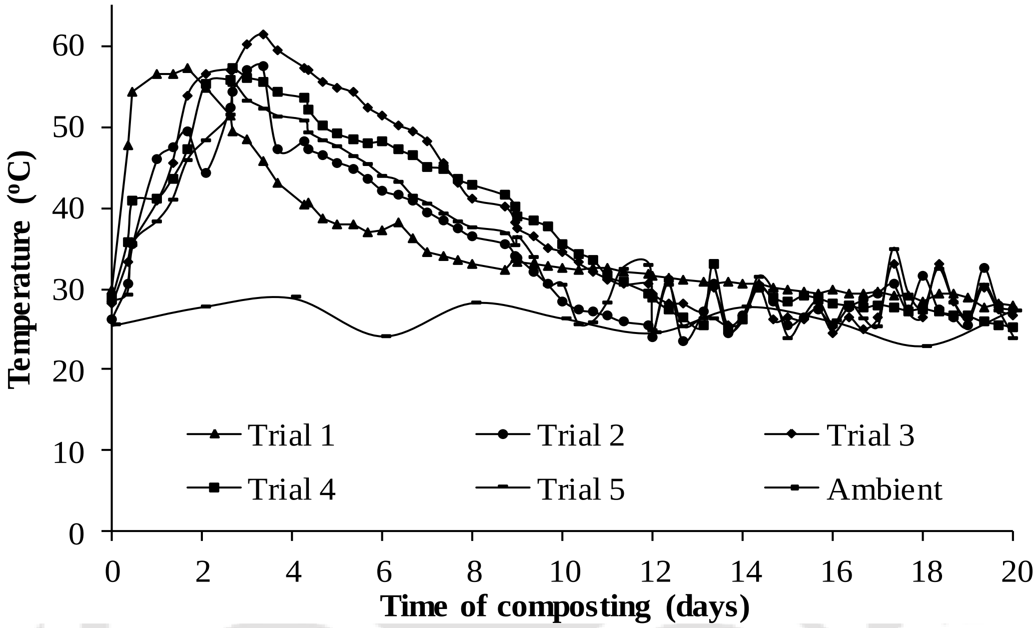

Fig. 4.5. Temperature profile during composting period

- **pH and electrical conductivity (EC)**

pH is considered as an important parameter in controlling the degradation process in composting. During the present study initial pH was in the range of 6.4 to 7.01 and was found to be 7.9 to 8.4 at the end of 20 days (Fig. 4.6). This rise in pH may be attributed due to the breakdown of organic fractions to simpler units and release as CO_2. Most composts have a pH value between 6 and 8; and the process is reported to proceed more efficiently at the thermophilic temperature when the pH is approximately 8 (Liao et al., 1996). Finally the pH values of composting were observed to remain constant, which may be due to the stabilization of compost and lower activities of microbial population. As the degradation of organic matter proceeds the compost will increase with mineral cation concentration which is not attenuated by salts leaching or by the binding to stable organic complex (Francou et al., 2005). Similar increase in EC was observed in the present study due to higher degradation of organic matter (Fig. 4.6). In initial day EC was in the range of 3.26, 3.67, 5.08, 5.67 and 4.58 (dS/m) and increased to 4.35, 5.38, 5.45, 6.36 and 4.58 (dS/m) respectively, in trial 1, 2, 3, 4 and 5 at the end of 20 days. The increase in EC due to degradation of organic matter during composting process was in accordance with the reports by Singh and Kalamdhad (2013).

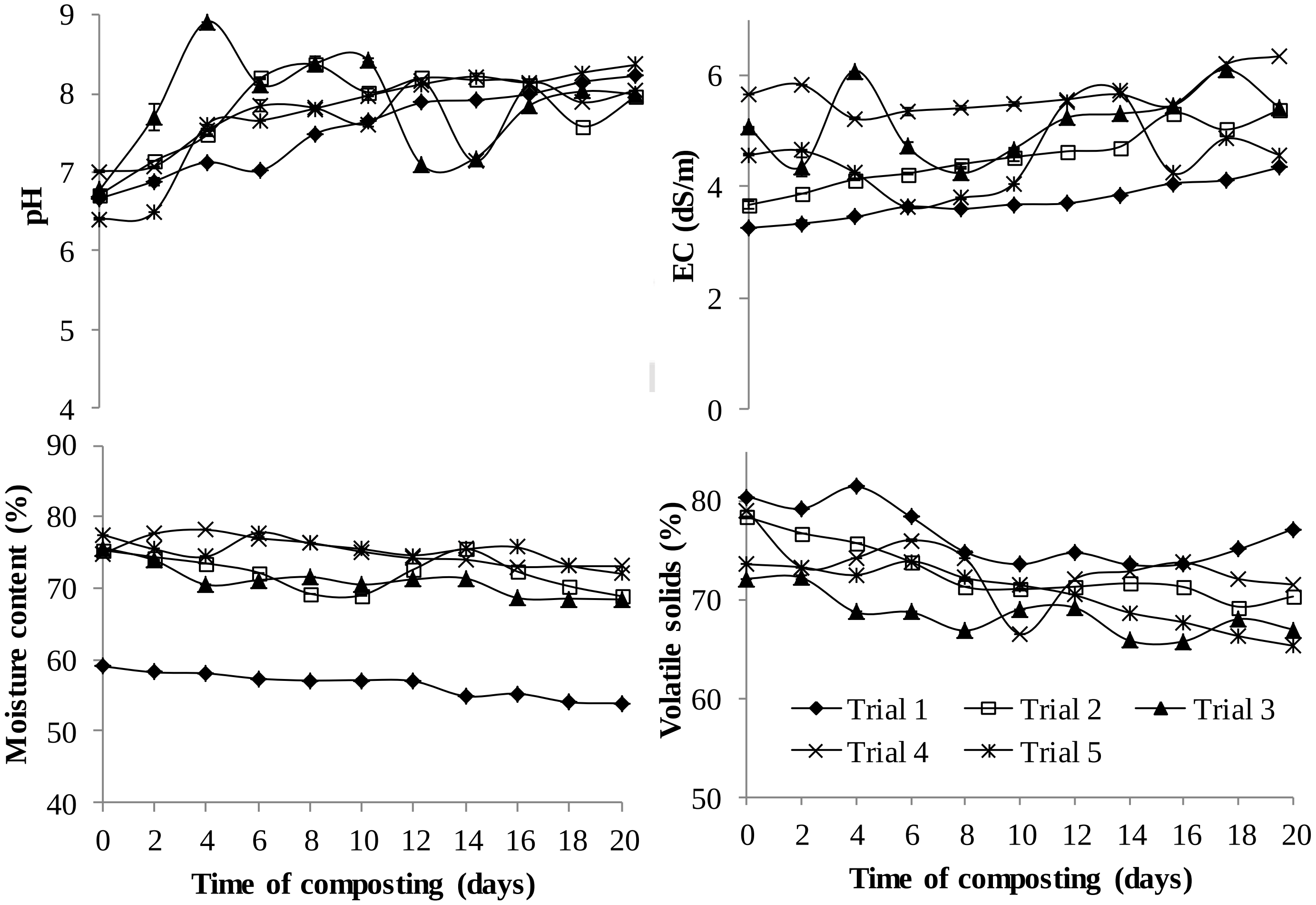

Fig. 4.6. pH, EC, moisture and volatile solids variation during composting period

- **Moisture and volatile solids (VS)**

Survival of microorganisms in composting system is primarily dependent on considerable amount of moisture content. As the degradation proceeds rise in temperature can be observed with loss of moisture and reduction in volatile solids. Hence moisture loss during the composting process can be viewed as an indicator of decomposition rate (Kalamdhad and Kazmi, 2009). Fig. 4.6 shows the reduction in moisture and volatile solids during the composting period. Due to readily degradable organic content, rise in temperature was observed within few days and no leachate was observed during the composting period. The added dry leaves were found appropriate in acting as a good bulking agent by providing better aeration and absorbing moisture. As the composting proceeded, the moisture and water vapors produced as a result of

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degradation was absorbed by dry leaves and trapped in the system. During rotation of the drum, the vapors were successfully released into the atmosphere, thereby drying out the compost. Hence addition of bulking agent plays a major role in controlling the leachate production during vegetable waste composting. Several studies were done on the usage of cattle manure, sawdust and rice straw as bulking agent in most of the composting process (Kalamdhad and Kazmi, 2009; Tang et al., 2007; Perez et al., 2009). Even though complete degradation of vegetable waste was achieved at the end of 20 days the added dry leaves contributed more TOC and VS, hence reuse of those leaves can be proposed. Hence removal of those dried leaves from compost would provide better reduction in actual TOC and VS. Similar studies were reported by Kalamdhad et al. (2009) on composting of vegetable waste by adding dry leaves in 3.5 m^3 full scale rotary drum for 150 days, in which the final compost were sieved by 6 mm sieve and the leaves were reused. Hence, VS reduction in the present study was observed from 80.47, 78.41, 72.16, 79.05 and 73.60% to 77.18, 70.33, 66.99, 71.55, 65.34% in trial 1, 2, 3, 4 and 5 respectively, at the end of 20 days composting period.

- **TOC and carbon/nitrogen (C/N) ratio**

During the composting process, carbon dioxide is emitted from the composting mass as a metabolic end product. Thus the total organic carbon content of the composting mass decreases as composting proceeds (Singh et al., 2009). TOC during composting period was initially in the range of 44.71, 43.56, 40.09, 43.92 and 43.12% and finally reduced to 42.88, 39.07, 37.22, 42.36 and 37.15% in trial 1, 2, 3, 4 and 5 respectively, at the end of 20 days. Due to addition of dry leaves as bulking agent for controlling moisture which are rich in cellulose and lignin it contributed more carbon content during the final stages of composting period. Eventhough degradation of cellulose and lignin is a long process by microbial action; the aim of adding dry leaves was only to serve as bulking agent, so it can be reused along with initial material after sieving. However, vegetable waste was completely degraded at the end of 20 days. Finally, trial 3 was found to have higher reduction in TOC when compared all other trials, in which same amount of dry leaves i.e. 10 kg were added. Hence it can be clearly stated that dry leaves contributed to TOC and VS more at the end of composting period. Compost having higher proportions of biodegradable matter may sustain higher microbial activity and further lead to odor generation and pathogen regrowth, therefore it may be considered unstable (Zucconi et al., 1985).

Organic matter decomposition and stabilization of the compost can be measured by analyzing the changes in C/N ratio of the compost (Kalamdhad et al., 2009). Thus, a C/N ratio between 10 and 15 in the compost indicates a good degree of maturity (Bhatia et al., 2012; Singh et al., 2009). Fig. 4.7 shows the changes in C/N ratio during composting period. Microorganisms utilize carbon as a source of energy and the nitrogen for building cell structures, thereby reducing the C/N ratio. In the present study, C/N ratio was reduced from 19, 17, 22, 23 and 23 to 18, 13, 12, 13 and 16 in trial 1, 2, 3, 4 and 5 respectively. However, combination of waste materials plays a major role in proper degradation with regard to C/N ratio. Out of all the trials, trial 3 was observed with higher C/N reduction, resulting in C/N 12 at the end of 20 days. A C/N ratio below 20 is indicative of proper compost maturity, with a ratio of 15 or less being preferred (Heerden et al., 2002).

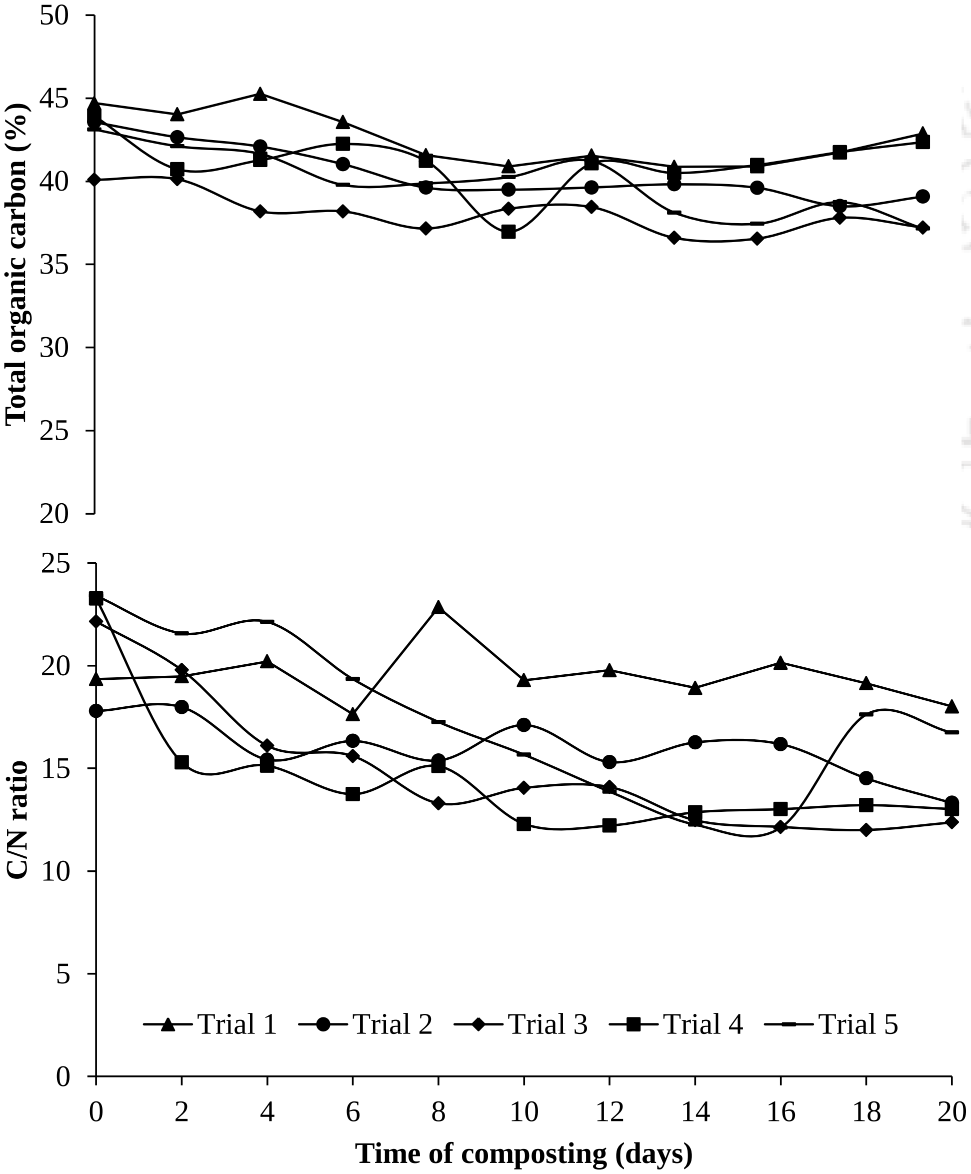

Fig. 4.7. TOC and C/N variation during composting period

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- **Nitrogen (TN and NH_4-N) and phosphorous (TP and AP) dynamics**

Nitrogen and phosphorous have been always considered as the essential nutrients in compost. Studies have shown that during composting, nitrogen is immobilized and converted to humus like material; furthermore it can be used as organic material with slow release of nutrients (Preusch et al., 2002). Fig. 4.8 depicts the time course of nitrogen and phosphorous turn over during the composting process. TN increased from 2.31, 2.45, 1.82, 1.89 and 1.75% to 2.38, 2.94, 3.01, 3.01 and 2.17% in trial 1, 2, 3, 4 and 5 respectively, at the end of 20 days. This result is in accordance with the reports by Vuorinen and Saharinen (1997) during drum composting, where increase in the TN from 1.6–2.0% to 2.8–3.0% was observed. Kalamdhad et al. (2009) also showed an increase in the TN from 1.4 to 2.6%. The increase in TN may be due to net loss of dry mass as CO_2 evolution and moisture loss by generation of heat by microbial action on organic matter. Furthermore, nitrogen fixing bacteria might also contribute to the increase in TN in later stage of composting (Bishop and Godfrey, 1983). NH_4-N was observed in the range of 0.7, 0.5, 1.4, 1.5 and 1.4% on day 0 and finally decreased to 0.6, 0.7, 0.4, 0.5, and 0.8% respectively, in trial 1, 2, 3, 4 and 5 at the end of 20 days. TP was observed in higher amounts in the range 3.42, 3.47, 3.26, 3.20 and 3.15 g/kg in day 0 and increased to 4.33, 3.81, 3.27, 4.21 and 3.51 g/kg in trial 1, 2, 3, 4 and 5 respectively, at the end of 20 days.

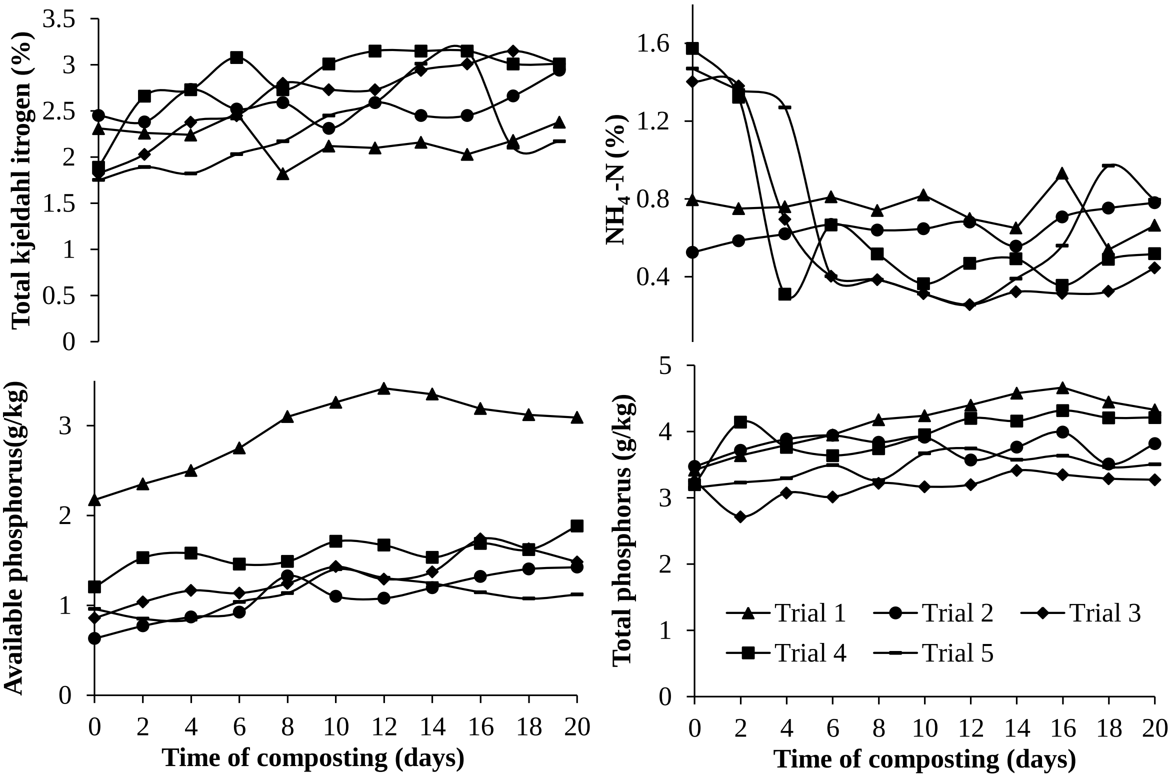

Fig. 4.8. Nitrogen and phosphorous dynamics during composting period

The increase in phosphorous content is considered to increase due to mineralization of organic material. However, higher amount of TP was observed in the initial days which may be attributed due to higher content of vegetable waste and cow manure (Kalamdhad et al., 2009). It is also reported that higher amounts of phosphorous may be contributed by the mix of waste materials in different combinations. AP was also observed to increase from 2.17, 0.63, 0.86, 1.20 and 0.96 g/kg respectively in day 0 to 3.09, 1.42, 1.48, 1.88 and 1.18 g/kg respectively, in trial 1, 2, 3, 4 and 5 at the end of 20 days. Even though nitrogen and phosphorus content was observed to be similar/high in trial 4 compared to trial 3, in terms of stability and pathogen removal trial 3 was observed better.

- **Nutrients and Heavy metals**

During composting, concentration of micronutrients and heavy metals has been observed to increase due to material weight loss caused by the mineralization of organic fractions (Liao et al., 1996; Fang and Wong, 1999). Hence, the total metal concentration is increased with loss of net initial waste materials (Amir et al., 2005). Table. 4.3 illustrates the total concentration of micro nutrients (Na. K, Ca and Mg). Table. 4.4 and 4.5 provides the total concentration of Cd, Pb, Ni and Fe; Mn, Cr, Zn and Cu in trial 1, 2, 3, 4 and 5 respectively, during the composting period. The increase in total metal concentration due to loss of net weight has reported by many authors during composting and the present study results were in accordance with the stated reports (Amir et al., 2005; Singh and Kalamdhad, 2013).

4.1.2.2 Biological Analysis

- **Soluble BOD and COD**

The biodegradable organic matter in compost is directly measured as soluble BOD and COD. As the organic fractions are decomposed by microbial populations, BOD and COD are decreased eventually (Kalamdhad et al., 2008). Fig. 4.9 shows the reduction in soluble BOD and COD during composting period. The reduction of soluble BOD was in the order of 73.86, 76.84, 93.40, 67.79 and 81.63% in trial 1, 2, 3, 4 and 5 respectively, at the end of 20 days of composting period. However, trial 3 showed the maximum reduction of 93.4% stating the higher degradation of organic matter and active microbial population due to proper combination of waste materials. The soluble COD values were observed to reduce from 3400, 3268, 3391, 3937 and 4662 mg/L to 1800, 1755, 1995, 2815 and 2832 mg/L in trial 1, 2, 3, 4 and 5 respectively at the end of 20 days. The higher values of COD may be due to the presence of complex material such as cellulose and lignin which are not easily available for microbial populations in the final stages.

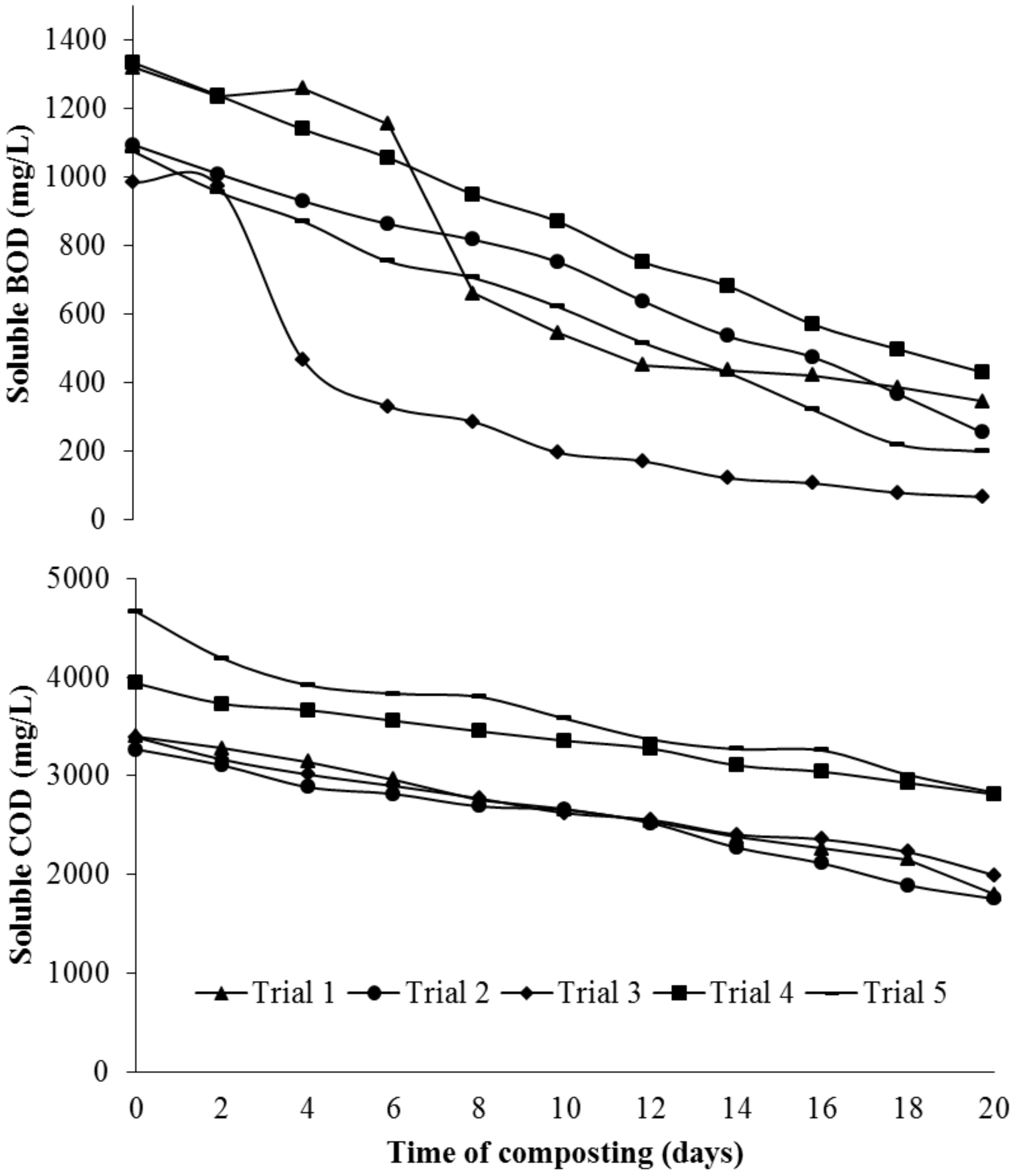

Fig. 4.9. Soluble BOD and COD variation during composting period

Hence the BOD was found very less as compared to COD. However, trial 3 was observed with higher reduction of maximum 41% when compared to all other trials. Decrease in BOD/COD ratio can be considered as the stabilization of the compost with more non-biodegradable content and furthermore no biodegradation can take place (Mangkoedihardjo, 2006). Similar findings were observed in the present study with BOD/COD reduction of 22, 18, 80, 33 and 45% in trial 1, 2, 3, 4 and 5 respectively. Trial 3 was observed with maximum BOD/COD ratio of 0.15, this may be due to appropriate addition of waste materials that lead to proper degradation and more stabilized compost. The above results were in accordance with reports by Kalamdhad and Kazmi (2009), where BOD/COD ratio was found to be 0.32 during the composting of green vegetables.

- **CO_2 evolution and Oxygen uptake rate (OUR)**

Compost stability is strongly related to the decomposition rate of organic matter and can be expressed by the biological activity. With lower biological action due to deprival of organic matter, emissions of CO_2 will be reduced thereby denoting the stability of compost. Fig. 4.10 shows the emissions of CO_2 during the process. In the initial stages higher amounts of CO_2 was observed due to the degradation of organic matter and it was found in the range of 13.75, 15.20, 7.11. 15.71 and 13.27 mg/g VS/day and finally reduced to 4.21, 1.31, 0.39, 0.48 and 3.39 mg/g VS/day at the end of 20 days.

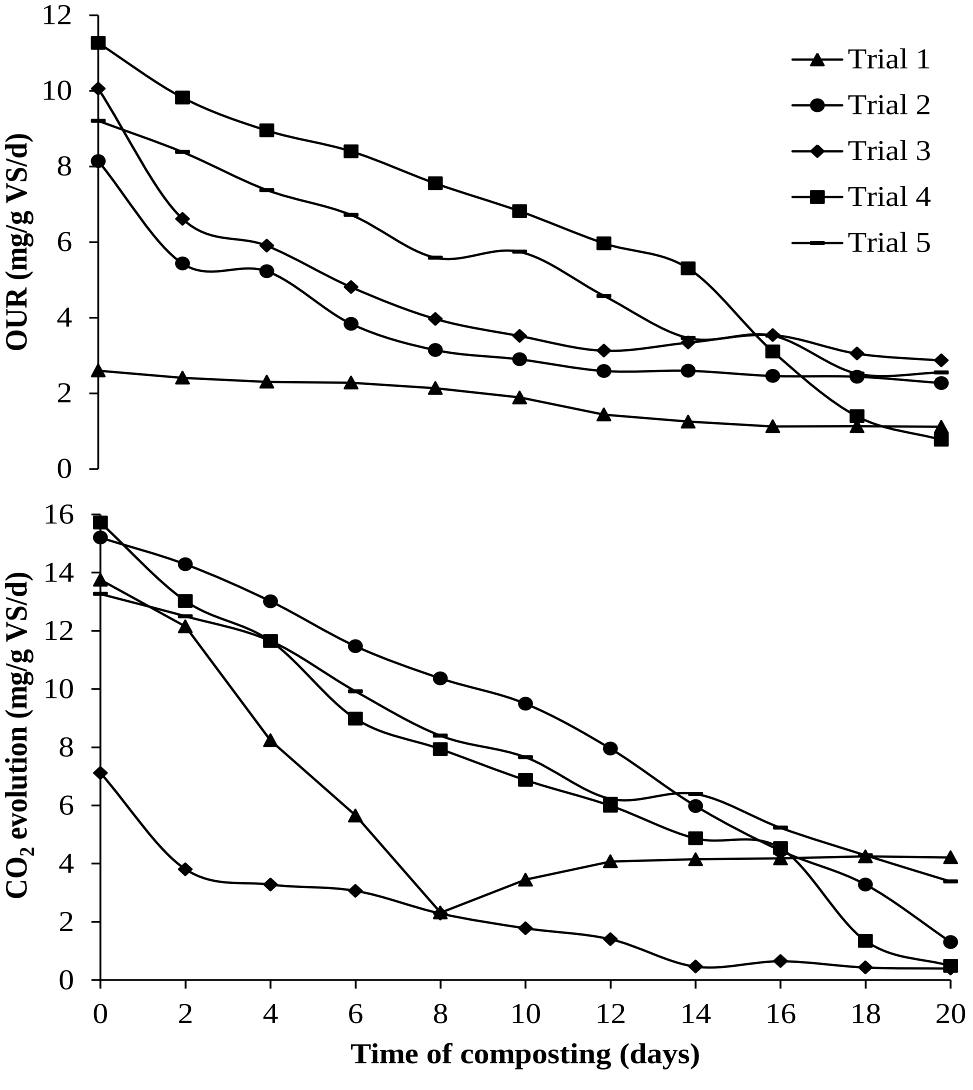

Fig. 4.10. OUR and CO_2 during composting period

The lower emissions of CO_2 at the final stages indicate the lack of degradable matter and lower activity of microbes. Eventhough CO_2 evolution was 0.39 and 0.48 mg/g VS/day in trial 3 and 4, trial 3 was found to be best in comparison with higher C/N ratio reduction and higher nutrient content.Due to propagation of microorganisms during composting process by feeding on the raw materials, OUR will be observed high (Iannotti et al., 1993). Similar results were observed during the present study due to high organic content of vegetable waste in the initial days. The OURs of trial 1, 2, 3, 4 and 5 decreased from initial values of 2.60, 8.14, 10.05, 11.27, and 9.21 mg/g VS/day to 1.11, 2.27, 2.87, 0.77 and 2.55 mg/g VS/day, respectively (Fig. 4.10). The reduction in OURs can be considered due to higher degradation of organic matter during the process. Eventhough trial 4 was observed with lower OUR than trial 3, it may be considered due to lower microbial action. As composting proceeds, large organic molecules are broken down to smaller and ones by the action of microbial communities. During the process higher oxygen demand is needed which is directly observed by the higher OURs during the initial stages of the composting and once the composting proceeded the final stages lower OURs were observed stating the deprival of readily available organic matter. The lower CO_2 evolution and OURs during the final stages in trial 3 denotes that the compost has matured with the proved days of composting.

4.1.2.3 Analysis of microbial community

Composting is essentially a phenomenon of microbial activity influencing and being influenced by the temperature during the process through biological degradation; and acting accordingly by change in different microbial communities. Based on the temperature, the rate at which the microbial population prevail and act will vary distinctly. In addition, monitoring the microbial succession during the composting process will be an effective management of the process and it further reflects the quality of maturing compost (Lasaridi and Stentiford, 1998). Therefore, microbial dynamics was observed during the different stages of composting and the effect of temperature and other external factors on the microbial community were discussed extensively.

- **Mesophilic and spore forming bacteria**

The indigenous population of total heterotrophic mesophilic bacteria in the initial raw material during day 0 was fairly high in all the trials and it was considered to be dependent on the total weight of waste residues used in each of the trials as reported by (Gazi et al., 2007). The initial amount of total heterotrophs was in the range of 7.7×10^9, Table. 4.3. Micronutrients during composting period

Day	Sodium (g/kg)					Potassium (g/kg)				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	1.69 ± 0.02	1.30 ± 0.07	2.14 ± 0.08	1.49 ± 0.03	1.35 ± 0.03	8.91 ± 0.12	11.11 ± 0.74	9.25 ± 0.05	16.96 ± 0.77	11.65 ± 0.60
2	1.71 ± 0.03	1.57 ± 0.04	2.16± 0.18	2.20 ± 0.04	1.48 ± 0.04	9.24 ±0.24	12.22 ± 0.33	10.94 ± 0.00	21.42 ± 0.00	12.37 ± 0.06
4	1.82 ± 0.02	1.66 ± 0.04	2.22 ± 0.07	2.07 ± 0.02	1.75 ± 0.09	9.73 ± 0.23	12.71 ± 0.30	12.93 ± 0.18	22.02 ± 0.29	12.67 ± 0.06
6	1.94 ± 0.04	1.60 ± 0.03	2.32 ± 0.11	1.82 ± 0.07	2.21 ± 0.02	10.28 ±0.25	13.16 ± 0.06	12.93 ± 0.14	21.05 ± 0.23	15.64 ± 0.07
8	2.09 ± 0.02	1.64 ± 0.03	2.36± 0.16	1.83 ± 0.06	2.30 ± 0.07	10.71 ± 0.04	14.16 ± 0.12	13.67 ± 0.15	22.02 ± 0.57	15.46 ± 0.05
10	2.12 ± 0.01	1.57 ± 0.02	2.38 ± 0.24	1.96 ± 0.00	2.36 ± 0.08	10.98 ± 0.12	13.83 ± 0.05	14.25 ± 0.11	22.24 ± 0.63	15.47 ± 0.04
12	2.02 ± 0.01	1.48 ± 0.02	2.47 ± 0.03	1.18 ± 0.00	2.19 ± 0.06	11.25 ± 0.14	13.66 ± 0.20	14.13 ± 0.02	22.49 ± 0.66	16.58 ± 0.05
14	2.14 ± 0.03	1.68 ± 0.01	2.54 ± 0.03	2.14 ± 0.01	2.20 ± 0.08	11.38 ± 0.26	19.21 ± 0.42	15.15 ± 0.09	21.91 ± 0.09	15.59 ± 0.04
16	2.28 ± 0.04	1.77 ± 0.03	2.66± 0.04	2.01 ± 0.04	1.96 ± 0.01	11.08 ± 0.01	17.88 ± 0.21	15.80 ± 0.09	22.20 ± 0.60	15.50 ± 0.04
18	2.36 ± 0.02	1.76 ± 0.02	2.72 ± 0.08	2.27 ± 0.02	1.93 ± 0.01	12.46 ± 0.24	19.15 ± 0.18	16.17 ± 0.04	27.66 ± 0.03	15.53 ± 0.19
20	2.57 ± 0.02	1.75 ± 0.01	2.84 ± 0.01	2.06 ± 0.01	2.00 ± 0.02	14.49 ± 0.16	19.40 ± 0.40	17.72 ± 0.03	21.15 ± 0.77	15.59 ± 0.13
Day	**Calcium (g/kg)**					**Magnesium (g/kg)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	5.68 ± 0.05	5.19 ± 0.06	4.88 ± 0.06	3.87 ± 0.00	4.90 ± 0.08	5.91 ± 0.04	5.966 ± 0.01	5.77 ± 0.03	8.03 ± 0.17	6.34 ± 0.05
2	6.41 ± 0.04	5.28 ± 0.06	5.59 ± 0.10	4.25 ± 0.06	5.07 ± 0.06	6.21 ± 0.06	6.135 ± 0.01	6.65 ± 0.06	8.61 ± 0.04	6.42 ± 0.04
4	6.10 ± 0.04	5.56 ± 0.08	5.57 ± 0.05	4.34 ± 0.03	5.14 ± 0.00	6.63 ± 3.42	6.432 ± 0.01	6.57 ± 0.05	8.69 ± 0.09	6.35 ± 0.06
6	6.24 ± 0.02	5.88 ± 0.17	5.47 ± 0.05	4.55 ± 0.08	6.50 ± 0.16	6.74 ± 0.05	6.583 ± 0.01	7.06 ± 0.09	9.13 ± 0.08	6.81 ± 0.04
8	6.32 ± 0.08	6.70 ± 0.04	5.03 ± 0.17	4.79 ± 0.00	15.46 ± 0.05	6.78 ± 0.01	6.950 ± 0.01	7.38 ± 0.05	8.73 ± 0.09	6.84 ± 0.08
10	6.54 ± 0.04	6.10 ± 0.05	5.76 ± 0.02	5.58 ± 0.07	15.47 ± 0.04	7.01 ± 0.06	8.191 ± 0.01	7.70 ± 0.12	9.21 ± 0.07	6.95 ± 0.05
12	7.04 ± 0.02	5.29 ± 0.01	5.97 ± 0.12	5.37 ± 0.03	16.58 ± 0.05	7.05 ± 0.07	6.649 ± 0.01	7.58 ± 0.05	9.47 ± 0.08	6.55 ± 0.14
14	7.24 ± 0.04	5.65 ± 0.12	5.62 ± 0.06	6.00 ± 0.18	15.59 ± 0.04	7.95 ± 0.25	7.509 ± 0.01	7.50 ± 0.05	10.21 ± 0.10	6.22 ± 0.14
16	7.80 ± 0.05	5.27 ± 0.02	5.41 ± 0.22	5.30 ± 0.06	15.50 ± 0.04	8.08 ± 0.01	7.490 ± 0.01	8.42 ± 0.03	10.44 ± 0.06	6.21 ± 0.08
18	7.91 ± 0.02	6.42 ± 0.04	5.41 ± 0.14	6.36 ± 0.05	15.53 ± 0.19	8.26 ± 0.54	7.250 ± 0.01	8.23 ± 0.09	10.70 ± 0.06	6.65 ± 0.05
20	8.18 ± 0.04	6.21 ± 0.03	5.64 ± 0.00	5.81 ± 0.19	15.59 ± 0.13	8.44 ± 0.01	7.726 ± 0.01	8.54 ± 0.06	10.30 ± 0.10	6.71 ± 0.11

Note: (mean ± SD, n=3) SD - standard deviation

Table. 4.4 Cd, Ni, Pb and Fe during composting period

Day	Cadmium (g/kg)					Nickel (g/kg)				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	58.25 ± 3.89	60.50 ± 0.71	70.25 ± 1.06	42.50 ± 0.71	72.50 ± 0.71	0.240 ± 0.001	0.217 ± 0.003	0.216 ± 0.004	0.247 ± 0.004	0.258 ± 0.011
2	57.26 ± 2.24	62.75 ± 1.06	66 ± 1.41	44.25 ± 1.41	75.50 ± 0.71	0.231 ± 0.002	0.242 ± 0.010	0.217 ± 0.011	0.246 ± 0.004	0.246 ± 0.001
4	49.35 ± 4.24	65.25 ± 0.35	71.5 ± 2.12	42.25 ± 1.06	79 ± 1.41	0.234 ± 0.002	0.251 ± 0.008	0.226 ± 0.015	0.247 ± 0.004	0.280 ± 0.034
6	51.28 ± 2.36	67.75 ± 0.35	69.25 ± 5.30	42.50 ± 0.71	73.5 ± 2.12	0.248 ± 0.001	0.252 ± 0.007	0.245 ± 0.013	0.265 ± 0.006	0.228 ± 0.031
8	56.25 ± 3.89	66 ± 1.41	75.75 ± 1.77	45.75 ± 0.35	77 ± 1.41	0.251 ± 0.002	0.227 ± 0.012	0.262 ± 0.046	0.274 ± 0.006	0.251 ± 0.008
10	61.29 ± 1.48	72.5 ± 0.71	69 ± 2.83	48.25 ± 1.77	80 ± 1.41	0.258 ± 0.001	0.241 ± 0.019	0.218 ± 0.003	0.255 ± 0.007	0.267 ± 0.002
12	59.75 ± 3.89	71.25 ± 1.06	71.75 ± 1.77	45.50 ± 2.12	83.5 ± 0.71	0.248 ± 0.018	0.245 ± 0.034	0.213 ± 0.001	0.286 ± 0.006	0.277 ± 0.004
14	67.26 ± 2.24	72.25 ± 1.06	69 ± 1.41	49 ± 1.41	86.5 ± 0.71	0.254 ± 0.024	0.253 ± 0.014	0.201 ± 0.021	0.277 ± 0.004	0.284 ± 0.004
16	75.25 ± 4.60	70.5 ± 0.71	64 ± 1.41	47 ± 0.71	85 ± 1.41	0.267 ± 0.002	0.238 ± 0.031	0.254 ± 0.005	0.269 ± 0.005	0.267 ± 0.005
18	78.24 ± 2.58	73.75 ± 1.06	70 ± 2.83	46.95 ± 0.78	72.5 ± 2.12	0.269 ± 0.001	0.215 ± 0.005	0.246 ± 0.005	0.260 ± 0.006	0.268 ± 0.002
20	80.50 ± 4.95	71.5 ± 0.71	75 ± 1.41	47 ± 0.71	84.5 ± 0.71	0.271 ± 0.003	0.223 ± 0.007	0.241 ± 0.007	0.269 ± 0.014	0.337 ± 0.004
Day	**Lead (g/kg)**					**Iron (g/kg)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	0.74 ± 0.02	0.66 ± 0.03	0.855 ± 0.021	0.94 ± 0.01	0.958 ± 0.032	6.47 ± 0.06	5.147 ± 0.003	6.914 ± 0.037	8.27 ± 0.10	8.70 ± 0.28
2	0.73 ± 0.01	0.78 ± 0.01	0.828 ± 0.018	0.92 ± 0.04	0.955 ± 0.014	6.54 ± 0.08	6.330 ± 0.022	8.681 ± 0.006	8.50 ± 0.08	7.45 ± 0.49
4	0.76 ± 0.01	0.86 ± 0.01	0.965 ± 0.000	0.95 ± 0.06	1.035 ± 0.035	6.74 ± 0.03	6.557 ± 0.016	7.780 ± 0.007	12.59 ± 0.11	9.06 ± 0.13
6	0.78 ± 0.02	0.92 ± 0.02	0.853 ± 0.011	0.94 ± 0.02	1.050 ± 0.035	6.86 ± 0.02	6.722 ± 0.005	8.738 ± 0.004	7.12 ± 0.10	17.17 ± 0.36
8	0.83 ± 0.03	0.87 ± 0.01	0.965 ± 0.007	0.92 ± 0.01	1.131 ± 0.008	6.90 ± 0.04	6.310 ± 0.007	8.153 ± 0.018	7.57 ± 0.08	15.29 ± 0.04
10	0.081 ± 0.02	0.95 ± 0.00	0.843 ± 0.053	0.85 ± 0.02	1.141 ± 0.009	7.24 ± 0.02	7.905 ± 0.008	7.923 ± 0.018	6.71 ± 0.10	14.33 ± 0.17
12	0.77 ± 0.01	0.98 ± 0.00	0.898 ± 0.067	0.87 ± 0.03	1.154 ± 0.008	7.68 ± 0.07	6.284 ± 0.008	7.312 ± 0.017	10.03 ± 0.16	14.72 ± 0.09
14	0.082 ± 0.02	1.00 ± 0.00	0.925 ± 0.035	0.89 ± 0.01	1.147 ± 0.001	7.88 ± 0.05	8.346 ± 0.006	8.770 ± 0.007	11.63 ± 0.84	13.66 ± 0.27
16	0.78 ± 0.01	0.94 ± 0.04	0.875 ± 0.000	0.84 ± 0.04	1.034 ± 0.003	8.24 ± 0.04	9.830 ± 0.008	8.323 ± 0.010	13.26 ± 0.43	12.39 ± 0.33
18	0.084 ± 0.02	0.93 ± 0.02	0.903 ± 0.032	0.93 ± 0.05	1.033 ± 0.025	8.54 ± 0.06	7.316 ± 0.015	8.828 ± 0.011	12.57 ± 0.09	11.16 ± 1.34
20	0.86 ± 0.01	0.92 ± 0.03	0.893 ± 0.039	0.91 ± 0.04	1.039 ± 0.009	9.62 ± 0.04	7.628 ± 0.025	10.782 ± 0.011	12.71 ± 0.42	12.25 ± 0.13

Note: (mean ± SD, n=3) SD - standard deviation

Table. 4.5 Mn, Cr, Zn and Cu during composting period

Day	**Manganese (g/kg)**					**Chromium (mg/kg)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	0.43 ± 0.002	0.67 ± 0.015	0.67 ± 0.005	0.80 ± 0.012	0.60 ± 0.003	7.50 ± 1.41	5.75 ± 1.77	26.5 ± 0.71	6.75 ± 0.35	13.5 ± 1.41
2	0.45 ± 0.001	0.68 ± 0.005	0.66 ± 0.005	0.82 ± 0.005	0.62 ± 0.004	7.64 ± 0.56	5.50 ± 0.35	36 ± 1.41	7.25 ± 0.35	13.65 ± 1.41
4	0.47 ± 0.002	0.72 ± 0.007	0.77 ± 0.004	0.78 ± 0.004	0.64 ± 0.004	8.24 ± 1.41	6.18 ± 0.25	36.8 ± 1.06	7.01 ± 0.71	13.81 ± 0.71
6	0.48 ± 0.004	0.74 ± 0.004	0.76 ± 0.004	0.79 ± 0.002	0.74 ± 0.010	8.46 ± 1.24	7.00 ± 0.71	44.3 ± 1.77	7.25 ± 0.35	14.01 ± 0.71
8	0.48 ± 0.012	0.81 ± 0.005	0.77 ± 0.004	0.83 ± 0.001	0.75 ± 0.006	8.68 ± 1.47	13.50 ± 2.66	52.0 ± 1.41	6.50 ± 0.71	14.51 ± 0.71
10	0.49 ±0.024	0.82 ± 0.011	0.86 ± 0.005	0.82 ± 0.006	0.70 ± 0.075	8.88 ± 0.58	14.00 ± 0.71	45.8 ± 0.35	7.50 ± 0.71	14.62 ± 0.71
12	0.51 ± 0.004	0.75 ± 0.009	0.86 ± 0.006	0.87 ± 0.005	0.77 ± 0.006	9.01 ± 0.71	15.66 ± 2.12	61.8 ± 1.06	7.75 ± 1.06	14.75 ± 0.71
14	0.52 ± 0.005	0.85 ± 0.004	0.83 ± 0.005	0.86 ± 0.010	0.78 ± 0.008	9.21 ± 0.52	17.88 ± 1.41	42.0 ± 1.41	7.55 ± 0.35	14.85 ± 0.71
16	0.69 ± 0.003	0.87 ± 0.010	0.89 ± 0.005	0.99 ± 0.005	0.78 ± 0.004	9.25 ± 1.77	19.20 ± 1.41	41.5 ± 0.71	7.75 ± 0.35	15.01 ± 1.41
18	0.72 ± 0.006	0.84 ± 0.002	0.82 ± 0.009	0.94 ± 0.006	0.72 ± 0.003	9.66 ± 0.66	27.50 ± 2.12	37.3 ± 2.47	7.64 ± 0.35	15.84 ± 0.71
20	0.79 ± 0.004	0.82 ± 0.010	0.86 ± 0.006	0.92 ± 0.004	0.73 ± 0.007	10.50 ± 2.12	30.50 ± 1.41	41.5 ± 3.54	8.45 ± 0.71	16.52 ± 0.71
Day	**Zinc (g/kg)**					**Copper (g/kg)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	0.31 ± 0.01	0.23 ± 0.001	0.26 ± 0.001	0.40 ± 0.007	0.28 ± 0.001	0.03 ± 0.002	0.02 ± 0.006	0.05 ± 0.00	0.58 ± 0.009	0.22 ± 0.002
2	0.34 ± 0.04	0.25 ± 0.001	0.31 ± 0.003	0.46 ± 0.013	0.28 ± 0.004	0.04 ± 0.001	0.03 ± 0.005	0.09 ± 0.00	0.47 ± 0.004	0.21 ± 0.004
4	0.29 ± 0.02	0.28 ± 0.001	0.32 ± 0.001	0.43 ± 0.005	0.28 ± 0.004	0.04 ± 0.002	0.05 ± 0.003	0.16 ± 0.00	0.23 ± 0.010	0.22 ± 0.005
6	0.34 ± 0.04	0.31 ± 0.001	0.30 ± 0.001	0.43 ± 0.010	0.34 ± 0.006	0.05 ± 0.001	0.06 ± 0.002	0.15 ± 0.00	0.27 ± 0.004	0.23 ± 0.005
8	0.32 ± 0.02	0.32 ± 0.007	0.31 ± 0.001	0.47 ± 0.005	0.35 ± 0.004	0.06 ± 0.002	0.06 ± 0.003	0.11 ± 0.00	0.06 ± 0.009	0.24 ± 0.006
10	0.36 ± 0.01	0.32 ± 0.001	0.33 ± 0.001	0.45 ± 0.004	0.39 ± 0.008	0.07 ± .001	0.05 ± 0.000	0.11 ± 0.00	0.09 ± 0.005	0.24 ± 0.005
12	0.34 ± 0.02	0.33 ± 0.001	0.33 ± 0.002	0.51 ± 0.005	0.35 ± 0.014	0.07 ± 0.001	0.05 ± 0.004	0.06 ± 0.00	0.07 ± 0.004	0.25 ± 0.006
14	0.35 ± 0.03	0.35 ± 0.001	0.33 ± 0.001	0.50 ± 0.004	0.35 ± 0.004	0.08 ±0.002	0.06 ± 0.001	0.07 ± 0.00	0.07 ± 0.008	0.25 ± 0.004
16	0.39 ± 0.02	0.34 ± 0.001	0.34 ± 0.001	0.56 ± 0.004	0.36 ± 0.005	0.08 ± 0.002	0.06 ± 0.001	0.07 ± 0.00	0.04 ± 0.008	0.27 ± 0.006
18	0.41 ± 0.05	0.35 ± 0.006	0.36 ± 0.001	0.48 ± 0.006	0.34 ± 0.005	0.08 ± 0.001	0.06 ± 0.000	0.08 ± 0.00	1.02 ± 0.009	0.32 ± 0.001
20	0.42 ± 0.01	0.35 ± 0.001	0.35 ± 0.004	0.50 ± 0.006	0.37 ± 0.004	0.08 ± 0.003	0.05 ± 0.001	0.55 ± 0.64	0.06 ± 0.001	0.33 ± 0.004

Note: (mean ± SD, n=3) SD - standard deviation

1.65×10^{10}, 7.1×10^{11}, 9.1×10^{11} and 8.1×10^{11} CFU/g in trial 1, 2, 3, 4 and 5 respectively. These mesophilic heterotrophs are responsible for the rise in temperature in composting systems by breaking down the organic material to simpler units by releasing CO_2 and heat as byproducts. Thermophilic stage was observed within 18 to 24 h of the process and reaching a maximum of 58 to 61.2°C in all the trials. Due to the rise in temperature the decline in mesophilic heterotrophs was observed drastically and found in the range of 3×10^{6}, 6.6×10^{6}, 2.65×10^{6}, 7.1×10^{6} and 3.3×10^{7} CFU/g in trial 1, 2, 3, 4 and 5 respectively (Fig. 4.11). The reduction in microbial count is considered due to the transition from mesophilic to thermophilic conditions and these mesophiles are not resistant to such temperatures leading to inactivation of their populations (Haug, 1993; Weppen, 2001; Sundberg et al., 2004). In addition reports by Ryckeboer et al. (2003) were supported during the present study on the relation between temperature and decline of the total microbial population. Furthermore, depletion of organic matter can also lead to the decline of bacterial count, as it was observed that lower CO_2 emissions and OUR during the final stages of the composting.

Hence, it can be considered that the populations of mesophilic bacteria were greatly influenced by higher temperature and readily available organic matter, rather than cellulose, hemicellulose and lignin. These mesophiles are not capable of forming spores as like fungi, yeasts and streptomycetes which can survive at high temperatures that can also add to the reduction in their numbers (Ryckeboer et al. 2003). With decline in mesophilic bacteria due to rise in temperature, proliferation of spore forming bacteria was observed extensively. These spore forming bacteria was found throughout the composting process in all trials and increased 100 fold in trial 3 due to higher temperatures in the composting system.

These results were supported by Gazi et al. (2007), where it was reported the same observation of spore forming units throughout the process, except the rise of population during the composting process. In the present study due to early thermophilic stage, rise in spore forming bacteria from 6.0×10^{7} to 3.82×10^{9} CFU/g in the order of 100 fold was observed within 3 days and was maintained for till day 8 in trial 3 of being highest compared to other trials, whereas from the above stated reports it was observed on day 63. However, the other trials also had considerable amount of populations in the range of 4.1×10^{6}, 4.4×10^{6}, 6×10^{7}, 5.35×10^{7} and 3.8×10^{7} CFU/g in trial 1, 2, 3, 4 and 5 and reduced to 3.4×10^{5}, 2.8×10^{6}, 2.9×10^{6}, 2.8×10^{5} and 2.9×10^{6} CFU/g at the end of 20 days.

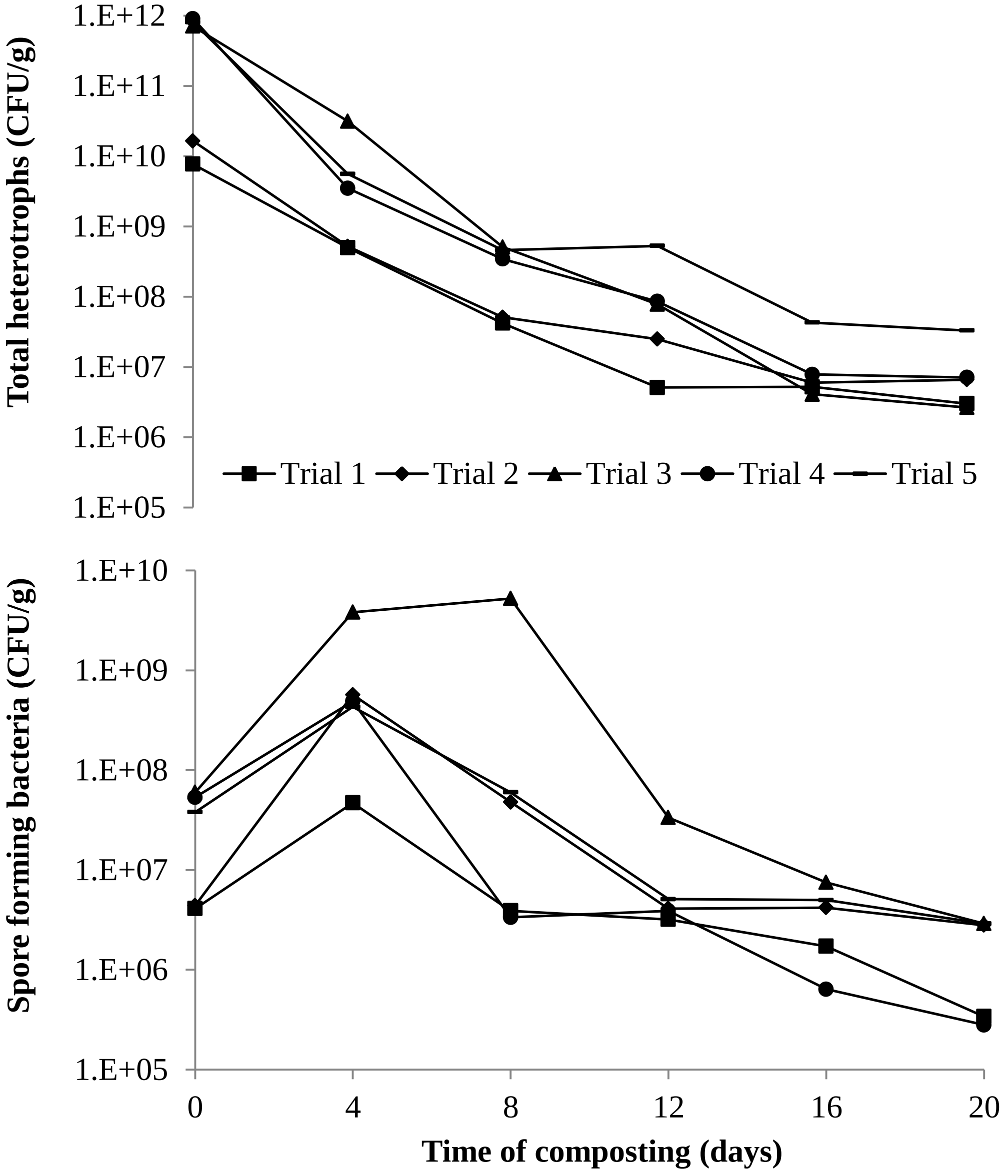

Fig. 4.11. Mesophilic and spore forming bacteria variation during composting period

Hence the relation between temperature and mesophilic bacteria survival can be related with each other from the present findings. Mesophilic bacteria dominant during the early stages of composting by degrading the organic matter; thereby raising the temperature of the system. Once the thermophilic stage has reached, spore forming bacteria are considered to take over and act upon the organic matter, which is clearly observed with the rise in population of spore forming bacteria especially in trial 3 and at the same time decline in mesophilic bacteria was observed in all the trials. The results were in accordance with the reports by Ishii et al. (2000) stating the significant role of temperature over the populations of one another. In comparison to all the trials, trial 3 reached a maximum reduction of bacterial count with highest temperature and with

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higher spore forming bacterial count during the process. The higher temperature may be observed due to the combinations of waste materials in appropriate amounts in trial 3, which favored the growth of mesophilic and spore forming bacteria to act effectively during the composting process.

- **Fungi**

Fungi have been reported to be an important group and are considered to play a very significant role in the biodegradation and conversion process during composting (Anastasi et al., 2005). This fungal diversity is reported to utilize many carbon sources mainly of lignocellulosic polymers and are mainly responsible for compost maturation (Miller 1996). In the present study, the populations of fungi were found in the range of 5.6×10^7, 3.8×10^7, 1.8×10^8, 8.25×10^8 and 7.4×10^8 CFU/g in trial 1, 2, 3, 4 and 5 and reduced to 7.7×10^4, 3.1×10^5, 2.85×10^4, 3.9×10^5 and 3.9×10^6 CFU/g respectively, at the end of 20 days (Fig. 4.12). The major reduction in fungal populations was observed during the thermophilic stage of composting process. This decline in numbers is mainly considered due to higher temperatures maintained in the order of 50 to 63.2°C during the composting process, which led to the death of most of the fungal species.

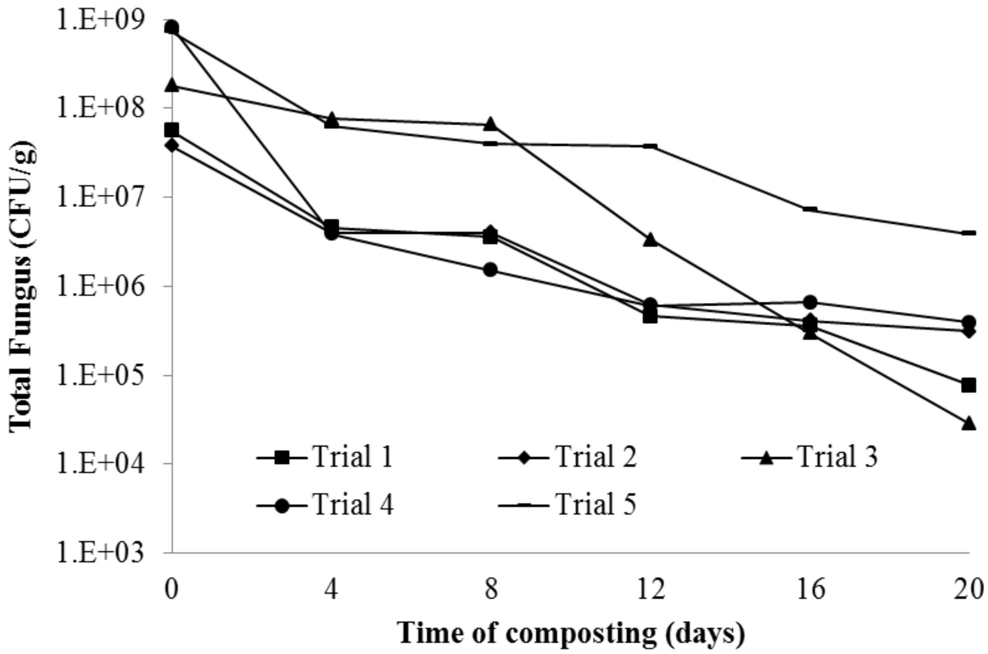

Fig. 4.12. Total fungus during composting period

The results were in accordance with the fact that fungi are generally more resistant to acids and less tolerant to temperatures greater than 35-40°C, when compared to bacterial species (Atlas and Bartha, 1998). Since the highest temperature was observed in trial 3 due to appropriate combinations of waste materials, major drop was observed during the thermophilic stage and finally fungal numbers was observed in the range of 2.85×10^4 CFU/g at the end of 20 days, being least in comparison to all other trials. Even though complete inactivation of fungi is reported in few studies during composting process (Bhatia et al., 2012), the present study observed considerable decrease in the amount of fungal numbers, with no such complete inactivation at the end of 20 days. The reason might be due to the presence of lignocellulosic carbon food available for the survival of fungi in the later stages. However the effect of temperature on the inactivation of fungi followed the same pattern as reported by Bhatia et al. (2012) in the present study. Ryckeboer (2003) also reported the death of fungi due to high temperature (76°C) to almost totally zero during thermophilic stage.

- **Actinomycetes and streptomycetes**

Actinomycetes are generally considered to act during the later stages of composting and are involved in degradation of recalcitrant organics such as lignocellulose and elimination of pathogenic and allergenic microorganisms (Rebollido et al., 2008). Higher population of actinomycetes were found in all trials in the range of 3.6×10^8, 6.1×10^8, 2.45×10^9, 4.55×10^9 and 6.7×10^9 CFU/g and reduced to 2.8×10^6, 3.1×10^6, 3.8×10^6, 3×10^6 and 3.6×10^7 CFU/g of compost respectively at the end of 20 days (Fig. 4.13). Actinomycete isolates were classified by observing the morphology of sporophores and color of aerial mycelium as reported by Miyashita et al. (1982). Only mesophilic actinomycetes were identified in the present study at 25°C, since most of the actinomycetes are thermo resistant and play an important role in the degrading natural polymers at higher temperature and aerobic conditions (Song et al. 2001). Hence due to their thermo resistivity and presence of lignocellulosic material, populations of actinomycetes were found in considerable amounts at the end of 20 days, however trial 3 was observed with higher reduction. The actinomycetes group has found potential use in biodegradation process and in the production of bioactive compounds such as antibiotics and enzyme. In addition, the population size and composition of actinomycetes are mainly dependent on the type of organic content materials and also on the physical conditions of the environment (Miyashita et al. 1982). In addition to actinomycetes, streptomycetes are also considered to play an important role in the degradation of recalcitrant macromolecules. They are also biologically important for their vast potential in producing a wide variety of secondary metabolites, including antibiotics and extracellular enzymes (Inbar et al., 2004). Since, ISP-4 medium is not strictly selective for streptomycetes; therefore only the colonies with aerial mycelium (powdery, wrinkled, or pasty) were counted sensibly as reported by Ryckeboer et al. (2003). The populations of streptomycetes were found in the range of 6.1×10^7, 4.1×10^7, 2.8×10^8, 3.8×10^8 and 3.9×10^8 CFU/g in trial 1, 2, 3, 4 and 5 and reduced to 4.7×10^5, 3.1×10^5, 4.1×10^5, 6.5×10^5 and 7.4×10^6 CFU/g of compost respectively at the end of 20 days (Fig. 4.13). These higher populations at the end of 20 days in all trials may be due to the presence of complex organic materials such as lignin and cellulose. However, streptomycetes are generally considered to secrete antibiotics for the control of pathogens, which might also be responsible for the reduction of mesophilic bacteria and certain species of fungi in addition to elevated temperatures.

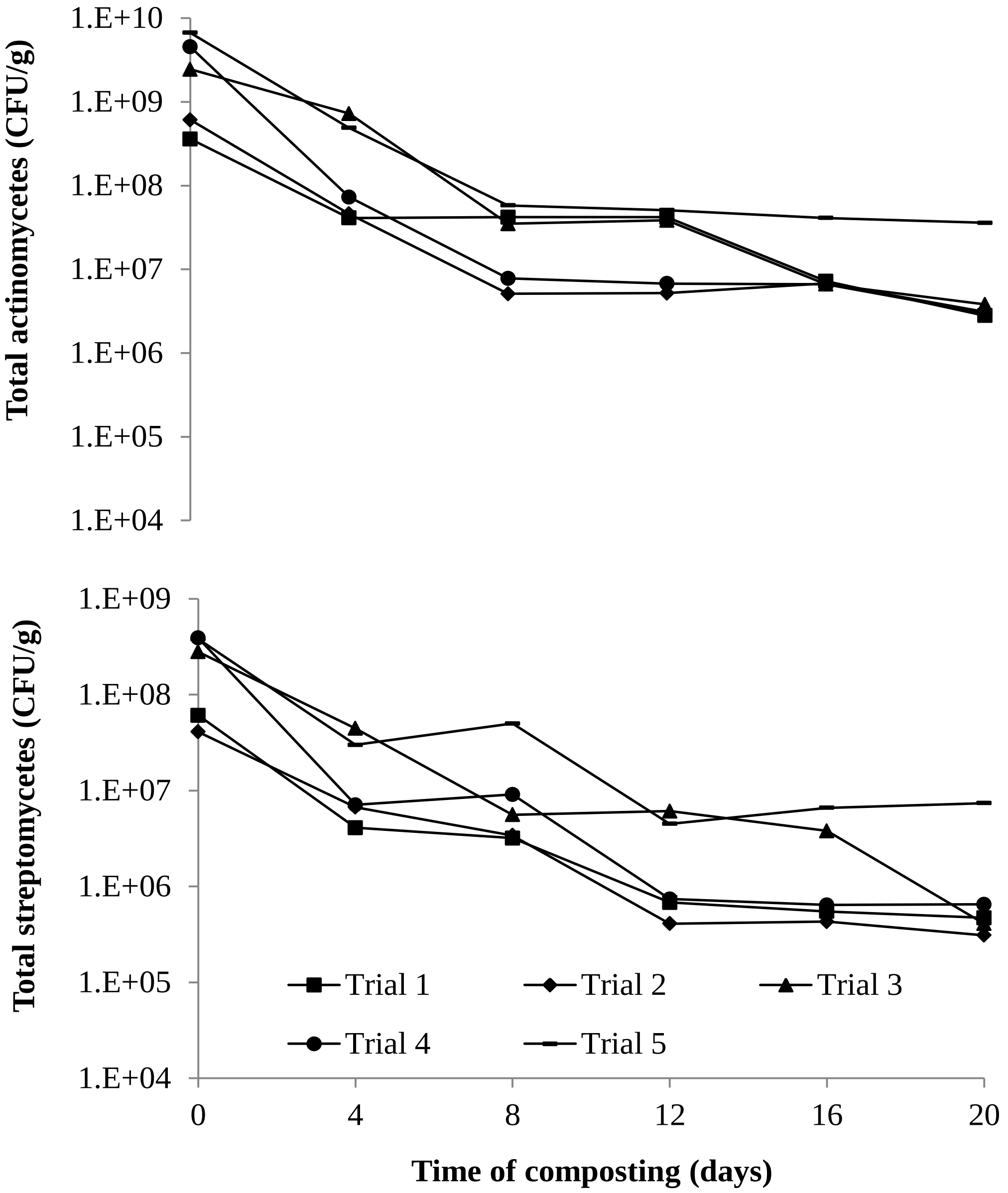

Fig. 4.13. Actinomycetes and streptomycetes during composting

- **Total and fecal coliform**

The recommended fecal coliform and streptococci densities for compost hygienization are 5.0×10^2 and 5.0×10^3 MPN/g, respectively (Vuorinen and Saharinen, 1997). The presence of coliform bacteria is often used as an indicator of overall sanitary quality of the compost (Kalamdhad et al., 2009). The control of these pathogens is carried out by two major factors i.e. temperature and the release of antibiotics as discussed earlier. The average number of total coliform bacteria was initially observed in the range of 1.5×10^{11}, 11×10^{11}, 4.6×10^{11}, 2.1×10^{12} and 11×10^{12} MPN/g in trial 1, 2, 3, 4 and 5 and finally reduced to 0.75×10^3, 2.4×10^3, 2.1×10^3, 4.6×10^4 and 4.6×10^4 MPN/g respectively, at the end of 20 d. However, the fecal coliform were in the order of 2.1×10^7, 11×10^7, 2.4×10^6, 4.6×10^7 and 4.6×10^7 MPN/g in trial 1, 2, 3, 4 and 5 and reduced to 0.091×10^2, 2.4×10^2, 2.4×10^2, 1.5×10^3 and 1.2×10^3 MPN/g respectively, at the end of 20 days. It is very clear from the results observed that the effect of elevated temperature and the presence of antibiotic releasing streptomycetes during the composting led to inactivation of these indicator organisms. The observed results were in accordance with the reports by Kalamdhad et al. (2009). Streptococci and enterococci are suspected pathogens in most groups of vertebrates. These are responsible for diseases in most of the birds, fishes and various mammals (Chanter, 1997). At the end of 20 days enterococci was found in the range of 4400, 290, 240, 340 and 580 CFU/g. Similarly streptococci were also observed to reduce in considerable amounts from 3.3×10^5, 4.4×10^5, 5.8×10^5, 5.5×10^4 and 6.4×10^5 CFU/g to 640, 940, 88, 120 and 890 CFU/g respectively at the end of 20 days.

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4.1.3 OPTIMIZATION OF DIFFERENT RATIOS AND EVOLUTION OF CHEMICAL AND BIOLOGICAL CHARACTERIZATION DURING THERMOPHILIC COMPOSTING OF VEGETABLE WASTE USING ROTARY DRUM COMPOSTER

The study dealt with the composting of vegetable waste using rotary drum in four different waste combinations including vegetable waste, cow dung and sawdust i.e. Trial 1 (5:4:1), Trial 2 (6:3:1), Trial 3 (7:2:1) and Trial 4 (8:1:1), by adding 10 kg of dry leaves in each of the trial as bulking agent (Table 3.4). The effects of bulking agent addition on the physico-chemical, microbial diversity and lignocellulose degradation was correlated and reported.

4.1.3.1 Physico-chemical parameters

- **Temperature**

Fig. 4.14 shows the temperature profile of different trials during rotary drum composting. In all the trials, a fast increase in temperature was recorded indicating a higher microbial activity. Due to the higher indigenous microbial populations the initial lag period was not recorded and early thermophilic period was observed in all the trials. Even though rise in temperature was recorded in the early stages of all the trials, the temperature pattern was completely different in all trials during the study, which might be due to the varying amount of vegetable waste added in all the trials. A maximum of 66.5^{o}C was observed in trial 1, 61.4^{o}C in trial 2, 60.9^{o}C in trial 3 and 57.4^{o}C in trial 4. Eventhough maximum temperature was observed in trial 1, maximum soluble BOD reduction was observed in trial 2, while TOC and VS reduction was observed high in trial 1.

Gray et al. (1971) has reported that temperature greater than 65^{o}C might inactivate the fungi, actinomycetes and most of the bacteria which play a major role in degradation during thermophilic stages and only spore-forming bacteria can be developed. As soon as the thermophilic stage was achieved in trial 1 and 2, the top layer of the compost was completely observed with the spores of microbial communities due to such elevated temperature. However such findings were not observed in trial 3 and 4. Trial 2 was observed to maintain a longer thermophilic period when compared to all other trials. Temperature greater than 55^{o}C for 2 days would be sufficient to maximize sanitation and destroy pathogens (Petrica et al., 2009). The present investigation was observed to maintain such elevated temperature more than 6 days and started to decrease, which might be due to the depletion of readily biodegradable components. Hence, trial 1 and 2 was observed to provide higher thermophilic conditions to the compost when compared to other two trials due to proper combinations of waste materials.

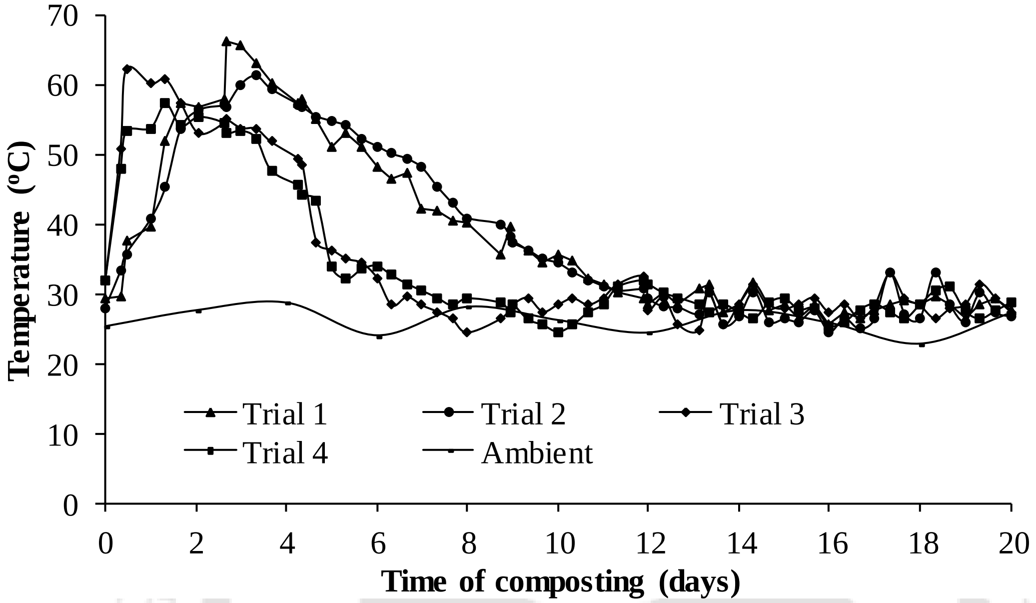

Fig. 4.14. Temperature profile during composting period

- **pH and electrical conductivity (EC)**

The changes in pH was observed to carry out the same pattern in all the trials, ranging from slight acidic to slight alkaline conditions (Fig. 4.15). Initially the pH was in the range of 6.7, 6.7, 5.8 and 6.4; and finally it increased towards alkaline conditions and was in the range of 7.7, 8.0, 7.9 and 7.8 in trial 1, 2, 3 and 4 respectively. The rise in pH towards alkaline conditions may be considered due to the degradation of carboxylic acids and phenol groups; and also due to the mineralization of other organic compounds i.e. proteins, amino acids and peptides to ammonia (Hachicha et al., 2009). Most compost has a pH value between 6 and 8 (Tiquia and Tam, 2000). The pH value was observed to maintain constant during the final stages of composting, which can be attributed to the stabilization of compost and with lower activities of microbial population. Due to higher temperatures during composting process, loss of organic matter was observed high that which gradually increased the value of EC in all the trials. Fig. 4.15 shows increase of EC in all trials. The increase in EC can be considered due to the increase of mineral cation concentration that is not attenuated by salts leaching or by binding to stable organic complex and majorily due to the loss of organic matter (Francou et al., 2005). The higher EC in the trials may be also due to higher release of ammonium ions throughout the composting process. The increase in EC due to degradation of organic matter during composting process was in accordance with the report by Singh and Kalamdhad (2013).

- **Moisture, volatile solids (VS) and total organic carbon (TOC)**

Organic matter degradation in composting can be viewed as a result of rise in temperature. As the temperature rises, the loss in moisture content can be observed. Fig. 4.15 depicts the loss of moisture content during the composting period. Hence loss of moisture during the composting process can be viewed as an index of decomposition rate, as the heat generated due to microbial metabolism accompanies decomposition as well as vaporization or moisture loss (Liao et al., 1996). However, the composting material should have minimum moisture content for the survival of micro-organisms (Kalamdhad et al., 2008). With higher temperature in trial 1, it was observed with higher moisture loss of 15.6% as compared to 9.6 and 7.8% in trial 2 and 3 respectively.

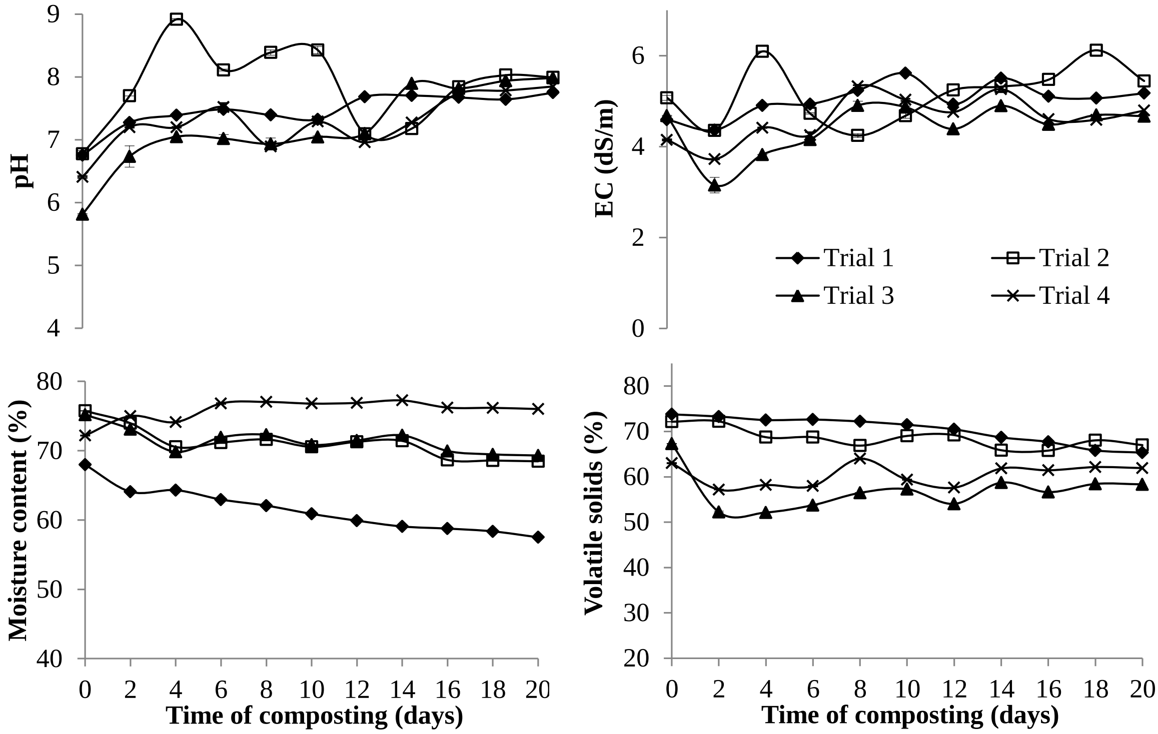

Fig. 4.15. pH, EC, moisture and volatile solids variation during composting

In addition, out of 15.6% reduction during the total 20 days, 10.5% was reduced within 10 days of the composting period in trial 1. This major reduction is mainly due to the higher temperature maintained in the compost. Therefore, it can be considered that higher temperature in the compost environment will lead to major reduction of moisture content. While due to large amount of vegetable waste addition in trial 4, release of moisture content was observed high during the process and the addition of 10 kg dry leaves were not sufficient. Hence, moisture content reduction was observed proper in trial 4. During microbial metabolism, heat and moisture are released as byproducts, in case of inadequate bulking material the moisture can be trapped in the system and it reduces the temperature in the composting system.

Along with moisture loss, VS reduction was also observed to reduce drastically due to higher temperature (Fig. 4.15). As the composting proceeded, due to active microbial action on organic content higher VS reduction was observed in trial 1 in comparison to all other trials. A maximum of 11.4% reduction of VS was observed in trial 1, as compared to 7.2, 13.3 and 1.7% in trial 2, 3 and 4 respectively. Even though complete degradation of vegetable waste was achieved at the end of 20 days, the added dry leaves contributed more TOC and VS, hence reuse of those leaves after sieving can be suggested. Hence at the end of 20 days with complete removal of vegetable waste, compost was full of partially degraded dry leaves. However, trial 1 was observed with higher reduction when compared to all other trials. Similar studies were reported by Kalamdhad et al. (2009) on composting of vegetable waste by adding dry leaves in 3.5 m^3 full scale rotary drum for 7 days, in which the final compost was sieved by 0.6 mm sieve and the leaves were reused. Hence it can be considered due to elevated temperature higher moisture loss and VS reduction was observed in trial 1 when compared to all other trials.

The changes in TOC during the composting period are detailed in Fig. 4.16. As the composting proceeds carbon dioxide is emitted from the composting mass as a metabolic end product. Thus the total organic carbon content of the composting decreased with mass reduction (Singh et al., 2009). TOC during composting period was initially in the range of 40.9, 40.0, 37.3 and 35.0% and finally reduced to 36.3, 37.2, 32.41 and 34.4% in trial 1, 2, 3 and 4 respectively, at the end of 20 days. Although dry leaves were partially degraded at the end of composting, the aim of adding dry leaves was only to serve as bulking agent, so it can be reused along with initial material after sieving. However, vegetable waste was completely degraded at the end of 20 days. Finally, trial 1 was found to have higher reduction in TOC when compared to all other trials. Hence it can be clearly stated that dry leaves and sawdust contributed to TOC and VS more at the end of composting period.

- **C/N ratio, nitrogen (NH_4-N and TN) and phosphorous (TP and AP) dynamics**

  C/N ratio between 10 and 15 in the compost indicates a good degree of maturity

(Singh et al., 2009). Similar results were observed in the present study with final C/N ratio of 15 and 12 in trial 1 and 2 respectively. The proper degradation in these two trials may be due to the appropriate combination of waste materials. Microorganisms are considered to utilize carbon as a source of energy and the nitrogen for building cell structures, thereby reducing the C/N ratio. However in trial 3 and 4, not much change was observed.

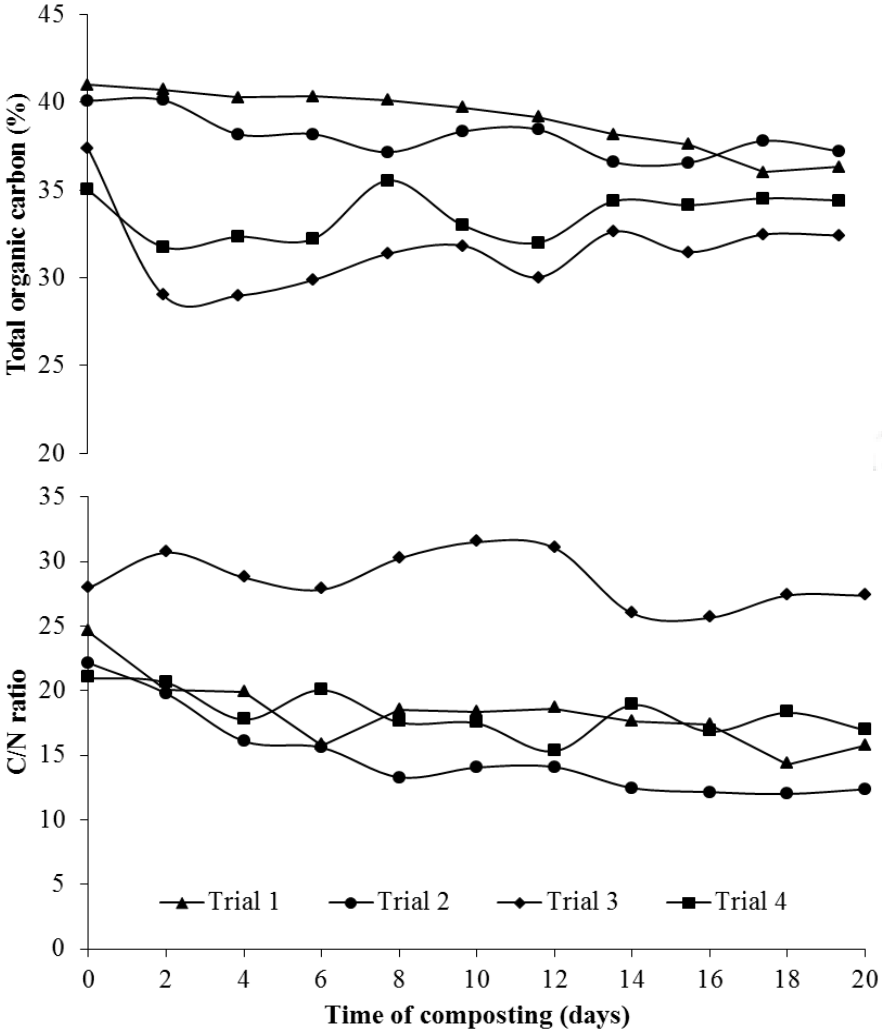

Fig. 4.16. Carbon and C/N variation during composting period

Moreover, a C/N ratio below 20 is indicative of proper compost maturity, with a ratio of 15 or less being preferred (Heerden et al., 2002). Similar results were observed during the present study in trial 1 and 2, stating the maturity of compost (Fig. 4.16). Fig. 4.17 shows the variation in the NH_4-N and TN content. Due to enhanced emissions of NH_3 and its transformation to gaseous state, NH_4-N was observed to decrease. The decrease might be due to NH_3 volatilization at high temperature and pH rise which was clearly

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observed in the study. In addition, decrease in NH_4-N can be considered due to immobilization as nitrogenous compounds such as amino acids, nucleic acids and proteins by microbes as reported by Sanchez-Mondero et al. (1999). The reduction in NH_4-N was observed from 1.31, 1.41, 1.24 and 1.41% to 0.85, 0.45, 0.80 and 0.78% at the end of 20 days in trial 1, 2, 3 and 4 respectively. During the thermophilic phase the reduction NH_4-N was observed to follow 42, 77, 19 and 49% in trial 1, 2, 3 and 4 respectively. From the results it can be clearly stated that the major reduction in NH_4-N has been occurred during the active thermophilic phase (initial 10 days) of the composting period. Hence for all the trials the emissions of NH_3 and so as reduction in NH_4-N coincided with the maximum of temperature during the composting period.

The maximum NH_4-N content in mature compost should be less than 0.4 % (Zucconi and de Bertoldi, 1981) and the present study values were not supported. Although lower levels of NH_4-N were observed at the end of 20 days due to pH higher than 7. Transformation of TN depends on the biodegradability of the materials and the ratio of C/N within the biodegradable fraction. Total nitrogen in all the trials was found to increase during the active thermophilic phase due to high NH_3 volatilization. The increase in TN may be considered due to net loss of dry mass as CO_2 evolution and moisture loss by generation of heat by microbial action on organic matter. Furthermore, nitrogen fixing bacteria might also contribute to the increase in TN in later stage of composting (Bishop, 1983). Therefore, TN increased from 1.68, 1.82, 1.40 and 1.68 % to 2.31, 3.01, 1.33 and 2.03 % in trial 1, 2, 3 and 4 respectively, at the end of 20 days (Fig. 4.17). Higher amount of TP and AP were observed in the initial period of composting which might be contributed by the mix of waste materials in different combinations (Fig. 4.17). However, TP was observed to increase from 3.41, 3.26, 2.84 and 2.86% to 4.30, 3.27, 4.14 and 4.13% in trial 1, 2, 3 and 4 respectively, at the end of 20 days. The increase in TP content is considered due to mineralization of organic material. The higher mineralization of organic can be presented well during the thermophilic phase of the composting period. Such elevated temperatures were observed during the initial 10 days of the composting period due to higher microbial activity. Hence during the thermophilic period maximum increase in TP was observed in all the trials. Similarly AP was observed to increase from 1.41 to 1.49%, 0.86 to 1.48%, 1.16 to 1.83% and 0.86 to 1.48% in trial 1, 2, 3 and 4 respectively at the end of 20 days.

- **Nutrients and heavy metals**

Even though nutrient content is low compared to synthetic fertilizers, usually compost is applied at greater rates with significant amount of nutrient content. These nutrients mainly include nitrogen, phosphorous and potassium (Darlington, 2001). Moreover, these micronutrients and heavy metals are observed in significant amount in vegetable waste and during composting it was observed to increase due to mass loss caused by the mineralization of organic fractions (Fang and Wong, 1999).

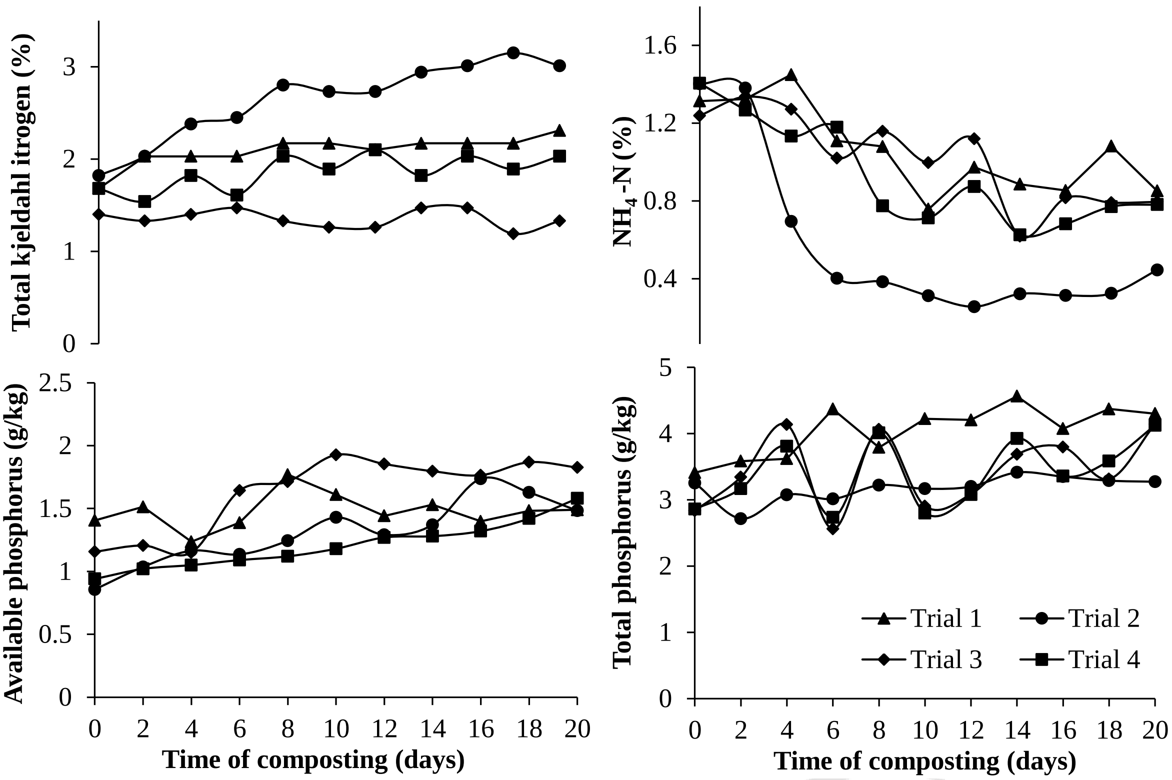

Fig. 4.17. Nitrogen and phosphorous dynamics during composting period

Although heavy metals which are referred as trace elements including arsenic, cadmium, chromium, copper, lead, mercury, molybdenum, nickel, selenium and zinc are actually required by plants for normal growth (Darlington, 2001). Table 4.6. illustrates the total concentration of micro nutrients (Na, K, Ca, Mg, Cr and Cd) and total concentration of Ni, Pb, Fe, Mn, Zn and Cu in trial 1, 2, 3 and 4 respectively, during the 20 days of composting period. The increase in total metal concentration can be considered due to loss of net weight during composting. Moreover, few metals i.e. Fe, Zn, Ca and Mg have been reported to be of bio-importance to humans in their daily medicinal and dietary allowances (Singh and Kalamdhad, 2011).

4.1.3.2 Biological analysis

- **Soluble BOD and COD**

The organic fractions in the compost mix can be directly measured as soluble BOD and COD. The percentage of the readily bioavailable organics has been considered important for the compost quality (Bernal et al., 1997). The organic fraction degradation can be measured by the decrease in soluble BOD and COD. The degradation of the organic fractions is mainly played by microorganisms during the composting process. The composting process is considered to proceed till the amount of these biodegradable organic matter is stabilized (Wang et al., 2004). With proper mixing and agitation higher degradation was carried out during the process by which the soluble BOD and COD are decreased drastically, resulting in decreased emission of carbon dioxide, ultimately indicating the stabilization of compost. Soluble BOD values decreased from 17.35 to 4.92 g/kg in trial 1, 9.85 to 0.65 g/kg in trial 2, 15.18 to 4.52 g/kg in trial 3 and 15.10 to 4.49 g/kg in trial 4 within 20 days of composting period. A maximum of 93% reduction of soluble BOD was observed in trial 2 as compared to 72% in trial 1, 70% in both trial 3 and 4. In addition out of 93% destruction of bioavailable organics in trial 2, around 80 % of the total soluble BOD reduced was observed during the initial 10 days i.e. during the active thermophilic phase. Correspondingly, soluble COD values decreased 44.41 to 23.21 g/kg in trial 1, 33.91 to 19.95 g/kg in trial 2, 42.22 to 25.55 g/kg in trial 3 and 38.01 to 19.94 g/kg of wet compost in trial 4 respectively (Fig. 4.18). When compared to all the trials, trial 1 was observed to have higher soluble COD reduction of 47.5%. In other trials, it was in the range of 41.2, 39.5 and 46.5% in trial 2, 3 and 4 respectively. In comparison to all trials an average of 20 to 22% of soluble COD reduction was observed during the active thermophilic phase (initial 10 days). In addition low soluble BOD/COD ratio may indicate the presence of either organic matter has stabilized such that microbial activities have been ceased due to hard materials at the end of composting period (Mangkoedihardjo, 2006). Similar findings were observed during the process with reduction of soluble BOD/COD from 0.39 to 0.21, 0.29 to 0.03, 0.35 to 0.17 and 0.39 to 0.22 in trial 1, 2, 3 and 4 respectively. Kalamdhad and Kazmi (2008) reported that final BOD/COD ratio of 0.02 stating the stability of the compost. Eventhough the trials in present study were not similar with such reports, but trial 2 was observed with final soluble BOD/COD of 0.03.

- **CO_2 evolution and oxygen uptake rate (OUR)**

The aerobic respiration during composting process can be directly related by the emissions of CO_2 by the microbial activity and it is measured as the direct method of compost stability, since it measures carbon derived from the compost material being carried out (Kalamdhad et al., 2008). The compost stability can be directly related to the decomposition rate of organic content present and expressed by the rate of biological activity. Fig. 4.19 shows the emissions of CO_2 during the process. Due to higher microbial action on organic content of vegetable waste mix, higher amounts of CO_2 was observed in the initial days.

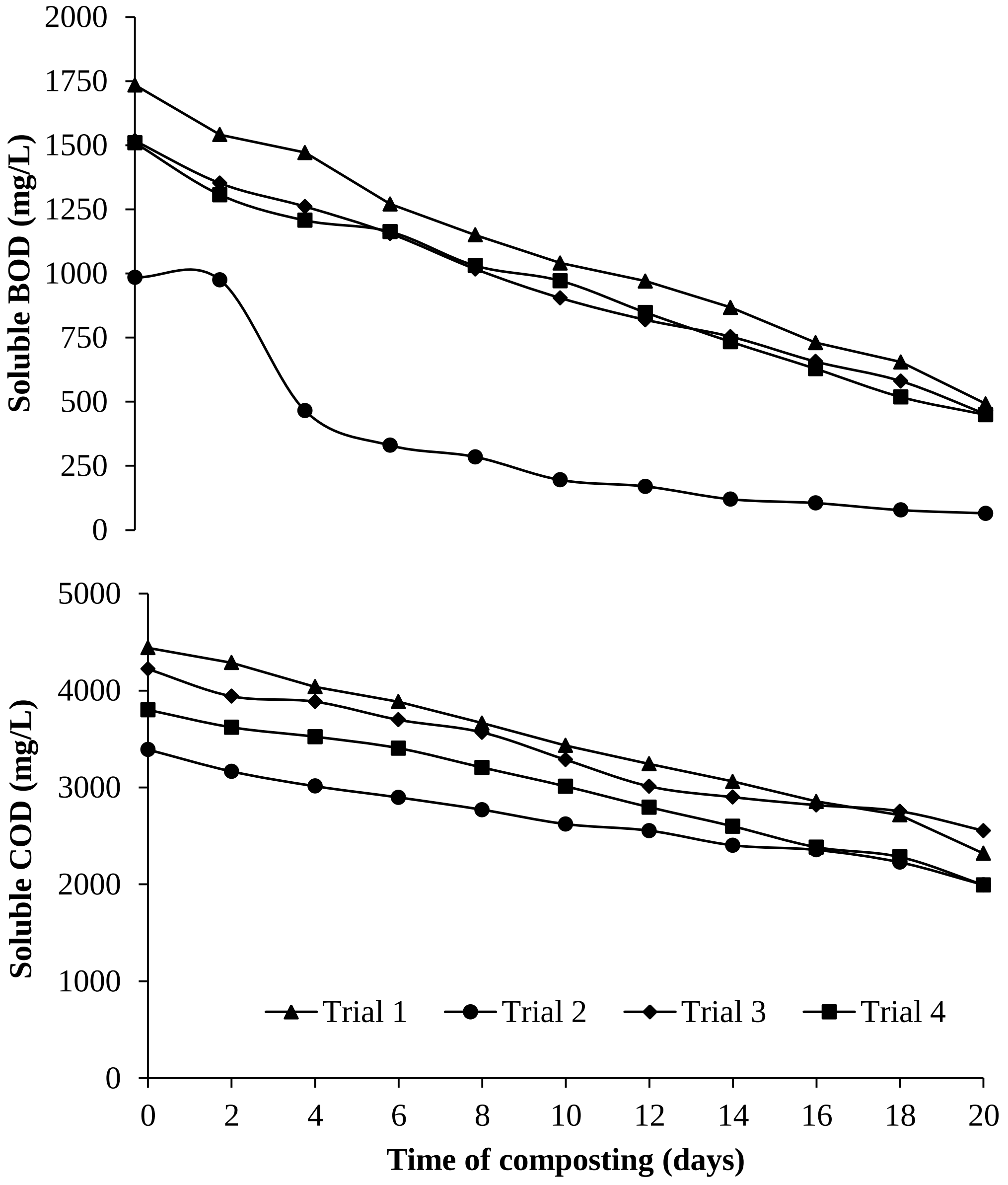

Fig. 4.18. Soluble BOD and COD variation during composting period

As the composting proceeded these emissions were drastically reduced from 14.9, 7.1, 22.8 and 23.0 mg/g VS/day to 2.2, 0.4, 4.1 and 4.8 mg/g VS/day in trial 1, 2, 3 and 4 respectively, at the end of 20 days. The decrease in CO_2 emissions was observed towards the end of composting period stating clearly the deprival of organic content resulting higher degradation. Similar reports were observed by Bhatia et al. (2012) on house hold 250 L rotary drum composter using vegetable waste mix. It has been reported that the different communities of microbes are involved in the degradation process till the end of 20 days playing a major role in degradation process by rising the temperature to more than 58°C.

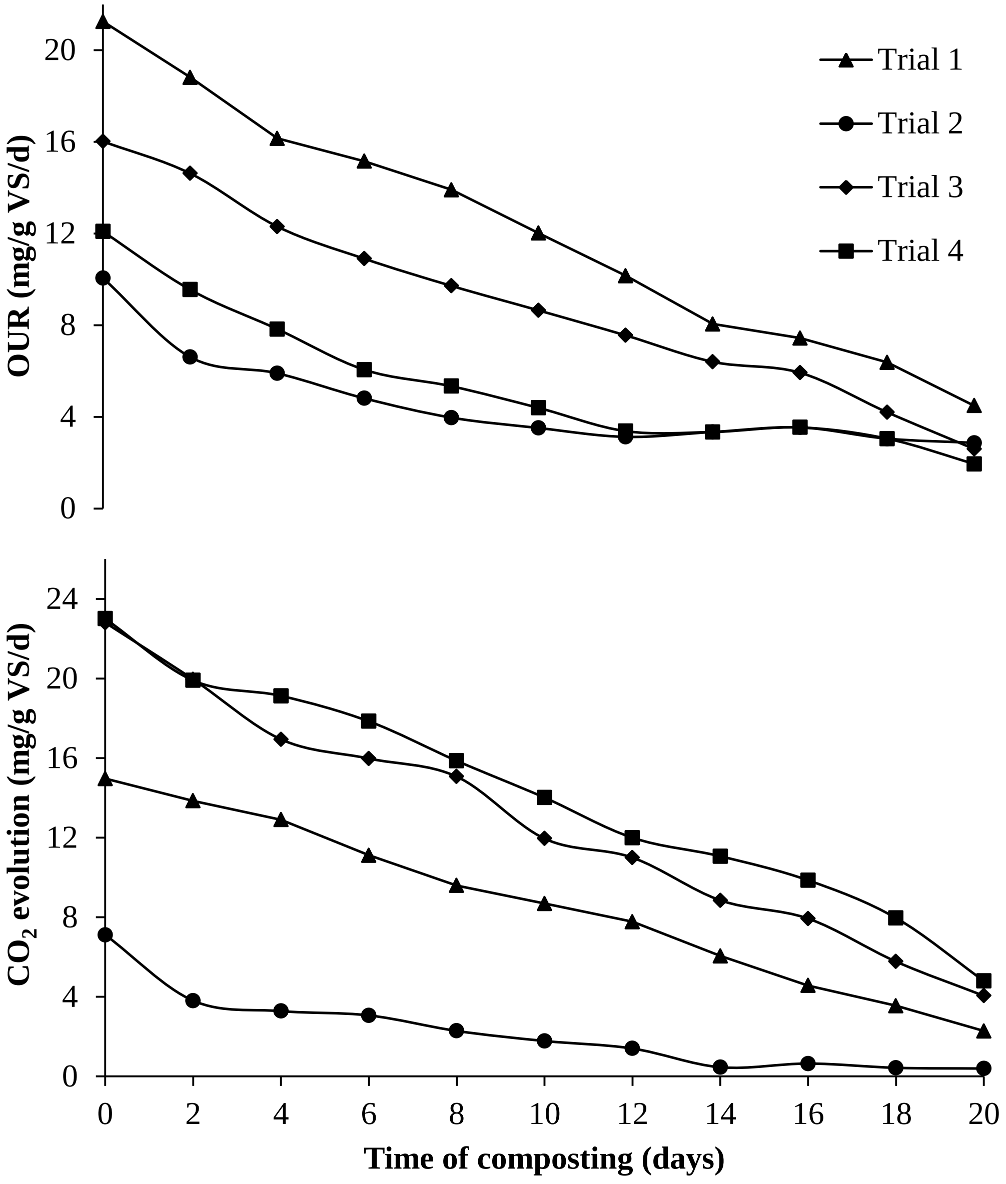

Fig. 4.19. OUR and CO_2 evolution rate during composting

With higher biodegradable matter in vegetable waste and propagation of microorganisms, OUR will be observed high during the composting process (Iannotti et al., 1993). The rates of oxygen demand will be reduced with lower food available to the microorganisms as the compost proceeds. Similar findings were observed in the present study with initial values ranging in the order of 21.3, 10.1, 16.0 and 12.1 mg/g VS/day denoting higher microbial activity and finally reduced to 4.5, 2.9, 2.6 and 1.9 mg/g VS/day in trial 1, 2, 3 and 4 respectively, at the end of 20 days. Hence at the end of 20 days the compost has been completely stabilized with lower CO_2 and OUR values.

- **Coliform**

In composting elevated temperatures and extended thermophilic stage is involved in the inactivation of pathogens and also by the competition with the favored thermophilic microbes (Yadav et al., 2010). Such elevated temperature is observed in trial 1 and 2, with highest of 66.5°C in trial 1 resulting in higher removal of total coliform (TC) and fecal coliform (FC). The average number of TC bacteria was initially observed in the range of 0.93×10^{11}, 11×10^{11}, 2.1×10^{11} and 1.6×10^{12} MPN/g of wet weight in trial 1, 2, 3 and 4 and finally reduced to 0.21×10^{3}, 2.4×10^{3}, 1.5×10^{3} and 0.93×10^{4} MPN/g respectively at the end of 20 days. However, the fecal coliform were in the order of 0.14×10^{7}, 11×10^{7}, 0.93×10^{6} and 2.9×10^{7} MPN/g in trial 1, 2, 3 and 4 and reduced to 0.15×10^{2}, 2.4×10^{2}, 0.75×10^{2} and 0.75×10^{3} MPN/g respectively at the end of 20 days. The recommended fecal coliform and streptococci densities for compost hygienization are 5.0×10^{2} and 5.0×10^{3} MPN/g, respectively (Vuorinen and Saharinen, 1997). Hence it can be concluded that the higher temperatures in trial 1 and 2 has played a major role in the destruction of pathogens.

4.1.3.3 Biochemical analysis

- **Lignocellulose degradation**

Biodegradation of organic matter during composting is greatly influenced by the lignocellulosic fractions of the waste materials and also the initial waste composition. Changes of hemicellulose (HC) and cellulose (C) fractions during composting are given in Fig. 4.20. A maximum of 29.7, 18.5, 19.6 and 14.8% reduction of HC and 44.2, 20.5, 26.1, 20.7% reduction of C was observed in trial 1, 2, 3 and 4 respectively at the end of 20 days (Fig. 4.20). Higher degradation of HC and C was observed between days 4 and 12, during the active thermophilic and post thermophilic stage of composting.

Table. 4.6. Micro-nutrient and heavy metal concentration during composting period

Day	**Sodium (g/kg)**				**Potassium (g/kg)**			
	541	**631**	**721**	**811**	**541**	**631**	**721**	**811**
0	1.25 ± 0.14	2.72 ± 0.07	2.64 ± 0.08	1.49 ± 0.03	14.08 ± 0.40	9.25 ± 0.05	16.73 ± 0.77	9.88 ± 0.12
20	1.36 ± 0.04	1.75 ± 0.01	2.43 ± 0.00	2.06 ± 0.01	25.67 ± 0.67	14.72 ± 0.03	13.16 ± 1.08	14.77 ± 0.35
Day	**Calcium (g/kg)**				**Magnesium (g/kg)**			
	541	**631**	**721**	**811**	**541**	**631**	**721**	**811**
0	8.27 ± 0.80	4.88 ± 0.06	11.13 ± 0.30	1.65 ± 0.07	5.40 ± 0.33	5.77 ± 0.03	5.43 ± 0.35	4.78 ± 0.09
20	10.36 ± 0.94	5.64 ± 0.00	28.83 ± 0.16	11.79 ± 0.13	7.41 ± 0.32	7.54 ± 0.06	7.04 ± 0.20	6.28 ± 0.10
Day	**Chromium (mg/kg)**				**Cadmium (mg/kg)**			
	541	**631**	**721**	**811**	**541**	**631**	**721**	**811**
0	54.75 ± 3.89	26.50 ± 1.41	66.50 ± 4.24	61.25 ± 2.47	71.00 ± 0.71	70.25 ± 2.12	68.00 ± 4.95	62.25 ± 0.35
20	38.50 ± 2.83	35.50 ± 1.41	44.00 ± 5.66	30.50 ± 2.83	68.50 ± 0.71	75.00 ± 2.83	55.25 ± 0.35	55.25 ± 1.77
Day	**Nickel (g/kg)**				**Lead (g/kg)**			
	541	**631**	**721**	**811**	**541**	**631**	**721**	**811**
0	0.184 ± 0.010	0.154 ± 0.002	0.154 ± 0.002	0.159 ± 0.004	0.99 ± 0.06	0.86 ± 0.02	0.83 ± 0.07	0.75 ± 0.04
20	0.191 ± 0.002	0.181 ± 0.007	0.181 ± 0.007	0.139 ± 0.010	0.96 ± 0.05	0.89 ± 0.04	0.83 ± 0.03	0.67 ± 0.13
Day	**Iron (g/kg)**				**Manganese (mg/kg)**			
	541	**631**	**721**	**811**	**541**	**631**	**721**	**811**
0	11.80 ± 0.62	6.91 ± 0.04	10.22 ± 0.15	9.77 ± 0.17	0.562 ± 0.005	0.674 ± 0.005	0.433 ± 0.006	0.438 ± 0.006
20	12.40 ± 0.05	10.78 ± 0.01	11.53 ± 0.26	8.19 ± 0.49	0.588 ± 0.004	0.865 ± 0.006	0.478 ± 0.005	0.481 ± 0.007
Day	**Zinc (g/kg)**				**Copper (g/kg)**			
	541	**631**	**721**	**811**	**541**	**631**	**721**	**811**
0	0.2353 ± 0.0006	0.2629 ± 0.0001	0.2202 ± 0.0008	0.2209 ± 0.0070	0.15 ± 0.03	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
20	0.2952 ± 0.0006	0.3531 ± 0.0045	0.2708 ± 0.0023	0.2474 ± 0.0004	0.05 ± 0.00	0.09 ± 0.01	0.03 ± 0.00	0.03 ± 0.00

Note: (mean ± SD, n=3) SD - standard deviation

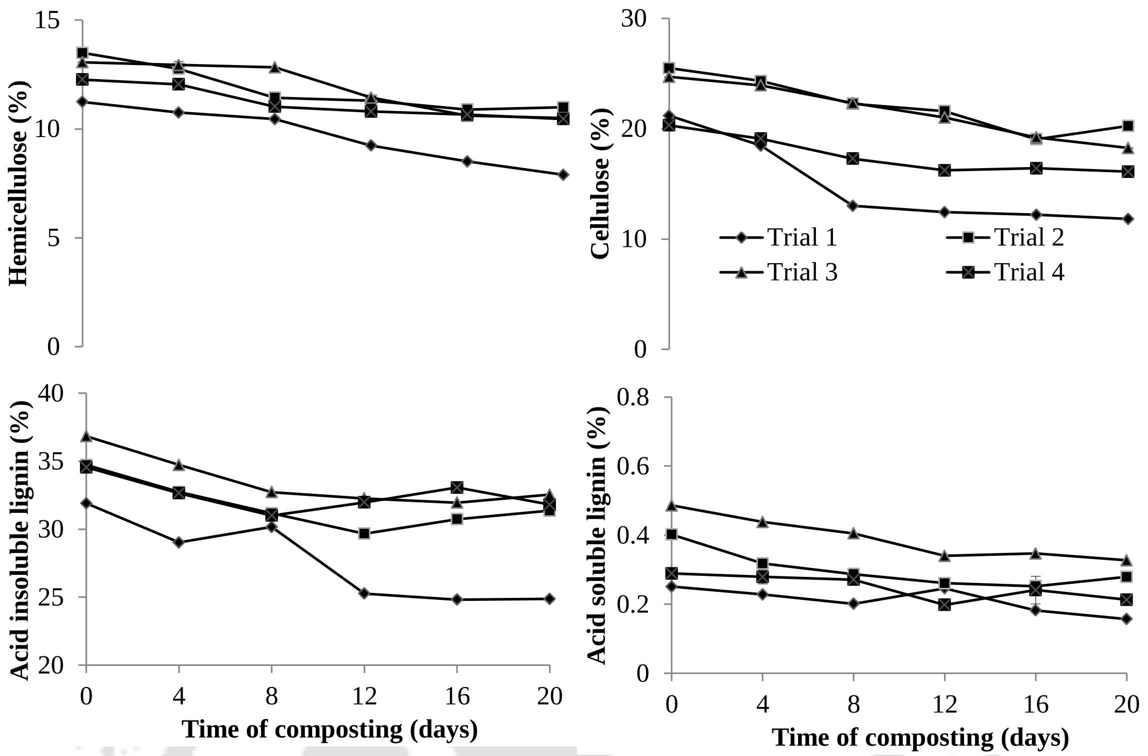

Fig. 4.20. Hemicellulose, cellulose and lignin variation during composting period

Due to proper combination of waste materials and active microbial degradation, maximum reduction of HC and C was observed in trial 1 as compared to other trials. During composting, HC is the first fraction utilized as carbon and energy source by the microorganisms (Serramia et al., 2010) and followed by cellulose. Higher temperature that prevailed during drum composting had a crucial effect on the degradation of HC and C, as major reduction of 17.8, 16.2, 12.4 and 12.0% in HC and 41.3, 15.3, 15.0 and 20.1% in C fractions was observed during the early and active thermophilic phase (day 1-12), out of the total loss observed from day 13-20 (final mesophilic stage).

The higher degradation of lignocellulosic fractions during thermophilic stage as compared to mesophilic stage during the study was supported by Serramia et al. (2010), Zeng et al. (2010) and Sarika et al. (2014). In addition, higher microbial population during drum composting (Bhatia et al., 2012) such as fungus and thermophilic bacteria also were responsible for major reduction of HC and C, which were observed in higher amount during the present study due to proper combinations of waste materials. Eventhough many studies have reported the difficulty of lignocellulose degradation during composting (Baca et al., 1992; Lynch, 1993), the present study was able to achieve higher degradation in lignocellulosic fraction within 20 days. Therefore, the results suggest that application of drum composter for the degradation of lignocellulose fractions can be much effective within short time period.

During composting, lignin is considered as the most difficult fraction to degrade due to its recalcitrant nature (Lynch, 1993) and it acts as an integral cell wall constituent. Quantitatively, Klason lignin is one of the most commonly used method and is determined gravimetrically by extraction with sulphuric acid (Dence, 1992; Toumela et al., 2000). Reports have suggested that elevated temperatures are able to degrade the lignin bonds and make them available for microbial degradation. With such elevated temperature greater than 55^{o}C for more than 5 days in trial 1, maximum of 22.1, 12.9, 11.7, 7.9% reduction of acid insoluble lignin (AIL) and 37.5, 30.6, 32.8 and 26.3% of acid soluble lignin (ASL) was observed at the end of 20 days. Similar to HC and C, AIL and ASL fractions were also observed with higher reductions during the thermophilic stage. The optimum temperature for mesophilic and thermophilic fungi involved in lignin degradation with lignocellulose substrate/compost environment ranges from 40–60^{o}C (Toumela et al., 2000). In addition, proper agitation and aeration also played a crucial role in degradation pattern, which was observed by turning the drum once in every 24 h. As many reports have stated that temperature had major effect in lignin degradation, the present study results also suggests that maintaining elevated temperature during lignocellulose fraction composting by proper combinations of waste material is very crucial for higher lignin degradation. There are many reports on the effect of temperature on lignin degradation during composting of industrial and agricultural wastes; however these studies were conducted up to a maximum of 224 days and minimum 140 days (Robinson et al., 1994; Tomati et al., 1995 and Franzluebbers et al., 1996). However, a maximum of 22.1 and 37.5% reduction of AIL and ASL was achieved within 20 days with the use of rotary drum composter which was the least reported elsewhere.

4.1.3.4 Microbial analysis

- **Total heterotrophic bacteria**

The initial amount of total heterotrophic bacteria was in the range of 4.8×10^{11}, 7.1×10^{11}, 5.4×10^{11} and 8.6×10^{11} CFU/g in trial 1, 2, 3 and 4 respectively (Fig. 4.21). The higher amount of indigenous microbial population during the initial period of composting might be due to the amount of initial waste material composition (Gazi et al., 2007). These mesophilic heterotrophs are considered to utilize the readily biodegradable matter, carbon as energy source and nitrogen for building materials; thereby increasing the temperature of the compost environment. Vegetable waste with high degradable organic matter was easily mineralized by these mesophiles during the initial stage and led to the rise of temperature. Hence there was a gradual decline in mesophiles during the 4th to 12th days and finally was in the range of 6.1×10^5, 2.6×10^6, 5.6×10^6 and 7.4×10^6 CFU/g in trial 1, 2, 3 and 4 respectively at the end of 20 days.

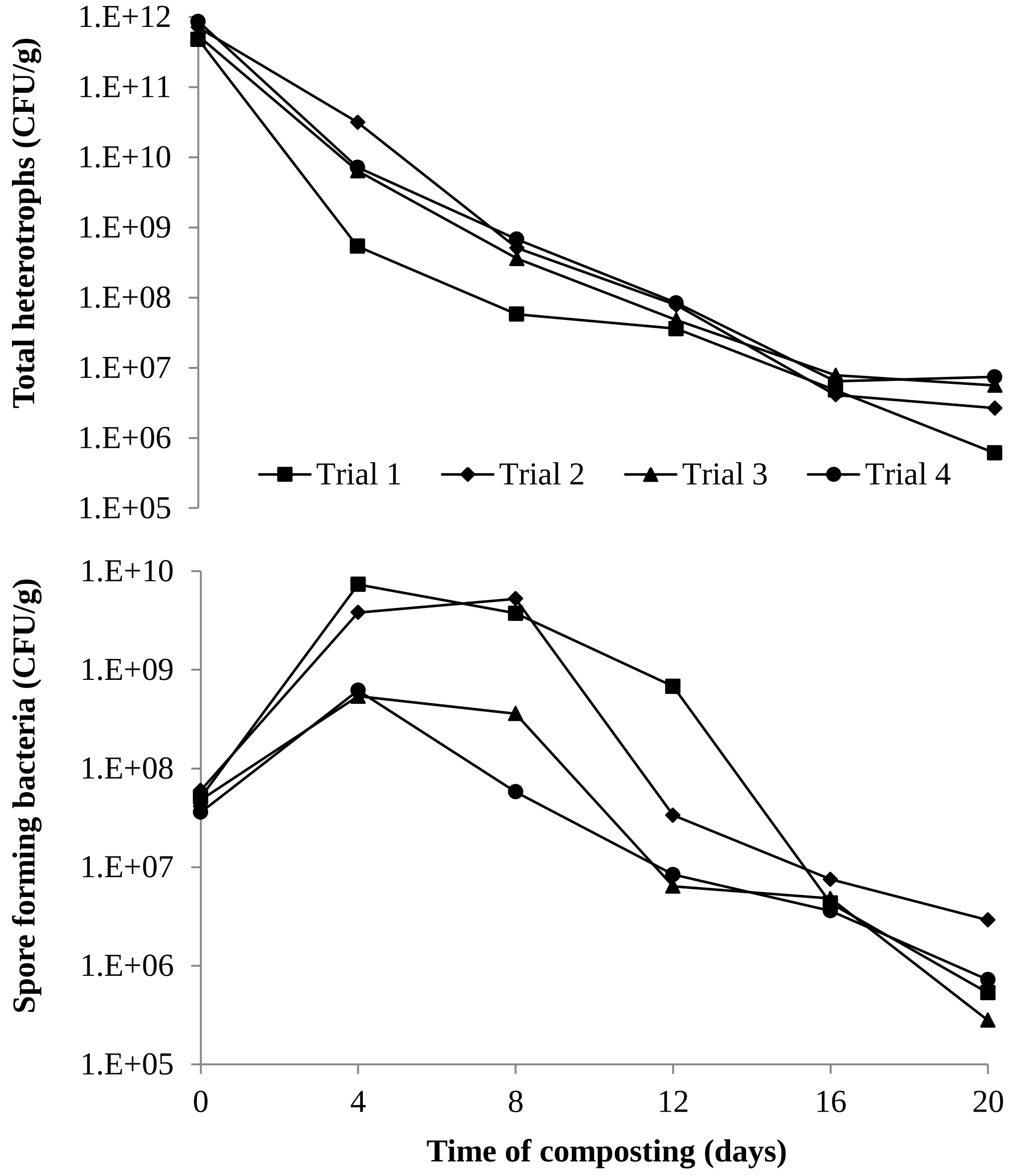

Fig. 4.21. Total heterotrophic and spore forming bacteria

The added sawdust and dry leaves were always considered to have high lignocellulose fractions as compared to vegetable waste and cattle manure which is highly resistant to bacterial degradation under mesophilic conditions. However with rise in temperature from day 1 to 8, during the active thermophilic stage there was a gradual rise in spore forming bacteria, which are considered to survive by forming spores at elevated temperatures in the compost environment. These elevated temperatures are also favorable in breaking the strong bonds of lignocellulose fractions and making them available for microbial degradation. These spore forming bacteria were in the range of 5.1×10^7, 6.0×10^7, 4.8×10^7 and 3.6×10^7 CFU/g during day 0 and were in the range of 5.3×10^5, 2.9×10^6, 2.8×10^5 and 7.2×10^5 CFU/g in trials 1, 2, 3 and 4 respectively at the end of 20 days. However, during day 4 which was at the active thermophilic stage there was rise in 100 fold of the spore forming bacteria and was observed to decrease with decline in temperature. Hence the effect of temperature on the growth of mesophilic and spore forming bacteria were clearly represented in the present study. Moreover the results were supported by the results of Ishii et al. (2000) stating the significant role of temperature over the populations of one another during composting.

Due to higher percentage of lignocellulosic fractions, the range of actinomycetes was in the order of 6.0×10^8, 2.5×10^9, 5.4×10^8 and 7.2×10^8 CFU/g and streptomycetes in the order of 6.6×10^7, 2.8×10^8, 5.8×10^8 and 4.2×10^8 CFU/g in trial 1, 2, 3 and 4 respectively, during the initial days of composting (Fig. 4.22). These species are generally involved in degradation of recalcitrant organics such as lignocellulose and elimination of pathogenic and allergenic microorganisms (Rebollido et al., 2008). From the results it can be concluded that the population size and composition of actinomycetes are mainly dependent on the type of organic content materials and also on the physical conditions of the environment. The final stage of composting was observed with considerable number of actinomycetes and streptomycetes in the range of 4.5×10^6, 3.8×10^6, 4.2×10^6 and 7.6×10^6 CFU/g and 5.2×10^5, 4.1×10^5, 7.2×10^5 and 3.6×10^5 CFU/g in trials 1, 2, 3 and 4 respectively which are mainly in degradation of lignocellulosic fractions as supported by Huang et al., (2010). In addition Gajalakshmi and Abbasi (2008) have also reported that actinomycetes species are observed to grow in numbers during later stages of composting utilizing the lignocellulosic fractions.

- **Fungi population**

The survival of fungi during composting is not only dependent on lignin source but also the hemicellulose and cellulose fractions that are utilized as growth substrate (Kirk and Darrell, 1987; Tuomela et al., 2000). With mixed lignocellulosic fractions of organic waste the population of fungi was in the order of 5.4×10^8, 1.8×10^8, 7.4×10^7 and 4.8×10^8 CFU/g in trials 1, 2, 3 and 4 respectively during the initial period of composting. These fungal species are considered to act when the easily biodegradable substances are acted upon by the bacteria and when the compost is left out with lignocellulosic fractions (Golueke, 1992). The growth of fungi in the compost environment is mainly dependent on the carbon and nitrogen sources, hence the addition of vegetable waste which is rich in nitrogen source played a major role in its survival and there by leading to higher degradation of lignin as discussed in the earlier part of the study.

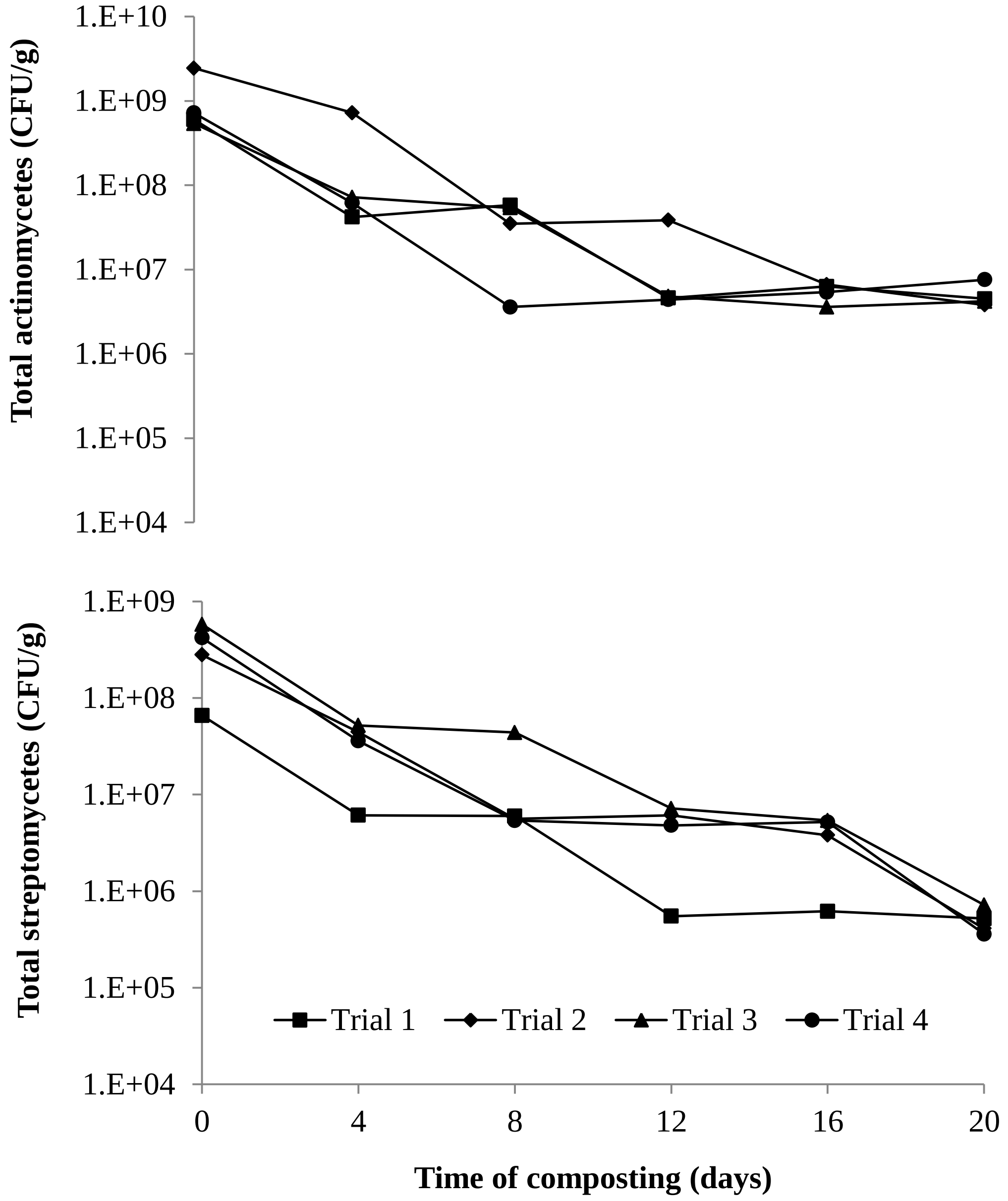

Fig. 4.22. Actinomycetes and streptomycetes during composting

The considerable amount of fungi at the end of composting period is supported by the results of Thambirajah et al. (1995) with 10^4-10^6 per gram of mesophilic and thermophilic fungi in mature compost. With several factors affecting the growth of fungi, mainly temperature and pH during the process, the final population of fungi was in the range of 4.1×10^4, 2.85×10^4, 3.6×10^5 and 4.2×10^5 CFU/g in trials 1, 2, 3 and 4 respectively at the end of 20 days (Fig. 4.23). However the results were not in accordance with the reports by Ryckeboer (2003), where complete inactivation of fungi was reported due to elevated temperature during the thermophilic stage. Enterococci have been considered as the indicator of health risk in salt water used for recreation and as a useful indicator in fresh water as well. The recommended populations of enterococci in moderate and lightly full body contact recreational water should be 124 and 276 densities per 100 mL (USEPA, 1986). At the end of 20 days enterococci was found in the range of 60, 240, 740 and 1200 CFU/g. Similarly streptococci were also observed to reduce in considerable amounts from 2.1×10^4, 5.8×10^5, 6.8×10^5 and 4.8×10^6 CFU/g to 64, 88, 540 and 940 CFU/g in trials 1, 2, 3 and 4 respectively at the end of 20 days.

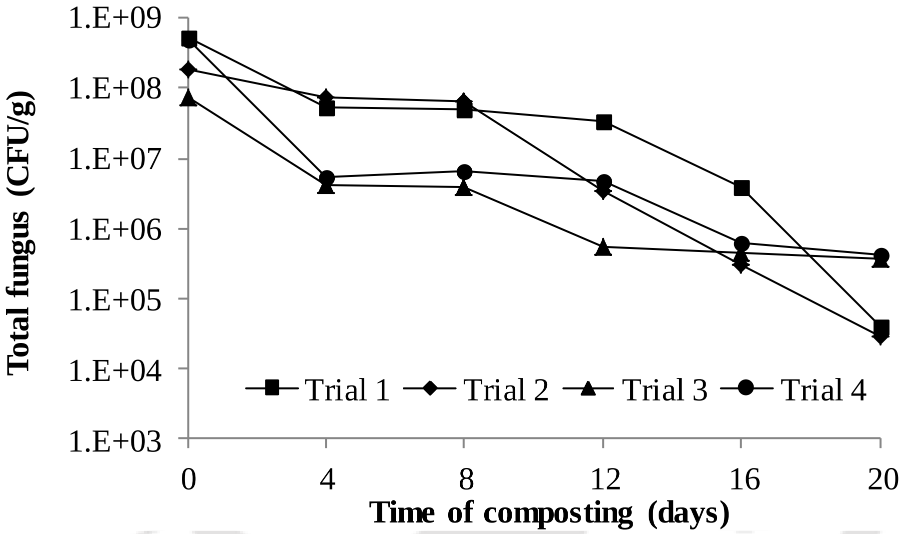

Fig. 4.23. Total fungus during composting

4.1.3.5 Instrumentation analysis

- **FTIR spectra**

The functional groups of organic content in the compost mix were identified by FTIR spectra during initial and final days of composting, reported in Fig. 4.24. The infrared spectra interpretations were based on the data of numerous studies by Quatmane et al. (2000), Droussia et al. (2009) and Kalyanaraman et al. (2013). The broad spectrum in the region of 3450 (3300-3500) /cm signifies the presence of OH stretching of the hydroxyl groups from alcohol and phenol; and also may be due to carboxylic groups and due to amide and amine N-H stretching. The two characteristic absorption in the region of 2860 and 2935 /cm represents the presence of long chain aliphatic compounds.

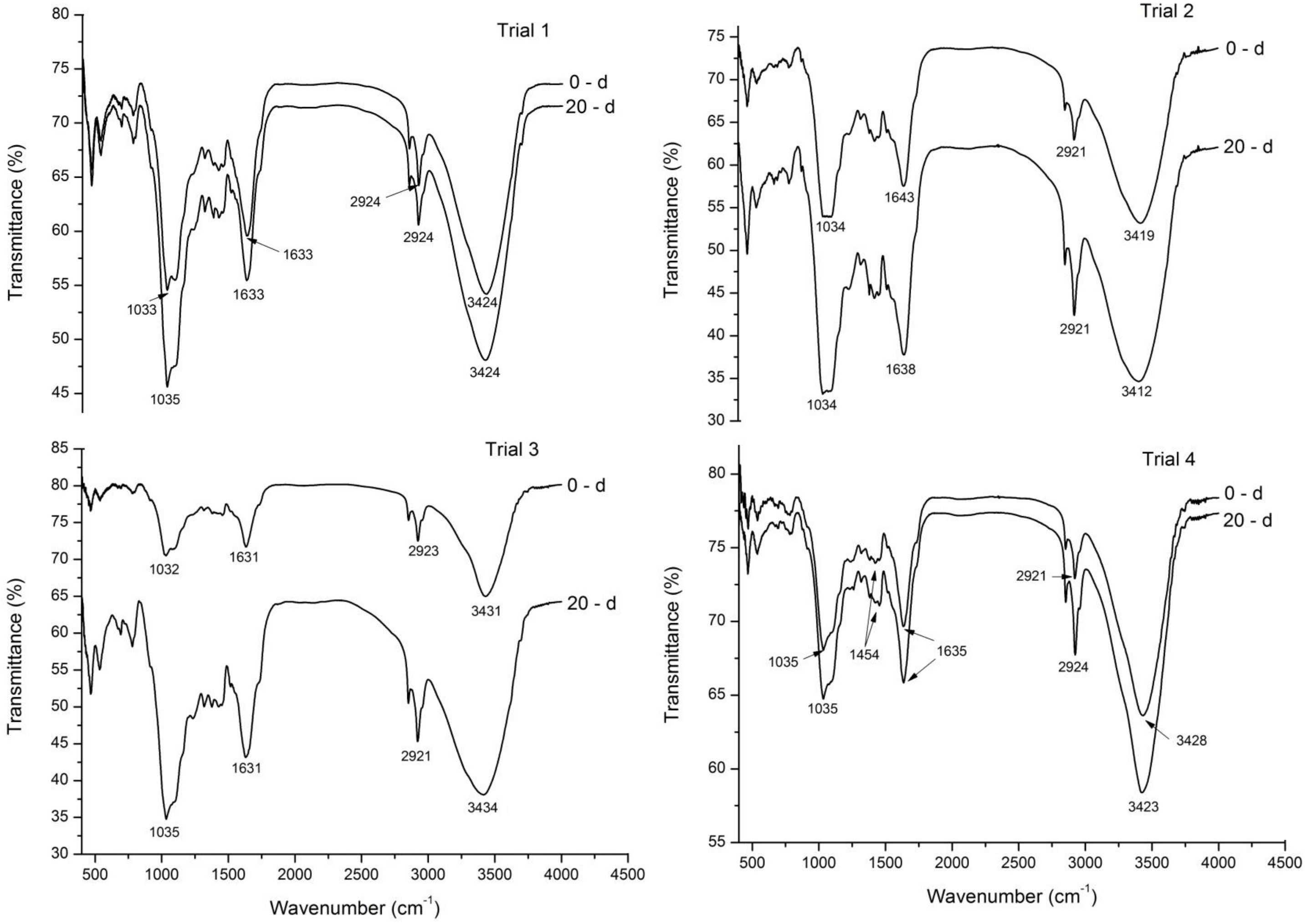

Fig. 4.24. FTIR spectra during composting

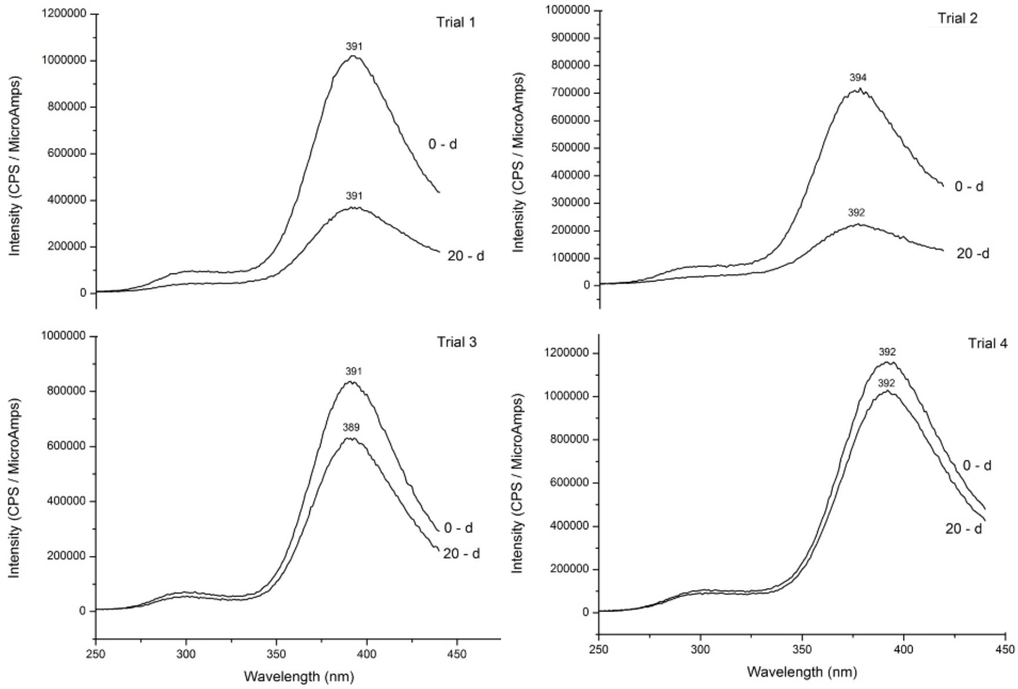

Fig. 4.25. Fluorescence excitation spectra of humic acids during composting

The absorption band at 1640–1620 /cm may be due to aromatic C=C stretching and C=O stretching of quinone and conjugated ketone and amide groups (Baddi et al., 2004). The weak bands in the range of 1250–1500 /cm are due to the presence of amides or ethers and aromatic esters. Well defined characteristic bands at 1126 and 1045 /cm are due to aromatic C-H in-plain deformation for syringyl and guaiacyl alcohols, which are the two structural components of lignin (Fuentes et al., 2007). The enrichment of aromatic C=C as compared to aliphatic carbon during composting process can be explained by the reduction in ratios of aliphatic/aromatic carbon to polysaccharide. Decrease in aliphatic carbon to polysaccharide (2930/1030) was observed from 1.332 to 1.181, with highest of 11.34% reduction in trial 1 as compared to all other trials. The ratio of intensities of aromatic carbon to polysaccharide (1630/1030) decreased from 1.204 to 1.086, with percentage reduction of 9.81% in trial 1. Similarly, the intensity ratio of aliphatic carbon to aromatic carbon (2930/1630) decreased from 1.106 to 1.087 with percentage reduction 1.75%, in trial 1. The ratio of characteristic bands at 1126 and 1045 /cm of trial 1, was observed to reduce from 1.103 to 1.051 at the end of 20 days of composting period with 4.64% reduction.

Therefore, it can be concluded that trial 1 was observed with higher reduction of aliphatic compounds and enriched with aromatic compounds after 20 days of composting period. The major excitation peak of fluorescence spectroscopy at a wavelength of about 390 nm and a number of secondary peaks occurring on either side of the major peak, at lower (360-345 nm) or higher wavelengths (455-470 nm) are mainly due to the humic acids (Sakellariadou, 2006) (Fig. 4.25). However in the present study only one major excitation peak was observed around 390 to 394 nm in all the four trials, representing the humic acids during composting (Hong-Li et al., 2009). The broad absorption band of humic acid observed may also contain the combinations of hydroxyl, methyl, carboxyl, phenol, alcohol, polysaccharide groups, aromatic bond and amide as well. The emission spectra around 390 nm was observed to decrease in all the trials during composting, thereby representing the increase of degree of polycondensation and aromatization of humic acids. Therefore, rotary drum composting can be considered to a viable option for composting of organic waste within shorter time period for producing a quality compost rich in humic acids.

4.1.4 CONCLUSIONS

The present study revealed that leachate production and high moisture content during the composting process had a huge effect on the microbial population and also adversely affected the composting parameters. Even though the process was normal during the early stages of composting, soon after the thermophilic phase huge amount of leachate production was observed due to insufficient bulking agent i.e. sawdust for vegetable waste composting. The reason for the leachate production might be due to saturation of sawdust with huge amount of moisture content during the metabolic process by active micro-organisms and once the saturation point has exceeded it started to leach out. It was also observed that higher diversity of microbial population prevailed during this rotary drum composting; however because of leachate production inconsistent degradation pathway was observed during the process. Finally authors would like to conclude that the presence of water is necessary for microbial activity and transport of soluble substances for organic matter destruction. However excess moisture had a major effect on oxygen uptake rate and heat release in the process. Therefore leachate production should be avoided during vegetable waste composting due to its major effect on the process. It is also suggested for the application of proper bulking should be of major concern during vegetable waste composting.

Due to proper combination of waste materials in trial 3 (6:3:1) i.e. vegetable waste, cow manure, sawdust and addition of 10 kg dry leaves, has produced a stabilized

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compost at the end of 20 days. Elevated temperature was observed in trial 3 due to proper aeration provided by adding dry leaves. As a result higher degradation of organic matter and inactivation of indicator organisms was observed. Longer thermophilic phase was observed due to active microorganisms by providing proper carbon and nitrogen ratio. With higher reduction in organic fractions, the final compost was completely stabilized with 0.39 and 2.87 mg/g VS/day of CO_2 evolution and OUR. Increase in heavy metals was also observed at the end of 20 days composting period due to loss of organic matter.

Finally it can be concluded from the observed results that addition of dried dry leaves for vegetable waste composting resulted in stabilized compost without any leachate production. Despite of various microbial communities during vegetable waste composting, each community is observed to act accordingly to temperature and nature of substrate available. Irrespective of the trials, microbial population growth was influenced by the temperature and also the effective organic matter degradation. However, combinations of waste materials played a major role in favoring microbial succession. Spore forming bacteria are majorly observed in the degradation process during thermophilic stage. Final stages of composting process were observed with considerable amount of fungi, actinomycetes and streptomycetes. These populations were considered to act more predominantly due to the presence of lingo-cellulosic material, even though lower CO_2 evolution and OUR was observed during the final stages. Hence, it can be concluded that from different trials it can be stated that trial 3>4>2>5>1 was observed the best in terms of stability and microbial dynamics. Overall, higher diversity of microbial community prevailed throughout the composting process resulting in high stabilized compost with higher degradation and pathogen free compost in shorter duration of composting.

Due to proper combination of waste materials in trial 1 (5:4:1) i.e. vegetable waste, cow dung, sawdust and addition of 10 kg dry leaves as bulking agent, has produced a stabilized compost at the end of 20 days. Elevated temperature was observed in trial 1 with a maximum of 66.5°C due to proper aeration provided by adding dry leaves. As a result higher degradation of organic matter and inactivation of indicator organisms was observed. Longer thermophilic phase was observed due to active microorganisms by providing proper carbon and nitrogen ratio. With higher reduction in organic fractions, such as VS and TOC, the final compost was completely stabilized in terms of lower CO_2 evolution and OUR. However trial 2 was also observed to have similar reductions in trial

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1. Increase in heavy metals was also observed at the end of 20 days composting period due to loss of organic matter.

The presence of lignocellulosic fractions during composting plays a key role in degradation of organic matter and is majorly affected by various process parameters such as temperature, physical properties of substrate and the type of composting employed. Due to proper combination of waste materials, higher degradation was observed in trial 1 followed by trial 3, 2 and 4. The degradation of lignocellulose using rotary drum can be considered mainly due to higher temperature and microbial diversity that prevailed during composting. Addition of vegetable waste with high biodegradable content led to higher temperature in the process resulting in proper degradation. Moreover, FTIR spectra were observed with many changes in the aliphatic and aromatic compounds throughout the composting period, however with higher reduction in trial 1. Fluorescence spectra also confirmed the increase of humic acids at the end of composting period in all the trials using rotary drum composter. Therefore, it can be concluded that the compost produced within 20 days was completely stabilized and the values were within the range of international compost quality standards. (Annexure I)

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4.2 PHASE II – COMPARISON OF DIFFERENT COMPOSTING TECHNOLOGIES

4.2.1 PILE COMPOSTING

The rate of aeration and mixing of the waste materials has been reported to be the most critical factor in maintaining the thermophilic phase in the composting system. However, the treatment process of vegetable waste in combination with other bulking agents has to be explored for the quality of compost in terms of stability and nutrient changes. The study compared the effects of pile composting on vegetable waste degradation operated at agitated pile (AP) (Trial 1), passive pile (PP) (Trial 2) and forced aerated pile (FAP) (Trial 3) conditions (Table 3.5). The effects of different operation mode on the degradation of organic matter and the changes in temperature pattern, moisture reduction, nitrogen and phosphorous dynamics was correlated during the 30 days of composting period.

4.2.1.1 Physico-chemical analysis

- **Temperature**

Fig. 4.26 shows the temperature pattern for three different pile composting methods. Due to high biodegradable nature of vegetable waste, raise in temperature up to 50°C was observed within 18-24 h of composting period in all trials. Temperature is an indicator for higher microbial activity which is an exothermic process, resulting in high release of CO_2 and degradation of organic matter. A maximum of 61.8°C, 67.6°C and 50.5°C was observed in trial 1, 2 and 3 respectively; due to different aeration rates and high degradation rate. A gradual decrease in thermophilic temperature was observed in trial 1 and 2 after 5th and 6th day of composting, which can be considered due to the depletion of readily degradable matter (Awasthi et al., 2014). The maximum temperature observed in trial 2, can be considered due to the optimum aeration provided by passive operation and also due to the proper combination of waste materials. However in trial 3, a sudden drop in temperature was observed which can be considered due to high aeration rate. The lower temperature and sudden loss of thermophilic phase can be considered due to the mass movement of materials in trial 1 and higher aeration in trial 3 (Haug, 1993).

- **pH and EC**

The changes in pH and EC during composting are considered due to the variation in chemical composition by organic matter degradation and release of mineral salts. Fig. 4.27 shows the pH and EC pattern during composting. The pH of all the trials was

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observed to increase from 6.4 to 7.6, 6.5 to 7.3 and 6.9 to 7.3 in trial 1, 2 and 3 respectively (Fig. 4.27). The rise is pH during composting can be considered due to the release of CO_2 by organic matter degradation and ammonification/mineralization of organic nitrogen (Haug, 1993; Kalamdhad et al., 2009). The CO_2 produced in the composting system could escape into atmosphere as gas or it might be dissolved in the substrates in the form of carbonic acid, bicarbonates and carbonates tending to neutralize the pH of the compost. In addition, under aerobic conditions the organic nitrogen is mineralized to form NH_3 or NH_4^+ during ammonification thereby increasing the pH of the pile (Wong et al., 2001). EC of the compost was observed to increase from 2.24 to 3.24, 2.86 to 4.29 and 2.26 to 3.31 in trial 1, 2 and 3 respectively (Fig. 4.27). The increase in EC can be considered due to the release of mineral ions such as phosphate, ammonium, potassium and other cations during the degradation of organic matter (Francou et al., 2005). Hence due to higher degradation of organic matter in the initial stages, the EC of all the piles was observed to increase as supported by Prasad et al. (2013).

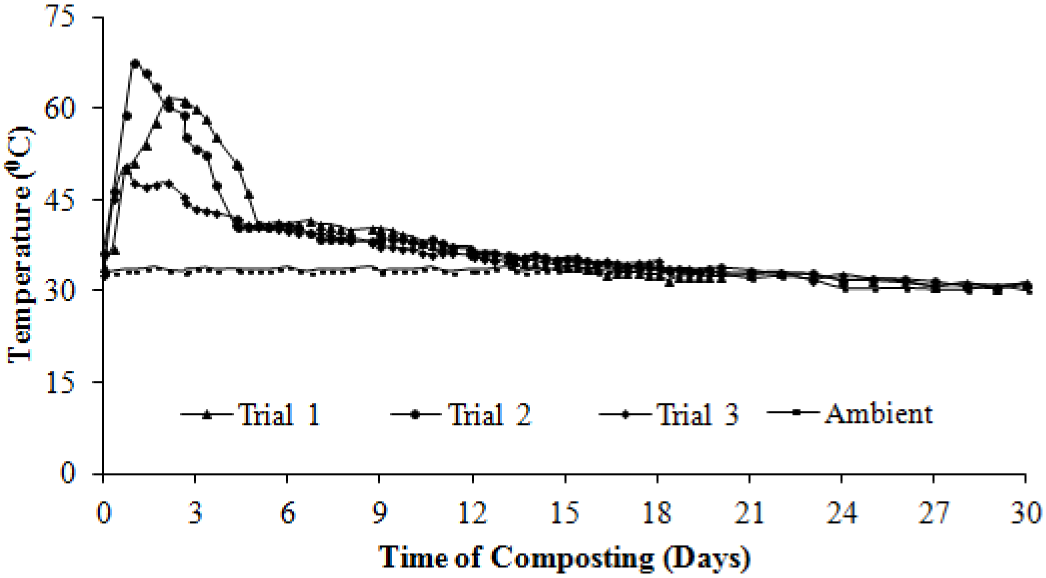

Fig. 4.26. Temperature profile during composting

- **Moisture content**

The optimum moisture content in the composting environment should be in the range of 60-80% for microbial activity (TMECC, 2002). During composting, loss of moisture can be viewed as the index of decomposition rate, due to thermophilic temperature (Kalamdhad et al., 2009). Due to higher degradation and elevated temperature in the piles, a maximum of 21.2, 24.4 and 28.9% reduction of moisture content was observed in trial 1, 2 and 3 respectively (Fig. 4.27). The thermophilic stage was attained within 24 h of composting period due to higher decomposition rate of raw materials. Hence, around 11.8, 8.9 and 17.1% of moisture reduction was observed within 9 days of initial period from the total reduction.

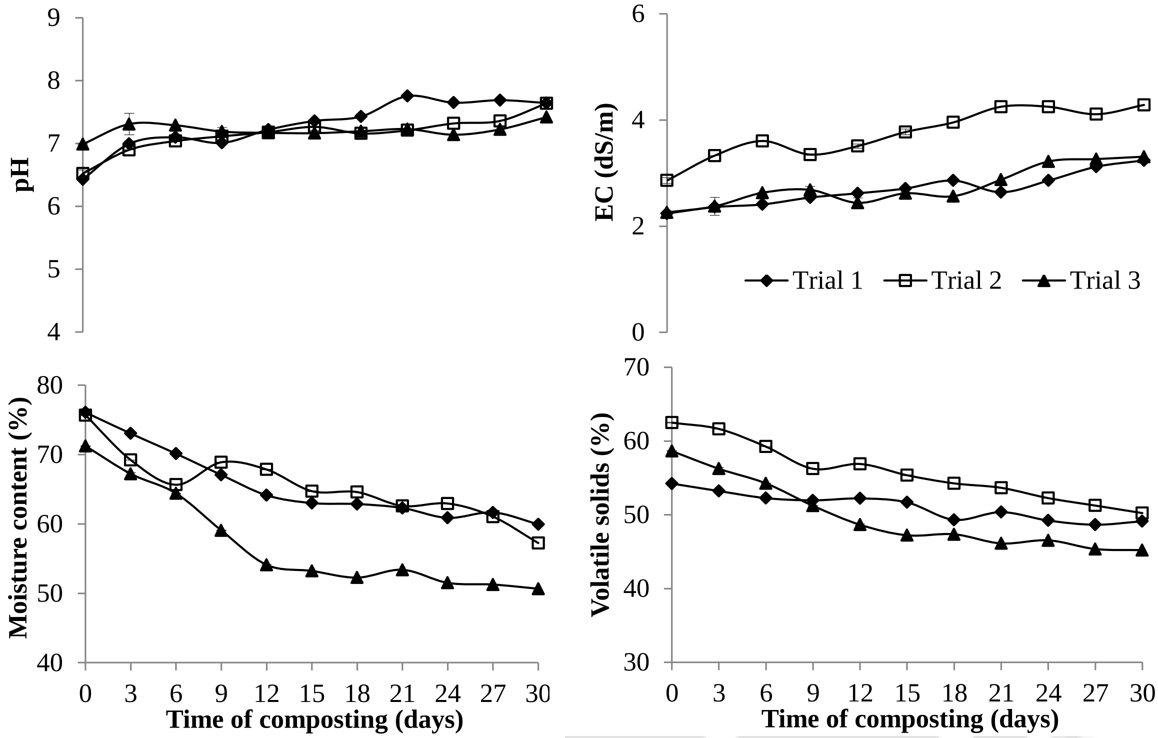

Fig. 4.27. pH, EC, moisture and volatile solids variation during pile composting

- **Volatile solids (VS)**

Due to higher microbial activity and the elevated temperature in composting piles, higher degradation of VS was observed during the study. A maximum of 22.9% of reduction was observed in trial 3 followed by 19.6 and 9.4% in trial 2 and 1 respectively (Fig. 4.27). Moreover, 50 to 60% of organic matter degradation was occurred within 9 days of the total composting period in all the trials. The higher degradation of volatile solids due to active microbial metabolism during thermophilic stage is supported by Sarika et al. (2014). The lower VS reduction in trial 1 can be considered due to poor activity of microbes and sudden drop of temperature that occurred after manual turning of pile after 3 days. Due to higher heat transfer by manual turning of pile, temperature dropped completely same as trial 3, where forced aeration reduced the temperature. But, no aeration was provided after three days in trial 3 and was operated as passive mode resulting in higher VS reduction. When compared to all the trials, trial 2 had the higher temperature and considerable VS reduction as compared to trial 3.

- **Nitrogen and phosphorus dynamics**

The Total Kjeldahl nitrogen (TKN) in the composting pile is generally increased due to the loss of organic matter as CO_2 evolution and by the action of nitrogen fixing bacteria (Nakasaki et al. 2005; Kalamdhad et al., 2009). Similar such observations were observed during the present study with increase in TKN from 1.12 to 1.45%, 1.46 to 1.82% and 0.98 to 1.36% in trial 1, 2 and 3 respectively (Fig. 4.28). The increased TKN during composting period were in accordance with the reports by Vuorinen and Saharinen (1997). During the decomposition of nitrogen compounds i.e. proteins and amino acids, the ammonia is released as gaseous form. Similarly, ammonium is produced by the decomposition of nitrogenous compounds and it is oxidized into nitrate by the action of ammonium oxidizing bacteria and nitrate oxidizing bacteria, thereby showing decrease in ammonium concentration (Tiquia et al., 2000). Similar such results were observed in the present study with decrease of NH_4-N from 0.48 to 0.22%, 0.44 to 0.28%, and 0.51 to 0.33% in trial 1, 2 and 3 respectively. Moreover, these emissions are observed majorly during the thermophilic stage as reported by Liang et al. (2006).

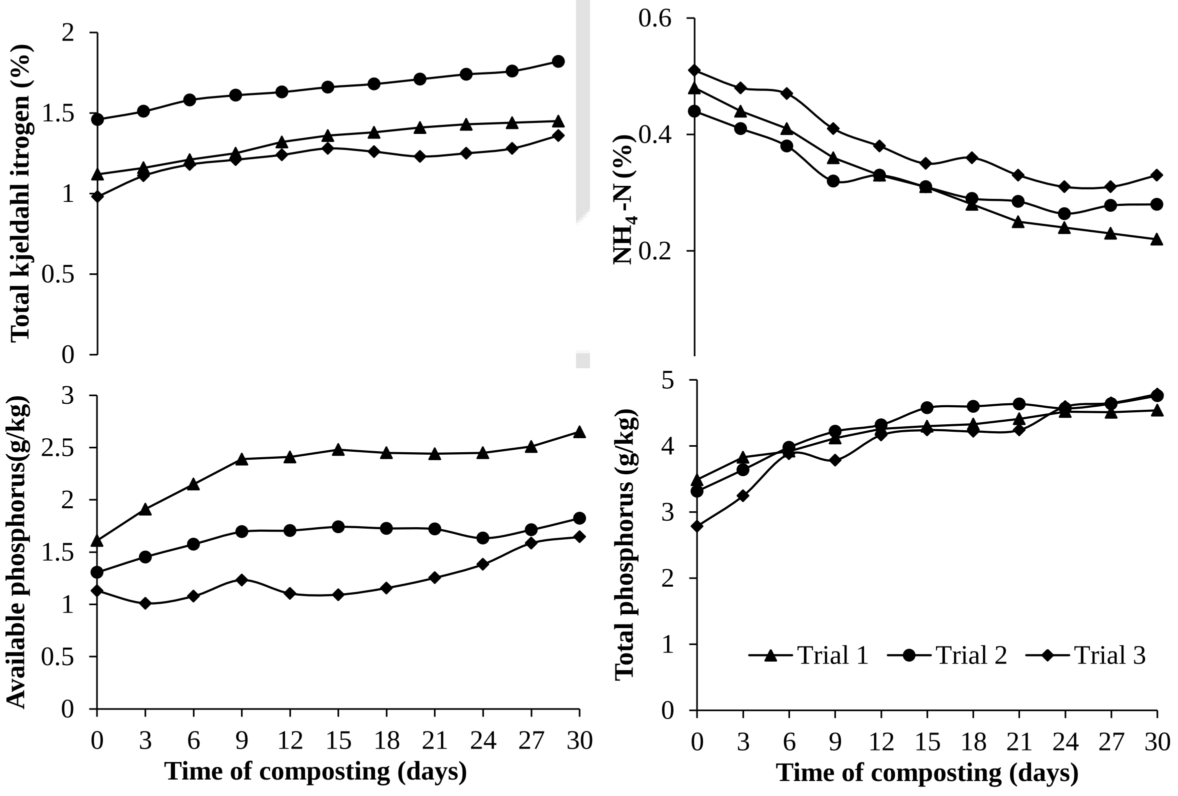

Fig. 4.28. Nitrogen and phosphorous dynamics during composting period

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The phosphorous content in the compost is considered to increase due to the mineralization of inorganic phosphate from organic phosphate and it is mainly dependent on the temperature and moisture content. The inorganic phosphorus is negatively charged and it reacts readily with positively charged metal ions to form relatively insoluble substances thereby fixing the phosphorus in compost. Hence, the available and total phosphorus were observed to increase in all the trials and it was found to be in the range of 2.65, 1.82 and 1.65 g/kg of AP; and 4.54, 4.76 and 4.79 g/kg of TP in trial 1, 2 and 3 respectively (Fig. 4.28).

4.2.1.2 Biological analysis

- **Soluble BOD and COD**

The soluble BOD and COD represent the organic fractions in the compost mix. Due to active microbial feed and degradation process, the values would be observed to reduce towards the end of composting. Similar such results were observed in the present study due to higher microbial activity. Soluble BOD values decreased from 11.1, 10.5 and 8.25 g/kg of wet compost to 3.6, 3.0 and 1.95 g/kg in trial 1, 2 and 3 respectively, at the end of 30 days of composting period (Fig. 4.29). Correspondingly, soluble COD values decreased from 28.4, 32.0 and 19.0 g/kg of wet compost to 5.81, 9.0 and 3.0 g/kg trial 1, 2 and 3 respectively (Fig. 4.29). CO_2 evolution rate will be observed higher during the degradation period and will be stable in the later stages of composting. The lower emission of CO_2 in the later stages represents the stability of compost (Bernal et al., 1997; Kalamdhad et al., 2008). The CO_2 evolution rates decreased from 4.88, 4.67 and 3.87 mg/g VS/day to 0.44, 0.16 and 0.76 mg/g VS/day in trial 1, 2 and 3 respectively, at the end of 30 days (Fig. 4.30). A maximum of 91.1, 96.5 and 80.3% reduction of CO_2 evolution rate was observed. Even though higher degradation of volatile solids was observed in trial 3, minimum reduction of CO_2 evolution rate was observed at the end collectively, which can be considered due to higher aeration rate during the initial period. Due to forced aeration higher heat transfer was observed; similarly it might have lead escape of CO_2 gas into the atmosphere.

- **Micronutrient and heavy metal concentration**

The micronutrients and heavy metals are observed to increase due to mass loss in the compost caused by the mineralization of organic fractions (Fang and Wong, 1999). Table 4.7 illustrates the increase in total concentration of micronutrients (Na, K, Ca and Mg) and metals (Cr, Cd, Ni, Pb, Fe, Mn, Zn and Cu) in trials 1, 2 and 3, respectively, during the 30 days of composting period. The total concentration of micronutrients of the final composts increased in the range of about 19-24% for Na, 31-54% for K, 55-119% for Ca and 27-35% for Mg, respectively.

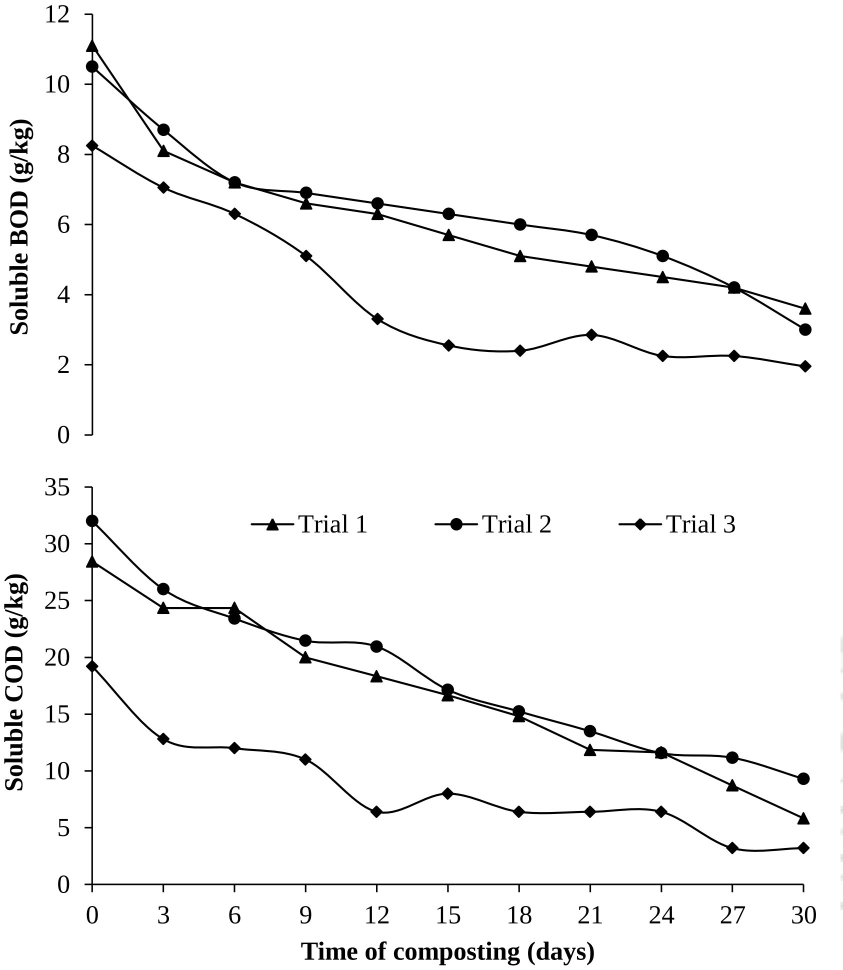

Fig. 4.29. Soluble BOD and COD variation during composting period

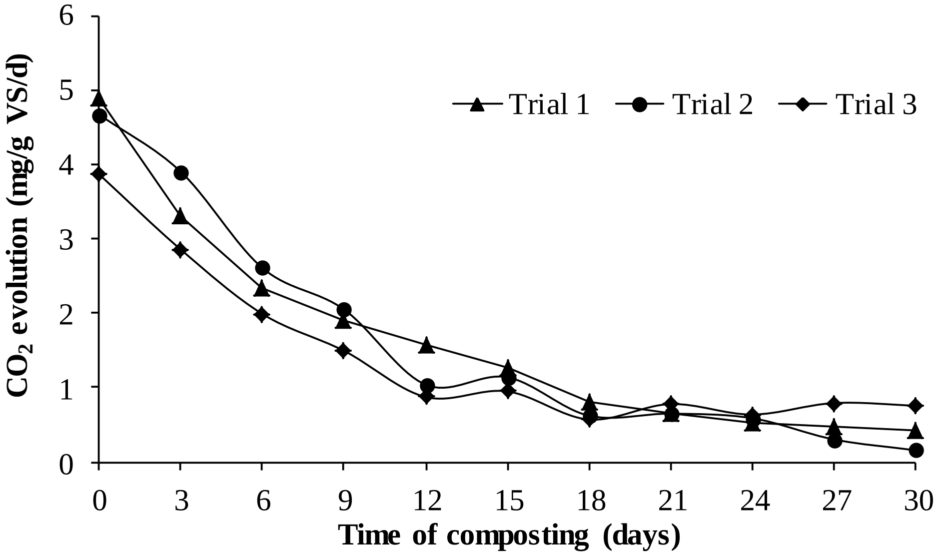

Fig. 4.30. CO_2 evolution rate during composting period

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Table. 4.7 Micronutrient and heavy metal concentration during pile composting period

Day	**Sodium (g/kg)**			**Potassium (g/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	1.14 ± 0.04	1.22 ± 0.06	1.49 ± 0.11	13.01 ± 0.26	11.64 ± 0.21	12.36 ± 0.32
30	1.36 ± 0.12	1.54 ± 0.14	1.86 ± 0.04	18.14 ± 0.31	17.22 ± 0.42	19.14 ± 0.16
Day	**Calcium (g/kg)**			**Magnesium (g/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	4.64 ± 1.11	8.42 ± 2.24	5.11 ± 1.24	3.12 ± 1.12	4.26 ± 0.42	5.36 ± 0.72
30	9.26 ± 2.16	13.12 ± 3.12	11.22 ± 2.36	4.22 ± 0.66	5.42 ± 1.12	7.24 ± 0.34
Day	**Chromium (mg/kg)**			**Copper (mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	32.12 ± 1.22	42.64 ± 3.32	54.22 ± 6.64	66.22 ± 2.24	54.26 ± 4.44	48.26 ± 2.26
30	43.22 ± 2.36	54.62 ± 1.36	72.22 ± 12.26	74.26 ± 3.34	66.24 ± 8.24	58.48 ± 5.54
Day	**Nickel (mg/kg)**			**Lead (mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	238.25 ± 1.25	194.71 ± 3.22	203.44 ± 4.52	564.22 ± 22.36	664.42 ± 24.12	774.12 ± 14.64
30	274.75 ± 5.75	245.56 ± 4.30	247.36 ± 1.22	721.32 ± 13.24	842.26 ± 32.33	942.62 ± 24.22
Day	**Iron (g/kg)**			**Manganese (mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	6.24 ± 0.62	4.62 ± 0.22	7.41 ± 1.26	591.52 ± 4.36	480.25 ± 12.36	383.32 ± 6.12
30	8.62 ± 1.22	9.11 ± 1.36	10.24 ± 0.61	632.22 ± 14.22	532.33 ± 18.20	550.54 ± 12.32
Day	**Zinc (mg/kg)**			**Cadmium (mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	390.52 ± 5.22	242.26 ± 6.75	216.56 ± 12.21	56.22 ± 4.13	42.36 ± 5.64	76.23 ± 2.64
30	302.34 ± 9.51	234.74 ± 14.26	289.52 ± 4.22	74.26 ± 6.24	84.62 ± 11.32	94.32 ± 6.54

Note: (mean ± SD, n=3) SD - standard deviation

4.2.2 CONCLUSIONS

From the study it can be concluded that pile composting of vegetable waste was found successful with the combinations of cow dung and other two bulking agents. However, comparing various operational methods showed different degradation pattern during the 30 days of composting period. Trial 2 and 3 operated in passive and forced aeration mode were observed with higher degradation of volatile solids (19.6 and 22.9%). In addition, the final compost was observed with higher nutrient value i.e. Na (1.36-1.86 g/kg), K (17.22-19.14 g/kg), Ca (9.26-13.12 g/kg) and Mg (4.22-7.24 g/kg) which can be used as soil conditioner. Trial 2 was observed highest temperature and considerable degradation as compared to trial 1 and 3. Further, the compost quality was well within the range of international compost quality standards. (Annexure I)

4.3 PHASE III - EFFICACY OF ROTARY DRUM COMPOSTING FOR STABILIZING VEGETABLE WASTE DURING PRE-COMPOSTING AND VERMICOMPOSTING TECHNOLOGY

In the present study direct utilization of vegetable waste using vermicomposting and efficacy of rotary drum composting followed by vermicomposting was studied for shortening the time duration of composting and producing nutrient rich compost. The experiments were conducted in three trials based on the (5:4:1) ratio. Trial 1 and 2 were performed for vermicomposting using *E. fetida* and *E. eugenia* for 45 days; trial 3 was performed for drum composting for 8 days and followed by vermicomposting using *E. fetida* for another 20 days (Table 3.6).

4.3.1 PHYSICO-CHEMICAL PARAMETERS

- **pH and EC**

The pH of the initial raw material greatly influences the composting and vermicomposting process. pH ranging from slight acidic (6.5) to neutral range (8.0) is optimum for composting and vermicomposting (Kalamdhad et al., 2009; Garg et al., 2012). The pH of all the trials was observed to increase due to the degradation of organic matter to the release of organic acids and by nitrogen and phosphorous mineralization. Initially, the pH values were in the range of 6.10, 6.36 and 6.40 and finally increased to 7.6, 7.5 and 7.08 in trials 1, 2 and 3 respectively (Fig. 4.31). There were not many changes in pH during the day 0 to 15 in trial 1 and 2, which can be considered due to the adaptation period of earthworms towards the waste. However in trial 3, gradual increase in pH was observed which might be due to proper combination of waste materials by which the microbial activity started very early to act upon the waste thereby cutting off the lag phase. The increase of pH in trial 3 can be considered majorily during the composting period of 8 days as compared to vermicomposting.

The pH increase in drum composting is dependent on the extent of organic matter degradation and the release of organic acids during the microbial degradation, CO_2 released during microbial decomposition, ammonification of organic nitrogen and proper agitation. Hence it can be stated that the increase of pH can be occurred during the 8 days of drum composting period. Moreover, these actions will result in the buffering capacity of the compost, thereby limiting the major fluctuation of the pH. The increase in pH can also be considered due to the formation of ammonium ions during the degradation of organic matter (Ndegwa and Thompson, 2000; Pramanik et al., 2007). EC of all the trials increased due to the release of minerals such as phosphate, ammonium and potassium due to the degradation of organic matter to the form of cations in the final compost (Kaviraj and Sharma, 2003). Initially EC was observed as 3.51, 3.58 and 3.7 dS/m in trial 1, 2 and 3 respectively; with the degradation of organic matter and release of mineral cations the value has increased to 4.0, 3.6 and 4.1 dS/m at the end of the process (Fig. 4.31). The increase in EC during vermicomposting of vegetable waste mix was supported by the reports of Garg et al. (2006a) and Khwairakpam and Kalamdhad (2011).

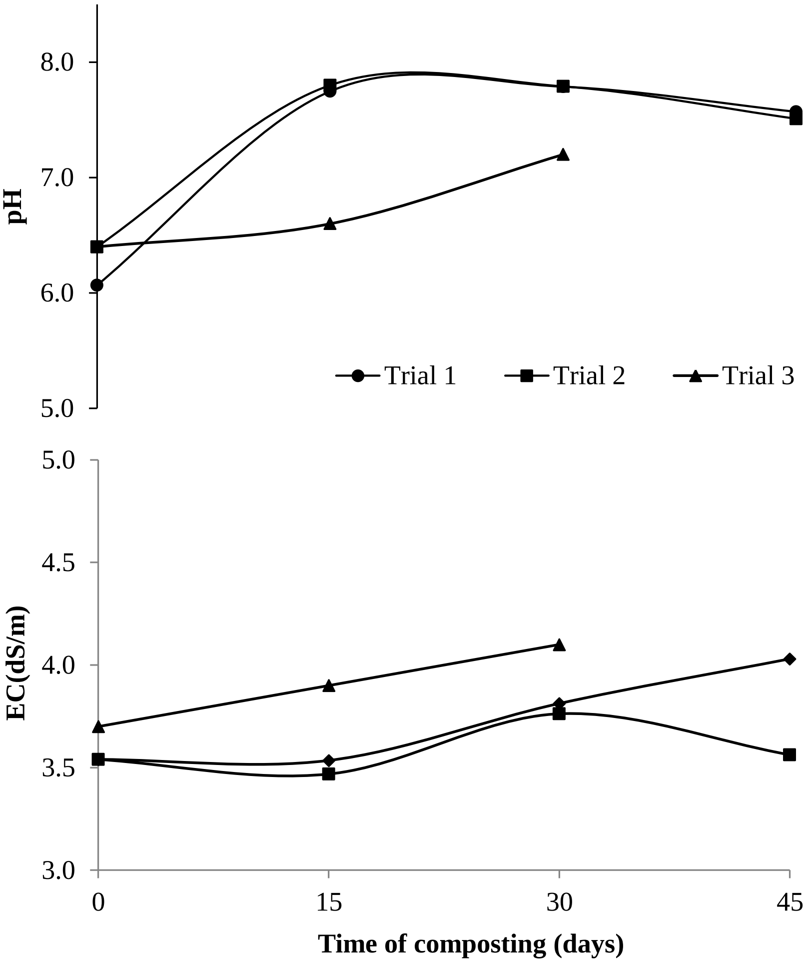

Fig. 4.31. pH and EC variation during composting period

- **Total organic carbon (TOC) reduction**

Fig. 4.32 shows the loss of TOC in all the trials during the process. The loss of TOC can be considered due to the degradation of organic carbon as CO_2 evolution by the synergetic action of microorganisms and earthworms during the process. Due to proper combination of waste materials and optimum growth conditions, it increased the activity of microorganisms and earthworms to utilize the waste materials more efficiently. Therefore, TOC was observed with a maximum reduction of 15.4 % in trial 3 followed by 10.4 and 9.2% in trial 1 and 2 respectively. The higher reduction of TOC in trial 3 can be considered due to the application of rotary drum composter for the stabilization of vegetable waste at thermophilic conditions. During the eight days of drum composting, a maximum of 63.4°C (Fig. 4.32) was observed due to higher microbial activity.

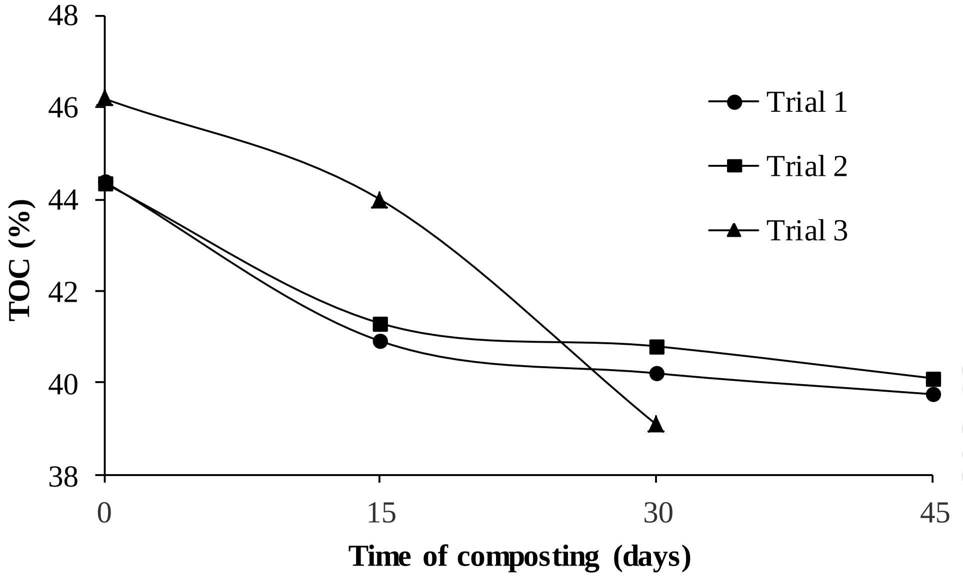

Fig. 4.32. TOC variation during composting period

It was found that, 3.14% of TOC was degraded within 8 days of composting period. The degraded portion contributes to about 20.2% of the overall reduction in 28 days. Moreover, the material has been partially degraded by the action of indigenous microorganisms and gone through higher thermophilic stage. Hence, applying this prestabilized and partially degraded waste material with optimum pH to earthworms, the earthworms were easily adopted to the environment to start away the degradation process. However with the direct utilization of vegetable waste for the vermicomposting required 15 days of acclimatization period, with only 2 and 3% TOC loss during that period in trial 1 and 2, respectively.

- **Nitrogen dynamics**

The initial TKN was in the range of 1.50, 1.52 and 1.62% in trial 1, 2 and 3 respectively. The higher percentage of TKN in the initial waste materials can be considered due the presence of vegetable waste and cow dung, which are rich in organic nitrogen. The higher nitrogen content in vermicompost is dependent on the initial nitrogen present in the feed stock and the degree of degradation (Crawford, 1983). Such increase in TKN was observed in the present study with 67.3, 47.3 and 63.5% increase in trial 1, 2 and 3 respectively. Hence at the end of process, the TKN was found to be 2.51 and 2.24% in trial 1 and 2. The increase in nitrogen levels during vermicomposting is attributed by the action of earthworms; during digestion in their gut they add their nitrogenous excretory products, mucus, body fluid, enzymes, and furthermore the decaying dead tissues of worms in vermicomposting subsystem (Suthar, 2007). Moreover, the biomass production in trial 1 is higher, which can be considered due to the proper availability substrate to the earthworms.

Therefore, it can be considered that *E. fetida* utilized the substrate for its growth and reproduction, thereby raising the levels of nitrogen in trial 1. In addition, the trial 3 is also observed with higher levels of nitrogen in which *E. fetida* was employed for 28 days of treatment. Hence, it can be concluded that *E. fetida* utilized the substrate better than the other worm. Hence during the process, due to the mineralization of carbon rich materials and also by the action of nitrogen fixing bacteria, the nitrogen content of the vermicompost can be increased (Plaza et al., 2007). However in trial 3, the TKN value after drum composting was observed to be 1.76% and after vermicomposting it was found to be 2.65% at the end of 28 days. NH_4-N showed a declining trend towards the end of process in all the trials (Fig. 4.33). The decrease in ammoniacal nitrogen might be considered due to the increase of pH and volatilization of ammonia (Kalamdhad et al., 2009). Similar results were observed in the present study with reduction in NH_4-N from 0.45, 0.51 and 0.73% to 0.21, 0.25 and 0.19% in trials 1, 2 and 3, respectively. Hence the application of rotary drum composter followed by vermicomposting can produce stabilized compost within shorter time period.

- **Phosphorous dynamics**

Phosphorous concentration in the final vermicompost plays as an essential nutrient for the photosynthesis of plants and it is available to plants as inorganic ions i.e. the orthophosphates (Hesse, 1971). During vermicomposting, even the unavailable forms of phosphorous can be converted to available forms in the final vermicompost that are easily available for the plants (Ghosh et al., 1999). Hence during the study, TP was observed to increase from 1.21, 1.29 and 2.52 g/kg to 1.71, 2.01 and 4.42 g/kg in trials 1, 2 and 3 respectively. Similarly, available phosphorous (AP) was observed to increase from 0.51, 0.53 and 1.82 g/kg to 1.19, 1.42 and 2.56 g/kg in trials 1, 2 and 3 respectively at the end of vermicomposting (Fig. 4.33). The increase in TP and AP can be considered due to the degradation of organic matter to organic acids, which further solubilizes the insoluble phosphorous and thereby increasing the phosphorous concentration in the vermicompost (Pramanik et al., 2007; Nayak et al., 2013). Acid phosphatases and alkaline phosphatases

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are the major earthworm gut enzymes involved in the conversion of phosphorous to more available forms. Also phosphorus solubilizing microorganisms present in earthworm's casts can also be considered for the release of phosphorus during vermicomposting (Le Bayon and Binet, 2006; Prakash and Karmegam, 2010).

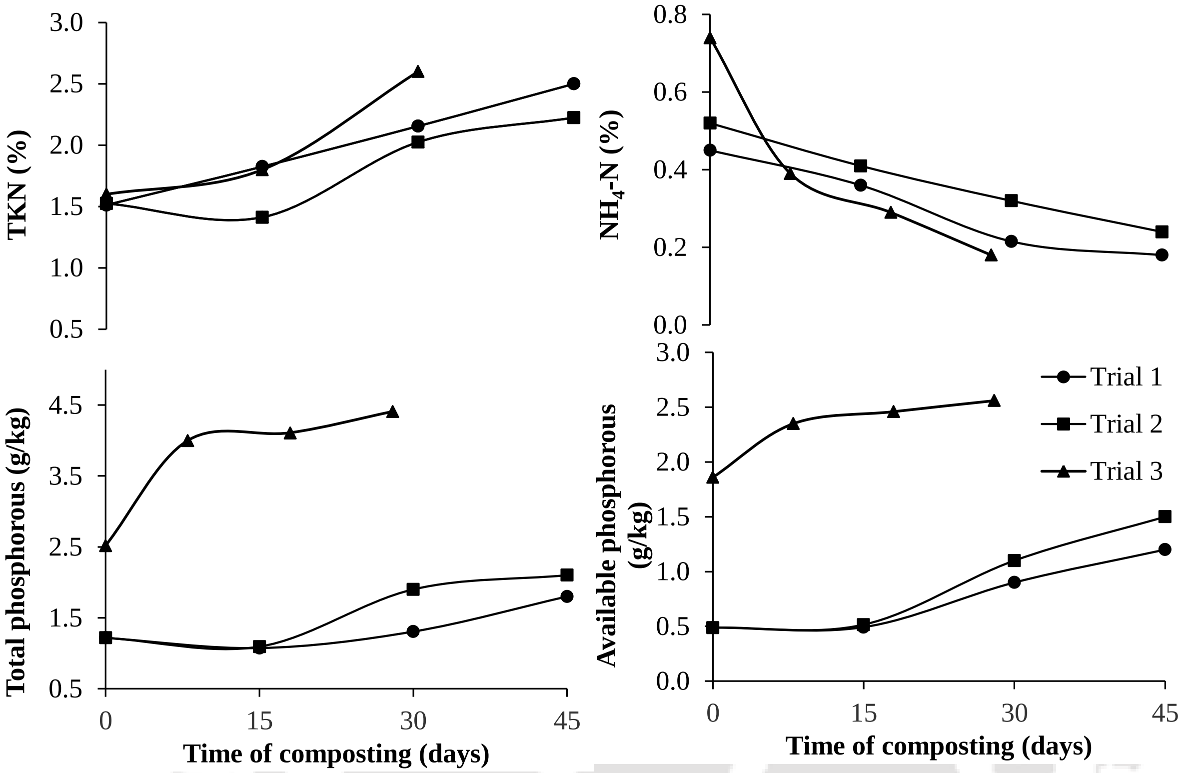

Fig. 4.33. Nitrogen and phosphorous dynamics during composting

- **Micronutrients and metals**

Table 4.8 provides the micronutrient and metal concentration during vermicomposting of all the three trials. These micronutrients and heavy metals were observed in considerable amount in the initial waste materials. It was observed that the micronutrients such as Na, K, Ca and Mg were observed to increase and heavy metal concentration of Cr, Cd, Co, Pb and Fe were observed to decrease towards the end of vermicomposting (Fang and Wong, 1999). The increase and decrease of the total micronutrient and heavy metal concentration during vermicomposting was in accordance with the reports of Kaushik and Garg, (2003); Suthar, (2009); Suthar and Singh, (2008). In addition, the decrease and increase in total metal concentration can be considered due to the uptake of metals into the earthworm gut and also by the degradation of organic matter and loss of net weight as supported by many reports (Garg and Gupta, 2011; Singh and Kalamdhad, 2013).

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Table 4.8 Micronutrient and metal concentration during vermicomposting

Days	**Na(g/kg)**			**k(g/kg)**			**Ca(g/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Initial	2.26 ± 0	2.26 ± 0	3.34 ± 0.12	10.41 ± 0	10.41 ± 0	14.86 ± 0.34	5.77 ± 0	5.77 ± 0	6.62 ± 0.22
Final	2.24 ± 0.11	2.35 ± 0.21	3.86 ± 0.14	10.33 ± 0.13	9.81 ± 0.18	16.45 ± 0.22	5.79 ± 0.16	5.99 ± 0.29	8.82 ± 0.32
Days	**Cd(mg/kg)**			**Cr(mg/kg)**			**Cu(mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Initial	61.25 ± 0.25	60.7 ± 0.25	52.26 ± 0.86	12.25 ± 0.75	12 ± 0.5	2.26 ± 0	77.25 ± 0.18	78.3 ± 0.13	66.4 ± 0.21
Final	59.63 ± 0.63	58 ± 1.75	51.22 ± 0.11	7.63 ± 0.63	17.88 ± 5.88	2.24 ± 0.11	49.25 ± 3.01	47.8 ± 5.13	52.2 ± 0.42
Days	**Mn(mg/kg)**			**Ni(mg/kg)**			**Pb(mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Initial	706.2 ± 0.5	707.5 ± 0.25	686 ± 2.24	263.2 ± 0.25	264.25 ± 0.25	221.6 ± 0.56	970.5 ±0.5	970 ±0.5	885 ± 0.86
Final	744.2 ± 4.7	767.5 ± 49.75	721 ± 0.23	280.2 ± 9.75	275.38 ± 15.63	236.2 ± 0.32	941.8 ±11.38	934 ±49.88	926.4 ± 0.54
Days	**Mg(g/kg)**			**Fe(mg/kg)**			**Zn(mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Initial	5.96 ± 0.01	5.71 ± 0.11	4.86 ± 0.32	4370 ± 8	4371 ± 2.75	4698 ± 2.24	171 ± 0.25	172.25 ± 0.25	152.2 ± 0.42
Final	7.25 ± 0.08	6.35 ± 0.06	5.24 ± 0.21	3339 ± 430	3502 ± 725	3689 ± 360	197 ± 11	188.38 ± 4.13	163.3 ± 0.26

Note: (mean ± SD, n=3) SD - standard deviation

The total concentration of micronutrients of the final compost increased in the range of about 5-16% for Na, 0-11% for K, 1-33% for Ca and 8-22% for Mg. The total concentration of metals in the final compost were observed to decrease in the range of about 2.5-3.3% for Cd, 1-37% for Cr, 21-36% for Cu, 2.5-3.7% for Pb and 19-24% for Fe. The increase in calcium content can be considered due to mineralization process by the earthworms, where they convert a proportion of calcium from binding form to free forms, resulting in its enrichment in worm casts (Yadav and Garg, 2011). Metals like Fe, Zn, Ca and Mg have been reported to be of bio importance to humans in their daily medicinal and dietary allowances (Duruibe et al., 2007).

4.3.2 Biological parameters

- **Earthworm count**

Initially 180 adult earthworms were added to all the trials. As the vermicomposting progressed, the number of earthworm count was increased towards the end of process. The total number of earthworms was 656, 307 and 388 in trial 1, 2 and 3 respectively at the end of vermicomposting process. The number of cocoons was in the range of 288, 12 and 132 in trial 1, 2 and 3 respectively. The number of juveniles and adult worms were in the range of 83 and 285 in trial 1, 20 and 275 in trial 2, 39 and 217 in trial 3 at the end of vermicomposting. The total number included the numbers of cocoons, juvenile and adult worms as explained above. The average initial weight of the earthworm i.e. on 0 day was 0.52 g (total weight 93.6 g) for trial 1 and 3 which was increased to 0.61 g (total weight 173.85 g) and 0.61 g (total weight 132.37 g) on the final day, respectively. The final average weight gain will be 1.78 and 1.94 g/earthworm day, respectively. Similarly, the average initial weight of the earthworm in trial 2 was 0.56 g (total weight 100.8 g) which was increased to 0.64 (total weight 176 g). The final average weight gain was observed to be 1.67 g/earthworm day. Even though the number of earthworm is huge in trial 1, the vermicomposting period was carried out for 45 days, however with the use of drum composting followed by vermicomposting, the number of worm count were found to be 380 which was achieved within 20 days as compared to trial 1. Hence it can be proposed that, pre-treatment of vegetable waste along with bulking agents using drum composting can be beneficial for successful treatment before vermicomposting instead of direct utilization.

- **Soluble COD and BOD**

The decomposition matter of vegetable waste mixture during composting can be directly measured as soluble COD and BOD. As the composting progressed, the biological organic content is degraded and the BOD and COD values were decreased. Soluble COD values decreased from 38.20, 38.60 and 39.37 g/kg to 13.6, 16 and 23.4 g/kg of wet waste in trial 1, 2 and 3 respectively.

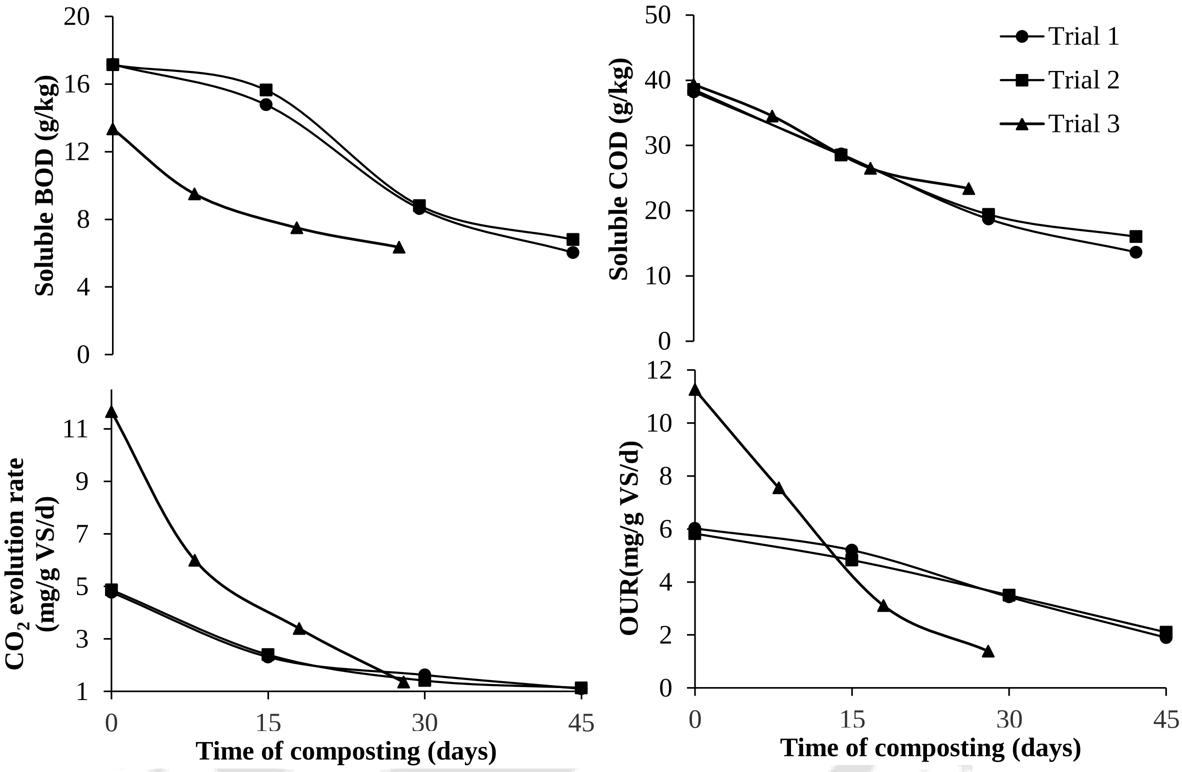

Fig. 4.34. Soluble BOD, COD, CO_2 and OUR variation during composting period

A maximum of 64.3 and 58.5% reduction was observed in trial 1 and 2 during the 45 days period, however trial 3 was observed with 40.6% reduction of soluble COD within 28 days of treatment period. Correspondingly, soluble BOD values decreased from 17.2, 17.1 and 14.35 g/kg to 7.41, 7.83 and 6.45 g/kg of wet waste in trial 1, 2 and 3 respectively, at the end of composting period (Fig. 4.34). A maximum of 56.9 and 54.2% reduction of soluble BOD was observed in trial 1 and 2, followed by 55.1% reduction in trial 3 within 28 days of treatment period. Therefore, with proper combination of waste materials and higher microbial activity in the gut of earthworms have played an important role in the degradation of organic content during the process.

- **Stability analysis**

CO_2 evolution and oxygen uptake rate (OUR) can be directly correlated to the stability of the compost during composting (Gomez et al. 2006; Kalamdhad et al. 2008). Compost stability is an important aspect of compost quality as it determines the compost nuisance potential, nitrogen immobilization and phytotoxicity (Lasaridi and Stentiford, 1998; Hogg et al., 2002). CO_2 evolution rates decreased from initial values 4.8, 4.9 and 11.65 mg/g VS/day to 1.1, 1.1 and 1.35 mg/g VS/day, respectively in trial 1, 2 and 3 (Fig. 4.34). The OURs of trial 1, 2 and 3 decreased from initial values of 6.0, 5.8 and 11.26 mg/g VS/day to 1.9, 2.1 and 1.39 mg/g VS/day in trial 1, 2 and 3 respectively (Fig. 4.34). The rate of evolution was found very low or proceeding the same manner during the final days of vermicomposting stating its stability. The lower CO_2 evolution rate and OUR in the final stages of composting was considered due to unavailability of readily available organic matter. A maximum of 88 and 87% reduction in CO_2 evolution and OUR was observed in trial 3 as compared to other two other trials, stating the stabilization of compost.

- **Total and fecal coliform**

The average number of total coliform (TC) was initially observed in the range of 2.1×10^{11}, 1.5×10^{11}, and 1.6×10^{12} MPN/g of wet weight in trial 1, 2 and 3 and finally reduced to 4.6×10^{4}, 2.4×10^{4}, and 2.1×10^{3} MPN/g at the end of vermicomposting. However, the fecal coliform (FC) were in the order of 2.4×10^{7}, 4.6×10^{7} and 1.5×10^{6} MPN/g in trial 1, 2 and 3 and reduced to 1.5×10^{2}, 2.1×10^{2} and 0.75×10^{2} MPN/g respectively at the end of vermicomposting. The recommended fecal coliform and streptococci densities for compost hygienization are 5.0×10^{2} and 5.0×10^{3} MPN/g, respectively (Vuorinen and Saharinen, 1997). The reduction of coliform bacteria can be considered due to the exposure to a microbiologically active environment and the secretion of virucidal enzymes by the earthworms during the digestion process. Moreover, higher temperature in the drum composter and lack of substrate to the coliforms also played a major role in the reduction of total number of pathogens.

4.3.3 CONCLUSIONS

From the study it can be concluded that pile composting of vegetable waste was found successful with the combinations of cow dung, sawdust and dry leaves. However, comparing various operational methods showed different degradation pattern during the 30 days of composting period. Trial 2 and 3 operated in passive and forced aeration mode were observed with higher degradation of volatile solids (19.6 and 22.9%). In addition, the final compost was observed with higher nutrient value i.e. Na (1.36-1.86 g/kg), K (17.22-19.14 g/kg), Ca (9.26-13.12 g/kg) and Mg (4.22-7.24 g/kg) which can be used as soil conditioner. Trial 2 was observed highest temperature and considerable degradation as compared to trial 1 and 3. Therefore, it can be concluded passive mode operation of vegetable waste composting could be more successful for higher degradation and waste minimization.

The efficacy of rotary drum composting over direct utilization of vegetable waste during vermicomposting by using *E. fetida* was proved efficient with higher loss of TOC and higher count of earthworm biomass at the end of process. Moreover the time duration of the process has been shortened to 28 days as compared to 45 days with direct utilization of vegetable waste. Higher reduction in organic carbon, soluble COD and BOD was observed with the application of rotary drum. Moreover the final end product was completely stabilized with lower OUR and CO_2 evolution at the end of vermicomposting. Therefore, it can be concluded that prestabilization of vegetable waste along with cattle manure and other bulking agents using rotary drum at thermophilic conditions proved as a beneficial alternative for the stabilization of vegetable waste before vermicomposting.

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Chapter 5

CARBIDE SLUDGE ADDITION AND FUNGAL INOCULATION DURING COMPOSTING

This chapter explained phase 4 as mentioned in chapter 3 (3.1 Experimental design section). The effects of waste carbide sludge addition and fungal inoculation were studied during rotary drum composting and vermicomposting. The study was conducted in three sections given as follows:

1. Effects of waste carbide sludge on nitrogen dynamics and stability of mixed organic waste using rotary drum composter
2. Carbon decomposition by inoculating *Phanerochaete chrysosporium* during drum composting
3. Effects of waste carbide sludge on earthworm growth and organic matter degradation during vermicomposting.

5.1 EFFECTS OF WASTE CARBIDE SLUDGE ON NITROGEN DYNAMICS AND STABILITY OF MIXED ORGANIC WASTE USING ROTARY DRUM COMPOSTER

The aim of the study was focused on the combination of different organic waste materials i.e., vegetable waste, cow dung, saw dust and dry leaves in the ratio 5:4:1 in four different trials by adding different proportions of waste carbide sludge (WCS); trial 1 (0%), trial 2 (1%), trial 3 (2%) and trial 4 (3%) as explained in Table 3.7. Effects of different proportions of waste WCS on physico-chemical changes and extent of degradation of organic matter was studied and successfully correlated with the stability of the compost i.e. OUR and CO_2 evolution.

5.1.1 PHYSICO-CHEMICAL PARAMETERS

- **Temperature**

Fig. 5.1 shows variation in temperature profile of all the four different trials during composting, indicating the effects of waste carbide sludge (WCS) amendment 0, 1, 2 and

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3% on the degradation pattern. A gradual increase in temperature was observed in all the four trials during the initial stage of composting.

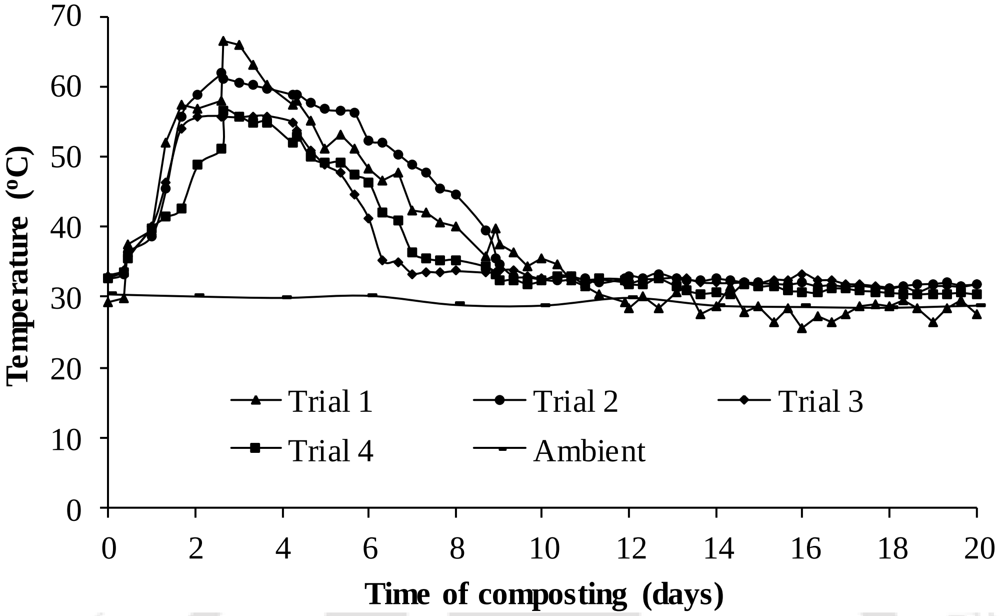

Fig. 5.1. Temperature variation during composting

However, as the composting progressed a variation in the temperature profile was observed which can be considered due to the addition of WCS. A maximum temperature of 66.5, 61.8, 55.8 and 51.4°C was observed in trial 1, 2, 3 and 4 respectively and started to decrease afterwards. During composting, temperature of 52 to 60°C was considered to maintain the greatest thermophilic activity (Mohee and Mudhoo, 2005). A sudden drop in temperature was observed in trial 3 and 4 during the second and third day of composting, in which higher amounts of WCS was added. But in case of trial 2, a maximum of 61.8°C temperature was achieved and observed to have prolonged thermophilic temperature above 50°C from day 2 to 7. However in trial 1, a maximum temperature of 66.5°C was observed, but the thermophilic phase did not continue for more than six days when compared to trial 2. Hence it can be considered that the prolonged thermophilic stage can be due to the increased metabolic activity of microbes by appropriate addition of WCS. Addition of lower WCS (1%) in trial 2, can be considered to provide a buffering capacity against pH drop and with suitable amount of Ca, which would have improved the metabolic activity of the microbes (Kubota and Nakasaki, 1991). Although higher temperature in compost environment was reported to have a major effect on pathogen control (Petrica et al., 2009); addition of lime was also

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reported to control their population by release of ammonia and higher pH (Wong and Selvam, 2009).

- **pH and EC**

In trial 1, the pH was around 6.7 which was observed to be least among all the other trials. With the addition of higher amount of WCS the pH was observed to increase and was in the range of 6.9, 7.1 and 7.6 in trial 2, 3 and 4 respectively. As the composting progressed there was a gradual increase in pH from 6.9 to 8.5 in all the trials (Fig. 5.2). Finally, it was in the range of 7.7, 7.8, 7.3 and 8.5 in trial 1, 2, 3 and 4 respectively at the end of 20 days. The increase in pH may be considered due to the release of ammonia, as the excess amount of organic nitrogen is not utilized by microbes and this might have increased the pH of compost (Rynk et al., 1992; Nayak et al., 2013). The similar pH increase and decrease during lime treatment of composting was reported by many researchers for different waste materials such as sewage sludge and water hyacinth (Fang and Wong, 1999; Wong and Selvam, 2006; Singh and Kalamdhad, 2013a). The pH of compost has a major effect on the survival of microbial communities with optimum range of 6.0 to 7.5 for bacteria and 5.5 to 8.0 for the growth of fungi (Golueke, 1972). But in case of trial 4, due to higher amount of WCS the pH level increased and thereby leading to the volatilization of ammonia.

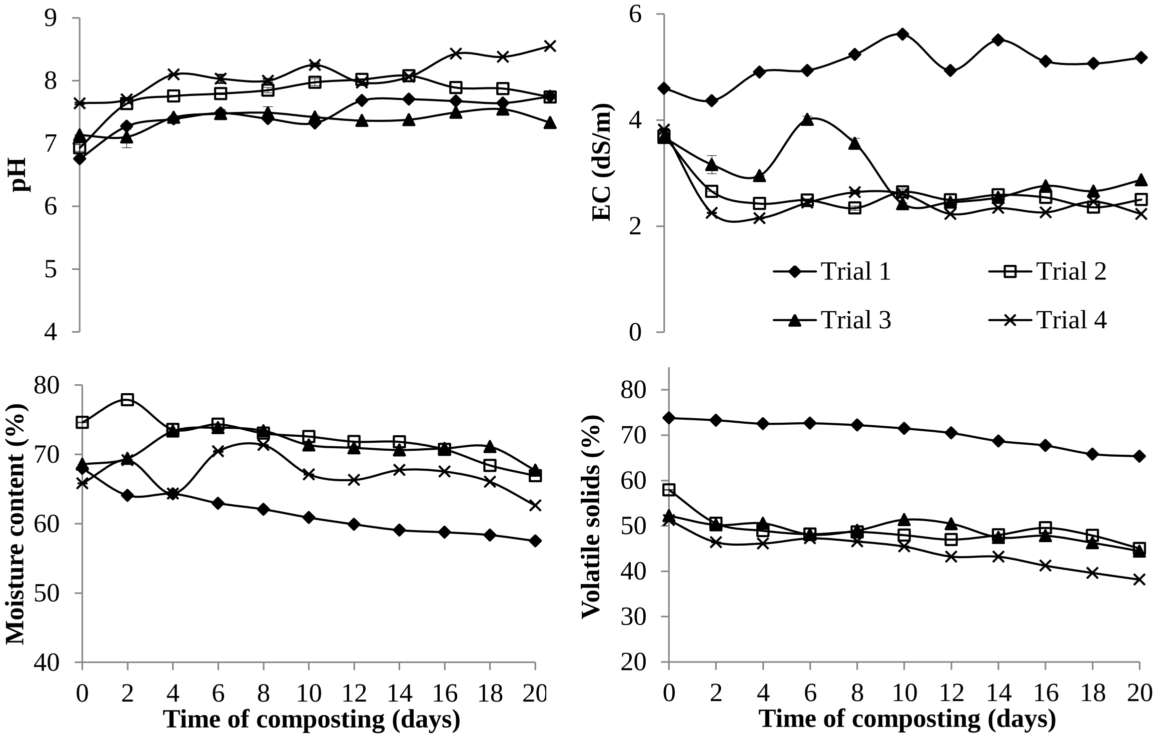

Fig. 5.2. Changes in pH, EC, moisture content and VS during composting

Hence, 1% WCS addition was observed to be optimum for the vegetable waste mixture composting, as higher amount of WCS leads to alkaline pH of the compost. The EC value of compost reflects the degree of salinity in the composting and its possible phytoxicity effects on the growth of plant. Higher EC in final compost will slow down plant rooting and reduce the transportation of water and nutrients into the plants (Chiang et al., 2007). However in the present study reduction in EC was observed in WCS added trials except trial 1. Similar trend of EC in lime amended compost was also monitored by Fang and Wong (1999). The final EC values of the all compost were about 5.18, 2.50, 2.87 and 2.23 dS/m in trial 1, 2, 3 and 4 respectively (Fig. 5.2). Richards (1954) has reported that EC values of all lime-amended sludge composts were lower than 3.0 dS/m, which would not affect the growth of slightly salt tolerant plants. Similar such results with final EC value less than 3.0 dS/m was observed in all the three lime amended compost and were also supported by the various literature (Fang and Wong, 1999; Singh and Kalamdhad, 2013b).

- **Moisture content**

Moisture loss during the composting process can be viewed as an indicator of decomposition (Kalamdhad and Kazmi, 2009). Due to the addition of WCS and high biodegradable organic matter of vegetable waste, rapid increase in temperature was observed in all the trials within few hours of composting process. Addition of WCS during composting process was reported to increase the metabolic activity without any negative effect on the microbial community (Fang and Wong, 1999; Gabhane et al., 2012). The reports were supported by the trial 2 results, where 1% WCS was added. However, in the case of trial 3 and 4, there was not much appreciable loss of moisture content. The reason might be due to the addition of excess amount of WCS, which had resulted in higher pH thereby causing the release of ammonia, since ammonium ions start to become unstable in the compost and is released as ammonia gas. In addition, the temperature profiles of trial 3 and 4 were also in accordance with the release of moisture content during the process. Fig. 5.2 shows the loss of moisture content during composting process. The moisture content was observed to reduce from 67.9, 74.6, 68.5 and 65.8% to 57.5, 66.9, 67.6 and 62.6% in the trial 1, 2, 3 and 4 respectively. Hence higher loss of moisture content (10.3%) was observed in trial 2 when compared to all other trials due to appropriate amount of WCS addition.

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- **Volatile solids reduction**

Fig. 5.2 shows the reduction in VS in all the four trials. The VS was observed to be in the range of 73.77, 57.9, 52.2 and 51.2% initially and finally reduced to 65.34, 45.1, 44.4 and 38.1% in the trials 1, 2, 3 and 4 respectively at the end of 20 days. The VS reduction was in the range of 11.4, 22.3, 24.9 and 25.6% in trial 1, 2, 3 and 4. Even though VS reduction was higher in trial 3 and 4 than the other two trials, the quality of compost with respect to pH, nitrogen and stability were not appreciable. The reason might be due to the addition of higher amount of WCS, alkaline hydrolysis of organic matter might have occurred instead of proper degradation, leading to higher VS reduction along with alkaline pH and loss of organic nitrogen at the end of composting period. Therefore, trial 2 can be considered to be the best trial with 1% addition of CS for higher degradation of vegetable waste mixture. A maximum of 22.1% of VS reduction was observed in trial 2 as compared to trial 1, where only 11.4% reduction was observed.

- **TOC reduction**

TOC is useful for estimating the age and physical properties of the compost. CO_2 is emitted from the composting mass as a metabolic end product during composting process. Thus, the organic carbon content decreases as the decomposition proceeds (Kalamdhad et al., 2009). Similar reports were observed in the present study with decrease in TOC as the composting progressed. Fig. 5.3 explains the significant reduction in TOC in all the four trials. Initially the amount of TOC was in the range of 40.9, 32.1, 31.1 and 31.3% and finally reduced to 36.3, 25, 24.6 and 21.1% in trial 1, 2, 3 and 4 respectively. Even though higher temperatures were not observed in trial 3 and 4, considerable amount of TOC reduction was observed in comparison to trial 2 which may be due to the addition of excess WCS in trial 3 and 4. However, trial 2 was observed to follow proper degradation pattern in comparison to all the other parameters. C/N ratio below 20 is indicative of proper compost, with a ratio of 15 or less being preferred (Van Heerden et al., 2002).

Fig. 5.3 shows the changes in C/N ratios during the composting period. In the present study, C/N ratio was reduced from 24, 22, 14 and18 to 16, 12, 19 and 14 in trial 1, 2, 3 and 4 respectively. However, combination of waste materials and addition of WCS played a major role in proper degradation with respect to C/N ratio. Out of all the trials, trial 2 was observed with higher C/N ratio reduction, resulting in C/N 12 at the end of 20 days composting period. C/N ratio between 10 and 15 in the compost indicates a good degree of maturity (Singh et al., 2009). The present study results were also observed with similar lower values of C/N ratio in trial 2 when compared to other three trials.

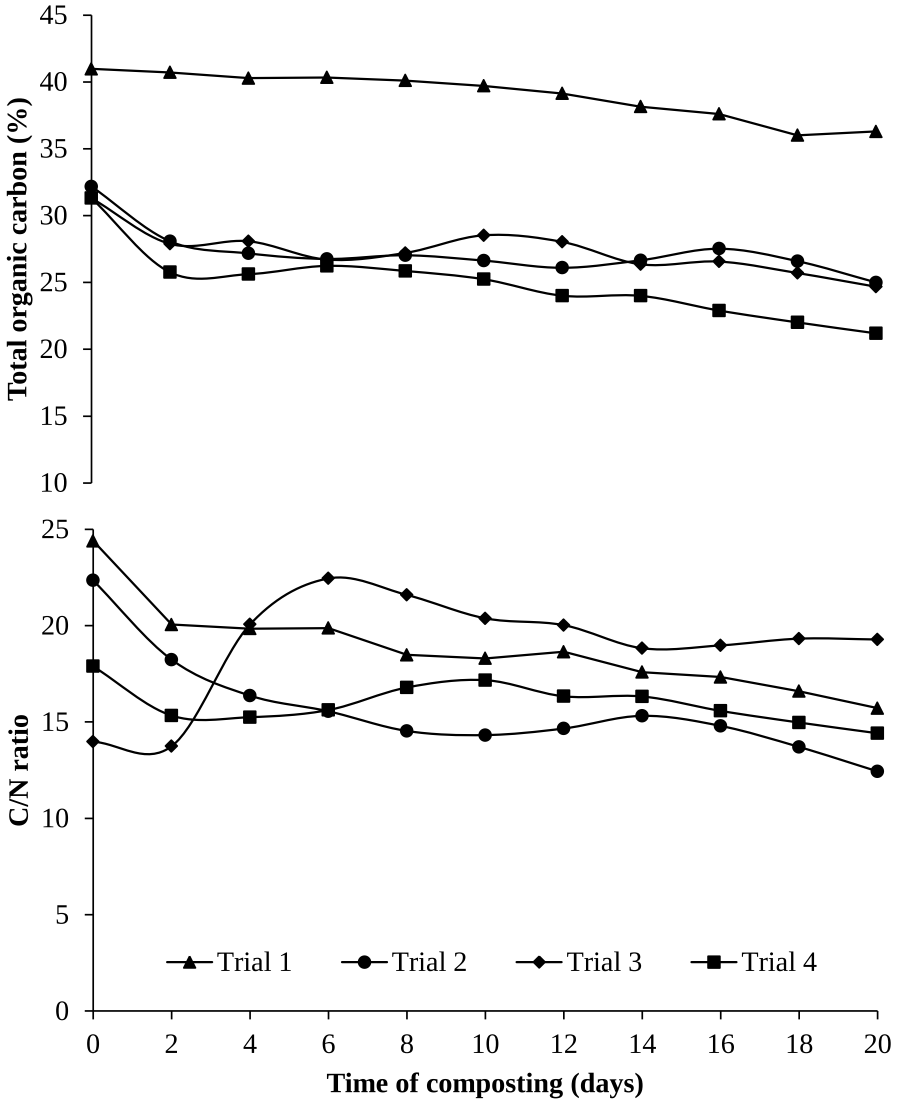

Fig. 5.3. Total organic carbon and C/N ratio during composting

- **Nitrogen dynamics**

During the composting process, organic nitrogen is considered to increase due to net loss of dry matter as CO_2 evolution and water loss by evaporation with the heat generated during oxidation of organic matter (Kalamdhad et al., 2009). In the present study, similar results were observed for organic nitrogen in the trial 1 and 2. However, in trial 3 and 4, gradual decrease in organic nitrogen was observed. The reason in reduction of organic nitrogen might be due to the excess amount of WCS addition that has increased the pH to alkaline conditions and favored the escape of ammonia gas. The decrease in organic nitrogen of trial 3 and 4 were in accordance with the increased amount of ammoniacal nitrogen during the composting period. Initially, organic nitrogen was observed to be in the range of 1.68, 1.44, 2.24 and 1.75% and finally was in the range of 2.31, 2.01, 1.28 and 1.47% in trial 1, 2, 3 and 4, respectively (Fig. 5.4). Except in trial 1 and 2, the other two trials were observed to lose organic nitrogen at the end of composting period which might be due to alkaline conditions created by the excess amount of LS addition. However, 39.5% increase of organic nitrogen was observed in trial 2, whereas in trial 3 and 4 reduction of 42.8 and 16% was observed. Correspondingly there was a gradual decrease of NH_4-N in trial 2 and increase in trial 3 and 4 was observed. During the initial stage of composting NH_4-N was in the range of 0.13, 0.26, 0.24 and 0.16% and finally was observed in the range of 0.09, 0.21, 0.33 and 0.25% in the trial 1, 2, 3 and 4 respectively at the end of 20 days (Fig. 5.4). The concentration of total nitrogen usually increases during composting when organic matter loss is greater and the present study results were in agreement with the data of Inbar et al. (1993) for trial 1 and 2. However in trial 3 and 4, there was a higher release of NH_4-N with gradual decrease in organic nitrogen. A maximum of 18% reduction of NH_4-N was observed in trial 2. However, in trial 3 and 4, 40 and 60% increase of NH_4-N was observed at the end of 20 days of composting period.

- **Phosphorous dynamics**

Addition of WCS had no effect on the available and total phosphorous (AP and TP) as the composting progressed. The release of phosphorous during composting process was considered due to the mineralization of organic matter (Kalamdhad et al., 2009). Higher amount of phosphorous was observed in the initial days of composting which might be due to higher amount of vegetable waste and cow dung. Hence, AP was observed in the range of 1.41, 1.49, 1.71 and 1.54%; and TP in the range of 1.49, 2.96, 2.75 and 3.06% in trial 1, 2, 3 and 4 respectively (Fig. 5.4). As the composting proceeded there was a gradual increase of both of AP and TP in all the four trials and was observed in the range of 1.49, 2.11, 2.34 and 2.16% in AP; and 4.3, 3.57, 2.94 and 3.78% in TP of trial 1, 2, 3 and 4, respectively at the end of 20 days. Overall there were 5.9, 41, 37 and 40% increase in AP and 26, 20, 7 and 23% increase of TP in trial 1, 2, 3 and 4, respectively at the end of 20 days. Table. 5.1 Illustrates the total concentration of micro nutrients (Na. K, Ca, Mg) and heavy metals (Cr, Cd, Ni, Pb, Fe, Mn, Zn and Cu) in trial 1, 2, 3 and 4 respectively. These nutrients and heavy metals were observed to increase during the composting with the loss of organic matter (Fang and Wong, 1999).

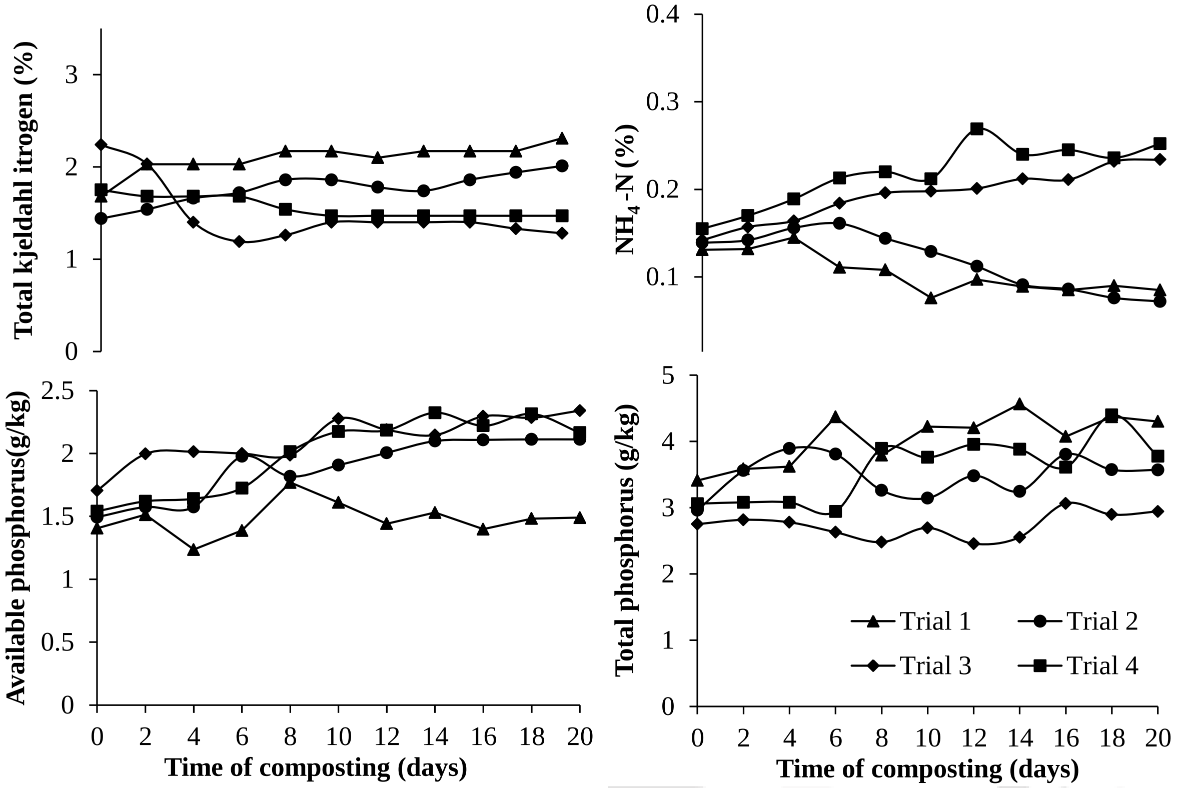

Fig. 5.4. Nitrogen and phosphorous dynamics during composting period

5.1.2 BIOLOGICAL ANALYSIS

- **Soluble BOD and COD**

The organic fractions in the compost mix can be directly measured as soluble BOD and COD. The percentage of the readily bio available organics has been considered important for the compost quality (Bernal et al., 1997). The organic fraction degradation can be measured by the decrease in soluble BOD and COD. The degradation of the organic fractions is mainly done by microorganisms during the composting process. The composting process is considered to proceed till the amount of these biodegradable organic matter is stabilized (Wang et al., 2004). Soluble BOD values decreased from 17.3, 12.6, 13.6 and 13.3 g/kg of wet waste to 4.9, 6.6, 7.5 and 6.4 g/kg in trial 1, 2, 3 and 4 respectively, at the end of 20 days of composting period (Fig. 5.5). Correspondingly, soluble COD values decreased from 44.4, 47.4, 47.4 and 45.6 g/kg of wet waste to 23.2, 16.5, 16.6 and 14.4 g/kg of wet waste in trial 1, 2, 3 and 4 respectively (Fig. 5.5). Therefore, with proper mixing and agitation higher degradation was carried out during the process by which the soluble BOD and COD were decreased drastically, resulting in decreased emission of carbon dioxide, clearly indicating the stabilization of compost.

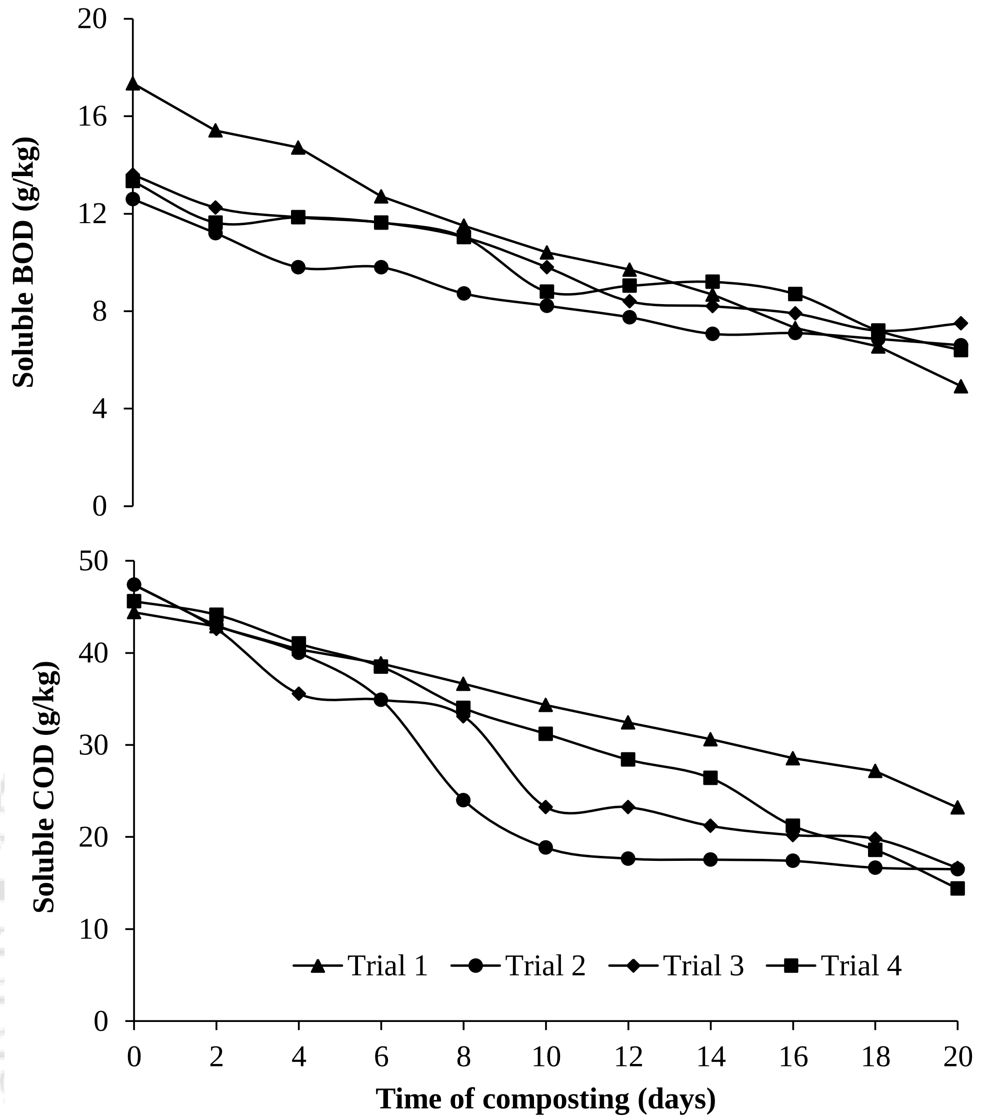

Fig. 5.5. Soluble BOD and COD variation during composting period

- **CO_2 evolution rate**

The microbial activity in the compost can be directly measured in terms of CO_2 evolution rate and the OUR. OUR and CO_2 evolution rate can be measured for stability of compost as unstable compost has high demand of oxygen and with high evolution of CO_2, thereby representing the instability of the compost (Bernal et al., 1997). Compost stability is an important aspect of compost quality as it determines the compost nuisance potential, nitrogen immobilization and phytotoxicity (Lasaridi and Stentiford, 1998). Hence with different combinations of waste material and WCS addition different degradation pattern was observed. The CO_2 evolution rates decreased from 14.97, 6.94, 5.64 and 7.24 mg/g VS/day to 2.28, 0.89, 2.30 and 3.11 mg/g VS/day in trial 1, 2, 3 and 4, respectively, at the end of 20 days (Fig. 5.6).

Table 5.1 Micronutrient and heavy metals during composting period

Day	**Sodium (g/kg)**				**Potassium (g/kg)**			
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
0	1.25 ± 0.14	0.834 ± 0.014	0.772 ± 0.061	0.807 ± 0.038	14.08 ± 0.40	5.527 ± 0.045	5.055 ± 0.049	4.922 ± 0.053
20	1.36 ± 0.04	1.447 ± 0.031	1.455 ± 0.056	1.297 ± 0.088	25.67 ± 0.67	5.210 ± 0.028	8.227 ± 0.314	7.681 ± 0.141
Day	**Calcium (g/kg)**				**Magnesium (g/kg)**			
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
0	8.27 ± 0.80	7.912 ± 0.053	13.910 ± 0.268	24.945 ± 0.219	5.40 ± 0.33	5.10 ± 0.21	6.42 ± 0.25	6.66 ± 0.33
20	10.36 ± 0.94	8.671 ± 0.134	15.482 ± 0.579	32.245 ± 0.572	7.41 ± 0.32	6.99 ± 0.33	7.85 ± 0.11	8.12 ± 0.45
Day	**Chromium (g/kg)**				**Copper (g/kg)**			
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
0	0.054 ± 0.004	0.062± 0.001	0.074 ± 0.002	0.084 ± 0.002	71.00 ± 0.71	0.077 ± 0.002	0.085 ± 0.001	0.142 ± 0.004
20	0.039 ± 0.003	0.071± 0.002	0.096 ± 0.003	0.091 ± 0.001	68.50 ± 0.71	0.089 ± 0.002	0.095 ± 0.001	0.328 ± 0.010
Day	**Nickel (g/kg)**				**Lead (g/kg)**			
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
0	0.184 ± 0.010	0.265 ± 0.005	0.310 ± 0.004	0.298 ± 0.004	0.99 ± 0.06	0.839 ± 0.002	0.917 ± 0.004	0.871 ± 0.004
20	0.191 ± 0.002	0.303 ± 0.003	0.277 ± 0.003	0.306 ± 0.002	0.96 ± 0.05	0.902 ± 0.003	0.844 ± 0.003	0.886 ± 0.010
Day	**Iron (g/kg)**				**Manganese (g/kg)**			
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
0	11.80 ± 0.62	5.511 ± 0.101	5.617 ± 0.031	5.671 ± 0.637	0.562 ± 0.005	0.443 ± 0.003	0.424 ± 0.004	0.404 ± 0.001
20	12.40 ± 0.05	5.682 ± 0.095	7.096 ± 0.033	5.642 ± 0.282	0.588 ± 0.004	0.419 ± 0.001	0.419 ± 0.001	0.493 ± 0.003
Day	**Zinc (g/kg)**				**Cadmium (g/kg)**			
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
0	0.235 ± 0.001	0.248 ± 0.010	0.193 ± 0.003	0.212 ± 0.005	0.15 ± 0.03	0.052 ± 0.001	0.058 ± 0.001	0.052 ± 0.001
20	0.295 ± 0.001	0.279 ± 0.002	0.168 ± 0.007	0.210 ± 0.014	0.05 ± 0.00	0.059 ± 0.001	0.047 ± 0.001	0.053 ± 0.002

Note: (mean ± SD, n=3) SD - standard deviation

- **Oxygen uptake rate (OUR)**

With higher biodegradable matter in vegetable waste and propagation of microorganisms, OUR will be observed high during the composting process (Iannotti et al., 1993). The rate of oxygen demand will decrease as less food will be available to the microorganisms as the compost proceeds. Similar findings were observed in the present study with initial values ranging in the order of 21.26, 10.05, 12.04 and 11.04 mg/g VS/day denoted higher microbial activity and finally reduced to 4.49, 3.21, 3.98 and 4.21 mg/g VS/day in trial 1, 2, 3 and 4 respectively (Fig. 5.6). The lower values of OUR at the end of 20 days clearly states that the vegetable waste has been degraded by the microorganisms and converted to a stabilized compost.

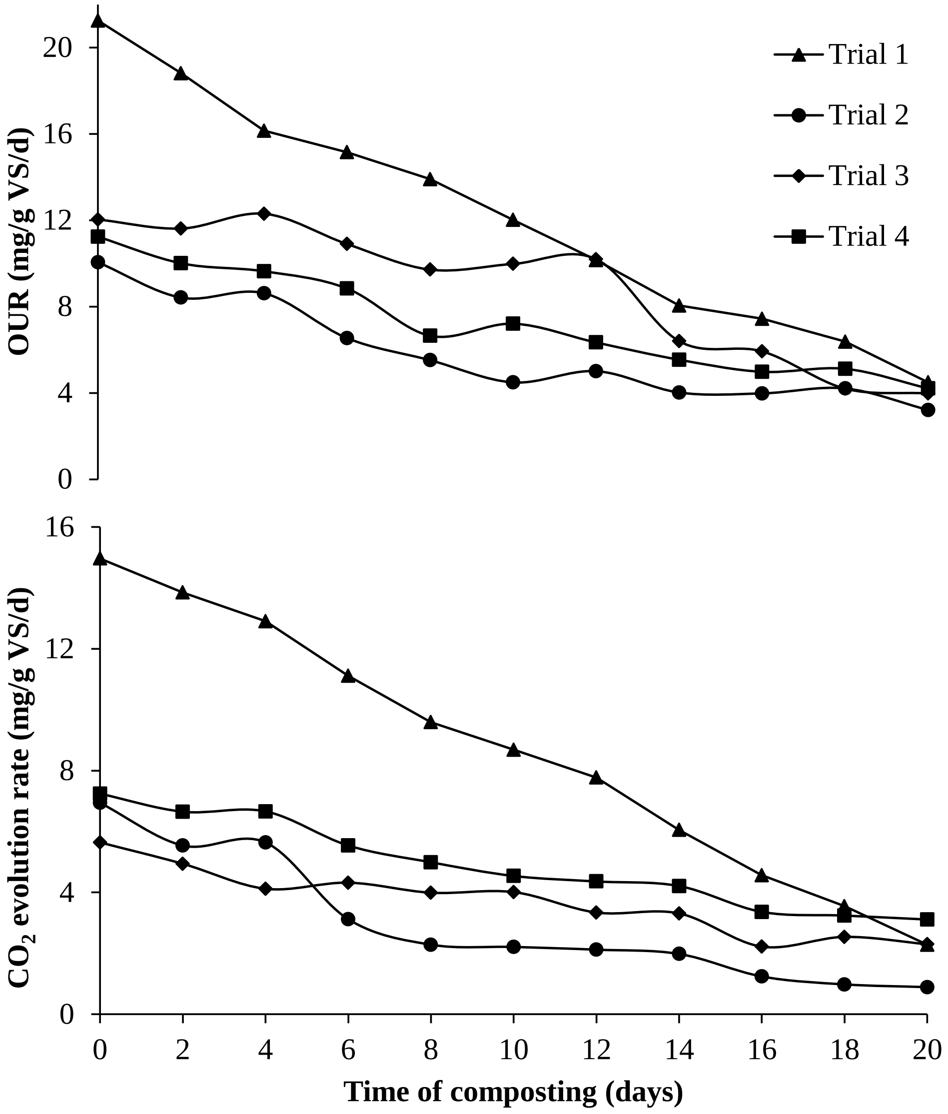

Fig. 5.6. Changes in CO_2 evolution and OUR variation during composting

- **Pathogens**

Higher temperature and addition of WCS during composting had major effect on the survival of indicator organisms (Wong and Selvam, 2009). The average number of total coliform (TC) was initially observed in the range of 0.93×10^{11}, 0.53×10^{12}, 0.14×10^{11} and 0.12×10^{11} MPN/g of wet weight in trial 1, 2, 3 and 4 respectively and finally reduced to 0.21×10^{3}, 0.15×10^{2}, 0.092×10^{2} and 0.11×10^{2} MPN/g respectively at the end of 20 days. However, fecal coliform (FC) was in the order of 0.14×10^{7}, 2.4×10^{6}, 0.75×10^{6} and 1.2×10^{5} MPN/g in trial 1, 2, 3 and 4 and reduced to 0.15×10^{2}, 0.93×10^{1}, 0.43×10^{1} and 0.06×10^{2} MPN/g respectively at the end of 20 days.

5.1.3 BIOCHEMICAL ANALYSIS

- **Hemicellulose (HC) and Cellulose (C)**

Lignocellulose is mainly composed of cellulose (C), hemicellulose (HC) and lignin. The lignocellulose is degraded by only a small group of microorganisms, which produce specific enzymes. These enzymes work by breaking the bonds that link the carbon atoms together within these molecules. During composting, HC is the first fraction utilized as carbon and energy source by the microorganisms and followed by cellulose. In contrast to cellulose, HC are easily hydrolysable polymers.

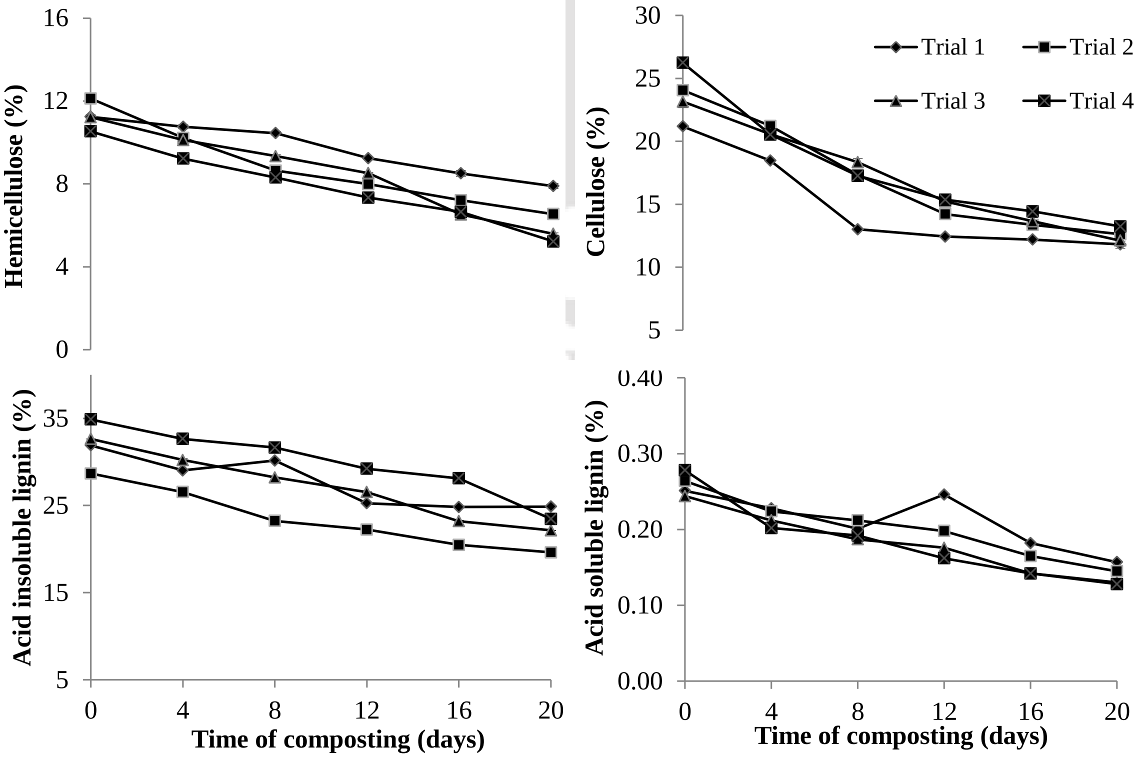

Fig. 5.7. Lignocellulose degradation during composting period

The ability to degrade hemicellulose is probably even more common than the ability to break down cellulose, although hemicellulose producing microbes commonly produce cellulase (Eriksson et al., 1990, Dix and Webster, 1995). Therefore, reduction in hemicellulose (HC) was found maximum in trial 4 (50.47%) followed by trial 3 (50.17%), trial 2 (46.03%) and trial 1 (29.7%) respectively due to higher microbial activity during the process. Most of the aerobic bacteria and fungi are reported to oxidize cellulose to CO_2 (Beguin and Aubert, 1994). The degradation is considered to carry out by endoglucanases and cellobiohydrolases, which hydrolyses the crystalline cellulose to cellobiose, and further the β- endoglucanase, degrades cellobiose to glucose. Therefore, with higher microbial activity during the process reduction in cellulose (C) was found maximum in trial 4 (49.54%) followed by trial 3 (47.66%), trial 2 (47.54%) and trial 1 (44.2%) respectively (Fig. 5.7). The higher reduction in HC during the present study was in accordance with the reports by Sarika et al. (2014).

- **Lignin**

Lignin is degraded to carbon dioxide, water and humus my most of the fungi. The degradation of lignin does not release any energy for the utilization of microorganisms, but the degradation enables efficient utilization of carbohydrates (Eriksson et al., 1990). Thus, microorganisms which degrade polysaccharides often secrete ligninolytic enzymes. Fig. 5.7 shows the degradation of acid insoluble lignin in compost environment. Lignin has a very complicated structure, which is highly resistant to microbial degradation (Hatakka, 2001). Degradation of lignin mainly occurs during thermophilic phase of composting. A maximum of 22.1, 31.56, 32.16 and 32.76% reduction of acid insoluble lignin was observed in trial 1, 2, 3 and 4 respectively. The higher degradation of acid insoluble lignin can be considered due to increased addition of carbide sludge. Although, higher reduction of lignin content was observed in trial 3 and 4, trial 2 was observed optimum with respect to prolonged thermophilic stage and higher nitrogen content without any loss. Hence, trial 2 can be recommended for higher reduction of lignocellulose with optimum addition of carbide sludge. Similarly, acid soluble lignin reduction increased with increasing lime amendment. The amount of acid soluble lignin present in compost was found to be very less when compared to acid insoluble lignin. The percentage reduction of acid soluble lignin at the end of 20 days was observed as 37.5, 45.07, 46.72 and 53.95% during trial 1, 2, 3 and 4 respectively.

5.1.4 MICROBIAL DIVERSITY

- **Mesophilic and spore forming bacteria**

The initial days of composting are dominated by mesophilic bacteria that are majorly involved in the breakdown of readily degradable organic matter and raising the temperature. These bacteria primarily has cellulolytic and pectinolytic activities, and limited efficiency in degrading lignin. Moreover they have a wider tolerance of temperature, pH, and oxygen limitations than fungi (Blanchette, 1995; Daniel and Nilsson, 1998). The initial load of mesophilic bacteria was in the range of 4.8×10^{11}, 5.6×10^{10}, 8.8×10^{10} and 9.6×10^{10} CFU/g and spore forming bacteria was in the range of 5.1×10^{7}, 4.4×10^{6}, 2.6×10^{7} and 3.2×10^{6} CFU/g in trial 1, 2, 3 and 4 respectively.

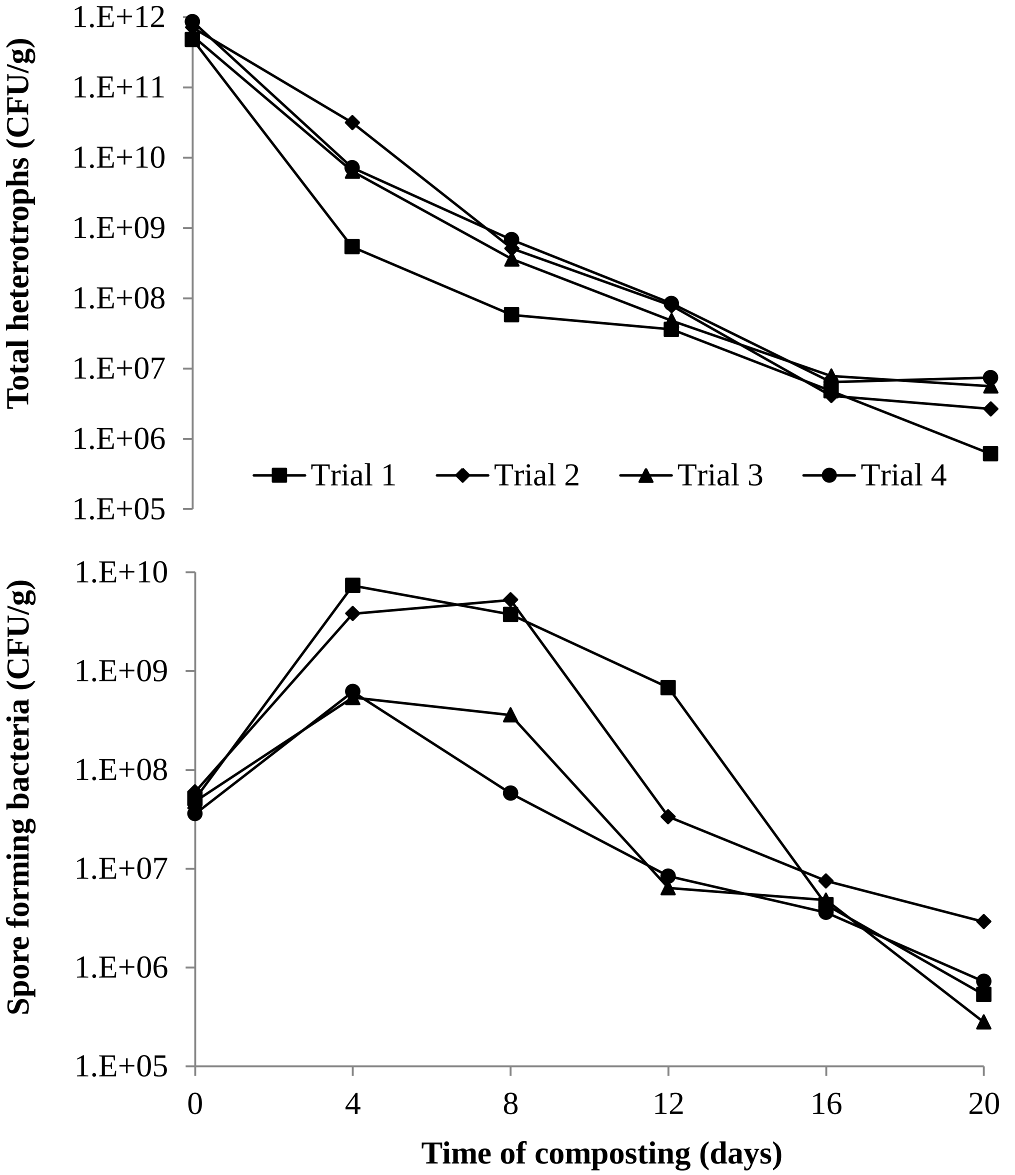

Fig. 5.8. Heterotrophic and spore forming bacteria during composting period

The higher population of these organisms during the initial days can be considered due to the higher addition of vegetable waste (45 kg) and cattle manure (36 kg) as supported by Gazi et al. (2007). Due to higher addition of highly degradable vegetable waste, the rise in temperature was observed within 18-24 h of composting with a maximum of 66.4 and 61.4^{o}C in trial 1 and 2 respectively. These higher temperatures are favourable in the breakdown of complex lignocellulosic fractions to simpler units. The higher temperature had an adverse effect in the growth of mesophilic and spore forming bacteria. With the rise in temperature, the mesophilic bacteria load reduced to 5.4×10^{8}, 4.2×10^{8}, 3.6×10^{9} and 7.4×10^{8} CFU/g; while the spore forming bacteria increased to 7.4×10^{9}, 4.6×10^{9}, 3.6×10^{8} and 5.2×10^{7} CFU/g in trial 1, 2, 3 and 4 during the 4th day of composting. Moreover, these bacteria are also involved in the degradation of wood including erosion, tunnelling and cavity formation (Blanchette, 1995; Daniel and Nilsson, 1998). Hence, with the depletion of readily degradable organic matter, the temperature of the compost started to reduce during the 7th day in trial 1 and 2; and 5 to 7 days in trials 3 and 4, so as the population of mesophilic and spore forming bacteria. Finally the load was in the range of 6.1×10^{5}, 2.4×10^{5}, 7.4×10^{5} and 2.6×10^{6} CFU/g for mesophilic and 5.3×10^{5}, 4.4×10^{5}, 3.2×10^{5} and 3.6×10^{5} CFU/g for spore forming bacteria in trial 1, 2, 3 and 4 respectively at the end of composting period (Fig. 5.8).

- **Actinomycetes and streptomycetes**

The population load of actinomycetes during composting is mainly dependent on the type of organic content material used and also on the physical conditions of the compost environment (Miyashita et al., 1982). Actinomycetes produce extracellular peroxidises i.e. lignin peroxidase-type enzyme (Mercer et al., 1996; Adhi et al., 1989) and *Streptomyces sp. EC1* produces peroxidise and cell-bound demethylase requiring H_2O_2 and Mn_2 which are involved in the degradation of lignocellulosic fractions (Godden et al., 1992). Due to higher percentage of lignocellulosic fractions in the compost mixture, the load of actinomycetes was found to be 6.0×10^{8}, 1.4×10^{8}, 3.6×10^{7} and 5.4×10^{7} CFU/g and streptomycetes as 6.6×10^{7}, 4.4×10^{7}, 6.4×10^{8} and 2.4×10^{8} CFU/g in trial 1, 2, 3 and 4 respectively, during the initial days of composting. These species are generally involved in degradation of recalcitrant organics such as lignocellulosic fractions (Rebollido et al., 2008).

Typically, Streptomyces spp. solubilizes part of lignin, and the end product is water-soluble acid-precipitable polymeric lignin (Crawford et al., 1983). The succession of actinomycetes during composting is observed similar to the reports of (Ryckeboer, 2003), and the similar growth pattern during composting can be considered mainly due to moisture content and C/N ratio. Hence at the end of composting, the load of actinomycetes and streptomycetes was in the range of 4.5×10^6, 4.4×10^5, 2.8×10^5 and 3.6×10^5 CFU/g; and 5.2×10^5, 3.6×10^4, 3.8×10^6 and 6.6×10^5 CFU/g in trial 1, 2, 3 and 4 respectively (Fig. 5.9).

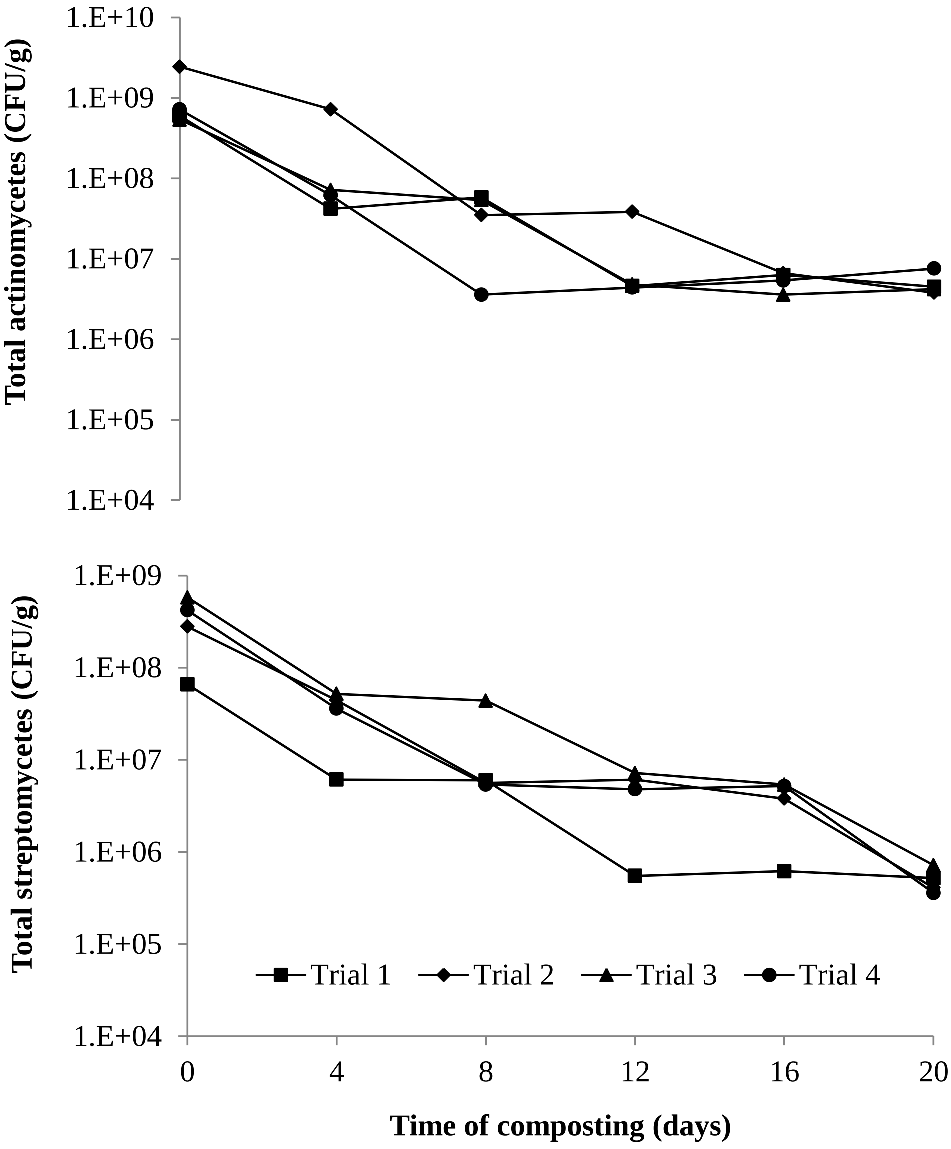

Fig. 5.9. Actinomycetes and streptomycetes variation during composting period

- **Fungi**

The survival of fungi in the compost environment is dependent on many carbon sources, mainly lignocellulosic fraction. They are mainly involved in the maturation of the compost and can survive at extreme conditions (Miller, 1996). Extracellular enzymes mainly involved in lignin degradation are lignin peroxidases (LiPs) and manganese peroxidases (MnPs), as well as laccases are secreted by most of the fungi. LiPs and MnPs are heme-containing glycoproteins which require hydrogen peroxide as an oxidant. LiP oxidizes nonphenolic lignin substructures by abstracting one electron and generating cation radicals that are then decomposed chemically (Kirk and Farrell, 1987). Due to higher nitrogen supplement from vegetable waste and lignocellulosic fractions from bulking agents, the initial fungal load was in the range of 5.4×10^{8}, 3.6×10^{7}, 5.4×10^{8} and 4.8×10^{7} CFU/g in trial 1, 2, 3 and 4 respectively. The higher load of mesophilic fungi during the initial days of composting were supported by the reports of Thambirajah et al. (1995) and Van Heerden et al. (2002); furthermore these populations might be dominated by *Aspergillus* and *Penicillium.* The rise in temperature during the thermophilic stage reduced the load of fungi up to 10^{2}-10^{3} CFU/g in all the trials. Even though decrease was observed in all the trials due to higher temperature and alkaline conditions, the remaining microbial population in the compost environment were resistant to a maximum growth temperature of about 40°C and prefers an alkaline environment (Dix and Webster, 1995; Deacon, 1997).

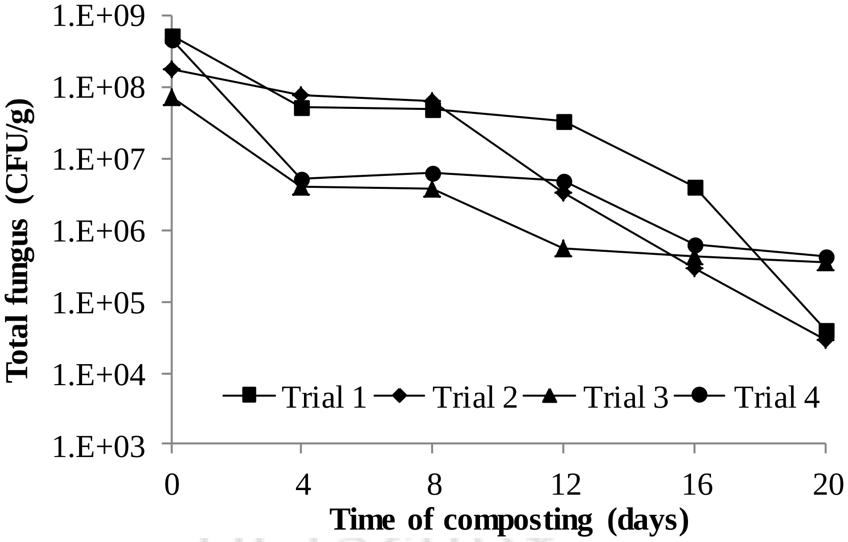

Fig. 5.10. Fungus variation during composting

Therefore, the final population of fungi was in the range of 4.1×10^{4}, 3.6×10^{4}, 3.6×10^{5} and 4.2×10^{5} CFU/g in trial 1, 2, 3 and 4 respectively at the end of 20 days due to optimum temperature and pH conditions favorable for their growth (Fig. 5.10). Thambirajah et al. (1995) have reported with 10^{4}-10^{6} per gram of mesophilic and thermophilic fungi at the end of composting stating the stability and maturity of compost. However, complete inactivation of fungi was not observed in the present study due to the presence of partially degraded lignocellulosic fractions in the compost. The depletion of fungi during thermophilic stage and later stages of composting can be considered due to the elevated temperature (76°C) (Ryckeboer, 2003), unavailability of carbon source and the type of composting method employed.

- **Pathogenic population**

The sanitary quality of the compost is indicated by the presence of pathogenic organisms. Temperature level above 50°C for more than 4 to 7 days during composting was reported to satisfy the regulatory requirements for Process to Further Reduce Pathogens (PFRP). Hence with such elevated temperature, *Salmonella* isolates reduced from 3.1×10^4, 2.5×10^4, 3.1×10^4 and 6.1×10^4 CFU/g to 36 CFU/g in trial 1, and zero in trial 2, 3 and 4 respectively; and Shigella isolates varied from 1.6×10^3, 1.1×10^4, 2.1×10^3 and 5.6×10^2 CFU/g to CFU/g in trial 1, and zero in trial 2, 3 and 4 respectively at the end of composting. Reduction in the number of pathogenic organisms can be considered due to the extended thermophilic stage and by the addition of waste carbide sludge which brought alkaline conditions of the compost. Higher temperature in compost environment and addition of CS was reported to control their population by release of ammonia and higher pH (Petrica et al., 2009; Wong and Selvam, 2009).

5.1.5 INSTRUMENTATION ANALYSIS

- **FTIR Spectroscopy**

FTIR spectra of compost provide reliable organic matter degradation with respect to aliphatic peak ratios and aromatic to polysaccharides with respect to the C/N ratio (Hsu and Lo, 1999). Fig. 5.11 provides the functional groups of the compost identified by FTIR spectra during initial and final days of composting. Interpretations of infrared spectra were based on the data of numerous studies by Quatmane et al. (2000), Droussia et al. (2009) and Kalyanaraman et al. (2013). Well defined characteristic bands at 1126 and 1045/cm are due to aromatic C-H in-plain deformation for syringyl and guaiacyl alcohols, which are the two structural components of lignin (Sjostrom, 1993). The enrichment of aromatic C=C as compared to aliphatic carbon during composting process can be explained by the reduction in ratios of aliphatic/aromatic carbon to polysaccharide. Decrease in aliphatic carbon to polysaccharide (2930/1030) was observed from 1.332 to 1.181, with highest of 11.34% reduction in trial 2 as compared to all other trials. The ratio of intensities of aromatic carbon to polysaccharide (1630/1030) decreased from 1.204 to 1.086, with percentage reduction of 9.81% in trial 2. Similarly, the intensity ratio of aliphatic carbon to aromatic carbon (2930/1630) decreased from 1.106 to 1.087 with percentage reduction 1.75%, in trial 2. The ratio of characteristic bands at 1126 and 1045 /cm of trial 1, was observed to reduce from 1.103 to 1.051 at the end of 20 days of composting period with 4.64% reduction. Therefore, it can be concluded that trial 2 was observed with higher reduction of aliphatic compounds and enriched with aromatic compounds after 20 days of composting period.

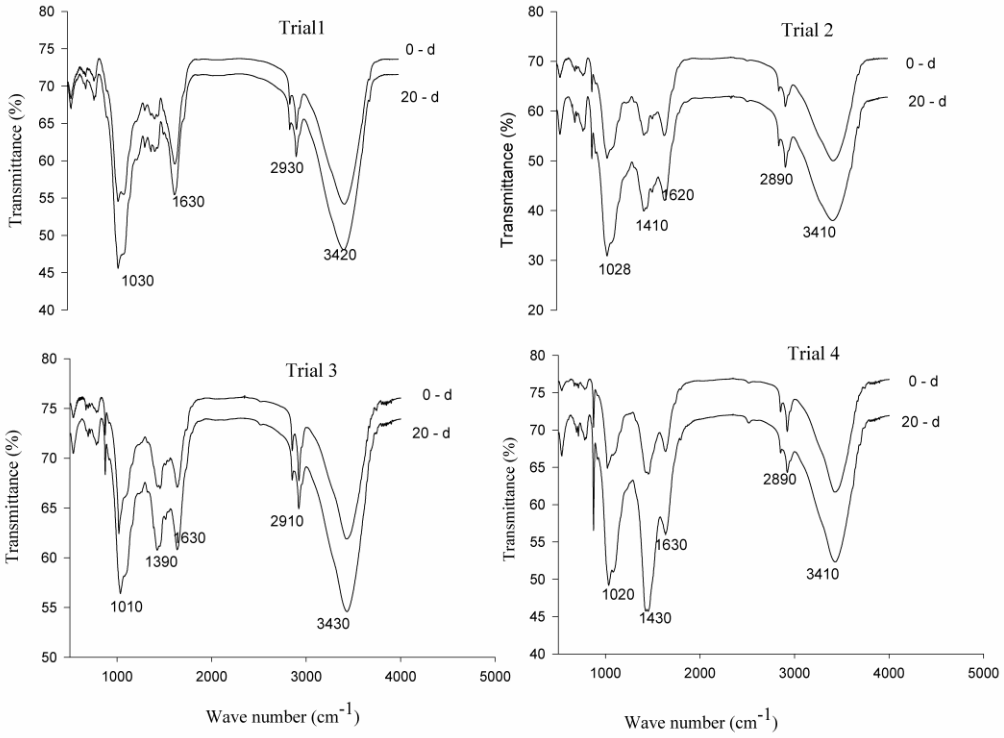

Fig. 5.11. FTIR spectra during composting period

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5.2 CARBON DECOMPOSITION BY INOCULATING *PHANEROCHAETE CHRYSOSPORIUM* DURING DRUM COMPOSTING

The white rot fungi are well known for lignocellulose degradation by producing a non-specific extracellular enzyme system consisting of manganese peroxidase, lignin peroxidase and laccases (Taccari et al. 2009). Hence, the present study was carried out to increase the volatile solids reduction by inoculating *Phanerochaete chrysosporium* during different stages of rotary drum composting in three different trials of (5:4:1) ratio with dry leaves. Trial 1 was control without inoculation of fungus, trial 2 was inoculated at 0th day of composting and trial 3 was inoculated after thermophilic stage i.e. at 8th day (Table 3.8). Finally all the three trials were correlated for the efficiency of *Phanerochaete chrysosporium* inoculation during drum composting and its possible effects on the physico-chemical and biological parameters of the compost.

5.2.1 PHYSICO-CHEMICAL PARAMETERS

- **Changes in temperature, moisture content, pH and EC**

Organic matter degradation during composting is directly indicated by the temperature pattern of the compost and it can be correlated to the rate at which the degradation is being carried out. Fig. 5.12 shows the temperature pattern of all the trials during composting period. With higher biodegradable and soluble organic matter in vegetable waste mixture, a rapid rise in temperature was observed within few hours of the process in all the three trials. A maximum of 66.4°C was observed in trial 1 followed by 62.4°C and 61.2°C in trial 2 and 3 respectively. Even though same combination of waste ratio (5:4:1) was maintained in all the three trials, early thermophilic stage with different temperature pattern was observed towards the end of composting period. This might be due to combination of vegetable wastes and higher indigenous microbial population and also due to the inoculation of fungus.

The drum was turned once in every 24 h for uniform mixing of waste material and to attain proper degradation. Even though maximum temperature was observed in trial 1, inoculation of *Phanerochaete chrysosporium* was observed to increase the overall microbial activity which was evident by the prolonged thermophilic phase in trial 2 and 3. This prolonged thermophilic stage can be attributed to the decomposition of soluble organic matter and moreover temperature ranging from 55 to 60°C is reported optimum for the degradation of lignocellulose content. Hence with the addition of saw dust and dried leaves which are rich in lignocellulose content, prolonged thermophilic phase can be found beneficial for higher degradation. Organic matter degradation in composting can be viewed as a result of rise in temperature. As the temperature rises, loss in moisture content can be observed. Figure 3 depicts the loss of moisture content during the composting period. Hence, loss of moisture during the composting process can be viewed as an index of decomposition rate (Liao et al., 1996). However, the composting material should have minimum moisture content for the survival of microorganisms (Kalamdhad et al., 2008).

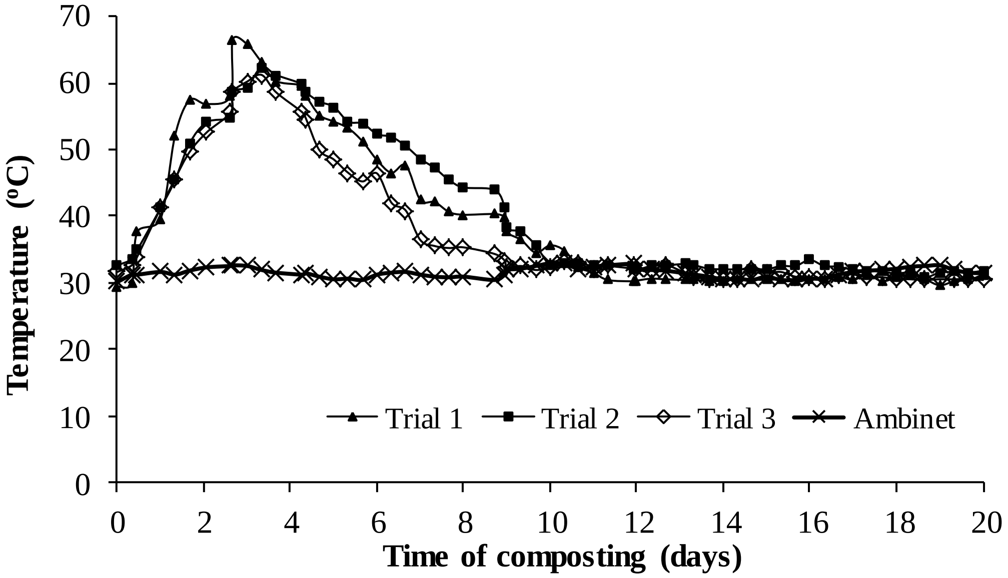

Fig. 5.12. Temperature profile during composting period

With higher temperatures in all the three trials, the moisture loss was found to be 15, 20 and 19% in trial 1, 2 and 3, respectively. This major reduction is mainly due to the higher and prolonged temperature maintained in the compost. Therefore, it can be considered that higher temperature in the compost environment will lead to major reduction in moisture content. Fig. 5.13 shows the variation in pH and electrical conductivity (EC) during the process. The pH of all the three trials increased towards alkaline conditions till the end of composting. The initial pH values were found to be 6.76, 7.18 and 6.54; and finally increased to 7.75, 7.22 and 7.7 in trial 1, 2 and 3 respectively. The degradation of organic nitrogen to the release of ammonia or ammoniacal nitrogen through ammonification can be considered for the increase in pH during composting. Proper aerobic conditions are required for the ammonification, which is maintained by turning the drum once in every 24 h for uniform mixing and release of trapped gases. With the volatilization of ammonia and precipitation of mineral salts, the EC of the compost could be decreased in the final stages of composting (Wong et al., 1995). Similar such trend was observed during the study in all the three trials. The initial values of the EC were found to be 4.16, 3.18 and 3.00 and finally were observed in the range of 5.18, 2.74 and 3.43 in trial 1, 2 and 3 respectively.

- **Carbon decomposition, nitrogen and phosphorous dynamics**

The decomposition of simple and complex organic compounds such as proteins, amino acids, lipids and sugars will lead to the loss of organic matter as CO_2 evolution and heat. With higher degradation, the total mass of the compost will be gradually decreased and the loss can be directly viewed by the decrease in TOC. During composting the microorganisms use carbon as a source of energy and nitrogen for building cell structure for the degradation of organic matter. The organic matter with excess carbon can utilize more nitrogen leading of robbing of soil nitrogen, while applying compost as soil conditioner. However, inoculation of white rot fungi lead to higher loss of TOC in the order 11.4, 16.4 and 19.3% in trial 1, 2 and 3 respectively (Fig. 5.14). The higher reduction of TOC in trial 2 and 3 can be considered due to the higher utilization of complex organic matter by the inoculated *Phanerochaete chrysosporium*.

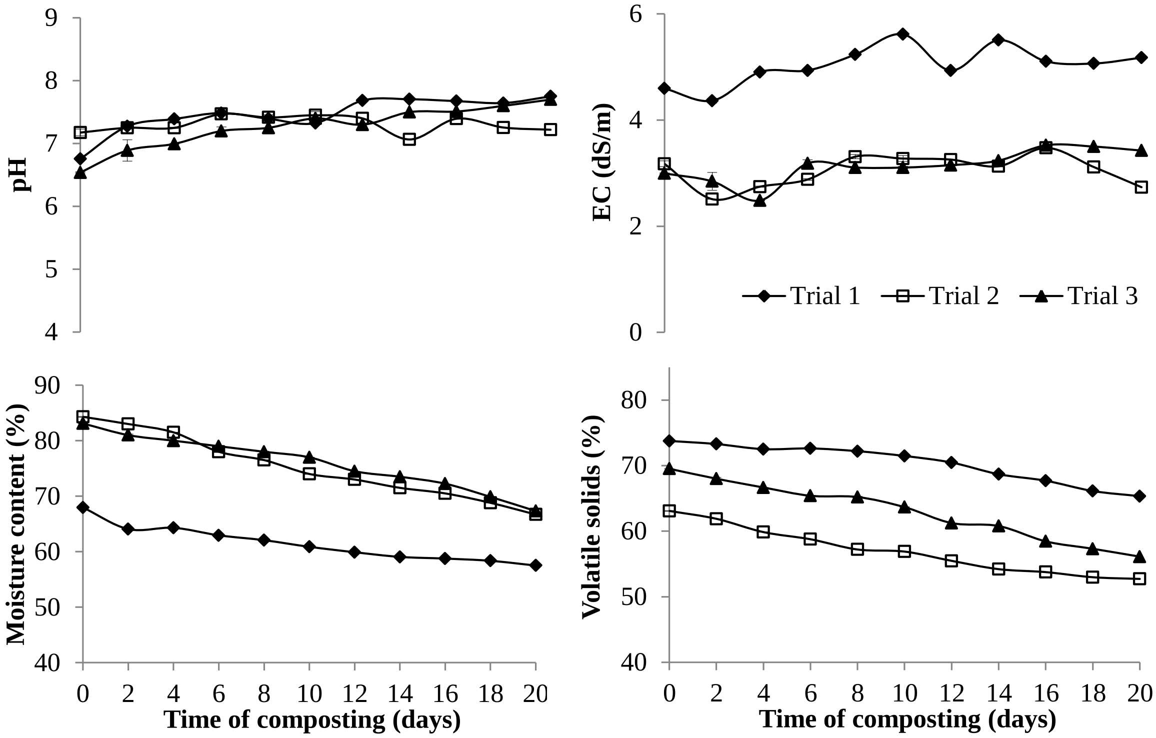

Fig. 5.13. pH, EC, moisture content and volatile solids reduction during composting

During composting lignocellulose degradation is considered as key process with the treatment of agricultural wastes. This polymer is difficult to be biodegraded and reduces the availability of lignocellulose to the microorganisms, while the ligninolytic enzymes secreted by white rot fungi have been widely used to degrade these recalcitrant environmental pollutants (Feng et al., 2011). The added basidiomycetes fungus secretes manganese peroxidase, lignin peroxidase and laccases enzymes which are majorly involved in the degradation of polymeric compounds i.e. lignocellulose. Even though the native indigenous microorganisms were able to degrade the organic matter to a maximum of 11.4%, but the added bulking agents were rich in lignocellulose so that higher degradation was not possible. During the initial period of composting, the soluble and easily degradable carbon sources such as monosaccharides, starch and lipids are degraded by indigenous microbial population followed by proteins. Finally, the more resistant compounds such as cellulose, hemicellulose and lignin are degraded and partly transformed into humus (Crawford, 1983). The inoculation of *Phanerochaete chrysosporium* during the 0 and 8th day in trials 2 and 3 was considered to increase the overall microbial activity thereby achieving higher TOC reduction. *Phanerochaete chrysosporium* has the optimum survival temperature of 20 to 50^{o}C as reported by many researchers (Mouchacca, 1997; Toumela et al. 2000). Since the thermophilic phase has reached 60^{o}C, there are chances of inactivation of the fungi due to such higher temperature.

But in the case of trial 3, where fungus was added after thermophilic phase, the TOC reduction was observed with 19.3%. The higher reduction of TOC in trial 3 can be considered due to the increased activity of *Phanerochaete chrysosporium* soon after the thermophilic stage, which was very optimum with the partially degraded lignocellulose content. The higher temperature can be considered to loosen the lignocellulose bonds, making them more available for the microbial degradation after thermophilic stage. Feng et al. (2011) has reported the increase in carbon decomposition by adding lignolytic enzymes during the lignocellulosic waste degradation. It was reported that the thermophilic temperature had a major effect in the degradation of hemicellulose and lignin, while cellulose was degraded in the initial phase of composting (Bolta et al., 2003).

Similar such results were observed in the present study with higher carbon degradation by inoculating white rot fungi. Therefore with the depletion of readily biodegradable organic matter till the end of thermophilic stage, the remaining lignocellulose was degraded further by the *Phanerochaete chrysosporium* with warm and moist environment. The population of fungi was in the range of 6.4×10^7 and 5.6×10^8 CFU/g in trial 2 and 3 respectively during the inoculation. Due to thermophilic temperature the growth of fungi was affected and was found to be in the range of 2.4×10^3 CFU/g at the end of composting in trial 2. However in trial 3 the growth was found to consistent without any gradual decrease in the population of fungi leading to higher loss of carbon as compared to other trials. Finally at the end of composting the population of fungi was found to be in the range of 4.6×10^5 CFU/g in trial 3. The considerable amount of fungi at the end of composting period was in accordance with the report by Thambirajah et al. (1995).

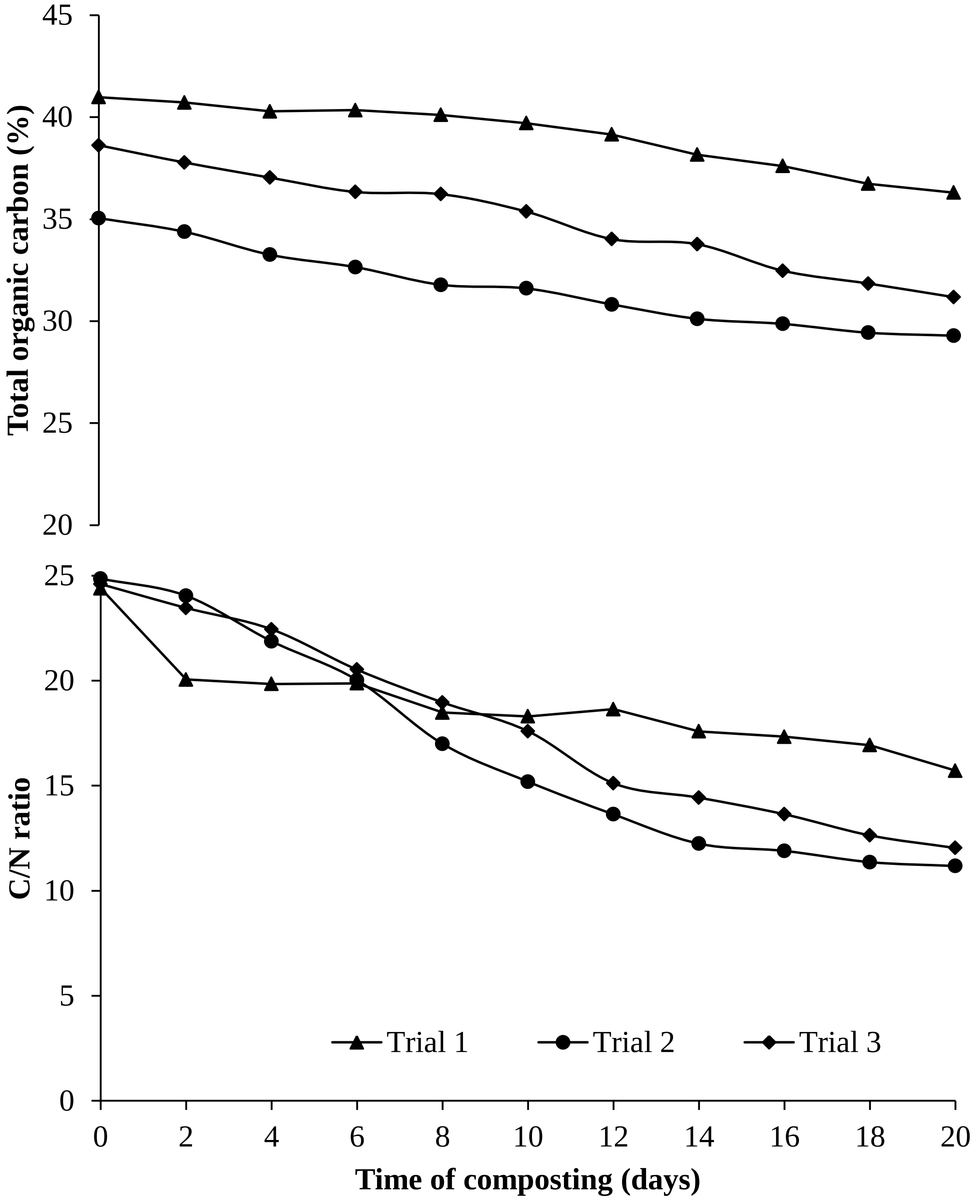

Fig. 5.14. Carbon and C/N variation during composting period

The nitrogen content of the compost mass usually increases with the loss of mass towards the end of composting (Kalamdhad et al., 2008). As the degradation of organic matter was higher with different inoculation stages, the nitrogen was observed to increase in all the three trials. Therefore, the TKN was increased from 1.68, 1.41 and 1.57% to 2.31, 2.62 and 2.59% in trials 1, 2 and 3 respectively, at the end of 20 days (Fig. 5.15). The increase in nitrogen can also be attributed to the nitrogen fixing bacteria in the later stage of composting (Sanchez-Monedero et al., 2001). Higher pH and temperature have been revealed to enhance ammonia loss and decrease NH_4-N during composting (Kalamdhad et al., 2009). Similarly, NH_4-N was observed to reduce from 0.31, 0.39 and 0.80% to 0.09, 0.27 and 0.35% in trials 1, 2 and 3, respectively.

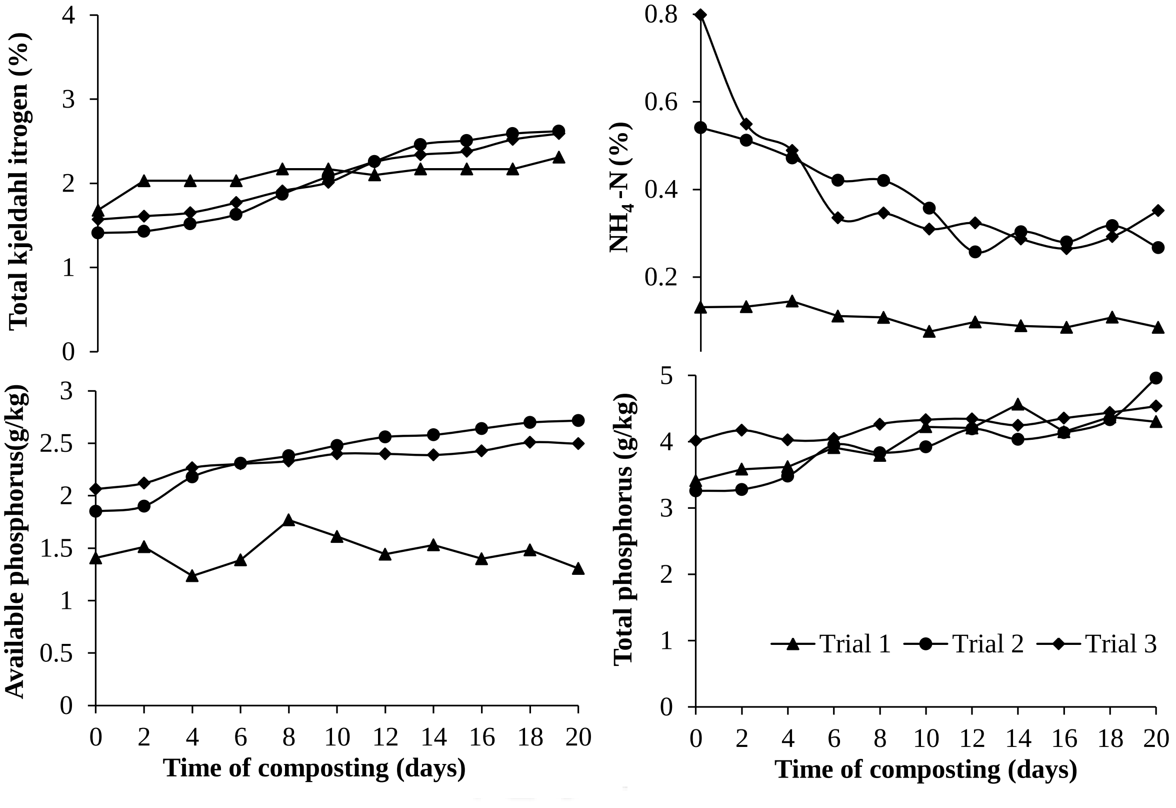

Fig. 5.15. Nitrogen and phosphorous dynamics during composting period

Decrease in NH_4-N can also be considered due to immobilization as nitrogenous compounds such as amino acids, nucleic acids and proteins by microbes (Sanchez-Mondero et al., 1999). The mineralization of inorganic phosphate from organic phosphate is mainly dependent on the temperature and moisture of the compost environment. The process is more rapid in warm and moist conditions which were observed during the process. Inorganic phosphorus is negatively charged, reacts readily with positively charged iron (Fe), aluminium (Al), and calcium (Ca) ions to form relatively insoluble substances (Bauer and Velde, 2014). When this occurs, the phosphorus is considered fixed. Higher amounts of TP and AP during the initial period of composting can be due to the mixture of waste materials in different combinations (Fig. 5.15). Due to higher degradation and elevated temperature during the process has increased the TP and AP values of the compost. TP was observed to increase from 3.41, 3.26 and 4.01 g/kg to 4.30, 4.96 and 4.54 g/kg in trials 1, 2, 3 and 4, respectively. Similarly, AP was observed to increase from 1.41. 1.85 and 2.06 g/kg to 1.49, 2.72 and 2.5 g/kg in trials 1, 2 and 3, respectively, at the end of 20 days (Fig. 5.15).

5.2.2 BIOLOGICAL PARAMETERS

- **OUR and CO_2 evolution**

During composting, large organic molecules are broken down to smaller and soluble ones by the action of microbial communities. During this process higher OUR is observed in the initial period and the final stages with lower OUR values due to the deprival of readily available organic matter (Iannotti et al., 1993). The reduction in OUR and CO_2 evolution values during the process can be considered due to degradation process. Hence with higher degradation, the OUR decreased from initial values of 21.26, 14.65 and 18.87 mg/g VS/day to 4.49, 2.21 and 1.98 mg/g VS/day, in trials 1, 2 and 3, respectively. Similarly, CO_2 evolution values decreased from 14.97, 12.24 and 13.64 mg/g VS/day to 2.28, 1.64 and 1.24 mg/g VS/day in trial 1, 2 and 3 respectively at the end of 20 days (Fig. 5.16). The lower OUR and CO_2 evolution at the end of composting period denotes the stability of compost and similar such values were observed during the process within 20 days (Kalamdhad et al., 2008).

- **Soluble BOD and COD**

The organic fractions in the compost mix can be directly measured as soluble BOD and COD. The percentage of the readily bioavailable organics has been considered important for the compost quality (Bernal et al., 1997). The organic fraction degradation can be measured by the decrease in soluble BOD and COD. With proper mixing and agitation, higher degradation was carried out during the process by which the soluble BOD and COD decreased drastically, resulting in decreased emission of carbon dioxide, ultimately indicating the stabilization of compost. Soluble BOD values decreased from 17.35 to 4.92 g/kg in trial 1, 19.36 to 5.51 g/kg in trial 2 and 16.24 to 3.25 g/kg in trial 3, respectively, within 20 days of composting period. Correspondingly, soluble COD values decreased from 44.41 to 23.21 g/kg in trial 1, 36.45 to 18.65 g/kg in trial 2, and 39.45 to 19.65 g/kg in trial 3, respectively (Fig. 5.17).

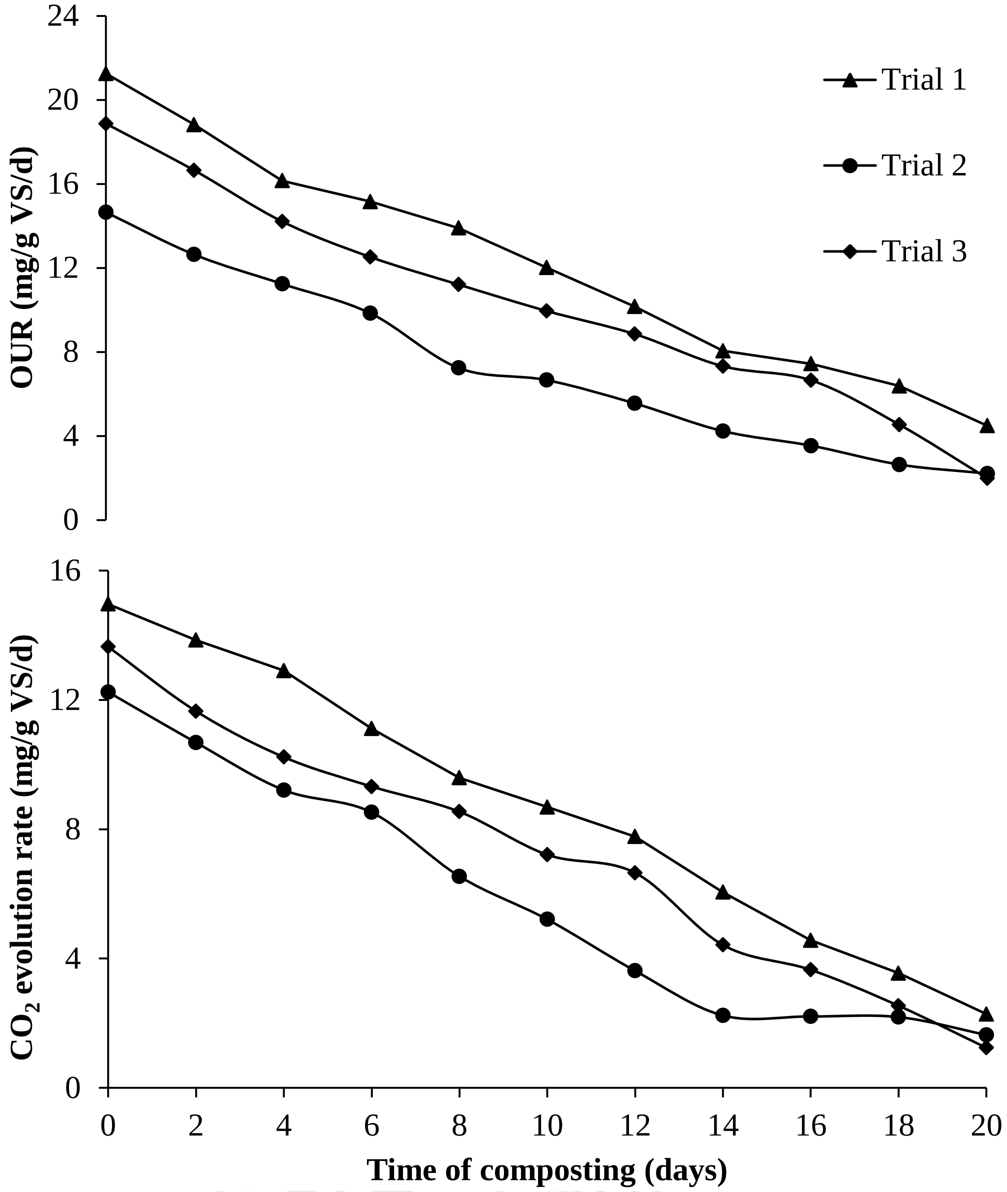

Fig. 5.16. OUR and CO_2 evolution rate during composting

- **Micronutrient and heavy metals**

Table 5.2 illustrates the increase in total concentration of micronutrients (Na. K, Ca, Mg) and heavy metals (Cr, Cd, Ni, Pb, Fe, Mn, Zn and Cu) in trials 1, 2 and 3, respectively, during the 20 days of composting period. During composting, micronutrients and heavy metals were observed to increase due to mass loss caused by the mineralization of organic fractions and they were found in significant amounts in vegetable waste and cattle manure (Fang and Wong, 1999).

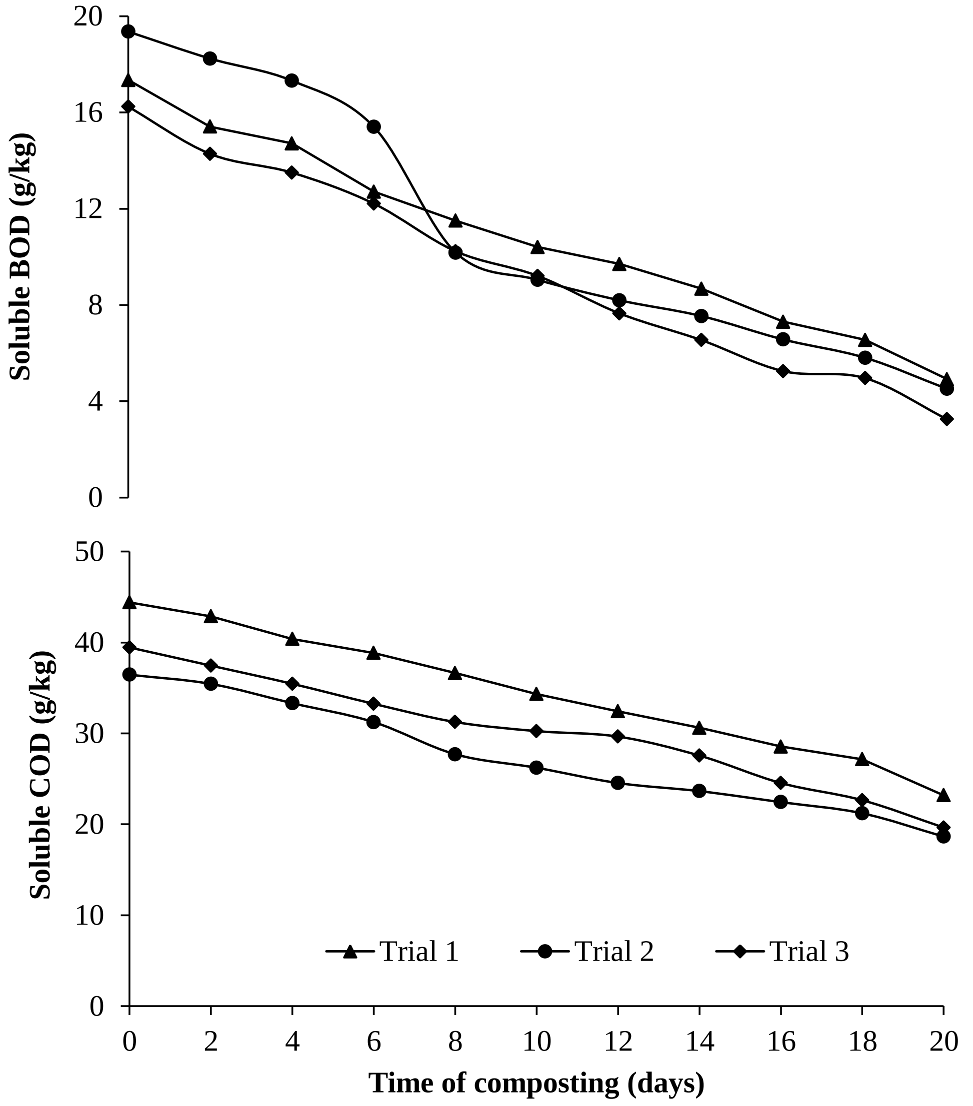

Fig. 5.17. Soluble BOD and COD variation during composting period

5.2.3 BIOCHEMICAL PARAMETERS

- **Hemicellulose (HC) and Cellulose (C)**

The initial period of composting is dominated by the mesophilic heterotrophs targeting the soluble and easily degradable carbon sources such as monosaccharides, starch, lipids and protein. This is followed by the degradation of more resistant compounds such as cellulose, hemicellulose and lignin to form humus (Crawford, 1983). During the degradation of lignocellulosic fraction, microbes initially attack hemicellulose (HC) for carbon and energy source, followed by cellulose (C) degradation (Serramia et al., 2010).

Table 5.2 Micronutrient and heavy metals during composting period

Day	**Na (g/kg)**			**K (g/kg)**			**Ca (g/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	1.25 ± 0.14	3.4 ± 0.08	2.43 ± 0	14.08 ± 0.4	35.59 ± 0.05	22.44 ± 0.77	8.27 ± 0.8	8.88 ± 0.06	8.49 ± 0.3
20	1.36 ± 0.04	2.67 ± 0	3.29 ± 0.04	25.67 ± 0.67	36.38 ± 0.03	31.82 ± 1.08	10.36 ± 0.94	10.41 ± 0	20.79 ± 0.16
Day	**Cd (mg/kg)**			**Cr (mg/kg)**			**Cu (mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	70.25 ± 1.06	57.5 ± 0.71	55.5 ± 1.06	26.5 ± 0.71	15.5 ± 1.77	5 ± 0.71	49 ± 0	40.5 ± 5.66	34.5 ± 0
20	75 ± 1.41	54.5 ± 0.71	56 ± 1.41	177.5 ± 3.54	17 ± 1.41	6.5 ± 3.54	549.5 ± 0.63	78.5 ± 1.06	25 ± 637.1
Day	**Mn (mg/kg)**			**Ni (mg/kg)**			**Pb (mg/kg)**		
	Trial1	Trial2	Trial3	Trial1	Trial2	Trial3	Trial1	Trial2	Trial3
0	673.5 ± 4.95	549.5 ± 14.85	712.5 ± 4.95	215.75 ± 3.89	290.5 ± 0.31	272 ± 0.32	855 ± 21.21	1035 ± 28.28	984.5 ± 21.21
20	865 ± 5.66	550 ± 9.55	802.5 ± 5.66	41 ± 7.07	277.5 ± 0.19	274.5 ± 0.25	892.5 ± 38.89	989.5 ± 31.82	990 ± 38.89
Day	**Mg (g/kg)**			**Fe (mg/kg)**			**Zn (mg/kg)**		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	5.10 ± 0.21	8.03 ± 0.17	5.55 ± 0.14	6914 ± 36.77	662.5 ± 0.45	3302.5 ± 0.37	262.9 ± 0.14	160 ± 1.41	141 ± 0.14
20	6.99 ± 0.33	10.44 ± 0.06	6.34 ± 0.05	10782 ± 11.31	708.5 ± 0.38	4583.5 ± 0.38	353.1 ± 4.45	150.5 ± 0.67	152.5 ± 4.45

Note: (mean ± SD, n=3) SD - standard deviation

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Hemicellulose degradation is reported to occur in two ways; either by the wood-rotting fungi secreting hemicelluloses, or the fungal lignin degradation is preceded by hemicellulolytic activity of bacterial consortia. HC are easily hydrolysable polymers consisting of five sugars D-xylose, D-mannose, D-galactose, D-glucose and L-arabinose. These sugars are linked together by b-1, 4- and occasionally b -1, 3-glycosidic bonds (Perez et al. 2002). HC is mostly degraded by the enzymes Endo-xylanases, beta-xylosidases and endo-mannanases produced by most wood-degrading fungi in addition to bacteria. Moreover, the inoculated *Phanerochaete chrysosporium* secretes xylanases and hemicellulases, which in together with peroxidases led to higher degradation of HC. Therefore, reduction in hemicellulose (HC) was found maximum in trial 3 (36.4%) followed by trial 2 (31.4%) and trial 1 (29.7%) respectively at the end of composting period.

The oxidation of cellulose to carbon-di-oxide is reported by most of the aerobic bacteria and fungi. Endoglucanases and cellobiohydrolases are two active enzymes mainly involved in the hydrolysis of the crystalline cellulose to cellobiose and further the β-endoglucanase, degrades cellobiose to glucose. Due to the higher microbial activity and inoculated *P. chrysosporium*, a maximum of 47.6% reduction of cellulose (C), was observed in trial 3 followed by trial 45.8 and 44.2% in trial 2 and 1, respectively. Higher temperature that prevailed during drum composting can also be considered to have a major effect on the degradation of cellulose. A major reduction of 45, 36 and 20.1% in C fractions was observed during the early and active thermophilic phase (day 1-12), out of the total loss observed from day 13-20 (final cooling stage). The effect of elevated temperatures on the degradation of lignocellulosic fractions as compared to mesophilic stage was supported by Zeng et al. (2010). Therefore, from the results it can be suggested that application of drum composter with fungus for the degradation of lignocellulose fractions can be much effective within short time period.

- **Lignin**

The degradation of lignin by microorganisms are reported to efficiently enable them to utilize the carbohydrates released, however this process does not release any energy for the utilization of microorganisms. Most of the fungi are reported to degrade fungi with by-products such as carbon dioxide, water and humus (Eriksson et al., 1990). Lignolytic activity in *P. chrysosporium* and other white-rot fungi is associated with multiple isoenzymes. At least 21 heme peroxidases are produced by *Phanerochaete chrysosporium* which are majorily involved in the degradation of lignocellulosic fractions (Leisola et al., 1987; Tien, 1987). A maximum of 26.4, 23.2 and 22.1% reduction of acid insoluble lignin was observed in trial 1, 2 and 3 respectively. The major extracellular enzymes involved in lignin degradation include lignin peroxidases (LiPs), manganese peroxidases (MnPs) that are secreted by *Phanerochaete chrysosporium* and laccases by most of the other fungi. Lignin peroxidases are reported to act by oxidizing non-phenolic lignin substructures by abstracting one electron and generating cation radicals, which are then decomposed chemically (Kirk and Farrell, 1987). Lignin has a very complicated structure, which is highly resistant to microbial degradation and mainly occurs during thermophilic phase of composting (Hatakka, 2001). Hemicellulases, primarily xylanases and mannanases of fungal origin are mainly involved in delignification process. The degradation of lignin by *Phanerochaete chrysosporium* was also reported to involve both oxidative and reductive conversions (Schoemaker et al., 1991). Initially, the lignin depolymerisation can be considered as an oxidative process followed by the metabolism of lignin fragments involving both oxidation and reduction mechanisms. The compartmentalization of oxidative and reductive conversions calls for a powerful transport mechanism through the cell wall (Tuor et al., 1993). Finally, the percentage reduction of acid soluble lignin was observed as 37.5%, 39.4% and 41.6% during trial 1, 2 and 3 respectively at the end of 20 days.

5.2.4 MICROBIAL DIVERSITY

- **Mesophilic and spore forming bacteria**

Microbial populations growing in composting mass mainly consist of bacteria including actinomycetes and fungi. During the initial period of composting, mesophilic bacteria are dominant in degrading the readily available organic matter and raising the temperature with a wide tolerance of pH and temperature. With different proportion of vegetable waste and cow dung, initial load of mesophilic bacteria was in the range of 5.6×10^{10}, 8.8×10^{10} and 9.6×10^{10} CFU/g in trial 1, 2 and 3 respectively. Due to the higher load of heterotrophs, rise in temperature was observed with 24-48 hrs of the composting period. These heterotrophs primarily has cellulolytic and pectinolytic activities, and limited efficiency in degrading lignin (Blanchette, 1995). A maximum temperature of 60 to 66.4^{o}C was observed in all the trials involved in the breakdown of complex lignocellulosic fractions to simpler units. However, the higher temperature reduced the load of mesophilic bacteria to 4.2×10^{8}, 3.6×10^{9} and 7.4×10^{8} CFU/g in trials 1, 2 and 3 respectively at the end of 20 days. However the, spore forming bacteria that are thermo resistant increased to 4.6×10^9, 3.6×10^8 and 5.2×10^7 CFU/g in trial 1, 2 and 3 respectively during the 4th day of composting (Fig. 5.18). The temperature drop in composting mass after 6 to 8 days can be considered as the depletion of readily biodegradable organic matter. Finally, due to reduced temperature the spore forming populations was in the range of 2.4×10^5, 7.4×10^5 and 2.6×10^6 CFU/g in trial 1, 2 and 3 respectively at the end of composting period (Fig. 5.18).

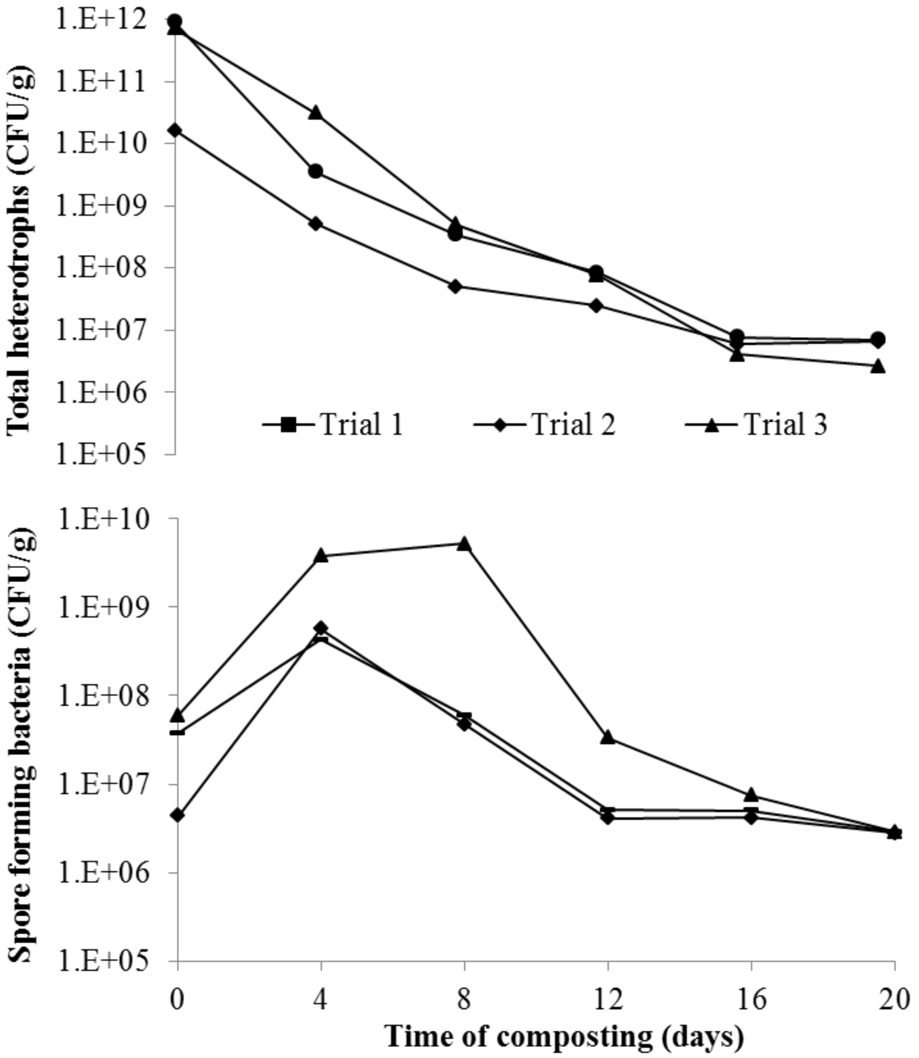

Fig. 5.18. Mesophilic and spore forming bacteria during composting period

- **Actinomycetes and streptomycetes**

According to Toumela et al. (2000) once the readily degradable organic matter is depleted by the heterotrophs, the polymers such as hemicellulose, cellulose and lignin will be partly transformed to humus. The degradation of the lignocellulosic fractions is majorily reported to happen by actinomycetes and fungi under aerobic conditions. Due to higher percentage of lignocellulosic fractions in the compost mixture, the load of actinomycetes was found to be 1.4×10^{8}, 3.6×10^{7} and 5.4×10^{7} CFU/g and streptomycetes as 4.4×10^{7}, 6.4×10^{8} and 2.4×10^{8} CFU/g in trial 1, 2 and 3 respectively, during the initial days of composting (Fig. 5.19).

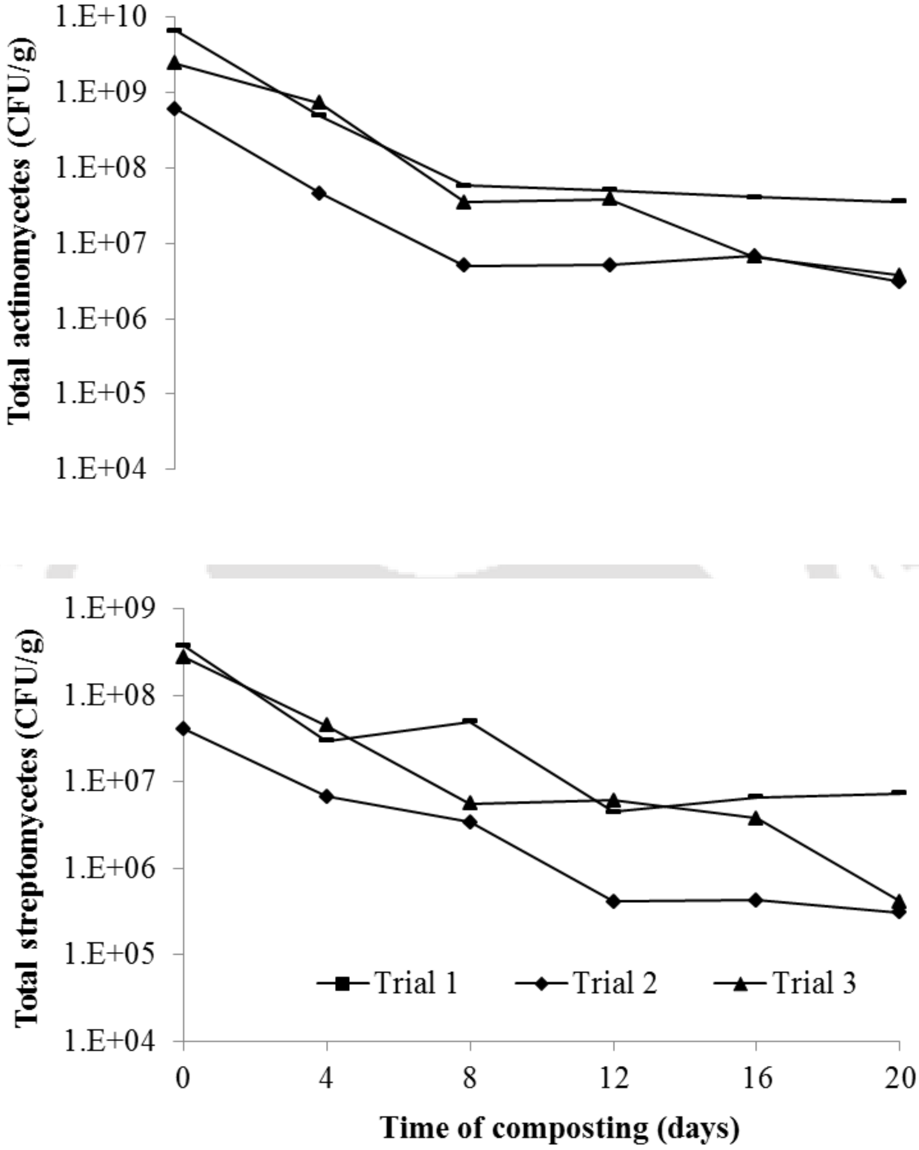

Fig. 5.19. Total actinomycetes and streptomycetes during composting period

The higher population of actinomycetes can be considered due to the type of waste materials used for composting and the physical conditions of the compost environment (Miyashita et al., 1982). These bacteria can degrade cellulose and solubilize lignin by secreting different cellulolytic and ligninolitic enzymes that are critical to the degradation of lignocellulosic materials in compost. Streptomyces species are reported to solubilize part of lignin to water-soluble and acid-precipitable polymeric lignin (Crawford et al., 1983). In addition, specifically actinomycetes are reported to produce extracellular peroxidises and streptomyces species to produce peroxidise and cell-bound demethylase involved in the degradation of lignocellulosic fractions (Adhi et al., 1989; Godden et al., 1992). Finally, the populations of actinomycetes and streptomycetes was in the range of 4.4×10^5, 2.8×10^5 and 3.6×10^5 CFU/g; and 3.6×10^4, 3.8×10^6 and 6.6×10^5 CFU/g in trial 1, 2 and 3 respectively at the end of composting period (Fig. 5.19).

- **Fungus**

Fungi are mainly involved in the degradation of lignin and maturation of compost during the later stages of composting. The higher activity of fungi is dependent on the carbon and nitrogen supplements of the compost. Due to higher lignocellulosic fractions and nitrogen content from vegetable waste, the load of fungi was in the range of 1.8×10^8, 7.4×10^7 and 4.8×10^8 CFU/g respectively during the initial period of composting. The inoculated *P. chrysosporium* is considered to produce lignin peroxidases (LiPs) and manganese peroxidases (MnPs); and laccase by other fungi. These enzymes are three major oxidative agents majorily responsible for the higher degradation of lignin and other wide range of lignin analogous compounds (Ruttimann-Johnson et al., 1993). The inoculated *Phanerochaete chrysosporium* growth was highly affected by the elevated temperatures during the drum composting. Therefore, the final population of fungi was in the range of 3.6×10^4, 3.6×10^5 and 4.2×10^5 CFU/g in trials 1, 2 and 3 respectively at the end of 20 days (Fig. 5.20). The considerable amount of fungi population at the end of composting can be considered due to the presence of partially degraded lignocellulosic fractions in compost mass (Thambirajah et al., 1995). Hence, it can be suggested that inoculation of *P. Chrysosporium* after thermophilic stage will be efficient for higher degradation of lignocellulosic fractions.

- **Pathogenic *population***

Temperature level above 50^{o}C for more than 4 to 7 days during composting was reported to satisfy the regulatory requirements for PFRP. Hence with such elevated temperature, *Salmonella* isolates reduced from 3.1×10^4 and 6.1×10^4 CFU/g to 2.1×10^4 in trial 1 and zero in trial 2 and 3 respectively; and Shigella isolates varied from 1.1×10^4, 2.1×10^3 and 5.6×10^2 CFU/g to 2.1×10^2, 1.2×10^2 CFU/g and zero trials 1, 2 and 3 respectively at the end of composting. Reduction in the number of pathogenic organisms can be considered due to the extended thermophilic stage and the alkaline conditions of

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the compost due to the release of ammonia (Petrica et al., 2009; Wong and Selvam, 2009).

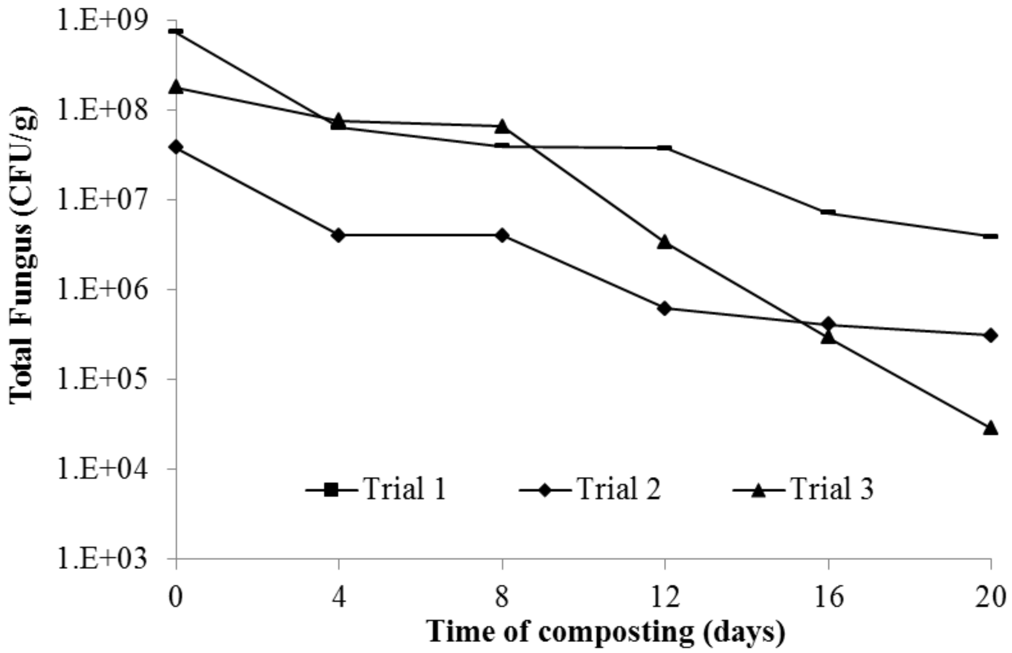

Fig. 5.20. Total Fungus during composting period

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5.3 POTENTIAL OF WASTE CARBIDE SLUDGE ADDITION ON EARTHWORM GROWTH AND ORGANIC MATTER DEGRADATION DURING VERMICOMPOSTING OF VEGETABLE WASTE

Different proportion of waste carbide sludge (WCS) addition during vermicomposting was experimented by employing *Eisenia fetida* for higher biomass production and higher volatile solids reduction. Totally 180 adult earthworms were employed in all the five trials with 0.5% WCS in trial 1, 1% in trial 2, 1.5% in trial 3, 2% in trial 4 and 0% in trial 5 respectively. Since vermicomposting is a combined microbiological and earthworm process, the use of waste carbide sludge will result in waste minimization as well as the treatment efficiency will improve. Hence, the present study focused on the effects of waste carbide sludge addition on the physico-chemical and biological characterization during vermicomposting of vegetable waste using *Eisenia fetida.*

5.3.1 PHYSICO-CHEMICAL ANALYSIS

- **pH and EC**

pH variation during vermicomposting is highly dependent on the waste materials used for the degradation process. The shift in pH towards acidic and alkaline conditions during the process can be considered due to the production of organic acids and ammonia production depending on the raw materials and the degradation pattern. But in the present study the pH was observed to increase till the end of the process. The initial pH value was in the range of 6.9, 7.2, 7.4, 7.5 and 6.9 in trials 1, 2, 3, 4 and 5 respectively. It can be observed that with higher addition of WCS the initial pH values were found higher starting from trial 1 to trial 4. Vegetable waste with acidic pH and higher biodegradable organic matter can lead to pH drop due to organic acid production. However such findings were not observed during the study, which can be considered due to appropriate addition of WSC. The added WCS was considered to act as buffering agent by maintaining the neutral pH during the process. Finally the pH values were found in the range of 7.5, 7.5, 7.6, 7.7 and 7.4 in trials 1, 2, 3, 4 and 5 respectively (Fig. 5.21). The EC values of all the trials were observed to reduce during the study.

The initial EC values were found to be 4.0, 2.7, 2.7, 3.0 and 3.8 dS/m and finally reduced to be 2.9, 2.6, 2.1 and 2.4 in trial 1, 2, 3, 4 and 5 respectively (Fig. 5.21). The EC values during vermicomposting for different waste materials were observed to increase at the end of process such as sewage sludge, textile sludge, fibre, kitchen waste and institutional waste (Garg et al., 2006; Nayak et al., 2013). The increase in EC was reported due to the release of freely available ions and minerals by the decomposition of organic matter. However in the present study the reduction in EC values was observed which can be considered due to the addition of WCS and uptake of the minerals by earthworms.

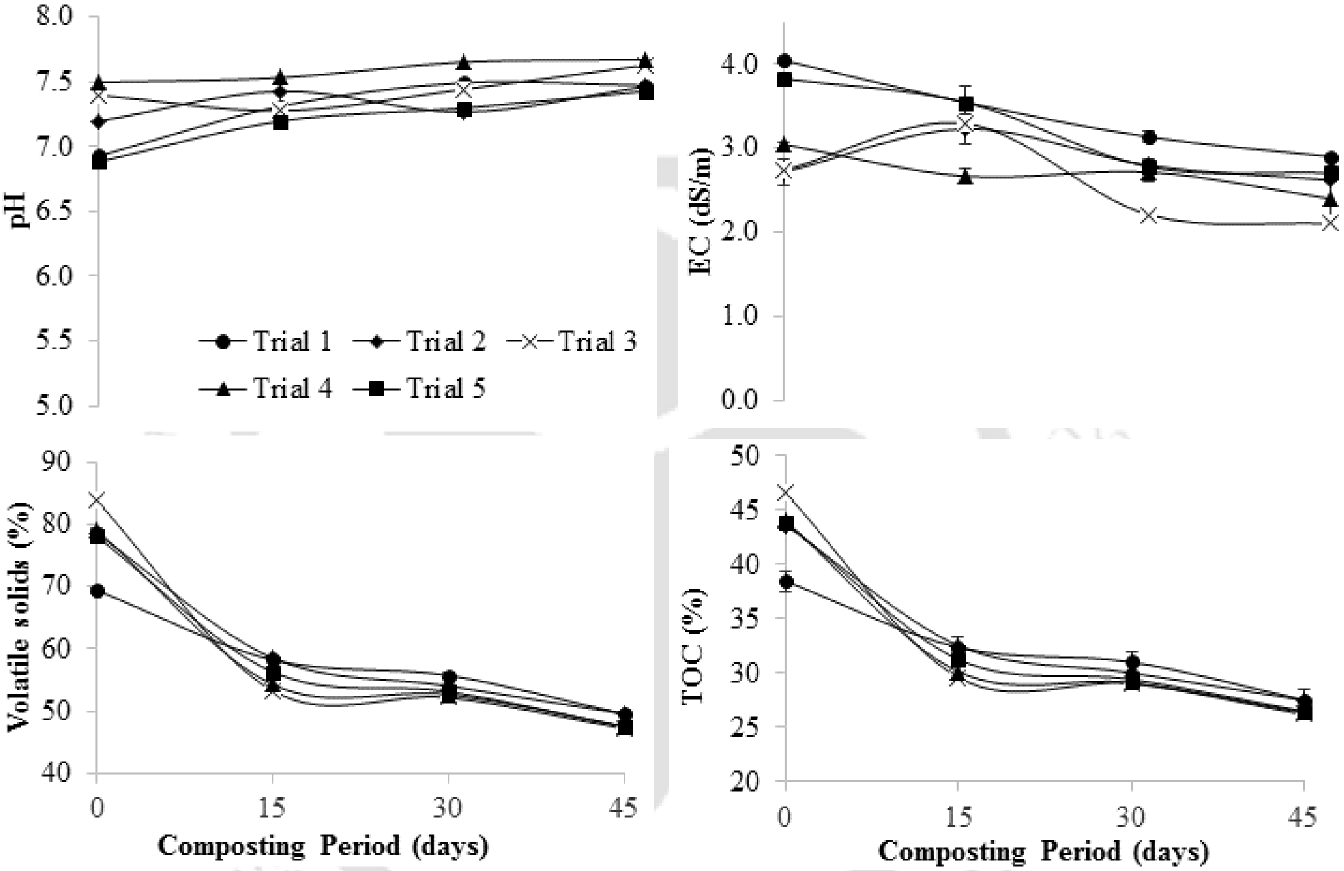

Fig. 5.21. pH, EC, Volatile solids and TOC variation during composting period

- **TOC**

The reduction in TOC during the process denotes the rate of degradation of organic matter. In all the experiments TOC reduction ranged from 28.3 to 43.7%, with trial 3 having the highest reduction. The reduction in organic carbon can be considered due to the loss of carbon in the form of CO_2 by the combined action of microbes and earthworms. The TOC reduction was observed with 28.3, 36.8, 43.7, 39.9 and 39.6% reduction in trial1, 2, 3, 4 and 5 respectively (Fig. 5.21). Generally, pure lime with higher Ca content is reported to increase the metabolic activities of microbes and earthworms

(Wong and Fang, 2000). The added WSC had 35-40% lime and 66 g/kg of Ca content, which might have increased the metabolic activity of both microbes and earthworms thereby resulting in higher organic matter utilization. In addition to higher degradation of organic matter, almost 50-80% of TOC reduction was observed within 15 to 30 days. The reason might be due to the buffering capacity of WSC towards the vegetable waste mixture during the process. There are many reports on the pre-treatment of vegetable waste for the stabilization of waste before being applied for vermicomposting for pH adjustment and partial degradation (Dandotiya and Agrawal, 2012; Chatterjee et al., 2014). Therefore the initial adaptation period can be reduced during the process. However in the present study, the added WSC with buffering capacity neutralized the pH against the organic acids production and ammonia release due to nitrogen mineralization.

- **Nitrogen dynamics**

The TKN of all the trials was observed to increase irrespective of the trials. This increase in nitrogen content during vermicomposting has been reported due to the release of excretory products by earthworms during the process such as mucus, nitrogenous excretory substances, growth stimulating hormones and enzymes (Tripathi and Bhardwaj, 2004). Therefore, due to active earthworm activity and increased metabolic activity by the addition of WCS to the process, higher TKN was observed at the end of 45 days. The initial TKN was observed to be 1.9, 1.8, 1.4, 1.5 and 1.3% in trial 1, 2, 3, 4 and 5 respectively. The higher amount of TKN during the initial stages might be due to the combination of different raw materials rich in nitrogen content. In addition, the final higher amount of TKN in the vermicompost is also dependent on the initial nitrogen content and the extent of degradation (Crawford, 1983; Gaur and Singh, 1995). Finally, the TKN was found to be 3.1, 3.2, 2.9, 3.1 and 2.9% in trial 1, 2, 3, 4 and 5 respectively (Fig. 5.22). Loss in total organic carbon might also be responsible for the increase in TKN (Viel et al., 1987). Such reports were supported in the present study with higher loss in TOC for the enhancement of TKN. Hence with higher degradation of organic matter and mineralization of organic nitrogen, the ammoniacal nitrogen was observed to decrease towards the end of process. The initial ammoniacal nitrogen was found to be 0.39, 0.29, 0.47, 0.38 and 0.35 % in trial 1, 2, 3, 4 and 5 respectively and finally observed to reduce to 0.22, 0.16, 0.15, 0.18 and 0.19 % in trial 1, 2, 3, 4 and 5 respectively at the end of 45 days.

- **Phosphorous dynamics**

TP and AP during the process was observed to increase in all the trials. The initial TP was found to be 5.31, 6.23, 6.09, 5.69 and 6.30 g/kg and AP was observed to be 2.69, 2.16, 2.77, 2.82 and 2.78 g/kg in trial 1, 2, 3, 4 and 5 respectively. Pramanik et al. (2007) have reported that acid production during organic matter decomposition by the micro-organisms is mainly responsible for solubilization of insoluble phosphorus, which subsequently results in the increase in TP in vermicomposts. Hence with higher organic matter degradation, enhanced TP and AP levels were observed in the present study at the end of 45 days. Finally, TP was found to be 7.95, 9.33, 8.47, 8.53 and 8.39 g/kg and AP was observed to be 5.23, 5.56, 5.52, 5.55 and 5.65 g/kg in trial 1, 2, 3, 4 and 5 respectively at the end of 45 days (Fig. 5.22). During vermicomposting, even the unavailable forms of phosphorous can be converted to available forms in the final vermicompost that are easily available for the plants (Ghosh et al., 1999). Table 5.3 provides the increase in micronutrient and metal concentration during vermicomposting of all the five trials.

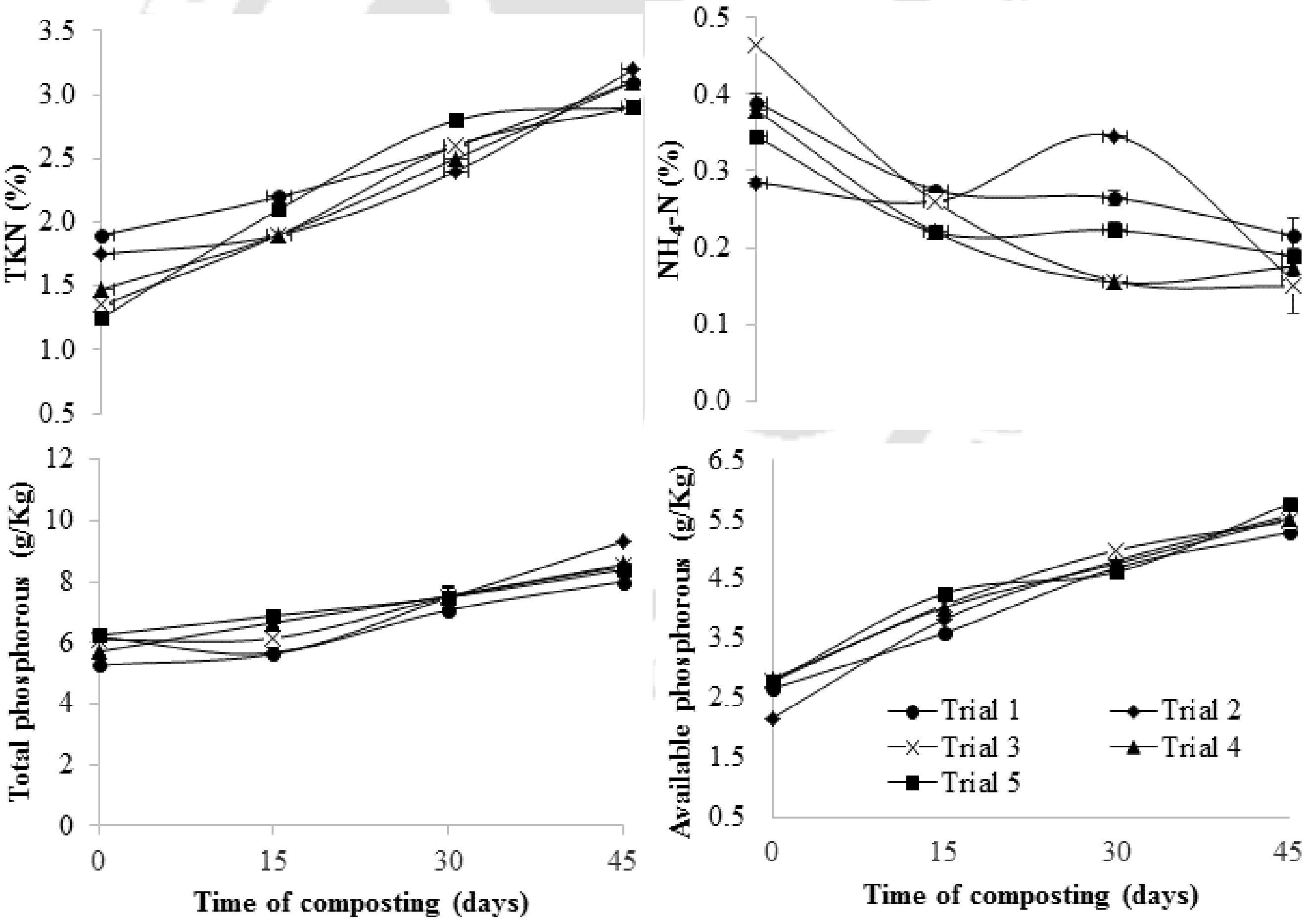

Fig. 5.22. Nitrogen and phosphorous dynamics during composting period

Table 5.3 Micro-nutrient and heavy metal concentration during composting

Days	**Sodium (g/kg)**					**Potassium (g/kg)**				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial	7.03±0.02	5.75±0.15	5.64±0.03	5.58±0.14	7.74±0.06	5.6±0.12	4.7±0.	6.4±0.3	5.9±0.14	5.8±0.22
Final	10.91±0.32	9.38±0.33	9.62±0.04	9.43±0.21	9.05±0.15	14.8±0.15	16.4±0.4	19.2±0.24	18.6±0.32	17.6±0.14
Day	**Calcium (g/kg)**					**Magnesium (g/kg)**				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial	5.6±0.02	5.7±0.1	5.8±0.1	5.9±0.03	5.6±0.1	6.45±0.2	5.9±0.1	7.12±0.1	6.84±0.6	8.34±0.4
Final	10.3±0.03	12.4±0.03	12.8±0.2	13.4±0.2	6.9±0.2	9.24±0.3	7.43±0.6	9.34±0.54	9.12±0.3	9.52±0.2
Day	**Chromium (mg/kg)**					**Copper (mg/kg))**				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial	6.4±1.2	6.84±1.4	7.21±1.2	7.44±2.6	12.4±1.6	52.7±6.4	44.6±0.6	54.8±1.2	58.4±2.2	46.4±1.2
Final	7.82±0.6	8.42±2.2	8.12±2.4	8.94±3.4	13.6±2.6	64.0±2.0	47.2±0.4	57.2±2.4	61.2±3.4	52.0±3.0
Day	**Nickel (mg/kg)**					**Lead (mg/kg)**				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial	218.4±3.6	198.2±2.2	224.6±3.4	242.4±1.8	236.2±1.4	244±10	262±12	209.12±12.8	184.2±1.2	242.4±1.4
Final	222.6±1.4	212.6±1.4	236.4±1.6	264.2±3.2	242.4±2.2	263.4±11.2	271.4±8.6	214.4±1.8	222.4±4.6	260.2±4.4
Day	**Iron (g/kg)**					**Manganese (mg/kg)**				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial	4.2±0.3	4.6±0.12	5.4±0.2	4.6±0.3	5.9±0.2	548.4±1.3	584.2±6.4	624.4±2.6	612.6±9.4	646.4±4.2
Final	7.6±0.20	6.4±0.1	9.6±0.3	10.2±0.2	8.6±0.4	644.2±9.6	722.4±12.6	844.6±3.4	744.8±3.4	844.61±1.4
Day	**Zinc (mg/kg)**					**Cadmium (mg/kg)**				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial	154.2±0.6	174.3±1.8	164.2±3.6	183.6±1.8	174.5±1.6	42.4±2.4	56.4±1.3	62.4±0.6	59.2±1.4	64.2±0.6
Final	184.6±0.8	194.6±3.2	189.1±4.8	264.8±4.2	224.6±2.8	51.0±1.6	64.4±2.0	63.6±3.0	68.4±2.2	69.2±1.2

Note: (mean ± SD, n=3) SD - standard deviation

5.3.2 BIOLOGICAL AND STABILITY ANALYSIS

- **Soluble BOD and COD**

The organic matter degradation during vermicomposting can be measured by the decrease in soluble BOD and COD. With proper mixing and agitation, higher degradation was carried out during the process by which the soluble BOD and COD were decreased drastically, resulting in decreased emission of carbon dioxide, ultimately indicating the stabilization of compost. Soluble BOD values decreased from 17.35 to 4.92 g/kg, 9.85 to 0.65 g/kg, 15.18 to 4.52 g/kg, 15.10 to 4.49 g/kg and 16.21 to 5.59 g/kg in trial 1, 2, 3, 4 and 5 respectively, within 45 days of vermicomposting. Correspondingly, soluble COD values were decreased from 37.35 to 11.92 g/kg, 39.85 to 08.65 g/kg, 15.18 to 4.52 g/kg, 15.10 to 4.49 g/kg and 16.21 to 5.59 g/kg in trial 1, 2, 3, 4 and 5 respectively (Fig. 5.23). A maximum of 93.9% reduction of soluble BOD and 93.2% of soluble COD was observed in trial 3 as compared to other trials due to the optimized addition of WCS.

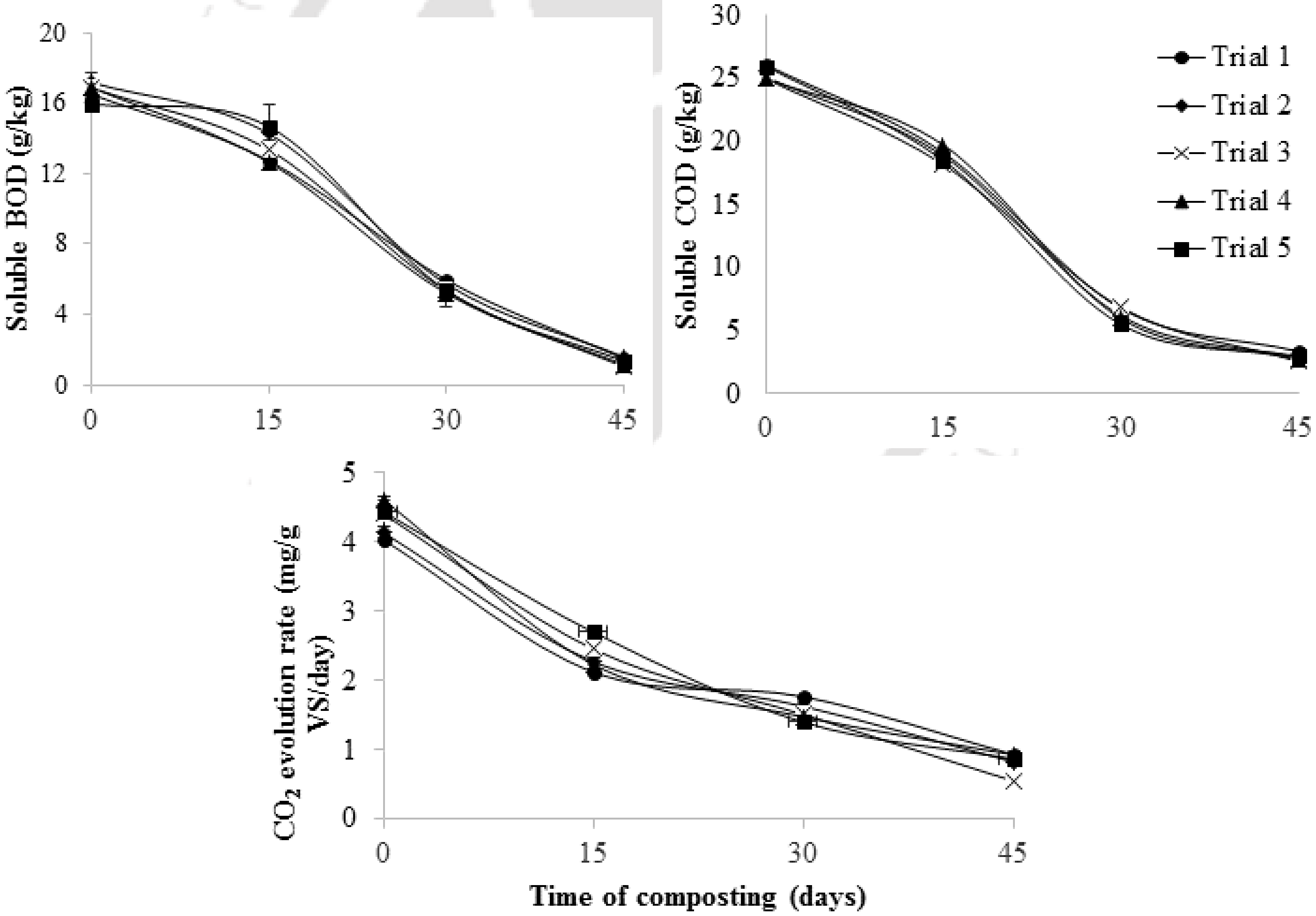

Fig. 5.23. Soluble BOD, COD and CO_2 evolution rate during composting period

- **CO_2 evolution rate**

The stability of the compost can be directly measured by the CO_2 evolution rate during the organic matter decomposition (Kalamdhad et al., 2008). Higher degradation of organic matter during the process will lead to lower emission of CO_2 at the end of the process. Similar such results were observed during the study due to higher carbon decomposition and stabilization of the vermicompost. A maximum of 87.5% reduction of CO_2 evolution rate was observed in trial 3 followed by 80.4, 80.1, 79.8 and 77.0% in trial 5, 2, 4 and 1 respectively. The stability of compost is determined by the lower CO_2 emission rates due to higher degradation of organic matter in the later stages of composting (Singh et al., 2013).

- **Earthworm population**

Initially a total of 180 adult earthworms were counted manually and were added to all the reactors. Due to proper combination of waste materials and appropriate addition of WCS, increase in growth of earthworm biomass was observed at the end of 45 days. Finally a maximum of 488, 434, 416, 314 and 290 earthworms were observed in trial 5, 4, 3, 2 and 1 (Table 5.4).

Table 5.4 Initial and final count of earthworms

Experiment	Initial count (Adult No's)	Final count (No's)			
		Cocoons	Juvenile	Adults	Total
Trial 1	180	54	38	198	290
Trial 2	180	66	42	206	314
Trial 3	180	94	36	286	416
Trial 4	180	86	44	304	434
Trial 5	180	136	54	298	488

The final total earthworm biomass count included the cocoons, juvenile and adult worms as explained in table 4. The higher growth in earthworm biomass can be considered due to the optimum pH and Ca content availability form the added WCS in trials 3 and 4. Due to higher addition of WCS, increased number of earthworm biomass were observed in trial 3 and 4 with higher organic matter reduction. Even though biomass growth was less as compared to control, WCS added trials demonstrated the positive approach for the utilization of WCS addition during vermicomposting. It is well established from the experiments that the addition of WCS in appropriate combination during vermicomposting of agricultural waste would have positive effect on the growth of earthworms.

- **Total and fecal coliform**

The initial load of TC and FC was observed high due to higher addition of vegetable waste and cow dung. TC was in the range of 2.4×10^8, 0.21×10^8, 0.15×10^8, 2.4×10^9 and 0.43×10^9 MPN/g, while FC was in the range of 0.19×10^5, 0.34×10^5, 0.26×10^4, 0.16×10^4 and 0.21×10^4 MPN/g in trial 1, 2, 3, 4 and 5 respectively. Due to unavailability of organic matter and by the release of extracellular enzymes the load of coliform bacteria was reported to diminish during vermicomposting. Similar such findings were observed in the present study with higher reduction of TC and FC in the final stages of vermicomposting. Finally the load of TC was found to be 2.1×10^2, 1.2×10^2, 0.23×10^1, 0.75×10^1 and 1.6×10^2 MPN/g, while FC was in the range of 0.21×10^1, 0.06×10^1, 0.19×10^1, 0.09×10^1 and 1.6×10^1 MPN/g, in trial 1, 2, 3, 4 and 5 respectively.

5.4 CONCLUSIONS

With different proportions of WCS addition, trial 2 was observed with higher degradation in volatile solids and high nutrient value at the end of composting period. Longer thermophilic phase was observed in trial 2 due to increased metabolic activity of microbes by appropriate addition of WCS. However, higher addition of WCS to compost materials led to volatilization of organic nitrogen as ammonia and raising the pH towards alkaline condition, thereby deteriorating the quality of final compost. However, phosphorous was not affected by the addition of WCS, as it had adverse effects on nitrogen and degradation pattern. Therefore, it can be concluded that addition of 1% WCS for composting of vegetable waste mixture can be suggested for higher organic matter reduction within shorter time period using rotary drum composter.

Appropriate addition of WCS (1%) in trial 2 was considered to increase the activity of the microbes during composting. Higher temperature and prolonged thermophilic phase observed due to proper combination of waste materials and addition of carbide sludge. Higher diversity of microbial population was observed throughout the process leading to higher VS reduction in trial 4, followed by 3, 2 and 1. Even though higher VS reduction was observed in trial 3

(24%) and 4 (25%), but it was found beneficial as compared to trial 2 where 22.4% VS reduction was observed. Moreover higher addition of carbide sludge had huge effect on the temperature and organic nitrogen loss. Hence it can be concluded that utilization of waste carbide sludge in appropriate addition (1%) during drum composting of mixed vegetable waste can lead to prolonged thermophilic stage and higher lingo-cellulose degradation within shorter time period.

Inoculation of white rot fungus for decomposition of agricultural waste was found effective in terms of higher organic matter degradation and nutrient rich compost. Application of rotary drum composter for agricultural waste composting was found beneficial in providing uniform mixing and proper aeration for higher decomposition within shorter time i.e. 20 days of composting period. A maximum of 19.3% reduction of TOC was found in trial 3 as compared to 16.4 and 11.4% in trial 2 and 1, respectively. In addition to higher degradation, the final compost of trial 3 was observed with 2.59% of TKN and 4.54 g/kg of TP respectively. Since, the thermophilic phase had effect on the growth of *Phanerochaete chrysosporium*; inoculation after the thermophilic phase could be beneficial for higher degradation of organic matter. Finally, it can be concluded that inoculation of fungal concentration at the level of 10^6-10^8 spores/g of compost after thermophilic stage would be effective during rotary drum composting of agricultural waste.

From the present study it can be concluded that the waste carbide sludge from acetylene gas production industries can be successfully utilized during vermicomposting of agricultural waste as an additive. Appropriate addition of WCS resulted in increase of earthworm biomass and so as higher organic matter reduction. Final vermicompost was rich in nitrogen, phosphorous and micronutrients. Low electrical conductivity and no loss of nitrogen was observed during the study with appropriate addition of WCS. Therefore, it is recommended to carry out appropriate addition of WCS for different organic materials, while 1.5 to 2% of WCS would be optimum during vermicomposting of agricultural wastes.

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Chapter 6

MICROBIAL DIVERSITY

This chapter dealt with the microbial diversity of rotary drum composting with the best combinations of waste materials in (5:4:1) ratio using the 16S Metagenome sequence method.

6.1 16S METAGENOME SEQUENCING OF ROTARY DRUM COMPOSTING

The aim of the study was focused on the enumeration and identification of different bacterial communities involved in the degradation of vegetable waste. The best combinations of waste materials i.e. vegetable waste, cow dung, sawdust and dry leaves (5:4:1) ratio from rotary drum composting was analyzed using the 16S Metagenome sequence method.

6.1.1 QUALITATIVE AND QUANTITATIVE ANALYSIS OF gDNA AND PREPARATION OF LIBRARIES FOR 2 X 300 bp RUN CHEMISTRY

DNA was isolated using modified xcelgen soil gDNA kit. Quality of gDNA was checked on 1% agarose gel (loaded 5 µl) for the single intact band. The gel was run at 110 V for 30 mins. 1 µl of each sample was loaded in nanodrop 8000 for determining A260/280 ratio. The DNA was quantified using Qubit dsDNA BR Assay kit (Thermo Fisher Scientific Inc.). 1 µl of each sample was used for determining concentration using Qubit® 2.0 Fluorometer. The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc.) as per the 16S Metagenomic Sequencing library preparation protocol (Part # 15044223 Rev. B). Primers for the amplification of the V3-V4 hyper-variable region (Table 6.1) of 16S rDNA gene of Eubacteria and Archaea were designed in Xcelris NGS Bioinformatics Lab. These primers were synthesized in Xcelris PrimeX facility. The amplicons with the Illumina adaptors were amplified by using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were purified by 1X AMpureXP beads and checked on Agilent DNA 1000 chip on Bioanalyzer 2100 and quantified on fluorometer by Qubit dsDNA HS Assay kit (Life Technologies).

Table. 6.1. Primers used in the present study

Sr.No.	Oligo Name	Oligo Sequence (5' to 3')	Length of primer	Product size (Approx.)
1.	V3 - Forward	CCTACGGGNGGCWGCAG	17	~ 460 bps
	V4 - Reverse	GACTACHVGGGTATCTAATCC	21	

- ***Cluster Generation And Sequencing***

After obtaining the Qubit concentration for the library and the mean peak size from Bioanalyser profile, the library was loaded onto MiSeq at appropriate concentration (10-20pM) for cluster generation and sequencing. Paired-end sequencing allows the template fragments to be sequenced in both the forward and reverse directions on MiSeq. The kit reagents were used in binding of samples to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

- ***QC on Agarose Gel***

Primers designed to amplify the eubacterial 16S rRNA gene sequences including the variable V3 and V4 regions yielded complex single-strand-conformation polymorphism (SSCP) patterns on polyacrylamide gels. The intensity of bands in the gel indicated good reproducibility of the DNA extraction method and PCR amplifications. With primers targeting the V3 region of actinomycete 16S rRNA genes, the SSCP patterns consisted of strong bands and also a cluster of other bacterial species of the V4 region (Fig. 6.1).

6.2 TAXONOMIC HITS DISTRIBUTION

The pie charts below illustrate the distribution of taxonomic domains, phyla, and orders for the annotations. Each slice indicates the percentage of reads with predicted proteins and ribosomal RNA genes annotated to the indicated taxonomic level. This information is based on all the annotation source databases used by MG-RAST.

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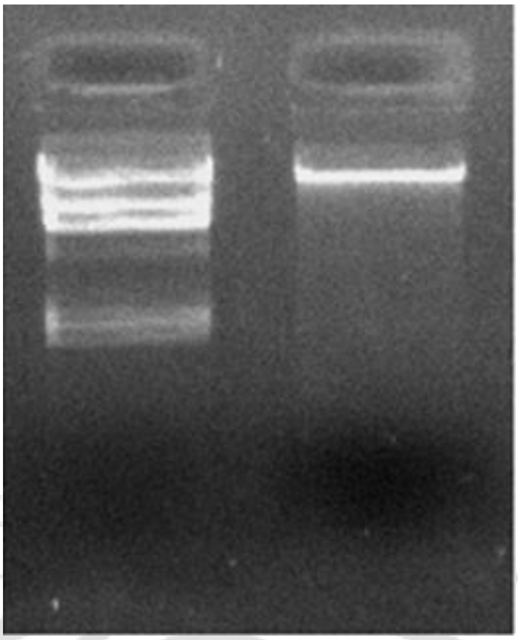

Fig. 6.1. QC of gDNA on 0.8% Agarose gel

6.2.1 TAXONOMIC HITS DISTRIBUTION AT DOMAIN LEVEL

Domain level taxonomic hits distribution shows that compost had 89.5% Bacteria, 9% Eukaryota followed by 1.4% Archaea as represented in Fig. 6.2. The higher abundance of bacteria can be considered due to the presence of readily available organic matter and lignocellulosic fraction. The early rise in the temperature of 5:4:1 combinations of drum composting can be considered due to these higher populations of bacteria.

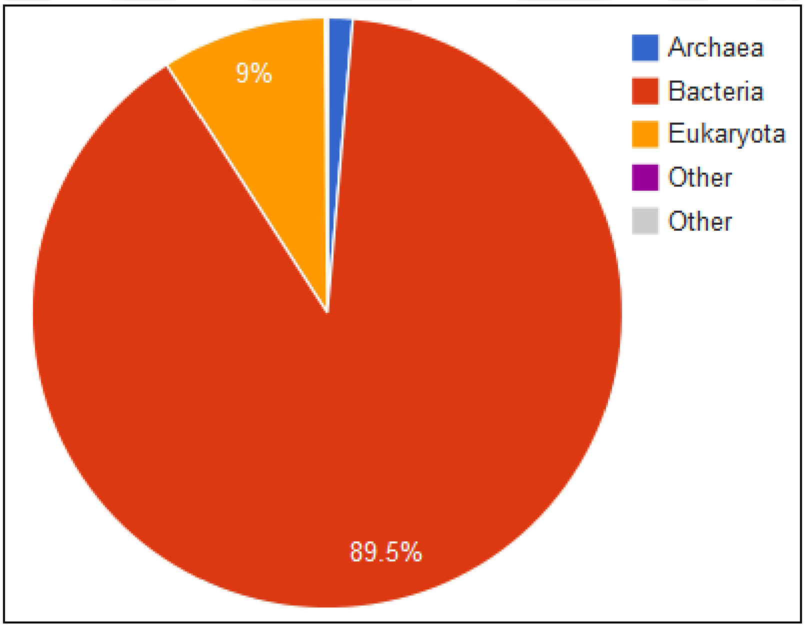

Fig. 6.2. Taxonomic hits distribution at domain level of compost

Bhatia et al. (2014) had also reported the higher abundance of bacteria during the composting of vegetable waste that were majorily involved in the degradation of organic matter. Eventhough, the population of eukaryota was observed low during the study, the importance of these organisms have reported crucial during composting and has been isolated during all stages of composting (Safika et al., 2013). The presence of archaea during composting are majorily involved in the oxidation of ammonia and it can successfully correlated to the higher reduction of ammoniacal nitrogen during the process.

6.2.2 TAXONOMIC HITS DISTRIBUTION AT PHYLUM LEVEL

Taxonomic hit distribution at phylum level shows that compost has 24.9% bacteroidetes, 21.6% firmicutes, etc as represented in Fig. 6.3. The bacteroidetes and firmicutes are facultative anaerobic and obligate anaerobic pathogens that are common in rumen.

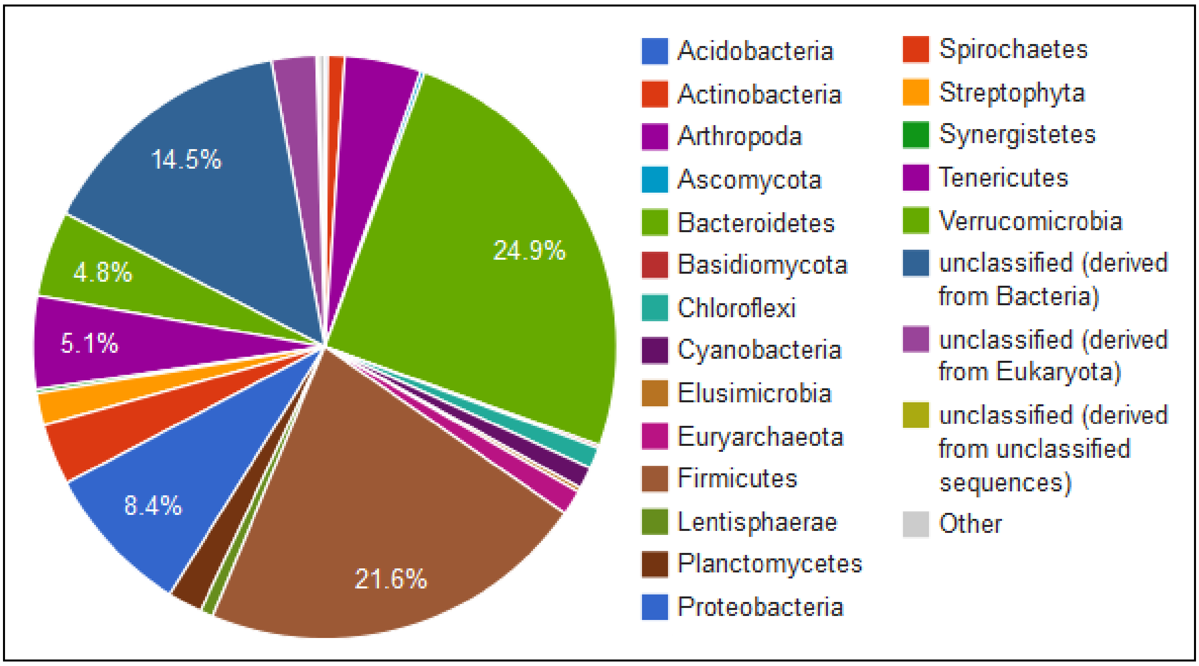

Fig. 6.3. Taxonomic hits distribution at phylum level of compost sample

The phylum bacteroidetes are composed of three large classes of gram-negative, non-spore forming, anaerobic or aerobic and rod-shaped bacteria that are considered important during the initial and final stages of composting. The addition of cow dung as bulking agent during composting can be considered as the major source for these microorganisms to the compost (Neher et al., 2013). Furthermore, relative abundance of γ-Proteobacteria, firmicutes and actinobacteria are reported as indicators of disease suppression during the process (Hadar and Papadopoulou, 2012). It was also reported that these organisms are highly involved in the maturation of composting during the final stage of the process.

6.2.3 TAXONOMIC HITS DISTRIBUTION AT CLASS LEVEL

Taxonomic hit distribution at class level shows that compost has 15.1% bacteroidia, 13.9% clostridia, 4.6% flavobacteria, etc as represented in Fig. 6.4.

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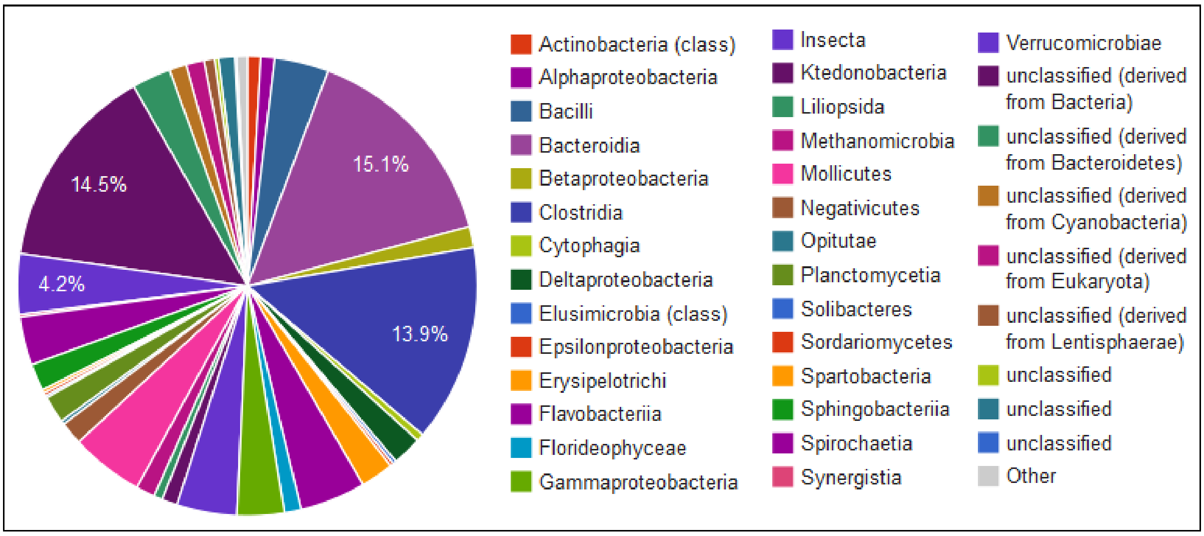

Fig. 6.4. Taxonomic hits distribution at class level of compost sample

The presence of flavobacteria was reported during the higher temperatures of natural compost, while *Arthrobacter sp.* was reported at the high-temperature process in cellulose-decomposing strain compost (Liu et al., 2011). *Bacillus sp.* was considered to be dominant species at middle and later stages of composting. Flavobacteria are common in composts and include opportunistic pathogens. Its presence in composts and the importance of these flavobacteria in the degradation of phenolic and chlorinated compounds has also been reported (Danon et al., 2008).

6.2.4 TAXONOMIC HITS DISTRIBUTION AT ORDER LEVEL

Taxonomic hit distribution at order level shows that compost has 15.1% bacteroidales, 13.8% clostridiales, 5.1% acholeplasmatales, etc. as represented in Fig. 6.5. The identified lactic acid bacterium is known to produce enzymes and natural antibiotics aiding effective digestion and has antibacterial properties, including control of salmonella and e.coli. This particular beneficial microorganism is often used in composting for stopping foul odors associated with anaerobic decomposition. Lactic acid bacteria thrive and feed on the ammonia released in the decomposition normally associated with foul odors. Rhizobiales is an order of alpha proteobacteria and are considered gram-negative. These rhizobia can fix nitrogen and are symbiotic with plant roots. The four families bradyrhizobiaceae, hyphomicrobiaceae, phyllobacteriaceae and rhizobiaceae contain at least six genera of nitrogen-fixing, legume-nodulating, microsymbiotic bacteria. The role of these rhizobiales in the denitrification of manure compost pellets and their relationship to N_2O emissions has been successfully correlated by Yamane et al. (2013).

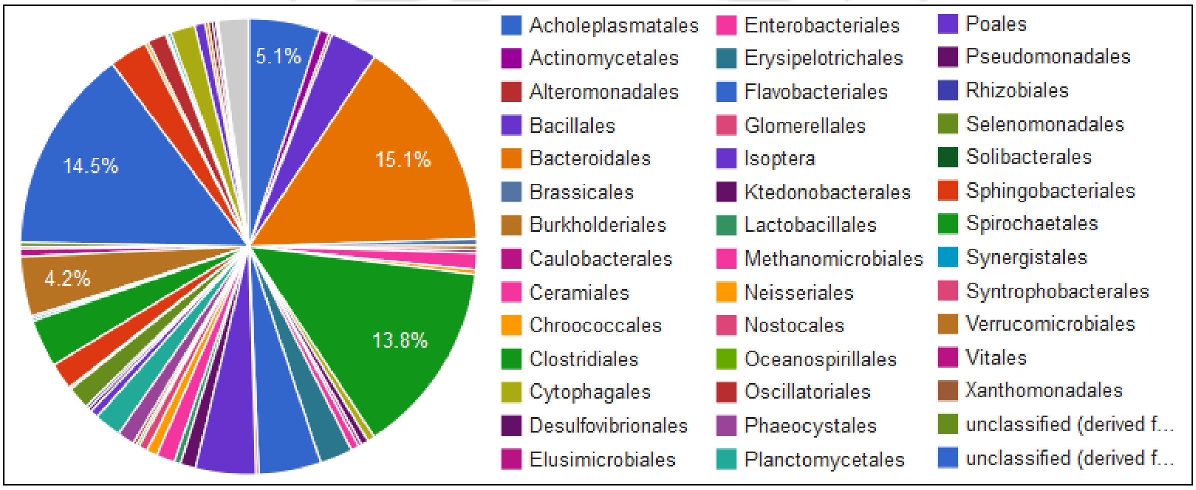

Fig. 6.5. Taxonomic hits distribution at order level of compost sample

6.3 LCA CLASSIFICATION PLOT OF COMPOST SAMPLE

The below LCA plot represents the high abundance of the phylum proteobacteria followed actinobacteria (Fig. 6.6).

6.4 CONCLUSION

Amplicon sequencing analysis has been carried out for compost sample on MiSeq platform, with following details. In total ~209 MB of data has been generated for Compost sample. MG-RAST analysis shows that compost sample shows α-Diversity=143.725 species. Taxonomic hits distribution at domain level shows that 89.5% sequences belonged to bacteria, 9% to eukaryota followed by 1.4% archaea in compost sample.

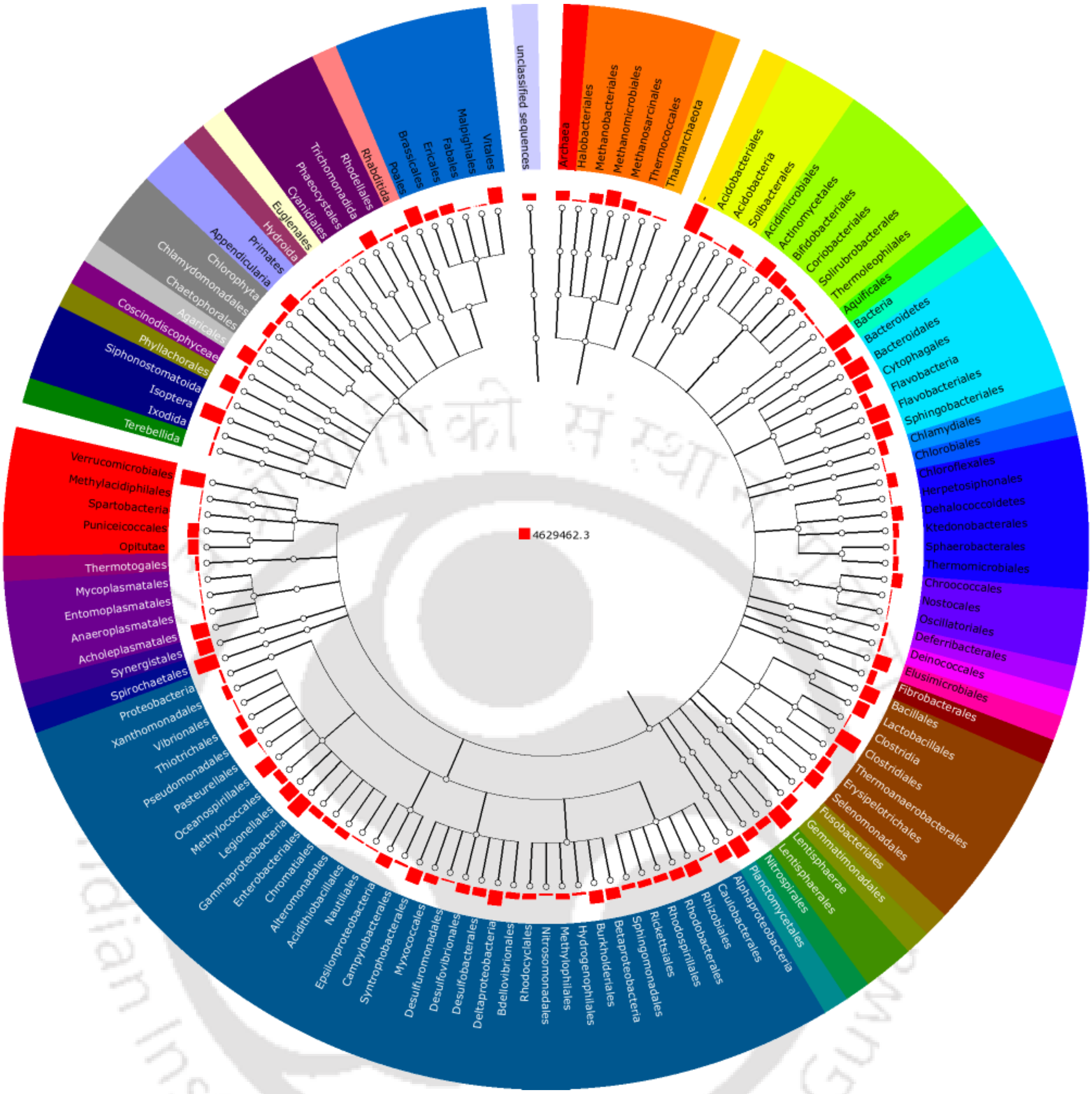

Fig. 6.6. Tree view at order level with colouring set to phylum level

The LCA classification plot shows the high abundance of the phylum proteobacteria followed by actinobacteria in compost sample. Actinobacteria play an important role in the later stages of composting and particularly for the degradation of relatively complex, recalcitrant compounds. The ability of actinobacteria to degrade lignocelluloses implies that this group of bacteria has potential to be useful indicators for compost maturity.

Taxonomic hit distribution at family level shows that compost sample has been enriched with *Thermomonosporaceae*. *Thermomonospora curvata* (strain ATCC

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19995/DSM 43183/JCM 3096/NCIMB 10081) is an aerobic, cellulolytic, thermophilic gram-positive bacterium which produces a number of industrially important compounds like cellulase, alpha-amylase, and polygalacturonate lyase. *T. curvata* can utilize many organic compounds present in the natural environment, such as cellulose starch, xylose or pectin.

According to details listed in Fig. 6.13, the sequences related to proteobacteria made up the largest fraction in compost sample, which included alpha, beta, gamma, delta and epsilon subclasses. The alphaproteobacteria comprising 165 OTUs were the most dominant subclass of proteobacteria followed by 156 OTUs of Gammaproteobacteria. The species like *Chelatococcus asaccharovorans, Rhodobium orientis, Pseudomonas thermotolerans, Pseudoxanthomonas sp. ITRH31, Pseudoxanthomonas dokdonensis, sulfur-oxidizing bacterium OAII2, Chondromyces apiculatus, Sorangium cellulosum,*etc were present in compost sample.

Firmicutes group was the second most frequent group. *Geobacillus thermodenitrificans* followed by *Geobacillus stearothermophilus* were the most abundant species in compost sample. *Bacillus licheniformis, Moorella thermoacetica, Brevibacillus thermoruber, Thermoactinomyces vulgaris, Thermobacillus xylanilyticus, Ureibacillus thermosphaericus, Bacillus pumilus, Laceyella sacchari, Ureibacillus thermosphaericus, Aneurinibacillus thermoaerophilus,* etc were the other species found in compost sample.

The other biggest group in compost sample was actinobacteria with *Thermoleophilum album* as the most abundant species followed by *Collinsella aerofaciens*. Thermomonospora was one of the common genera including species like *Actinomadura vinacea, Thermomonospora curvata, Actinoallomurus spadix, Actinomadura rubrobrunea*. The other species like *Pseudonocardia thermophila, Streptomyces thermovulgaris, Streptomyces thermoviolaceus, Thermobispora bispora,* etc were found in compost sample.

Strains belonging to the moderately thermophilic species have been detected during the study and are mainly involved in the lignocellulose degradation. *Pseudoxanthomonas spp*. has been reported to enhance cellulose degradation by their acetate-consuming effect and consequent pH neutralization (Farris and Olson, 2007). Moreover, the most common genera of actinobacteria in the compost were *Thermobifida* and *Thermomonospora*, which are well known for their cellulose- and hemicellulose-degrading ability (Steger et al., 2007). Since the temperature during composting had

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reached upto 66°C, the compost explained the dominant presence of these bacteria that are extremely survival at higher temperature and involved in degrading lignocellulose. Therefore, it can be concluded that the higher percentage of lignocellulose degradation observed in the present study can be successively correlated to these microorganisms as supported by the literatures.

Chapter 7

CONCLUSIONS AND RECOMMENDATIONS

This chapter dealt with conclusion achieved from the all phases conducted with different composting methods, effects of waste carbide sludge addition and fungal inoculation during composting process and finally the recommendations for the future scope.

7.1CONCLUSIONS

Rotary drum composting of vegetable waste was successful with the combinations of cow dung, saw dust and dried leaves. The 20 days of operation was found highly efficient for producing stabilized compost within shorter time. Appropriate addition of waste materials played a major role in the degradation process. The successful operation of vegetable waste composting is followed out by adding appropriate quantity of bulking agents such as saw dust and dry leaves to maintain the thermophilic phase and increase the efficiency of process. Since, lower addition of bulking agents during the process might lead to the production of leachate thereby deteriorating the quality of compost (loss of micro and macro nutrients). However, higher addition of bulking agents might add more lignocellulose concentration to the end product, which might take longer time for further degradation.

Maintenance of longer thermophilic phase during the process proved to be crucial in the elimination of pathogens and degradation of lignocellulosic fractions. Despite of various microbial communities during vegetable waste composting, each community was observed to act accordingly to temperature and nature of substrate available. Microbial population growth was influenced by the temperature and also the effective organic matter degradation. However, combinations of waste materials played a major role in favoring microbial succession. The final end product was completely stabilized with lower oxygen uptake rate and CO_2 evolution rate at the end of composting period.

Agitation, mixing and aeration of the composting materials was found crucial during pile composting of vegetable waste operated at agitated, passive and forced aeration condition. The degradation pattern of organic matter was completely different in comparison of all the three operated conditions. Higher aeration rate to the composting mass resulted in loss of thermophilic temperature and drying of materials.

Vermicomposting of vegetable waste by using *Eisenia fetida* was very effective as compared to *Eudrilus eugeniae*, with waste combinations in 5:4:1 ratio. Higher biomass production was observed during the 45 days of vermicomposting. However the efficacy of rotary drum composting over direct utilization of vegetable waste during vermicomposting by using *E. fetida* was proved efficient with higher loss of TOC and higher count of earthworm biomass at the end of process. The overall composting period was reduced to 28 days as compared to 45 days by pretreating the vegetable waste using drum composter followed by vermicomposting.

The addition of waste carbide sludge (WCS) increased the overall activity of microbes during the process, thereby resulting in higher organic matter degradation. Higher addition of WCS resulted in increase of pH of the composting mass, thereby volatilizing the nitrogen. However, phosphorous was not affected by the higher addition of WCS, as it had adverse effects on nitrogen and degradation pattern. With different proportions of WCS addition, 1% addition of WCS was observed to be optimum for vegetable waste composting with higher degradation in volatile solids and increase in nutrient value at the end of composting period.

Inoculation of white rot fungi was found effective in higher degradation of organic matter. However, thermophilic phase had effect on the growth of *Phanerochaete chrysosporium*; hence inoculation after the thermophilic phase could be beneficial for higher degradation of organic matter. Addition of WCS during vermicomposting was also observed successful. Appropriate addition of WCS i.e. 1.5 to 2% WCS resulted in increased biomass growth and higher organic matter degradation.

16S Metagenome sequencing of composting was observed with 89.5% sequences of bacteria, 9% of eukaryota followed by 1.4% of Archaea. The compost was enriched with different communities of microbial species. Higher abundance of proteobacteria was observed during the study followed by actinobacteria. Actinobacteria are majorily involved in the degradation of lignocellulosic fractions and helps in the maturity of the compost. In addition, a number of thermophilic bacteria were also observed which are majorily involved in the degradation during thermophilic stage of composting.

Therefore, it can be concluded that rotary drum composting of vegetable waste was found successful with the combinations of cow dung, sawdust and dry leaves within 20 days of composting period compared to pile composting. In addition, pre-stabilization of vegetable waste in drum composter followed by vermicomposting was also found successful for producing nutrient rich end product and reducing the overall composting period. The compost produced was well within the range of compost standards.

7.2 RECOMMENDATIONS FOR FUTURE WORK

- Evolution of composting dynamics by adding other bulking agents, agricultural and industrial waste materials could be carried out.
- Studies could be carried out on availability and speciation of heavy metals during vegetable waste composting.
- Persistent organic compounds degradation through composting.
- The potential role of composting in greenhouse gas mitigation - comparison of rotary drum and windrow composting methods.

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ANNEXURE I

1. Solid Waste Management Rules, 2015. Ministry of Environment, Forest and Climate Change

These rules shall apply to every urban local body, all statutory towns, outgrowths in urban agglomerations as declared by the registrar general & census commissioner of India, notified areas/notified industrial townships, notified area committees, area under Indian railways, defense cantonments, special economic zones in the country and every waste generator

In order to ensure safe application of compost, the following specifications for compost quality shall be met, namely:-

Parameters	**Organic compost (FCO 2009)**	**Phosphate rich organic manure (FCO 2013)**
Arsenic (mg/Kg)	10.00	10.00
Cadmium (mg/Kg)	5.00	5.00
Chromium (mg/Kg)	50.00	50.00
Copper (mg/Kg)	300.00	300.00
Lead (mg/Kg)	100.00	100.00
Mercury (mg/Kg)	0.15	0.15
Nickel (mg/Kg)	50.00	50.00
Zinc (mg/Kg)	1000.00	1000.00
C/N ratio	<20	Less than 20:1
pH	6.5-7.5	(1:5 solution) maximum 6.7
Moisture, percent by weight, maximum	15.0-25.0	25.0
Bulk density (g/cm^3)	<1.0	Less than 1.6
Total organic carbon, per cent by weight, minimum	12.0	7.9
Total Nitrogen, per cent by weight, minimum	0.8	0.4
Total Phosphate (as P_2O_5), percent by weight, minimum	0.4	10.4

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Total Potassium (as K_2O), percent by weight, minimum	0.4	-
Colour	Dark brown to black	-
Odour	Absence of foul odour	-
Particle size	Minimum 90% material should pass through 4.0 mm IS sieve	Minimum 90% material should pass through 4.0 mm IS sieve
Conductivity (as dsm^{-1}), not more than	4.0	8.2

* Compost (final product) exceeding the above stated concentration limits shall not be used for food crops. However, it may be utilized for purposes other than growing food crops.

2. Hong Kong Organic Resource Centre, Compost and Soil Conditioner Quality Standards, 2005.

Parameters	**Range (unit)**
pH	5.5 - 8.5
Moisture content	25 – 35 %
Temperature	< 8^o C to ambient temperature
C/N ratio	15 - 25
Oxygen uptake rate	$\leq$ 0.4 g O_2 /kg TS/h
CO_2 evolution rate	$\leq$ 2 g C/kg VS/day
E. Coli	$\leq$ 1000 MPN/g

3. Guidelines for Compost Quality, Canadian Council of Ministers of the Environment Maturity/Stability of Compost

Characteristics of mature and stable compost include biostabilization and humus formation. Guidelines for compost maturity are necessary as unstable/immature product has the potential to cause adverse effects on plants when applied in large amounts or attract vectors, such as flies, and to cause odour. Compost shall be mature and stable at

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the time of sale and distribution. To be considered mature and stable, compost shall be cured for a minimum of 21 days and meet one of the following three requirements:

a) The respiration rate is less than, or equal to, 400 milligrams of oxygen per kilogram of volatile solids (or organic matter) per hour; or,

b) The carbon dioxide evolution rate is less than, or equal to, 4 milligrams of carbon in the form of carbon dioxide per gram of organic matter per day; or,

c) the temperature rise of the compost above ambient temperature is less than 8 °C

- **Pathogens in Compost**

As pathogenic organisms may be present in the compost feedstock, the compost itself may also contain pathogenic organisms and, as a result, may pose a risk to human health. To adequately reduce these health risks, the compost shall conform to the criteria outlined in either a) or b) depending on the feedstock source.

When compost contains only yard waste the following criteria shall be met:

The compost shall undergo the following treatment or other process recognized as equivalent by the relevant province or territory.

- ➢ Using in-vessel composting method, the material shall be maintained at operating conditions of 55 °C or greater for three days.
- ➢ Using the windrow composting method, the material shall attain a temperature of 55° C or greater for at least 15 days during the composting period. Also, during the high temperature period, the windrow shall be turned at least five times.
- ➢ Using the aerated static pile composting method, the material will be maintained at operating conditions of 55°C or greater for three days. The preferable practice is to cover the pile with an insulating layer of material, such as cured compost or wood chips, to ensure that all areas of the feed material are exposed to the required temperature.

OR

Organism content shall meet the following: Fecal coliforms 2 < 1000 most probable number (MPN)/g of total solids calculated on a dry weight basis,

AND

No Salmonella sp. with a detection level < 3 MPN/4g total solids calculated on a dry weight basis.

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4. Ontario Compost Quality Standards, Ontario Ministry of the Environment Waste Management Policy Branch, 2012.

- **Maturity**

Generally, the term “mature” is used in reference to compost that exhibits limited biological activity, and which has degraded to the point where it can be stored and used without risk of odour and adverse effects, such as risk to plants from residual phytotoxic compounds. ‘Stability’ is different from ‘maturity’. ‘Stability’ generally refers only to reduced biological activity. Compost becomes more stable when it has completed the thermophilic phase of microbial decomposition. However, compost could also appear to be stable as a result of a nutrient imbalance or a lack of moisture, and therefore might not demonstrate extensive decomposition at the time of testing. It could become ‘unstable’ again if any of the limiting conditions are removed. All mature compost is stable, but not all stable compost is mature. In order for compost to meet Categories AA, A and B standards for maturity, it must be cured in accordance with the maturity criteria described below. The curing process is considered to have commenced immediately after the final quantity of compost has been discharged from the processing operation and added to the lot of compost to be cured. The compost shall be maintained at $\geq$ 40% moisture during curing. Compost is mature if:

The compost has been cured for a minimum period of 21 days from the day the last portion of material went into the batch, and the respiration rate is:

- less than, or equal to, 400 milligrams of oxygen per kilogram of volatile solids (on a dry weight basis) per hour; or,
- less than, or equal to, 4 milligrams of carbon in the form of carbon dioxide per gram
  of organic matter (on a dry weight basis) per day.

OR

The compost is made from leaf and yard waste only, and has been cured for a minimum period of 6 months.

Maximum Concentration for Metals in Feedstock

Item	Column 1	Column 2	Column3
	Metal	Category AA compost	Category A and B compost
		mg/kg dry weight	
1.	Arsenic	75	170
2.	Cadmium	20	34
3.	Chromium	1060	2800
4.	Cobalt	150	340
5.	Copper	760	1700
6.	Lead	500	1100
7.	Mercury	5	11
8.	Molybdenum	20	94
9.	Nickel	180	420
10.	Selenium	14	34
11.	Zinc	1820	4200

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PUBLICATIONS

INTERNATIONAL JOURNALS (PUBLISHED OR ACCEPTED)

1. Varma, V.S., Chatuphale, M., Kalamdhad, A.S. (2013). "Effects of bulking agent in composting of vegetable waste and leachate control using rotary drum composter." *Sustainable Environmental Research*, 24(4), 245-256.
2. Varma, V.S., Kalamdhad, A.S. (2014). "Bio-conversion of organic waste using composting technologies - a review." *International Journal of Environmental Technology and Management*, 17(6), 183-507.
3. Varma, V.S., Ramu, K., Kalamdhad, A.S. (2014). "Effects of waste lime sludge on nitrogen dynamics and stability of mixed organic waste using rotary drum composter." *International Journal of Environmental Research*, 9(1), 395-404.
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6. Varma, V.S., Kalamdhad, A.S. (2014). "Stability and microbial community analysis during rotary drum composting of vegetable waste." *International Journal of Recycling of Organic Waste in Agriculture*, 3:52.
7. Varma, V.S., Ramu, K., Kalamdhad, A.S. (2015). "Carbon decomposition by inoculating Phanerochaete chrysosporium during drum composting of agricultural waste." *Environmental Science and Pollution Research*, 22(10), 7851-7858.
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ARTICLES (SUBMITTED REVISION/UNDER REVIEW)

1. Varma, V.S., Kalamdhad, A.S. (2016). "Microbial degradation of lignocellulosic fractions during drum composting of mixed organic waste." *Sustainable Environment Research* (Under review)

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3. Varma, V.S., Kalamdhad, A.S. (2016). “Efficacy of rotary drum composting for stabilizing vegetable waste during pre-composting and vermicomposting technology.” *Environmental Engineering Research* (Under review)
4. Varma, V.S., Kalamdhad, A.S. (2016). “Changes in physico-chemical parameters during pile composting of vegetable waste operated at agitated, passive and forced aerated condition.” *Environmental Science and Pollution Research* (Under review)
5. Varma, V.S., Kalamdhad, A.S. (2016). “Microbial community changes and lignocellulose degradation by inoculating white rot fungi during In-vessel composting of biowaste.” *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* (Under review)

INTERNATIONAL CONFERENCE AND PROCEEDINGS

1. Varma, V.S., Kalamdhad, A.S. (2013). “Chemical and microbial aspects during rotary drum composting of vegetable waste.” *Proc., International conference on technologies for sustainable waste management in developing countries*, Guntur, India, 64. **(Best paper award)**
2. Varma, V.S., K. Ramu, Kalamdhad, A.S. (2014). “Decentralized composting of mixed organic waste using in-vessel system.” *Proc., International conference on harnessing natural resources for sustainable development: global trends*, Cotton College, Guwahati, India, 260.
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NATIONAL CONFERENCE AND PROCEEDINGS

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