Composting of Organic Wastes for use in Agriculture

A

DISSERTATION REPORT

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR

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CERTIFICATE

Certified that the thesis "**Composting of Organic Wastes for use in Agriculture**" submitted by Ms. Drishti Sharma, in fulfilment of the requirement for the award of the degree of **Masters in Science** in the Department of Biotechnology, Thapar University, Patiala, Punjab, India is the record of the candidate's own independent and original research work carried out by her under my supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree.

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DECLARATION

I hereby declare that the work which is being presented in this thesis **"Composting of Organic Wastes for use in Agriculture"** submitted by me for the award of the degree of **Masters in Science** in the Department of Biotechnology, Thapar University, Patiala, is true and original record of my own independent and original research work carried out under the supervision of Dr. Dinesh Goyal, Professor & Head, Department of Biotechnology, Thapar University, Patiala, Punjab, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

Place: Patiala (Drishti Sharma)

Date: July 14, 2015

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Life itself is a unique project, the objectives of which can never be accomplished alone despite putting our best endeavours in different phases of life. To taste the success, everyone requires perennial support, guidance, motivation, logical feed-back and healthy critical analysis of guides, teachers, mentors, supporters, well-wishers and parents.

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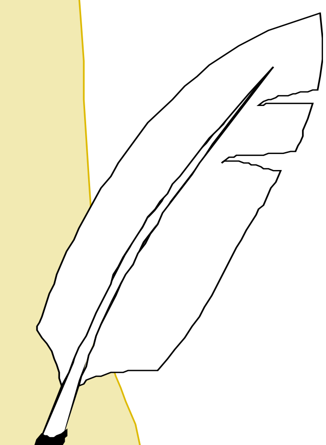
Date: Drishti Sharma

Place: Patiala

Dedicated

To

My Parents



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List of Symbols

%	Percent
°C	Degree Celsius
θ	Angle of diffraction
α	Alpha
C	Carbon
H	Hydrogen
O	Oxygen
N	Nitrogen
P	Phosphorus
K	Potassium
Mg	Magnesium
Na	Sodium
Cu	Copper
Zn	Zinc
Ca	Calcium

List of Abbreviations

et al	And others
etc	And other things
g	Grams
mg	Milligrams
Kg	Kilograms
h	Hour
min	Minutes
s	Second
M	Molarity
rpm	Revolutions per min
CO_2	Carbon dioxide
sp.	Species
MSW	Municipal solid waste
$K_2Cr_2O_7$	Potassium dichromate
DPA	Diphenylamine
FAS	Ferrous ammonium sulphate
H_2SO_4	Sulphuric acid
DNS	Dinitro-salicylic acid
w/v	weight by volume
HgO	Mercuric Oxide
$CuSO_4$	Copper sulphate
K_2SO_4	Potassium sulfate
CH_4	Methane
NH_3	Ammonia
MTCC	Microbial type culture collection
NCIM	National collection of industrial microorganism

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Abstract

Present research work was aimed at developing a system for recycling and utilization of compost derived from kitchen wastes for soil amendment to improve plant growth. Treatment of kitchen waste with a consortium of fast degrading microbes was done. Various parameters were monitored to check the degradation. Kitchen waste was collected from Thapar University, hostel mess and kinnow peels were collected from fruit shop at Thapar University, Patiala. Composting of kitchen waste and kinnow peels was done in pits and plastic tubs respectively using a consortium of microbes, comprising *Bacillus subtilis* (NA 15), *Paenibacillus polymyxa*, *Saccharomyces cerevisiae* and *Trichoderma reesei* (MTCC 164).

Using this consortium, various parameters such as pH, EC, organic carbon, nitrogen, reducing sugar, ash content and volatile solids were monitored at different intervals for 90 days. The carbon content was reduced to 36.31% from 69.03% in 56 days of composting while in case of kinnow peels it reduced to 48%. Simultaneously there was a drop in C: N ratio, optimum C: N ratio was obtained in just 56 days while in the case of kinnow peels the C: N ratio dropped to optimum range of 27.09 in 42 days. Loss in moisture content was monitored in kitchen waste, which reduced from 55.5% to 21.1% over the period of 90 days. On the other hand moisture content in kinnow peels dropped to 18.5%. Reducing sugar yield was observed to have an increasing trend to 65.4 mg/mL from 3 mg/mL while in case of kinnow peels it increased to 107.3 mg/mL from 8 mg/mL. The total nitrogen values tend to increase from 0.063% on day 0 to 2.15% in 90 days of composting. Also the kinnow peels showed the trend, where the nitrogen content increased from 0.057% to 3.66%. Scanning electron microscopy (SEM), X-ray diffraction (XRD) and FTIR was done to check the physical and chemical properties of the treated and untreated biomass. Bio-efficacy trials of the compost prepared from kitchen waste and kinnow peels was checked for its effect on plant growth in pots using brinjal and ladyfinger respectively. Soil to compost ratio was maintained as 3:1 in pots and their performance was compared to plants growing in soil without addition of compost. Physico-chemical test for soil like pH, total carbon, and total nitrogen was checked to evaluate the efficiency of the compost followed by destructive analysis in which biomass produced, root and shoot length along with germination index was analyzed. The Germination Index in case of brinjal with compost was 70 % and in case of ladyfinger it was 66 %. Thus as compared to control the plants grown with compost were much healthier.

Chapter- 1

Introduction

Waste volume continues to increase, which leads to loss of resources and increased environmental risks. Landfill and incineration, until now have been the most widely used means of solid waste disposal throughout the world, the land filling of biodegradable waste is proven to contribute to environmental degradation, mainly through the production of highly polluting leachate and methane gas. Human activities have always generated waste. This was not a major issue when the human population was relatively small and nomadic, but became a serious problem with urbanisation and the growth of large conurbations (Kotovicova et al., 2010). According to FAO, "No economic, environmental or ethical argument can be made to justify the extent of food waste and loss currently happening in the world. 1.3 billion tonnes of food is wasted or lost every year". The problems are varied depending from one place to another and even affect human health. The increase in the production of wastes in a society could be brought down if an added value is attributed to them.

The benefits derived from utilization of organic materials for improvement of soil fertility and crop production have been well discussed by many authors (Tandon, 1992; Tian et al., 1992; Parr et al., 1986; Parr et al., 1984). The importance of balanced fertilization using organic waste materials, supplemented with other nutrient sources including employment of appropriate cultural practices, cannot be overemphasized (Lombin et al., 1994).

Composting is biological process, which turns food waste into fertilizer. Application of compost to agricultural land is required to be carried out in such a manner that it ensures sustainable development. Management systems have to be designed to maximize agronomics benefit, while ensuring the protection of environmental quality. The main determinant for efficient agronomics use is nitrogen availability, from mineral fertilizers as well as increasing the nitrogen use efficiency of organic fertilizers (Amlinger et al., 2003).

In the present study degradation pattern of kitchen waste (mixed waste) along with specific kinnow peels was done using consortium of microbes. Microbially treated biomass was subjected to many physico-chemical tests. Consortia treated biomass and untreated biomass were subjected to SEM, XRD and FTIR for the analysis of physical and chemical changes.

Compost prepared from kitchen waste and kinnow peels was subjected to bio-efficacy trials using seasonal vegetables such as brinjal (egg-plant) and lady in pots containing soil and compost (3:1) and also control was set up containing only soil. Destructive analysis was done on the test plants to check the amount of biomass obtained from each pot. Also the comparison on the basis of root and shoot length were done for the same. The plants grown in compost showed better shoot length i.e. 15.5 cm in brinjal and 16.7cm in ladyfinger.while the shoot length in case of control was 6.9 cm and 14.5 cm respectively. Along with biomass produced, the soil parameters (pH, Organic carbon, Nitrogen, and C: N ratio) were monitored before and after compost application.

Chapter- 2

Review of Literature

Composting helps to optimize nutrient management and the land application of compost may contribute to combat soil organic matter decline and soil erosion (Camp et al., 2004). Compost land application completes a circle whereby nutrients and organic matter which have been removed in the harvested produce are replaced (Diener et al., 1993). The recycling of compost to land is considered as a way of maintaining or restoring the quality of soils, mainly because of the fertilizing or improving properties of the organic matter contained in them. Furthermore, it may contribute to the carbon sequestration and may partially replace peat and fertilizers (Smith et al., 2001). Organic waste management strategies can help to utilize this leftover kitchen waste in making up of nutrient rich compost. However in order to obtain a product with high value as fertilizer, the composting process should be performed adequately. It can destroy pathogens and converts nitrogen from unstable ammonia to stable inorganic forms, reduces the volume of waste, and satisfies the needs of fertilizer for agricultural use seasonally (Zhu, 2007). Waste volumes continue to shoot up the values, which leads to loss of resources and increased environmental risks. Open dumping and sanitary landfill is a major method for waste disposal.

The biological treatment of biodegradable waste involves either aerobic or anaerobic techniques. Biological processing includes composting, anaerobic digestion and mechanical biological treatment (Tweib et al., 2011)

2.1 Types of composting

Composting is extensively practised in two ways depending upon availability of oxygen for effective micro-organisms present in the environment.

2.1.1 Aerobic composting: Composting refers to the break-down of organic wastes in the presence of oxygen (air). Products evolved in this process include CO_2, NH_3, water and heat. Thus, appropriate blend of ingredients and environmental conditions complete successful composting process. The parameters like moisture contents of around 60-70% and carbon to nitrogen ratios (C: N) of 30/1 are desirable. Any significant variation inhibits the degradation process (Tweib et al., 2011). Sufficient amount of oxygen is made to circulate in the organic waste through ventilation or forced and passive aeration.

2.1.2 Anaerobic composting: Composting without oxygen releases products like CH_4, CO_2, NH_3 and trace elements. Anaerobic composting is primarily used for the composting of animal waste manure and sewage sludge but now it is widely being used in treatment and management of municipal solid (Tweib et al, 2011).

2.2 Phases of composting

The time–temperature course of the composting process can be divided into four phases as discussed by Tweib et al., 2011:

2.2.1 Mesophilic phase: Onset of the first phase a distinct population of mesophilic bacteria and fungi proliferates, reducing primarily the readily available nutrients and thereby increasing the temperature to about 45 °C. At this point their activities stops, the vegetative cells and hyphae die and eventually breakdown, and only heat resistant spores survive.

2.2.2 Thermophilic phase: After the short span of lag phase, there occurs the more or less steep rise in temperatures. The onset of this phase is marked by the development of the thermophilic species of bacteria, actinomycetes and fungi. The optimum range for this microbial population is 50-65°C and their activity stops at range 70-80°C (Tweib et al., 2011).

2.2.3 Stationary phase: It is regarded as the third phase, without any significant change in temperature as the heat produced by microbial diversity and heat dissipated is balanced during this phase. While, the microbial population continue to have thermophilic bacteria, actinomycetes and fungi.

2.2.4 Maturing phase: Drop in temperature, signifies the maturation period of the composting process. The mesophilic microbes get activated again and invade the activity of thermophilic microbes, which in turn fasten the degradation process.

Table 1: **List of materials that can be used for composting**

Sewage sludge	Kitchen waste (fruit and vegetable peels, egg shells, tea bags and left over food waste.
Municipal solid waste	Soft prunings
Industrial waste	Grass clippings
Poultry waste	Leaf litter
Agricultural waste (rice and wheat straw)	Shredded paper

Composting is majorly done to replenish the soil nutrients. Composting has many beneficial properties (Fig. 1). Nowadays along with improving physico-chemical properties of soil composting is the best alternative for waste management. All sort of organic and biodegradable wastes can undergo composting (Table 1).

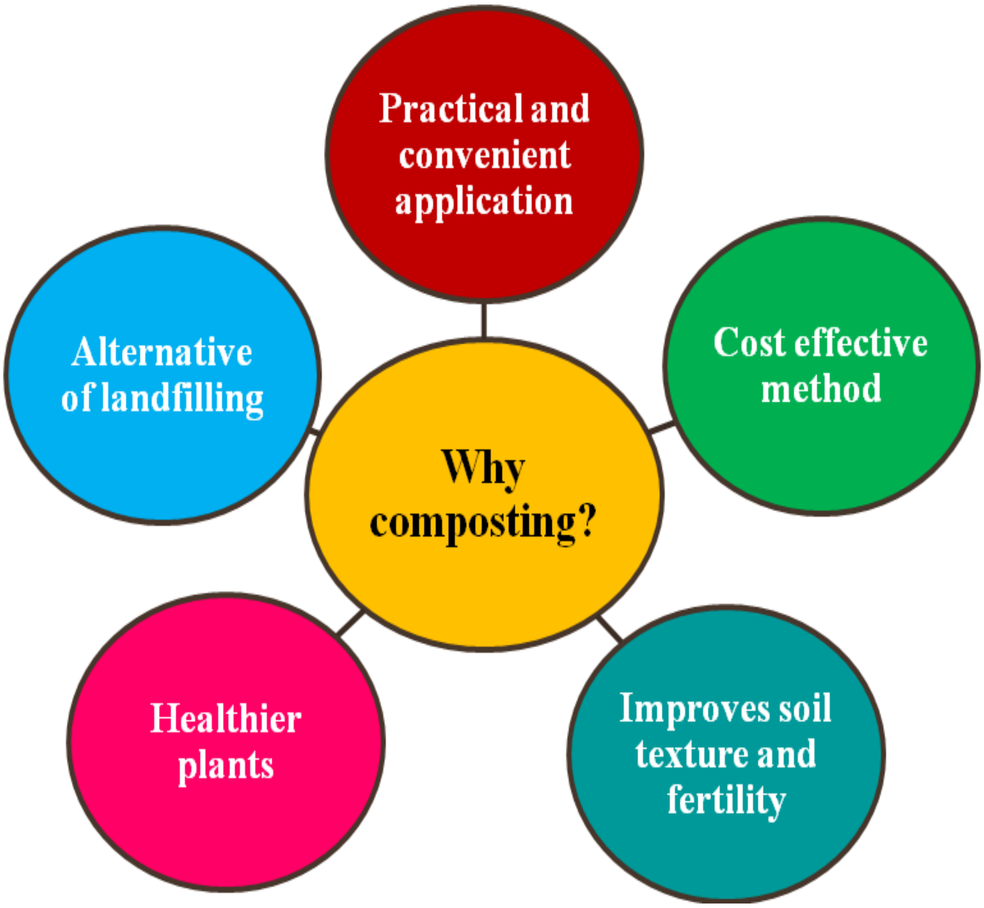

Fig 1: Benefits of composting.

2.3 Methods of composting

(Source: On farm hand-book of composting by William F. Brinton, 2000)

2.3.1 Aerated static pile: This method was developed in United States, Department of Agriculture. The pile of biomass is constructed over an air source such as, aeration cones, perforated plastic pipes or a perforated floor; and aeration is accomplished either by forcing air inside the pile or drawing air through the compost pile. The monitoring equipment determines the timing, duration and direction of air flow. Air flow requirements will depend on the types of biomass to be composed, the size of the pile, and age of the compost. The top of the pile after addition of biomass is covered with the insulation sheet and left for composting process as shown in Fig 2.

2.3.2 Turned windrow: It involves the production of compost in windrows using mechanical aeration. The compost mix is aerated by a windrow turner, which can be powered by a farm tractor, self-powered or self-propelled. Turned windrow composting represents a low technology and medium labour approach and produces uniform compost.

2.3.3 In-vessel composting: It refers to degradation of biomass within a building, container, or vessel. In-vessel methods depend upon a variety of forced aeration and mechanical turning techniques to brush up the composting process. Number of methods combine techniques from the windrow and aerated pile methods in an attempt to overcome the deficiencies and exploit the attributes of each method. There are different combinations of vessels, aeration devices, and turning mechanisms. This method of composting requires consistent level of management/ product flow to be cost efficient (Table 2).

2.3.4 Bin composting: It is some-what similar to in-vessel composting. It includes the production of compost in a bin. The compost is produced by natural aeration and through turning at fixed intervals using a tractor front-end loader. Bin composting represents a low technology, medium labour approach producing a medium quality product. This option is primarily used for mortality composting.

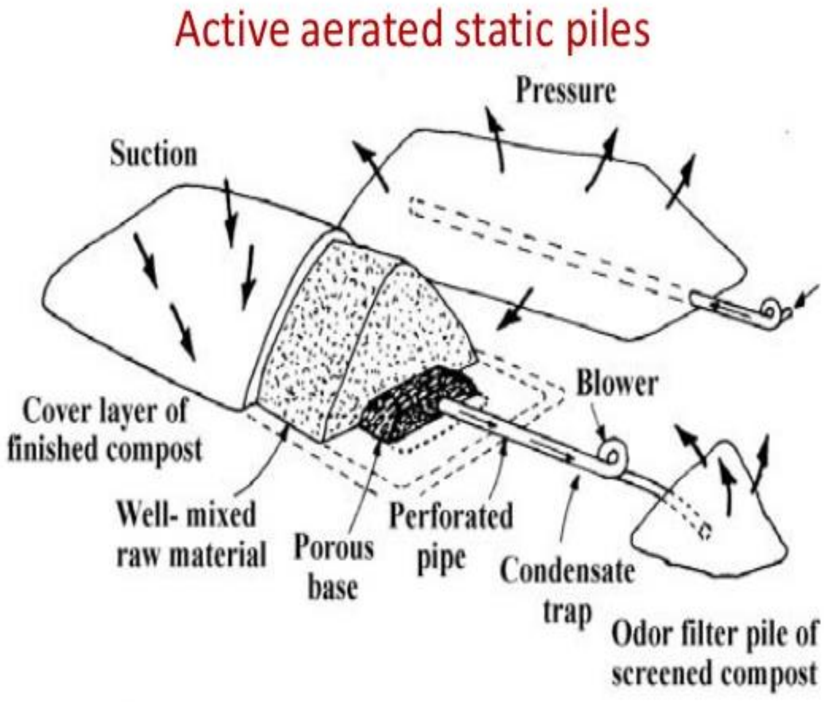

Fig 2: Aerated static composting pile

Table 2: Methods of composting and their properties

	Passive windrow	Aerated windrow	Aerated static pile	In-vessel method
General	Low technology Quality problems	Active systems most common on farms	Effective for farm and municipal use	Large-scale systems for commercial applications
Labour	Low labour required	Increases with aeration frequency and poor planning	System design and planning important. Monitoring needed	Requires consistent level of management/ product flow to be cost efficient
Site	Requires large land areas	Can require large land areas	Less land required given faster rates and effective pile volumes	Very limited land, due to rapid rates and continuous operations
Bulking agent	Less flexible	Must be porous Flexible	Less flexible	Must be porous Flexible
Active period	Range: 6-24 Months	Months Range: 21-40 days	Range: 21-40 days	Range: 21-35 days
Curing	Not applicable	30+ days	30+ days	30+ days
Size: Height: Width: Length:	1 - 4 metres 3 - 7 metres Variable	1 - 2.8 metres 3 - 6 metres Variable	3 - 4.5 metres Variable Variable	Dependent on bay design Variable Variable
Aeration system	Natural convection only	Mechanical turning and natural convection	Forced positive/ negative air flow through pile	Extensive mechanical turning and aeration
Process control	Initial mix only .	Initial mix Turning	Initial mix. Aeration, temperature and/or time control	Initial mix. Aeration, temperature and/or time control. Turning
Odour factors	Odour from the windrow will occur. The larger the windrow the greater the odours.	From surface area of windrow. Turning can create odours during initial weeks.	Odour can occur, but controls can be used, such as pile insulation and filters on air system	Odour can occur, but controls can be used, such as pile insulation and filters on air system

Source: British Columbia, Composting factsheet, 199

2.4 Composting parameters

In order to fulfil the conditions of composting various physical and biochemical parameters need to be satisfied before the onset of action of composting process. Any alteration in these parameters can deteriorate the microbial population which in turn can slow down the degradation process.

The list of parameters to be monitored is:

1. pH
2. Temperature
3. Moisture content
4. C: N ratio

2.4.1 pH: It determines the activity of different fungi, bacteria and actinomycetes present in the environment. The recommended range is 4.2 – 7. The acidic pH is observed due to the production of lactic acid and acetic acid during initial phase of biodegradation of biomass. (Dickson et al., 1991)

2.4.2 Temperature: Heat generated by the microbes in the pile increase the temperature of the composted pile. The temperature range between 32-65° C indicates the fast processing of biomass in the pile. Thus temperature above 65°C can hinder the activity of some microbes. Temperature probes can be used on regular basis to keep the track record of pile and maintain the compost micro-flora for rapid composting (Dickson et al., 1991).

2.4.3 Moisture content: The optimum moisture range for compost is 40-60%. Thus, if the moisture content falls from 40%, the bacterial population will reach the dormant stage and if it exceeds 60%, the nutrients are leached and anaerobic conditions will prevail, affecting degradation process. In case of excess moisture bulking agents like grass clippings, paper cutting and leaf litter can be used to maintain moisture content (Dickson et al., 1991).

2.4.4 C: N ratio: The carbon source present in biomass is the real energy booster for the micro-organisms. Moreover, a very small fraction of carbon is incorporated into microbial cells. Meanwhile, nitrogen is essential for growth of microbes. If nitrogen is deficient, microbial populations will remain lesser and decomposition rates for available carbon will be lower. According to Golueke (1992), rapid and entire humification of substrates by the microorganisms primarily depends on it, initially having a C: N ratio between 25 and 35.

Several authors have concluded that using a single parameter as a maturity index is insufficient and that amalgamation of several parameters is usually needed (Table 3). The influential factors affecting composting include temperature, moisture content, C: N ratio, degree of aeration, pH level, and the physical structure of the waste material. Furthermore, compost maturity and stability are the significant factors of the bioprocess.

Table 3: Different composting parameters with their optimum range

Parameter	**Standardised range during composting**
pH	Acidic to neutral
Moisture content (%)	40-60
C:N ratio	30:1
Temperature (°C)	32-60
Oxygen (%)	5
Organic matter (%)	>20
Total N (%)	Less than 2

(Source: Dickson et al., 2011).

Bernal et al., 1998 discussed Rutgers static pile composting; this method was used to maintain the ceiling temperature of the static pile providing a high decomposition rate. The organic matter and carbon content reduced during this process pointing towards the biodegradation of the organic matter. Also, the C: N ratio decreased to 9.4 during the composting process. The Germination Index exceeded 50%, which indicated the lack of phytotoxicity (Table 3).

According to the studies of Jusoh et al., 2013, the effective microbes (EM) fasten the degradation process based on the study of different parameters. Composting of rice straw with effective microbes was monitored, which aims to evaluate the quality of both compost treatments. The parameters for this process such as temperature, pH, Total organic Carbon and C: N ratio, show that decomposition of organic matter occurs during the 90-day period. The decrease in Total organic Carbon values and C: N ratio also shows that an organic compound is being consumed by microorganisms as discussed in the Table 3.

Anaerobic biodegradation of kitchen waste was assessed by Neves et al., 2008, in batch assays, under different solid contents between 1.8 and 24%. Methanization rate and cumulative methane production from synthetic wastes simulated with different blends of protein, carbohydrates, fat and cellulose were compared. They concluded that the real kitchen waste was more biodegradable than the synthetic waste. However both produced methane at similar rates in batch assays for a waste/seed ratio of 1.35 gVS/gVS (Neves et al., 2008).

Compatible lignocellulolytic fungal consortia can be introduced for rapid composting of rice straw. Forty-nine isolates of fungi were isolated from several natural and induced rice straw composting sources Kausar et al., 2010 excised four isolates (F26, F28, F29, and F44) were selected as potential lignocellulolytic agents for in-vitro compatibility study based on their optimum growth rate, biomass production, and lignocellulolytic activities. The fungal consortium was able to decompose cellulose, hemicelluloses, lignin, and total carbon significantly ($p < 0.05$) over the control. The C: N ratio was reduced to 19.5 from an initial value of 29.3 in three weeks of the biodegradation process. Thus, it shows the potential of this method, for use in large-scale composting of rice straw. (Kausar et al., 2010)

Imam and Sharanappa (2002) reported that, composting of poultry manure with different crop residues (wheat and ragi straw) at varying ratios like 0.25:1, 0.5:1, 1.75:1 and 1:1 ratio for 3 months under vat method recorded high nutrient content in 1:1 proportion with the values of 3.5 % N, 4.94% P and 2.1 % K and C: N ratio of 6:1.

Ahmad et al. (2007) reported that organic waste materials can be converted into value-added organic fertilizer by the addition of lower doses of nitrogen as well as biologically active substances. It is possible to get higher yield with complimentary use of organic and inorganic (chemical) fertilizers than the application of organic or chemical fertilizers alone. The improvement in soil health and reduction in piling of organic wastes could be extra benefit.
The increase in total nitrogen may have been due to the net loss of dry mass as the loss of organic carbon as CO_2 during composting (Abdelhamid., 2008). Moreover total N can be increased by the activities of nitrogen-fixing bacteria at the end of the composting process. The same authors found that the C: N ratio decrease to 13.3 -8.9 from initial C: N ratio of 19.2.

In order to fasten the process of composting, inclusion of a higher proportion of sappy green matter with higher nitrogen content (lower Carbon/Nitrogen ratio) such as grass and other plant cuttings and poultry manure is essential (Misra and Roy 2004). To prevent loss of nitrogen due to turning, that provide greater opportunity for ammonia volatilization (Harrison, 2005), the compost pile was left unturned for a composting period of 36 days in this study. The disturbance occurred only during the addition of green biomass for the 3:2 and 3:3 treatments.

Rich et al., (2014) explained the concepts of composting Municipal solid waste (MSW) by studying temperature, aeration rate, moisture content, porosity, C: N ratio, micro-organisms. pH range between 6.2 -9 supported microbial activity. The leachate produced in the compost of MSW can be collected safely and disposed properly through in-vessel composter.
Pineapple leaves and chicken manure was composted to obtain high quality organic fertilizer. Ch'ng et al., 2013 inferred that N and P content increased while C content decreased and the pH of the compost increased from 6.14 to 7.89. These factors concluded the quality of the compost as quality fertilizer.

The appropriate balance of carbon and nitrogen in a system builds up the C: N ratio. Every organic matter is made up of substantial amounts of carbon combined with lesser amounts of nitrogen. It is expected that as composting proceeds to maturity, the C: N ratio gradually decreases from around 30:1 to 10-15:1 for finished compost. According to Chen et al., 2011 this occurs because each time organic compounds are consumed by micro-organisms, two-thirds of the carbon is converted and given off as CO_2. Fall of C: N ratio during composting and in its final value can even be used as one criterion of relative maturity of the product (Hubbe et al., 2010). However Chazirakis et al., 2011 indicated that it is necessary that caution is taken before using C: N ratio as criteria for relative maturity since not all carbon is available for microbial use.

Richard and Trautmann (1996) indicated that, although the usual recommended range for the C: N ratios at the start of a composting process should be about 30:1, depending on the bioavailability of the carbon and nitrogen, this recommendation may vary. Depending upon the nature of raw material the C: N ratio varies (Table 4). The nitrogen content of composts varies depending upon the source of material and how it is composted (Mangan et al., 2013).

The availability and binding dynamics of total compost Nitrogen in soils during the long and short term is strongly linked to the soil organic matter and the relative magnitude of the active pool and the more passive pool of the soil organic matter (Nortcliff, 1999; Amlinger et al., 2003). Compost addition to soils was shown not to increase mineralization of soil organic matter (Sikora and Yakovchenko, 1996). Figure 3 illustrates the potential fate of compost N in the soil.

Table 4: Estimated C: N ratio of different organic wastes

High carbon materials browns	High nitrogen materials green	C: N ratios for browns	C:N ratio for greens
Ashes, wood	Alfalfa	25:1	12:1
Cardboard, shredded	Clover	350:1	23:1
Corn stalks	Coffee grounds	75:1	20:1
Fruit waste	Solid waste	35:1	20:1
Leaves	Garden waste	60:1	30:1
Newspaper, shredded	Grass clippings	175:1	20:1
Peanut shells	Hay	35:1	25:1
Pine needles	Manures	80:1	15:1
Sawdust	Seaweed	325:1	19:1
Straw	Vegetable scraps	75:1	25:1
Wood chips	Weeds	400:1	30:1

(Source: Composting 101, 2006).

Knowledge of the short and long term availability of Nitrogen following biowaste and vegetable waste compost application is essential in order to meet crop requirements, whilst ensuring environmental protection from excessive nitrate leaching. Increasing the Nitrogen use efficiency of organic amendments and understanding the Nitrogen dynamics in compost amended soils remain important issues for research (Amlinger et al., 2003; Gutser et al., 2005).

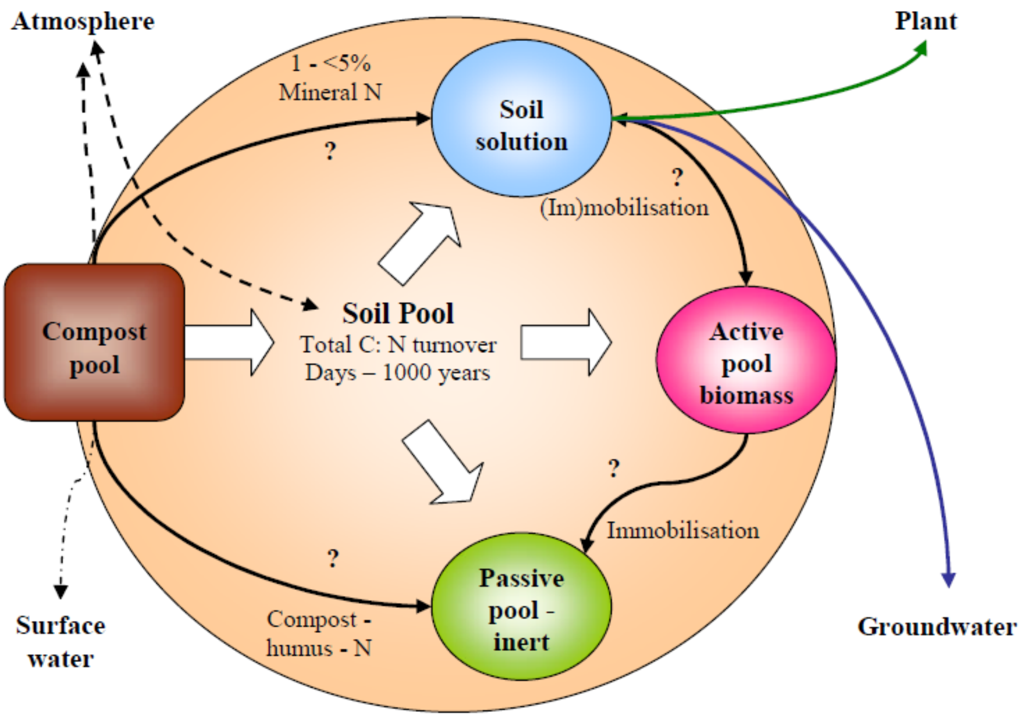

Fig. 3: Sparely known fate of compost nitrogen in the soil organic matter pools (Adopted from Amlinger et al., 2003).

The total nitrogen present in the organic sample shows the increasing trends as the degradation rate increases. The organic portion of compost total nitrogen which is not readily available to plants, can be mineralized, and then potentially taken up by the plants, immobilized, denitrified, volatilized, fixed within the clay minerals and/or leached. Fig.3. illustrates the main transformations of compost nitrogen applied to the soil.

Nitrogen dynamics in compost amended soils may be influenced by various factors related to compost parameters, climatic conditions, crop types, soil properties and soil management practises (Amlinger et al., 2003) as summarized (Fig.4.)

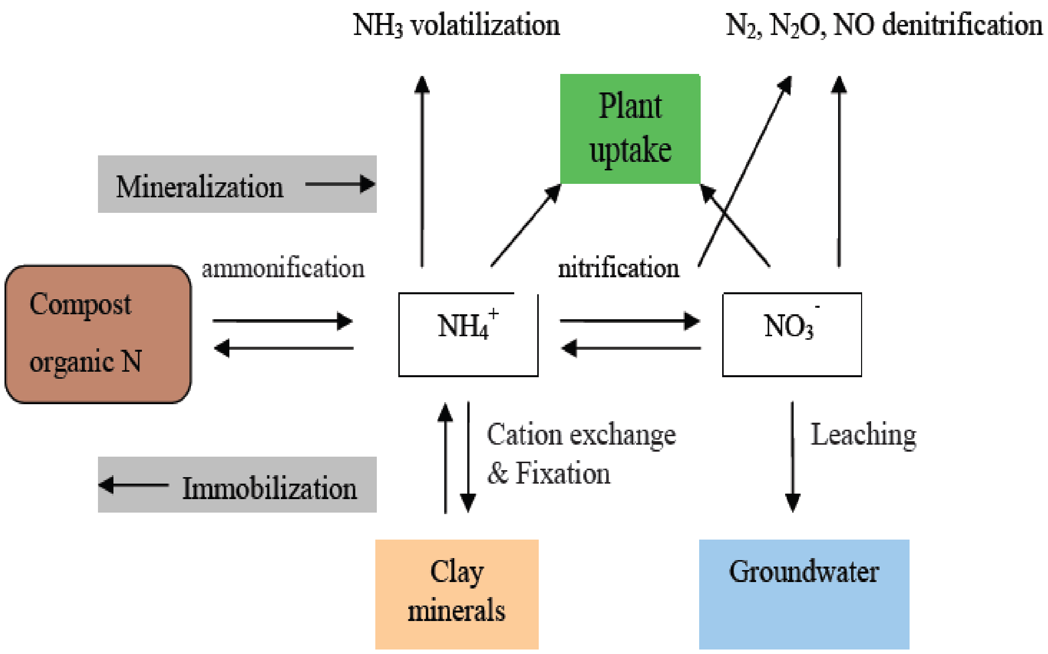

Fig. 4: Schematic diagram of the main organic compost nitrogen transformations in the soil

Table 5: Various substrates used for composting and effected parameters

S No.	Raw material	Method of composting	Influence factor	Results	References
1.	Sewage sludge and cotton waste	Rutgers static pile system	Organic carbon concentration, C:N ration, nitrogen content	Sewage sludge can provide N rich compost.	Bernal et al., 1998
2.	Pig faeces and amended wheat straw	Tunnel reactor composting system.	Degradation of biomolecules	The degradation rate of cellulose was much lower and degradation was not completed within the four weeks of composting	Veeken et al., 2001
3.	Rice straw		Sewage sludge	The optimum results was obtained at lower C:N value (17)	Iranzo et al., 2004
4.	Pig manure and sawdust		Different C:N	In optimum condition was obtained at low C:N ratio after 49 days	Huang et al., 2004
5.	Rice straw with poultry Manure		Poultry manure and oilseed rape cake	After 90 days C:N ratio and GI reach to 13.3–8.9 and 71.1–81.6%	Abdelhamid et al., 2004
6.	Mixed food waste	Turned windrow and passively aerated windrow	Compost Stability Interpretation. Efficacy of Food Waste Composting Methods	Lower respiration rates for the LTW method than for the PAW method were observed throughout both winter and spring composting trials.	Matteson et al., 2006
7.	Poultry waste and olive-mill waste	Turned windrows	Exposure to thermophillic phase, manure toxicity	The results showed that the humidification with olive mill wastewater improved the quality of the compost.	Hachicha et al., 2006
8.	Cattle manure with rice straw		Influences of temperature	Thermophilic condition is more significant on enhancement of composting	Tang et al., 2007
9.	Jatropha hulls		Effective lignocellulolytic fungal consortium,	Inoculation of lignocellulolytic fungi resulted in better compost of jatropha hulls within 1 month.	Sharma et al., 2008
10.	Rice straw with dairy manure	-----	Size reduction of raw materials, MC and aeration rate	C:N, VS and total solid values in final compost 52.3, 75.7 and 59.2% was reduced respectively	Li et al., 2008
11.	Rice straw and fresh farmyard manure		Quantity and particle size	C:N ratio of final product reached to 13:1 from 30:1 in initial C:N after 75 days	Hatem et al., 2009

12.	Rice straw		Lignocellulolytic fungal consortium	A consortium of *A. niger* (F44) and *T. viride* (F26), which gave a partial compatible interaction, has the potential to be developed as a lignocellulolytic consortium for rapid and efficient composting of rice straw into a value-added product of agro waste materials.	Kausar et al., 2010
13.	A mixture of air-dried rice straw, fresh buffalo's manure		10% of rock phosphate and MC 50–60%	The EC and OM were declined. TKN increased. GI for optimum condition was 83.38% after 90 days. The minimum pH at the end of composting process	Rashad et al., 2010
14.	Pineapple waste and chicken manure		Stability and maturity of manure. Waste management, C:N ration and germination index	The initial C: N ratio of co-compost was approximately 30.0, and it decreased to 19.8 at the end of co-composting.	Ch'ng et al., 2013
15.	Green waste		Quality improvement	Increased crop production	Taguiling et al., 2013
16.	Rice straw	Static pile	Effective microbes	TOC of treated pile decreacsed to 36.3% from initial value of 47.6%	Jusoh et al., 2013
17.	Combination of waste sludge and water hyacinth or rice straw		To identify the suitable C: N ratio for composting products that consists of a combination of sludge from catfish ponds and water hyacinth or rice straw.	Sludge-straw compost with C: N ratio of 30 and sludge hyacinth compost with C: N ratio of 25 contained the highest amount of nutrients	Bui. et al., 2014
18.	Municipal solid waste	In vessel method	bulking agent of different types and size for reducing N-losses	In MSW with a low initial C: N ratio the degradable carbon exceeds inorganic N which leads N loss by ammonia volitilisation or by leaching. These can be corrected by adding bulking agent with adequate degradable C.	Rich et al., 2014

2.5 Compost – Habitat of effective microbes

2.5.1 Source: Micro-organisms which enhance the biodegradation process are found throughout the natural environment. They are present in compost feedstock as well as in water, air, soil, and machinery. The feedstock and compost are exposed to them during processing. In order to maintain an active microbial population during the dynamic chemical and physical processes of composting various parameters such as shifts in pH, temperature, water, organic matter, nutrient availability and C: N ratio are regularly monitored.

2.5.2 Micro-organism types and their requirements: The micro flora of compost comprises various bacteria and fungi. Interestingly in present day studies yeast (esp. *Saccharomyces* sp.) have performed quite well in degrading the organic waste. The majority of microorganisms responsible for the formation of compost are aerobes; they work best in the presence of oxygen. These microbes also require a moist environment because they live in the water films surrounding organic matter particles. The moisture content of 50-60% is considered optimal for the process.

2.5.3 Bacteria: The most numerous and dominant biological entity of compost is the bacteria whereas, the actinomycetes and fungi typically proliferate in the later stages. The inevitable role played by bacteria basically lies in considerable degradation of cellulose based organic wastes (Table 6). In addition many studies of aerobic cellulolytic microbes have focused on improving the yield and characteristics of cellulase enzymes. The availability of easily usable organic substances enables the proliferation of the fastest-growing microorganisms, the bacteria. Mesophilic bacteria, therefore, dominate initial decomposition. These bacteria release heat from the breakdown of large amounts of easily degraded organic matter. Application of natural isolates; *Bacillus* sp. (AS1, AS2 and AS3) for recycling of cellulosic agricultural waste biomass was reported by Akhtar et al., 2012. Treatment of leaf litter biomass by *Bacillu*s sp. AS3 led to decrease in cellulose content.

Table 6: Cellulose degrading bacteria

Bacteria	Optimum pH	Optimum Temperature (°C)	Temperature Range (°C)	Reference
Acidothermus cellulolyticus	5	55	37-65	Mohagheghi et al., 1986
Caldibacillus cellulovoran	6.5 - 7	80	65-89	Huang and Monk 2003
Sinorhizobium fredii	7	35	30-45	Chen et al., 2004
Pseudomonas fluorescens	10	35	30-35	Bakare et al., 2005
Bacillus pumilus EB3	6	60	30-70	Affrin et al.,2006
Caldibacillus cellulovoran	6-7.5	50-60	<50	Li J.Y., 2010
Bacillus thuringiensis	4	40	20-70	Lin et al., 2012
Cellulomonas sp. ASN2	7.5	60	40-60	Irfan et al., 2012
Paenibacillus barcinonensis	7	35	30-65	Asha et al., 2012
Bacillus coagulans Co4	7.5	60	35-75	Adeleke et al.,2012
Bacillus subtilis AS3	9.2	20-45	>60	Deka et al., 2012
Paenibacillus terrae ME27-1	5.5	50	--	Liang et al., 2014

2.5.4 Fungi: Fungi form their individual cells into long filaments called hyphae. Fungal hyphae are larger than actinomycetes and may be more easily seen with the naked eye. These hyphae play significant role in degradation process of biomass. About 70,000 different species of fungi have been described worldwide, but an estimated 1 million additional species remain undiscovered and undescribed. Ecologically, fungi play a vital role in breakdown of dead plant materials. Commonly highlighted studies are on fungi of class *Soradiomycetes,* belonging to *Trichoderma* sp. namely *Trichoderma viride* and *Trichoderma reesei*. Cellulose degradation in plant remnants was found to depend on the duration of micromycete growth. The lowest cellulose levels after 60 days were detected after cultivation of *Galactomyces geotrichum* (5.48%) and *Myrothecium verrucaria* (12.34%), (Varnaitė et al, 2008). Based on their studies Kumar et al. 2011 concluded that better quality compost can be prepared by enriching raw material with some sources of plant nutrients. They further reported that the *Trichoderma* sp can also be used to enrich the compost and can be seen where phosphorus content was found maximum (1.35%) and it may be because of phosphorus solubilizing action of fungus.

In an attempt to improve the quality of compost from rice straw, Goyal and Sindhu (2011) used biogas slurry, consortium of fungi with *Trichoderma sp*. and cattle dung as compost mixture (Table 7). In the prepared compost, the total Nitrogen (N) content varied from 1.15 in paddy straw alone to 2.17% in paddy straw amended with cattle dung after 90 days of decomposition.

Table7: Cellulose degrading fungi

Fungus	Substrate	Reference
Trichoderma harzianum	Wheat bran	Ahmed et al (2009)
Phlesbia fascicularia	Wheat straw	Arora et al (2009)
Aspergillus awamori	Rice straw	Goyal and Sindhu (2011)
Phanerochaete chrysosporium	Wheat straw	Kuhar et al (2008)
Trichoderma reesei	Cellulosic biomass	Kumar et al (2008)
Coriolus versicolor MTCC 138	Paddy straw	Phutela et al (2011)
Rhizopus oryzae	Paddy straw	Viji et al (2014)
Aspergillus fumigates	Paddy straw	Viji et al (2014)

Maximum increase in total Phosphorus (P) content was found with fungal culture treatment followed by inoculation of cattle dung and it varied from 0.062 in paddy straw alone to 0.164 by inoculation of fungal consortium. The total potassium (K) content also varied from 0.134 to 0.169% and maximum increase in total K was observed by inoculation of the fungal consortium. Thus, amount of N, P and K contents were found more in compost prepared with consortium of fungi and cattle dung than the compost prepared from biogas slurry.

The individual effect of all biomass degrading microbes have shown considerable results, thus this process of degradation can further be designed such that the consortium i.e. the combination of two or more of these effective microbes when introduced to specific organic waste shoot up the degradation process. This degradation process would lead to lowering of C content and increase in N content; these alterations mark the significant C: N ratio. Compost quality is dependent on these C: N ratio values. Lesser is the ratio more intense is the quality of compost. From the list of cellulose degrading microbes we can use permutations and combinations to check the viability and degradation tendency with different consortia. The consortia may include a group of 2 or more species including *Bacillus* sp., *Trichoderma* sp., *Saccharomyces* sp. and *Aspergillus* sp. The organic waste inoculated with these species is monitored for minimum 90 days. Shredding of organic waste fasten up the degradation process.

Biodegradable wastes must be separated prior to composting: only pure food waste, garden waste, wood chips and to some extent paper waste are suitable for producing good quality compost (Crowe et al., 2002). However, for composting to occur in an optimum manner, five key factors need to be controlled: temperature, moisture content, oxygen content, material particle size and nature of the feedstock with particular importance to carbon over nitrogen ratio (Pace et al., 1995).

During composting of biodegradable municipal waste (predominantly kitchen and garden waste) 20% to 40% of the nitrogen contained in the waste is lost as ammonia, and 40% to 60 % of the carbon as carbon dioxide (Crowe et al., 2002). Komilis & Ham (2006) showed that food waste has a higher potential for ammonia volatilization during composting than yard waste, amounted to 65% and 25% of the initial total N, respectively. Organic matter generally breaks down more efficiently and completely in conditions of ready oxygen availability, largely as a result of the energy produced from the aerobic respiration (Evans, 2001).

2.6 Agricultural prospects of compost

Compost provide a ready source of carbon and nitrogen for microorganisms in the soil, improve its structure, reduce erosion and lower the temperature at the soil surface and also aid in seed germination and increase its water holding capacity (Adebayo *et al.*, 2011).

Stable and mature compost can be applied to soil as an organic amendment to improve plant growth and soil fertility, as well as enhancing the function of soil for carbon sequestration (Guo *et al.*, 2012).

A field experiment carried out to evaluate the growth of *Brassica chinensis* and *Zea mays* proof that, addition of manure compost increased total organic matter, macro-nutrients (N, P, Mg, Na, Ca and K) and micro-nutrients (Cu, Zn and Mn) in the amended soils according to the rate of compost application. It also improved soil physical properties with a significant increase in soil porosity and hydraulic conductivity, but a decrease in bulk density. The dry weight yields of both plant species were much higher in soils receiving manure compost amendment and plots with 50 and 25 tonnes ha^{-1} compost had the highest yields of *Z. mays* and *B. chinensis*, respectively. An increase in dry weight yields indicated a better nutrient status in compost-amended soil which was supported by the higher tissue nutrient contents of N, P and K of plants grown in soil with manure compost amendment (Wong et al., 1999).

Compared to some chemical fertilizers which release nutrients so quickly that rain can leach them away even before plants derive benefit in compost, most of the nitrogen and phosphorus are held in organic form and released slowly. The nutrients in compost are therefore available throughout the growing season (Dickson et al., 1991).

The advantage of readily available materials for compost preparation, gradual release of plant nutrients without being wasted through leaching or erosion, destruction of harmful weed and toxic materials during preparation and environmental friendliness have made organic amendments, particularly composted manure popular among farmers (Adebayo et al., 2011).

Ullah et al. (2008) reported that combined treatment (60% organic +40% inorganic) showed the best performances in terms of maximum branching (20.1), highest number of fruits/plant (15.2), fruit length (14.1 cm), fruit diameter (4.3 cm), and yield (45.5 t ha^{-1}) of eggplant (brinjal).

Compost made from households and cafeteria refuse and chemical fertilizers or chemical fertilizers alone, significantly gave higher yield of brinjal compared to the plots amended with combined application of high rate of compost and chemical fertilizers that was statistically similar to high rate of compost alone treatment (Munshi, 2014).

According to the Taguiling, crop residue and green biomass combinations at 3:0, 3:1, 3:2 and 3:3 ratios subjected to rapid composting method with the aid of *Trichoderma harziamum* as activator. Using the compost products mixed with pure soil at 1:1 ratio, pot experiment was conducted to test the effect of green biomass-enriched compost on the early growth of eggplant (*Solanum melongena*) and green pepper (*Capsicum annuum*). The results indicated that, gradual enrichment of crop residue compost with green biomass especially those prepared at 3:3 and 3:2 crop residue-green biomass combinations gave significant increase in the Nitrogen, Phosphorous and Potassium contents of the compost. It also showed significant improvement in the percent organic matter, pH level, and weight of the compost product.

Chapter 3

Materials and Method

3.1 Micro-organisms

3.1.1. Bacteria

Bacillus subtilis (NA 15)

*Paenibacillus polymyxa (*MTCC 3088)

*(*Bacterial isolates were procured from IMTECH, CHANDIGARH*)*

3.1.2. Yeast

Saccharomyces cerevisiae (NCIM 3215)

3.1.3. Fungi

Trichoderma reesei (MTCC 164)

3.2 Bacterial inoculum

The strains of *Bacillus* sp. were grown on nutrient broth and later on used for the biodegradation of kitchen waste. 1% inoculum was added to 100 mL of Nutrient broth and incubated at 37°C for 24 h at 120 rpm. After 24 h this nutrient broth was stored at 4°C to be used later for preparing the consortia.

3.3 Yeast inoculum

The strain of *Saccharomyces cerevisiae* was grown in Yeast extract peptone dextrose medium (YEPD). YEPD was inoculated with 1% inoculum in 100 mL of media and kept at 28° C for 48 h at 120 rpm. This nutrient broth was stored at 4°C to be used later for preparing the consortia.

3.4 Fungal inoculum

Fungus used for the degradation of kitchen waste was *Trichoderma reesei* (MTCC 164), 1% of the spore suspension was added to 100 mL of PDB for mass cultivation and incubated at 28°C for 48 h at 120 rpm. After 48 h the culture was stored at 4°C for mass production of consortium.

3.5 Collection of kitchen waste and Kinnow peel waste

Kitchen waste from different hostels and juice shops of Thapar University was collected regularly for the period of 15 days. This mixed waste consisted majorly fruit and vegetable peels, leftover food waste and fruit pulp. This waste was dumped in pits at STEP, Thapar University Patiala, later on treated with consortia.

3.6 Experimental set up for composting

Kitchen waste collected was in a pit of known dimension (30×30×0.61) metres. 15 days of kitchen waste was dumped in the pits. The total kitchen waste dumped was 160 Kg (approximately). This pit containing kitchen waste was equally divided into 2 parts where one part contained control and other part contained treated biomass. Kinnow peels were collected in small tubs with diameter of 0.3 metres and each tub (one as control and other treated) contained 7 kg of the biomass.

3.6.1 Processing and pulverization of biomass

The biomass sample after every 7 days was air dried followed by oven drying at 105°C for 4 hours. Dried sample was then grinded using mechanical blender and sieved to mesh size of 0.5 mm. The sieved biomass samples were stored in air tight containers so as to avoid moisture for further study.

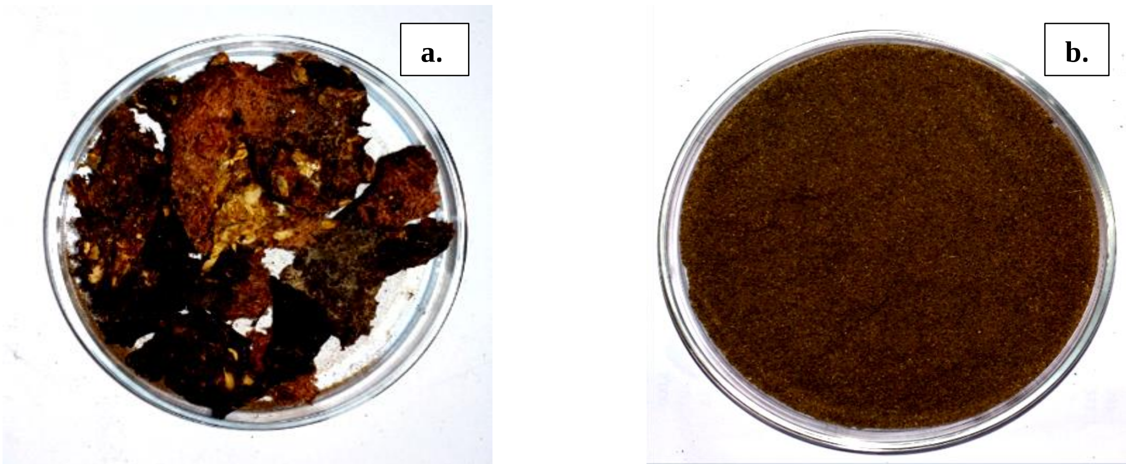

Figure 1: a) Oven-dried waste sample b) Grinded and sieved (0.5mm) biomass.

3.6.2 Maintenance of culture consortia

Jaggery based media was used to collectively grow cultures of bacteria, fungi and yeast which was made upto volume of 10 L and was maintained at l28°C room temperature on a rotary shaker at 120 rpm for 3 days. After 3 days this consortia containing namely, *Bacillus subtilis* (NA 15), *Paenibacillus polymyxa (*MTCC 3088) *Saccharomyces cerevisiae* (NCIM 3215) and *Trichoderma reesei* (MTCC 164) were added to the waste containing pits and biodegradation process was checked after every 7 days followed by turning of the waste in the pits at regular intervals of 3 days.

3.7 Measurement of pH

Measurement of pH was done by **Potentiometric method.**

a) 1g of completely dried waste sample was taken into 100mL beaker.
b) Then 20mL of distilled water was added to it.
c) This mixture was stirred thoroughly for 10s using a glass rod.
d) Further stirred the suspension for four to five times during next 30 min.
e) Allowed the suspension to settle for next 30 min.
f) Meanwhile pH electrode was washed with distilled water and wiped with tissue paper carefully.
g) The calibration of pH meter was checked before proceeding for further experiment.
h) pH was measured by immersing the electrode in the supernatant of the suspension.
i) The stabilized readings shown by the pH meter were recorded.

3.8 Measurement of Electrical Conductivity

a) 1g of completely dried waste sample was taken into 100mL beaker.
b) Then 20mL of distilled water was added to it.
c) This mixture was stirred thoroughly for 10s using a glass rod.
d) Further stirred the suspension for four to five times during next 30 min.
e) Allowed the suspension to settle for next 30 min.
f) Meanwhile EC electrode was washed with distilled water and wiped with tissue paper carefully.
g) The calibration of EC meter was checked before proceeding for further experiment.
h) EC was measured by immersing the electrode in the supernatant of the suspension.

i) The stabilized reading shown by the EC meter was recorded.

3.9 Measurement of temperature

Temperature of kitchen waste and orange peels was recorded after every 7 days using digital thermometer. The thermometer was inserted in pile of waste and the increase in temperature was noted regularly.

3.10 Determination of Moisture content

a) 5g of the waste biomass was dried at 105°C in a hot air oven till a consistency in weight was observed.
b) Following drying, the sample was moved to a desiccator for cooling, without absorbing moisture.
c) After cooling the dry weight was noted.
d) The percentage difference of weight is expressed as the moisture content of the sample.

$$\textbf{Moisture content (\%)} = \frac{A-B}{B-C} \times 100$$

Where A is the weight of the crucible and sample, and B is the constant weight of crucible and sample after drying, C is the weight of empty crucible.

3.11 Determination of ash content (in percent %)

a) Ash content in the biomass was adopted as per the protocol described by ASTM D3174-04.
b) For determination of ash content, already oven dried samples of waste (1g) was taken in a crucible and mass of the crucible along with the sample was determined.
c) Crucible was placed in muffle furnace maintained at 575 ± 10˚C for 4 hrs.
d) Crucible was removed from the muffle furnace and placed in a dessicator.
e) The above process of heating and cooling was repeated until constant weight was obtained. This process allows removal of the volatiles and un-burnt carbon.

$$\textbf{Ash}\ (\%) = \left[\frac{\mathbf{Wt - W1}}{\mathbf{W2 - W1}}\right] \times \mathbf{100}$$

Where W_1 is the weight of the silica crucible, W2 is the weight of the crucible and the oven-dried sample, and Wt is the constant weight after combustion.

3.12 Determination of Volatile matter

a) The volatile matter in kitchen waste was determined by the procedure given in ASTM D3175-07.
b) The biomass sample (1.0 g) was taken in crucible with cover and intial weight was determined the covered crucible was placed in muffle furnace regulated at 950 ± 10˚C for 7 min to obtain rapid heating.
c) Then the covered crucible was removed from the furnace and cooled to room temperature in a desiccators and weight was recorded.
d) The percentage weight loss was regarded as the percentage of volatile matter.

$$\textbf{Volatile matter}\ (\%) = \frac{\mathbf{Wt - W1}}{\mathbf{W2 - W1}} \times \mathbf{100}$$

Where W_1 is the weight of the silica crucible, W2 is the weight of the crucible and oven dried sample, and Wt is the constant weight after heating the sample.

3.13 Estimation of Organic carbon

Percent organic carbon was measured by **Walkley and Black, 1934.**

a) 0.01g of dried waste sample was taken in 500 mL conical flask and 10 mL of 1N $K_2Cr_2O_7$ was added to it. Swirled the flask for mixing the waste and the reagent.
b) 20mL of H_2SO_4 was added and allowed the flask to stand undisturbed for 30 min after which 200mL of distilled water was added.
c) To this mixture, 10 mL of 85% Orthophosphoric acid and 0.5g of NaF was added.
d) 1mL of diphenylamine DPA indicator was added just prior to titration.
e) Finally titrated with 0.5 N ferrous ammonium sulphate FAS till the end point was observed from violet blue to green
f) Also a reagent blank was run by using above mentioned procedure but without sample.

Calculation:

$$\textbf{\%Oxidisable organic carbon} = \mathbf{10}(\boldsymbol{B - T}) \times \mathbf{0.003} \times \frac{\mathbf{100}}{\boldsymbol{B}} \times \boldsymbol{g\ of\ sample}$$

Where B= Volume of ferrous ammonium sulphate consumed for blank titration.

T= Volume of ferrous ammonium sulphate consumed for sample titration.

3.14 Determination of total nitrogen (Piper, 1960)

a) 1 g sample was mixed thoroughly with sulphuric- salicyclic acid (1 g salicyclic acid mixed with 30mL concentrated H_2SO_4) followed by 5 g sodium thiosulphate.
b) The mixture of sample was heated for 5min and allowed to cool at room temperature for 30 min.
c) To this, 10 g of digestion mixture [10 g HgO, 5 g $CuSO_4$ and 100 g K_2SO_4 (2:1:20)] was added and contents were mixed well in a kjeldhal flask.
d) The flask was kept in the digestion chamber at 100˚C for 2 h.
e) Further, the gradual colour change was monitored from dark brown to greenish white after that the contents were cooled and 300mL distilled water was added.
f) Then 20 mL of the digested sample, 15-20 mL NaOH (50% w/v) and glass beads were added to the distillation flask to avoid bumping through the open end of the condenser attachment and stoppered.
g) Water flow was maintained through the condenser. The distillate was collected through a receiver tube in a beaker containing 15 mL boric acid (4%, w/v) and 2 drops of mixed indicator (0.066 g of methyl red and 0.099 g of bromo-cresol green dissolved in 100 mL of ethyl alcohol) till the end point changes from pink to green. The distillate was titrated against 0.02 N H_2SO_4 until the endpoint colour changed from green to pink.

$$\boldsymbol{Total\ N\%} = \frac{[(\boldsymbol{T} - \boldsymbol{B}) \times \mathbf{0.0014} \times \mathbf{100}]}{\boldsymbol{Weight\ of\ sample}}$$

Where, T is the titre value for sample and B is for blank.

3.15 Determination of reducing sugar

Reducing sugar in kitchen waste and orange peels were estimated using DNS (3, 5 dinitrosalicyclic acid) method as per Miller (1959).

a) Dried biomass (0.1 g) was taken in a test tube containing 1 mL of distilled water.
b) In each test tube 3 mL of DNS reagent was added and test tubes were capped.
c) These test tubes were then kept in water bath at 90°C for 10 min.
d) The solution was cooled down to room temperature and absorbance was recorded at 540nm
e) Reducing sugar concentration was determined using standard glucose curve.

3.16 Bio-efficacy trials

Compost prepared from kitchen waste and kinnow peels was mixed with soil. The soil to compost ratio was maintained as 3:1 respectively. Growth of brinjal (egg-plant) was monitored in kitchen waste compost and in case of kinnow peel compost lady finger was grown. Soil parameters were checked before and after compost application.

3.17 Analysis of untreated and treated biomass

3.17.1 *SEM analysis*

Physical changes in native and consortia treated biomass were observed by Scanning electron microscope (SEM). Images of native and treated biomass were taken using SEM (Model: JEOL JSM-6510 LV, USA) at magnification 1,000 X. The samples were coated with gold using sputter at a voltage of 10-15 kV. Gold sputtering provides conductivity to the samples, and then the SEM micrograph was obtained.

3.17.2 *XRD analysis*

Crystallinity of native and consortia treated biomass were analysed at room temperature in the scanning angle of 2-20°C at the scan speed of 5° min^{-1} using a PANanalytical X'pert PRO diffractometer (Netherlands) with Ni-filter, operated at 45 kV and 40 mA with λ (Cu Kα) = 1.504 Å. The crystallinity index *(CrI)* of the biomass samples were determined as described by Segal et al. (1959) as follows:

$$CrI = [(I_{002}-I_{am})/I_{002}] \times 100$$

Where I_{002} is the intensity for the crystalline portion of biomass at about 2θ of 80° and I_{am} is the peak for amorphous portion.

3.17.3 FTIR analysis

The structural changes of native and treated biomass at different intervals were obtained using Nicolet Instrument (Model: MAGNA 550, USA). The biomass (10mg) was well mixed with 200 mg of KBr and the mixture was compressed for preparation of pellets. Each spectrum was the average of 64, co- addition of scans with a total scan time 15 s in the IR range of 400-4000 cm^{-1} at 1 cm^{-1}

Chapter- 4

Results and discussions

4.1 Collection of organic wastes and its recycling

Kitchen waste and kinnow peel waste were collected from hostel mess and fruit shops at Thapar University, Patiala. Kitchen waste was collected for 15 days in pits (Fig. 5) in shade provided by covering with dark blue plastic sheets. Pit was divided into two parts one for control and other part was treated with specially designed microbial consortium containing *Bacillus subtilis* (NA 15), *Paenibacillus polymyxa, Saccharomyces cerevisiae and Trichoderma reesei*. 50 % moisture content was maintained in pits under shade conditions. Turning of the organic waste was done after every 3 days.

Fig. 5: Experimental set up for Kitchen waste

Similarily, kinnow peels waste was collected in plastic tubs of diameter 0.3 m, one as control and another was treated with same consortia and 40% moisture content was maintained (Fig. 6). Turning of the waste was done after every 3 days. After fixed interval of 7 days sample from each experimental set up was drawn, dried and pulverized to particle size 0.5 mm and stored in air tight container for further analysis

The effect of microbial consortium was justified by studying various parameters to convert the mixed kitchen waste and kinnow peel waste into compost. Various parameters such as pH were monitored during 90 days and later the compositional and structural characteristics of degraded biomass were studied using SEM, XRD and FTIR and compared with untreated control biomass.

Fig. 6: Experimental set up of Kinnow peel waste in tubs

4.2 pH

pH plays a significant role in microbial. pH of the kitchen waste pile was recorded to be 6.77 in the initial stages later in the process fluctuations in the values were observed in case of microbially treated pile as well as control pile. While in case of kinnow peels the pH was more on the acidic scale due to its citrus nature. The initial pH recorded for kinnow peels was 3.84 and later on it showed increasing trend in pH during composting and finally stabilized pH at of 7.1 (Table 8).

Table 8: pH at different intervals of Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	6.77±0.03	6.77±0.03	3.84±0.04	3.84±0.04
7	6.92±0.03	7.00±0.006	4.15±0.03	4.42±0.01
14	7.05±0.05	7.34±0.04	4.25±0.03	4.83±0.02
21	7.52±0.09	7.39±0.01	4.51±0.02	5.13±0.01
28	7.82±0.03	7.56±0.04	4.68±0.01	5.62±0.01
35	8.02±0.03	8.16±0.05	5.16±0.06	5.62±0.06
42	8.20±0.10	7.40±0.10	5.40±0.17	6.10±0.01
48	7.86±0.07	7.60±0.05	5.79±0.02	6.28±0.03
56	7.60±0.01	7.48±0.02	6.10±0.10	6.81±0.07
63	8.22±0.01	7.40±0.01	6.31±0.02	6.83±0.04
70	8.11±0.02	7.58±0.03	6.21±0.01	7.16±0.06
77	7.95±0.05	7.21±0.01	6.52±0.02	7.12±0.03
84	7.43±0.15	7.15±0.09	6.81±0.01	7.02±0.03
91	7.31±0.08	7.10±0.10	6.59±0.10	7.13±0.04

± Values of Standard Deviation

Variation in pH levels of piles may have been due to variations in pH levels of the different raw material used for mixing the compost piles (Fig 7 & Fig 8). This was indicated by Graves and Hattemer, (2000), that there is variation in pH levels of raw materials being used for a compost mix. However, these variations do not impact significantly on the composting process because different micro-organisms thrive at different pH levels of the compost. Maximum increase in the pH in case of kitchen waste was observed till 7.56 in case of consortia treated pile and 8.22 in case of control pile during composting. Increase in pH could be due to excess of nitrogen content in composting material (Smith and Friend, 2013). Although most pH values were above the ideal pH range, normal composting was observed (Ahmed et al., 2007) as there is no specific pH required for composting, since different organic materials that are suitable for composting have pH range of 5.0 to 12.0. Various authors claim that the pH tend to approach neutral state as compost reaches stability (Gowda 1996, Tennakoon and Bandara 2003, Harrison 2005, Fening 2010).

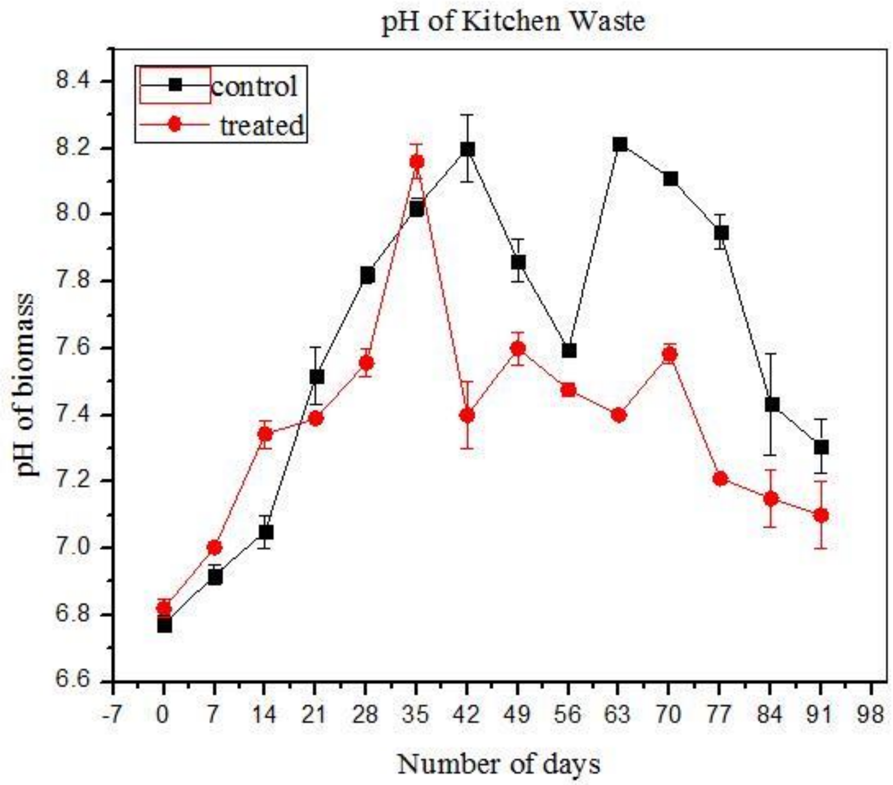

Fig.7: pH at different intervals of untreated (control) and treated kitchen waste

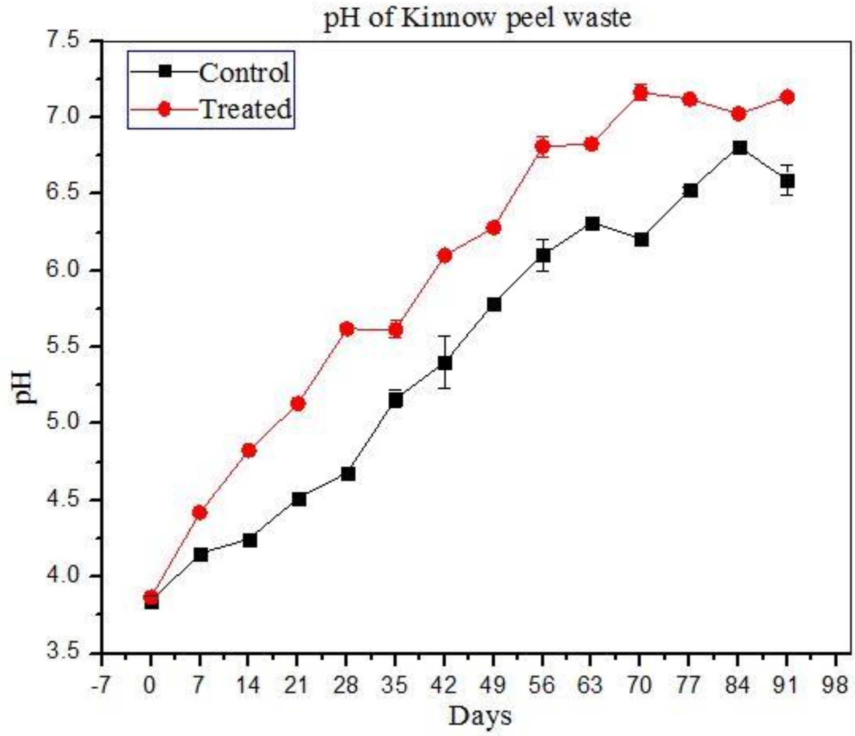

Fig. 8: pH at different intervals of untreated (control) and treated Kinnow peel waste

4.3 Electrical conductivity

In case of kitchen waste the electrical conductivity increased from 0.77 dS/m on day 0 to 4.8 dS/m in consortia treated pile while 2.45 dS/m was observed in control pile at the end of 90 days (Table 9).

Table 9: Electrical Conductivity at different intervals of Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	0.77±0.01	0.77±0.01	0.47±0.02	0.47±0.02
7	0.89±0.02	0.98±0.006	0.34±0.01	0.96±0.02
14	0.95±0.01	1.33±0.01	0.52±0.02	1.33±0.02
21	1.02±0.02	1.44±0.02	1.14±01	1.47±0.02
28	1.24±0.01	1.48±0.01	1.41±0.01	1.47±0.01
35	1.35±0.02	1.72±0.01	1.42±0.01	1.76±0.01
42	1.43±0.02	1.87±0.006	1.65±0.02	1.87±0.02
48	1.46±0.01	1.93±0.02	1.75±0.01	1.88±0.02
56	1.68±0.01	2.83±0.03	1.81±0.02	2.79±0.03
63	1.81±0.01	3.4±0.01	2.5±0.10	3.42±0.02
70	2.03±0.02	3.89±0.01	2.7±0.10	3.84±0.06
77	2.17±0.01	4.3±0.01	3.47±0.04	4.31±0.01
84	2.35±0.02	4.33±0.20	3.94±0.03	4.50±0.02
91	2.45±0.04	4.8±0.10	4.2±0.10	4.58±0.06

± Values of Standard Deviation

Increase in soil EC due to the application of mixed green and animal waste compost and also vinasse compost has been reported (Stamatiadis et al., 1999; Madejón et al., 2001). Although the increase of EC was not found capable of causing a sodium hazard to the soil, it indicates potential problems following the repeated application of compost to agricultural soil. While in case of kinnow peels the conductivity reached 4.58 dS/m from 0.47 dS/m in treated tub and 4.2 dS/m in control (Table 9).

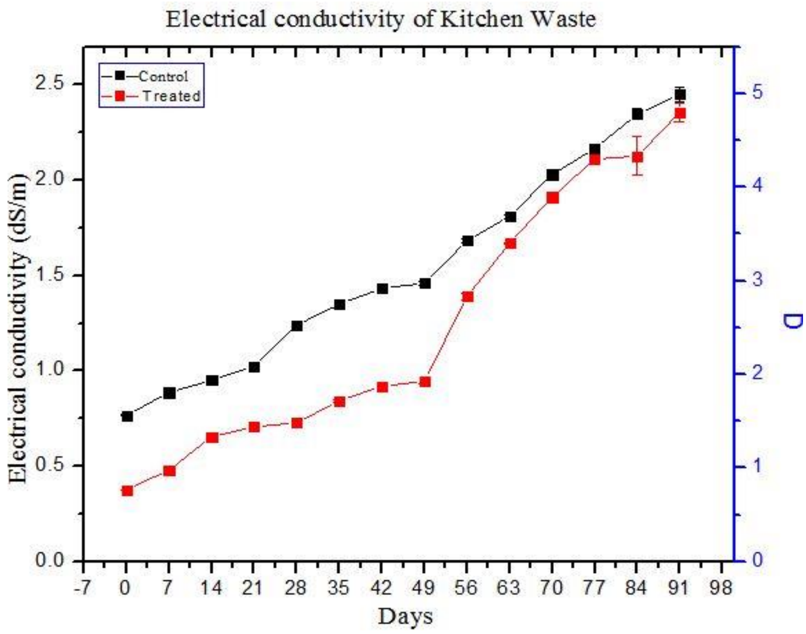

Fig. 9: Electrical conductivity at different intervals of untreated and treated Kitchen waste

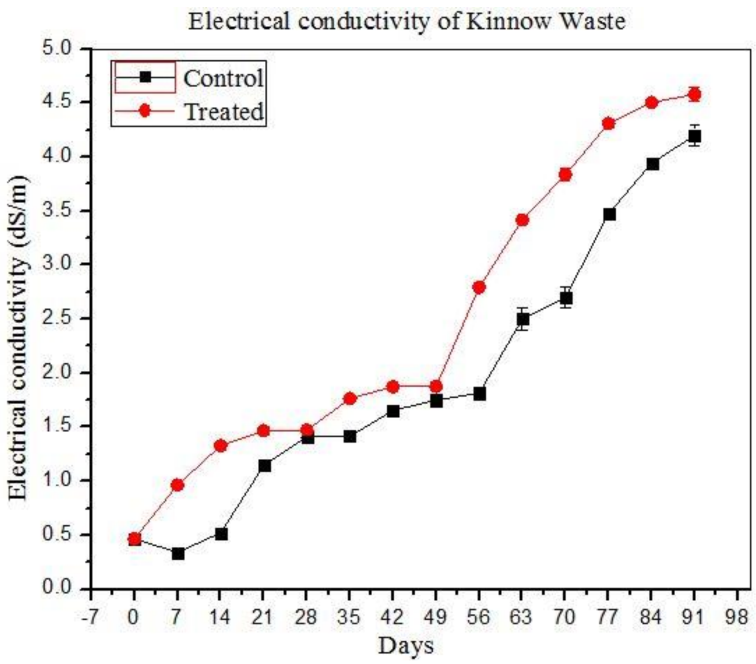

Fig. 10: Electrical Conductivity at different intervals of untreated and treated Kinnow peel waste

Fig.9 & Fig 10 demonstrates the levels of E.C in Kitchen waste and kinnow peel waste. Sánchez-Monedero et al. (2001) demonstrated that the increase in compost EC values was significantly correlated with the increase of NO_3-N concentration.

4.4 Temperature

During the composting process of kitchen waste and kinnow peels the temperature followed the increasing trend initial temperature (0 day) was recorded to be 19.17°C for control pile and treated pile (Table 10). During this process, due to climatic changes, rainfall led to drop in temperature which showed fluctuations in the temperature pattern. Later the temperature was increased till 56.47±0.45°C in consortia treated pile, while in case of control the temperature raised up to 43.53±0.47°C. We can say that the consortia treated pile showed vigorous microbial activity to change the waste in to compost (Fig. 11 & Fig 12). The temperature showed stabilization as it reaches 90 days of degradation. The drop in temperature indicated the curing phase of composting.

Table 10: Temperature at different intervals of untreated and treated Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	19.17±0.21	19.17±0.21	14.2±0.20	14.2±0.20
7	19.3±0.26	22.5±0.10	15.23±0.21	15.43±0.25
14	20.6±0.10	23.17±0.21	21.13±0.15	21.47±0.06
21	22.4±0.20	26.27±0.25	21.47±0.06	22.3±0.20
28	22.17±0.20	27.7±0.17	22.47±0.15	24.17±0.12
35	23.63±0.20	29.4±0.10	23.17±0.21	24.47±0.15
42	21.2±0.20	33.63±0.12	23.63±0.15	24.73±0.12
48	30.3±0.20	38.67±0.21	24.23±0.15	26.13±0.15
56	31.8±0.10	43.33±0.25	25.47±0.21	26.5±0.20
63	33.53±0.15	45.53±0.32	27.8±0.26	27.27±0.21
70	33.23±0.20	43.17±0.21	31±0.10	33.27±0.38
77	36.23±0.20	48.3±0.20	31.93±0.21	35.57±0.35
84	40.5±0.20	53.4±0.35	32.67±0.06	38.5±0.10
91	43.53±0.47	56.47±0.45	33.1±0.10	38.73±0.31

± Values of Standard Deviation

Kinnow peels in tubs showed the initial temperature of 14.2±0.2°C on day 0. The rapid increase in temperature was monitored in consortia treated peels, temperature rose up to 38.73±0.31°C, while in case of control the temperature at the end of 90 days was 30°C (Table 10). This was studied by Cooperband (2001), that mesophilic bacterial whose working temperature range is generally between 21ºC to 38ºC colonize the compose again and rapidly decompose organic matter, producing acids, carbon dioxide and heat. Temperature is a vital parameter used in determining the success of a composting process. Increasing temperature in the composted pile is due to the microbial activity. During the vigorous degradation process this heat generated by micro-organisms stays in the pile and leads to increase in temperature. Composting process is carried out in three different phases, which is conventionally defined in terms of the kinds of microbial population that thrive at different temperature ranges. According to Hubbe et al. (2010), these phases are the psychrophilic, mesophilic and thermophilic.

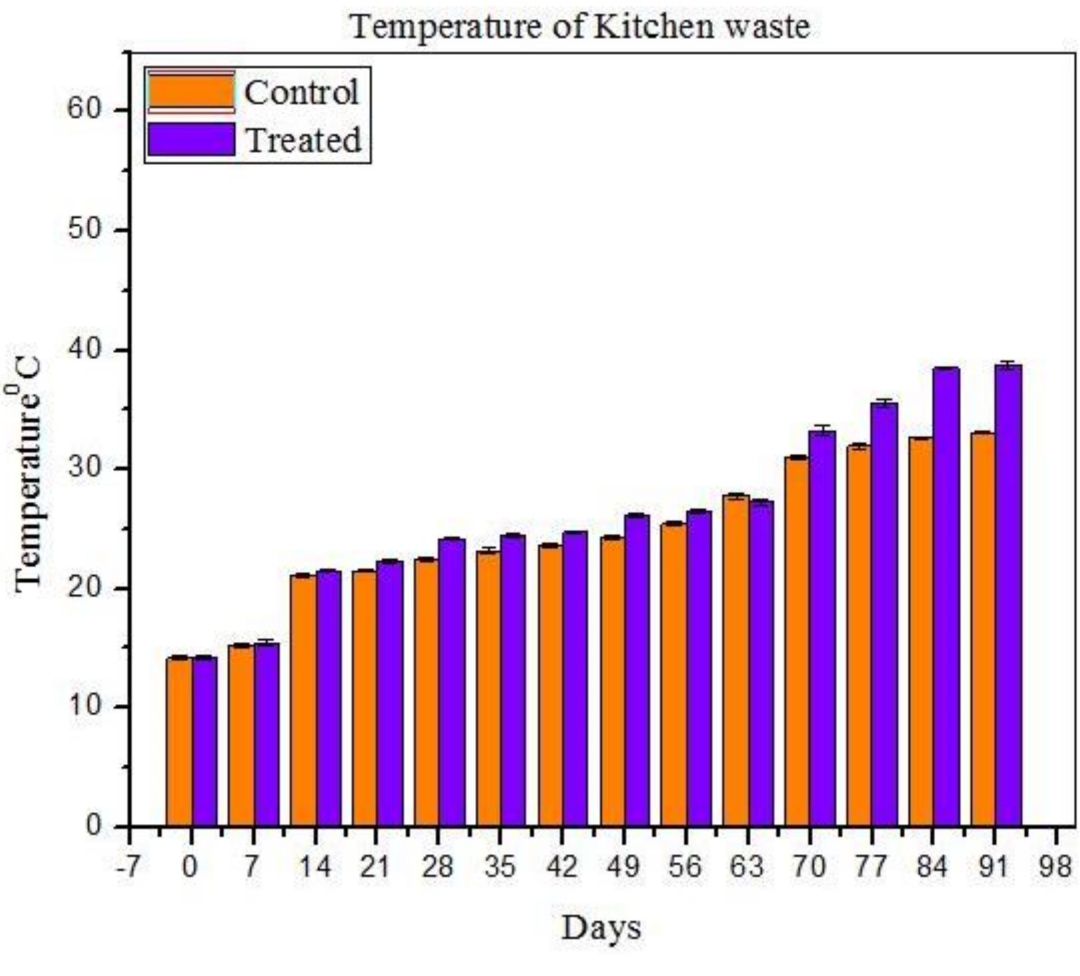

Fig.11: Temperature at different intervals of untreated and treated Kitchen waste.

Smith and Friend (2013) showed that the drop in compost pile temperature is not a sign that composting is complete, but rather an indication that the compost pile is entering into another phase of the composting process, hence the changes in temperatures of the process was an indication of lag periods.

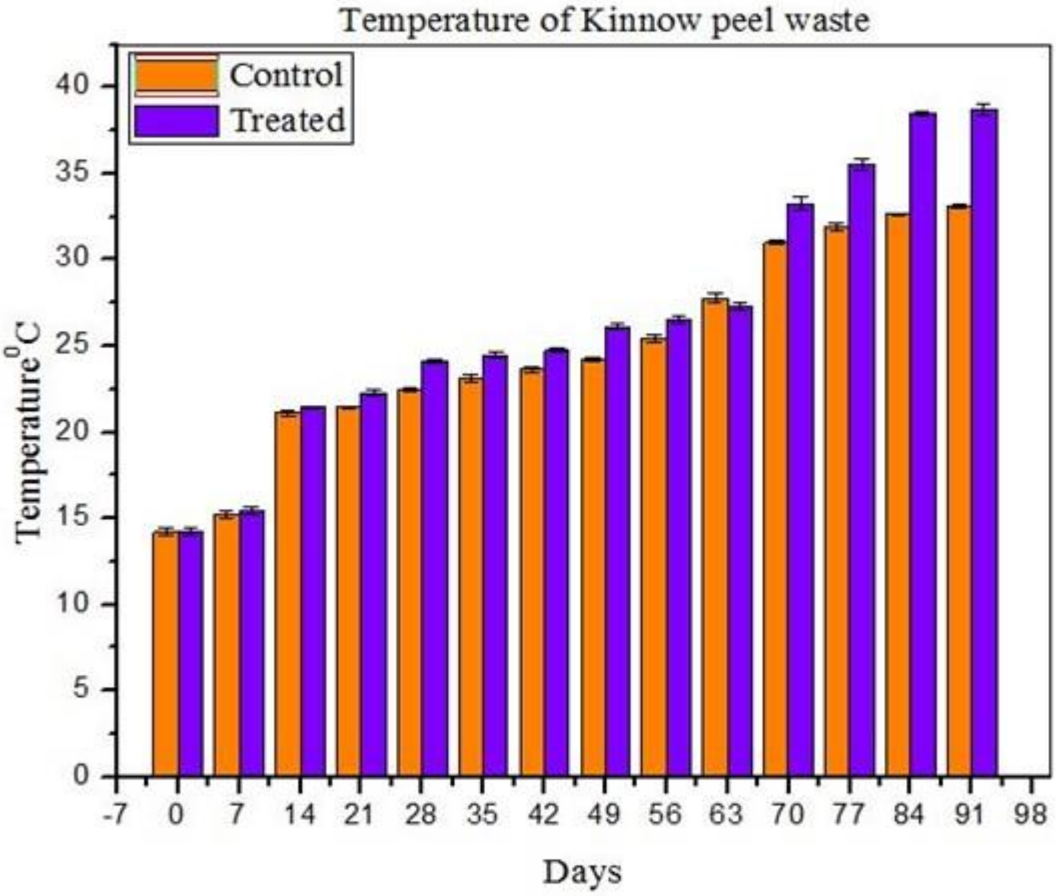

Fig.12: Temperature at different intervals of untreated and treated Kinnow peel waste.

4.5 Moisture content

Moisture is necessary to support the metabolic activities of the micro-organisms in compost. Adequate moisture is essential for microbial activity hence balanced moisture content encourages the growth of microorganisms that break down the organic matter into humus (McLaurin and Wade, 2012). The changes in microbial activities and the composting process are also affected by many environmental factors, especially moisture content, since water availability is directly related to oxygen supply (Tiquia et al., 1996). Moisture content decreased in later stages of composting. Initial moisture content for kitchen waste was recorded as 55.57±0.09% and it dropped to 19.51±0.18% in treated and 21.47±0.049 in cast of control (Table 11). The optimum moisture content for efficient composting is recommended between 40% and 60% (Troy et al., 2012). But most literature recommends a moisture content of 50%-60% for optimal composting condition (Trautmann and Richard, 1996).

Table 11: Moisture content (%) at different intervals of untreated and treated Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	55.57±0.09	55.57±0.09	58.07±0.06	58.07±0.06
7	54.21±0.09	53.40±0.10	57.90±0.10	57.35±0.13
14	52.67±0.05	49.50±0.26	57.30±0.10	55.79±0.01
21	50.4±0.13	46.13±0.15	55.96±0.05	53.75±0.05
28	52.54±0.06	47.96±0.06	55.82±0.08	51.83±0.06
35	51.72±0.04	46.44±0.29	53.73±0.07	49.82±0.03
42	52.52±0.03	44.47±0.15	52.07±0.06	45.71±0.01
48	50.17±0.15	41.39±0.53	49.84±0.29	41.91±0.03
56	45.54±0.05	38.50±0.2	47.62±0.11	38.54±0.04
63	43.53±0.15	35.47±0.06	43.67±0.06	34.71±0.05
70	40.45±0.18	33.67±0.15	40.04±0.07	31.73±0.21
77	37.65±0.08	30.49±0.02	35.72±0.03	27.87±0.06
84	31.47±0.25	24.30±0.20	30.11±0.01	22.17±0.12
91	21.47±0.049	19.51±0.18	25.64±0.08	18.57±0.12

± Values of Standard Deviation

Moisture content generally decreases as composting proceeds; hence the need to add additional water to the compost (Pace et al., 1995). Hence, from Fig. 13 & Fig 14 we can analyse the amount of decrease in moisture content.

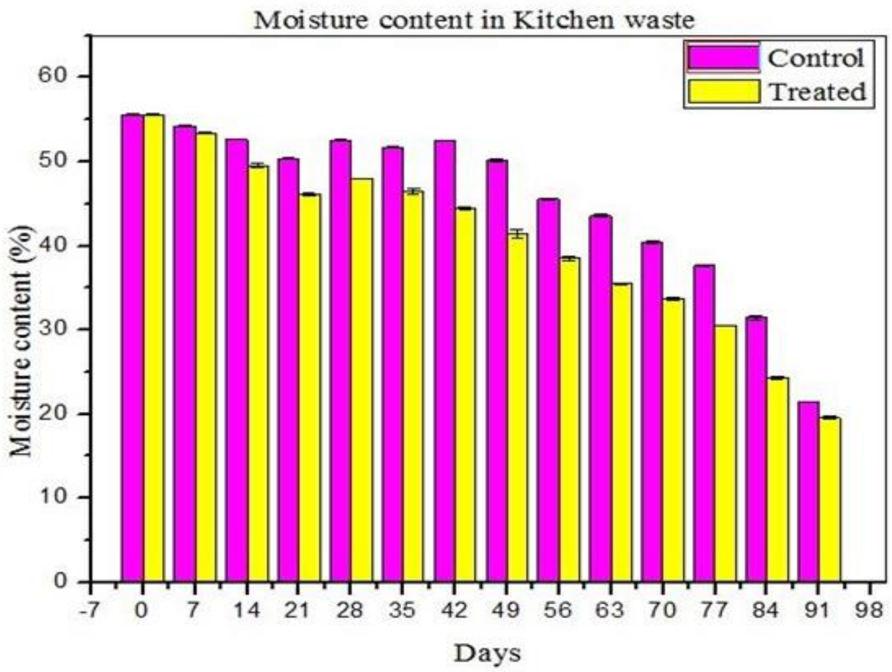

Fig. 13: Moisture content (%) at different intervals of untreated and treated Kitchen waste

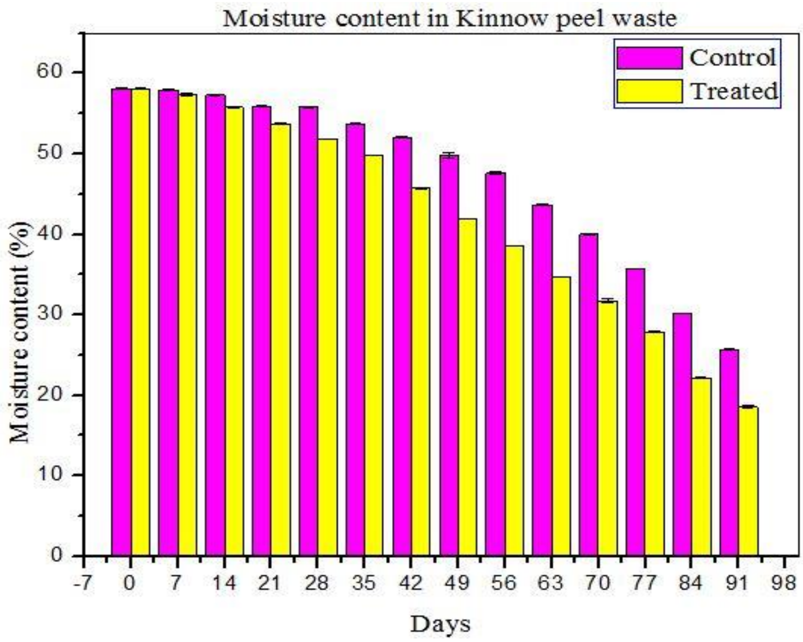

Fig. 14: Moisture content (%) at different intervals of untreated and treated Kinnow waste.

4.6 Carbon content

The organic carbon content reduces as the decomposition progressed. Initially, amount of total organic carbon was 69.03% which was reduced to 41 % in just 49 days of composting by microbial consortia as compared to control which was 50.6% (Table 12).

Table 12: Carbon content (%) at different intervals of treated and untreated Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	69.033±0.24	69.033±0.24	59.957±0.03	59.957±0.03
7	63.577±0.07	60.333±0.20	57.93±0.05	59.167±0.04
14	57.9±0.08	57.4±0.14	56.333±0.09	57.267±0.04
21	56.033±0.04	52.35±0.10	56.383±0.15	55.6±0.14
28	56.417±0.11	51.033±0.02	55.733±0.12	52.767±0.09
35	53.083±0.31	46.797±0.07	54.333±0.04	50.05±0.03
42	52.447±0.39	42.263±0.12	53.5±0.16	48.4±0.16
49	50.683±0.18	41.133±0.12	53±0.08	48.073±0.05
56	48.667±0.04	36.31±0.16	52.533±0.24	46.063±0.01
63	47.533±0.20	29.403±0.02	51.833±0.12	43.55±0.14
70	45.167±0.09	29.553±0.01	51.323±0.12	42.197±0.02
77	44.367±0.09	28.19±0.01	49.6±0.21	40.057±0.01
84	42.85±0.10	27.3±0.14	46.5±0.08	38.533±0.33
91	40.103±0.01	25.133±0.02	44.467±0.17	35.777±0.02

± Values of Standard Deviation

A good decreasing trend in organic carbon was observed over 90 days. While similar trend was observed in case of kinnow peels in treated tubs which was initially observed to be 60% and the treated peels considerably showed decrease by 13.95% in 48 days of composting while in case of control the value decreased by 7.2%. Thus, faster rate of degradation was observed in case of peels treated microbial with consortia (Fig. 15 & Fig 16).

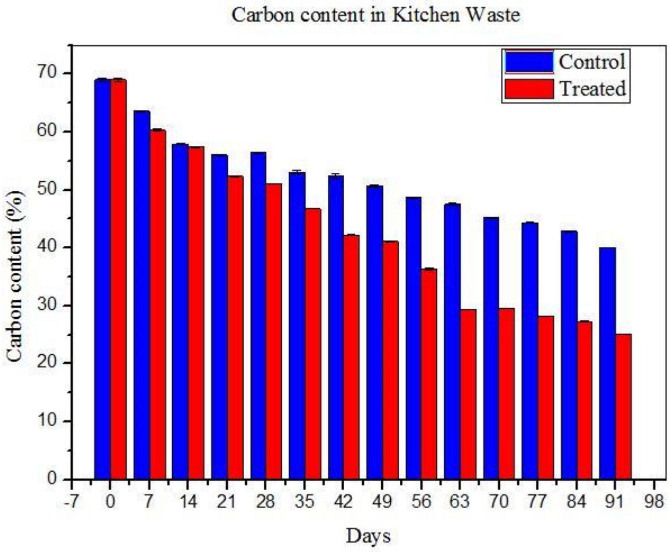

Fig.15: Carbon content (%) at different intervals of treated and untreated Kitchen waste

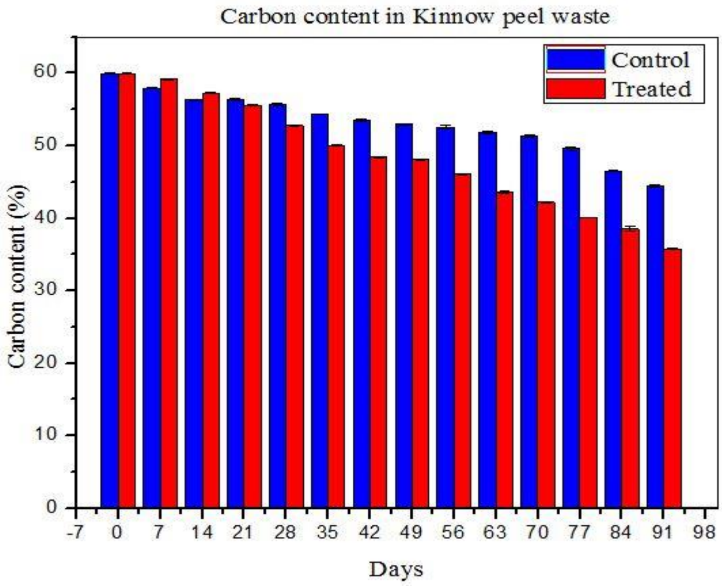

Fig.16. Carbon content (%) at different intervals of treated and untreated Kinnow peel waste

A good proportion of carbon and nitrogen in a composting substrate provide a balanced diet for micro-organisms along with enough energy source and protein for optimal growth and reproduction.

4.7 Total Nitrogen content

The Nitrogen content increases as the degradation proceeds (Fig 17 & Fig 18). Initially the nitrogen content was 0.07% and when the kitchen waste was treated with consortia for 90 days, the nitrogen levels increased to optimum range in 56 days of composting while in case of control it increased to 1.3%.

Table 13: Total Nitrogen (%) at different intervals of treated and untreated Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	0.0633±0.01	0.0633±0.01	0.057±0.01	0.057±0.01
7	0.783±0.01	0.847±0.01	0.18±0.02	0.833±0.02
14	0.863±0.01	1.105±0.01	0.729±0.01	1.096±0.03
21	0.954±0.01	1.197±0.01	0.847±0.01	1.215±0.01
28	1.032±0.01	1.249±0.01	1.257±0.01	1.459±0.02
35	1.067±0.01	1.35±0.01	1.677±0.01	1.645±0.01
42	1.11±0.01	1.56±0.01	1.833±0.01	1.827±0.02
48	1.151±0.02	1.623±0.01	1.97±0.01	2.162±0.02
56	1.247±0.01	1.763±0.01	2.243±0.01	2.36±0.03
63	1.423±0.01	2.087±0.01	2.357±0.01	2.483±0.13
70	1.5±0.01	2.177±0.01	2.463±0.01	2.853±0.02
77	1.677±0.01	2.357±0.01	2.68±0.01	3.037±0.02
84	1.94±0.01	2.45±0.01	2.827±0.01	3.24±0.02
91	1.357±0.01	2.45±0.01	3.01±01	3.677±0.01

± Values of Standard Deviation

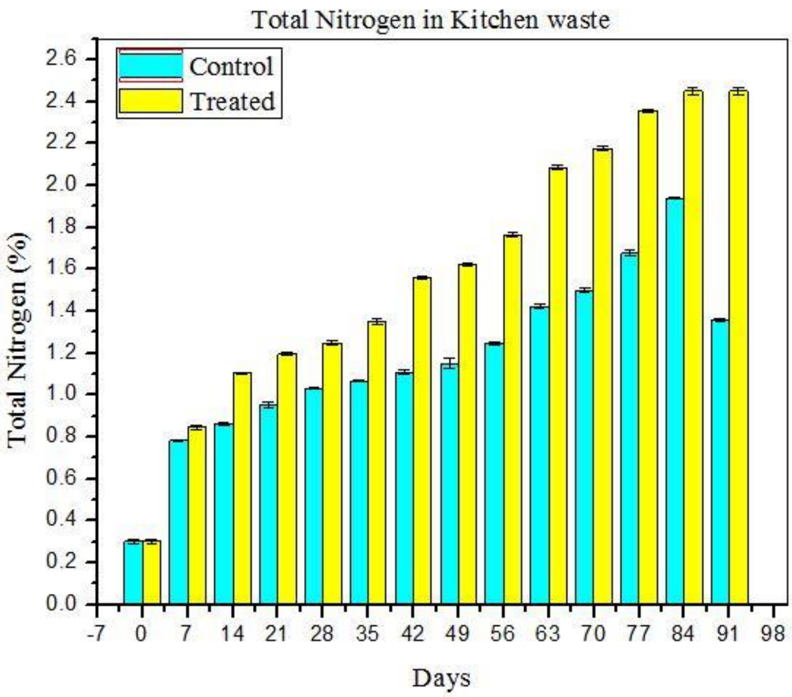

Fig.17: Total Nitrogen (%) at different intervals of treated and untreated Kitchen waste

Meanwhile, in case of kinnow peels nitrogen content increased from 0.057% to 3.67% in treated case and 3.02 % in case of control (Table 13). Typical values of compost total N in the literature may vary from 0.8% to 3% (Iglesias-Jimenez & Alvarez, 1993; Wolkowski, 2003; Zmora-Nahum et al., 2007).

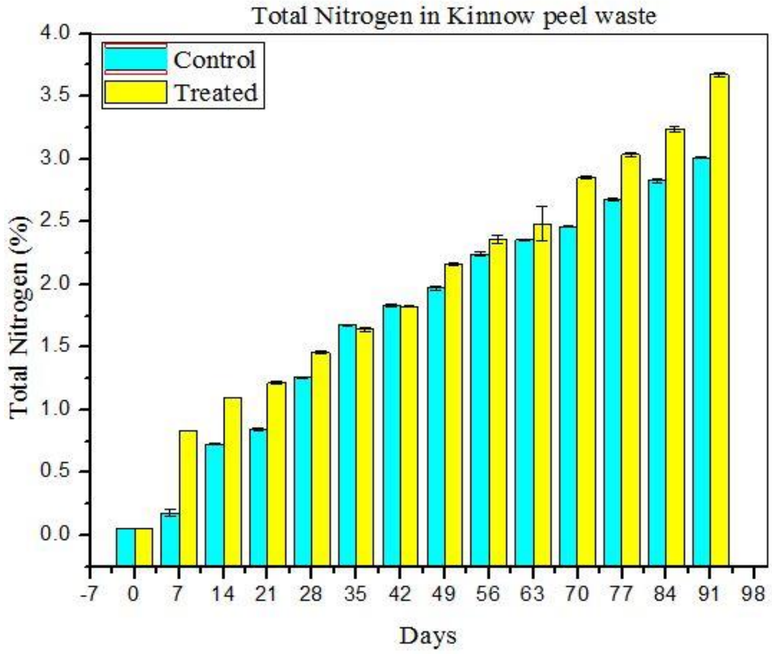

Fig. 18: Total Nitrogen (%) at different intervals of treated and untreated Kinnow peel waste

4.8 Carbon: Nitrogen ratio (C: N ratio)

C: N ratio is one of the most important parameters that determine the extent of composting and degree of compost maturity (Shyamala and Belagali, 2012). Compost C: N ratio is a commonly used indicator of the compost N mineralization potential (Sullivan et al., 2002; Wolkowski, 2003; Flavel & Murphy, 2006).

The C: N ratio of the kitchen waste treated with consortia on day 0 was noted to be 1117.81 which showed the decreasing trend and reached till 10.21 till the end of 90 days. The optimum C: N ratio was attained in 42 days of composting process i.e. 27.09. While on comparing the results with the control the initial ratio decreased to 30.02 in 90 days of degradation (Table 14). Thus, C: N ratio followed decreasing trend as in Fig. 19 & Fig. 20. A low C: N ratio in ecosystem increased the microorganism growth (Begone et al., 1997) and therefore the O_2 uptake is increased.

Table 14: C: N ratio at different intervals in treated and untreated Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	1117.8±186.84	1117.8±186.84	1046.2±22.77	1046.25±22.77
7	82.93±0.41	71.27±0.99	331.56±59.68	71.00±0.38
14	70.33±0.41	51.93±0.32	77.31±0.13	52.27±0.20
21	60.48±0.81	43.75±0.26	66.60±0.56	45.78±0.46
28	55.35±0.26	40.87±0.27	44.35±0.18	36.18±0.26
35	50.24±0.33	34.67±0.43	32.41±0.08	30.43±0.29
42	48.71±0.06	27.09±0.18	29.18±0.29	26.50±0.15
48	43.78±0.73	25.34±0.15	26.90±0.16	22.24±0.04
56	41.71±0.13	20.59±0.15	23.42±0.05	19.52±0.30
63	33.87±0.14	14.09±0.05	21.99±0.06	17.60±1.07
70	30.94±0.18	13.58±0.06	20.84±0.08	14.79±0.04
77	28.08±0.23	11.96±0.06	18.51±0.13	13.19±0.09
84	22.75±0.01	11.14±0.04	16.45±0.04	11.89±0.17
91	19.66±0.07	10.26±0.07	14.77±0.04	9.73±0.05

± Values of Standard Deviation

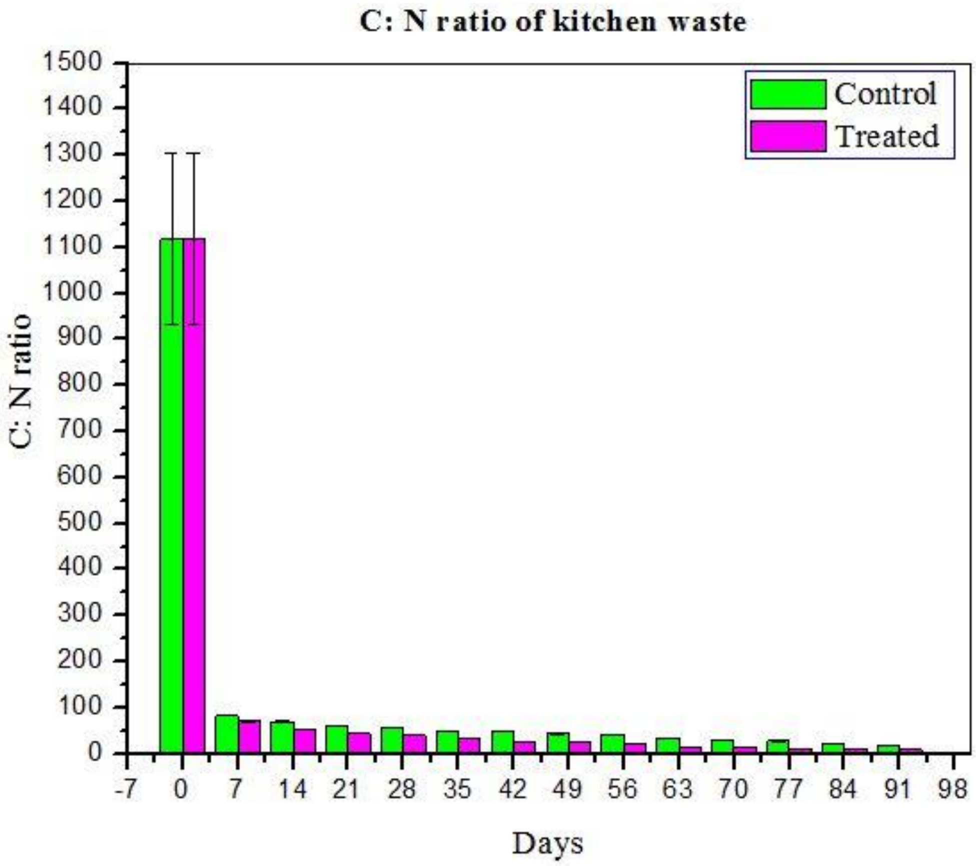

Fig. 19: C: N ratio at different intervals in treated and untreated Kitchen waste

The contribution of compost organic nitrogen to the nitrogen mineralized following the application of different types of composts with different C: N ratios to poor acidic, clay, and sandy loam soils, was shown in other work to range from negative to lower than 12% (Beloso, 1993; Gagnon et al., 1998; Gagnon and Simard, 1999; Mamo et al., 1999). Eriksen et al. (1999) also reported an early season immobilization of soil N following the application of about 310 kg total N ha^{-1} of MSW compost with a C: N ratio of 40. Thus C: N ratio is the most important parameter to analyze the quality of the compost from any organic waste.

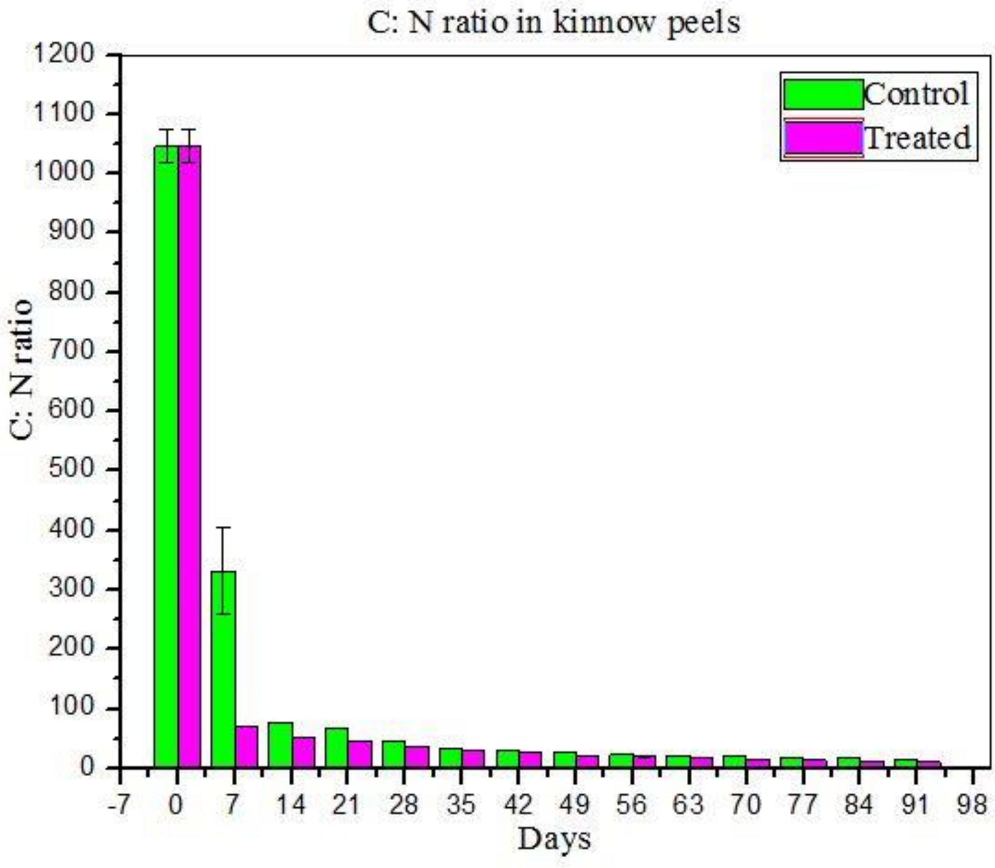

Fig. 20: C: N ratio at different intervals in treated and untreated Kinnow peel waste

4.9 Reducing sugar

Estimation of reducing sugar showed increasing trend from 3 mg/mL on 0 day to 126.5 mg/mL in 90 days when consortia treatment was given to the kitchen waste while in case of control the reducing sugar increased to 65.3 % (Table 15). In case of kinnow peels the reducing sugar increased from 8 mg/mL to 107.3 mg/mL while in control the increase was by 48.9 % (Fig. 21 & Fig 22). Increase in reducing sugar was also reported by Oberoi et al., 2012.

Table 15: Reducing sugar at different intervals in treated and untreated Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	3.033±0.06	3.027±0.04	8±0.13	8.06±0.06
7	13.04±0.05	25.17±0.21	9.1±0.10	9.79±0.02
14	20.81±0.02	40.60±0.006	10.8±0.21	11.15±0.13
21	25.73±0.42	51.10±0.1	11.8±0.03	13.86±0.05
28	35.24±0.06	56.60±0.1	12.9±0.05	14.75±0.05
35	43.93±0.03	63.17±0.11	13.3±0.10	19.33±0.03
42	45.20±0.10	78.27±0.25	18±0.05	20.06±0.05
48	45.04±0.05	79.23±0.08	20±0.07	38.06±0.06
56	50.09±0.02	80.43±0.42	20.70±0.11	40.40±0.13
63	53.92±0.02	82.3±0.2	30.10±0.05	50.69±0.25
70	56.06±0.06	82.70±0.1	44.30±0.05	55.47±0.08
77	57.11±0.01	93.70±0.2	45.10±0.03	66.13±0.06
84	61.82±0.07	102.63±0.15	55.70±0.04	73.14±0.04
91	65.40±0.10	126.50±0.1	56.90±0.13	107.35±0.05

± Values of Standard Deviation

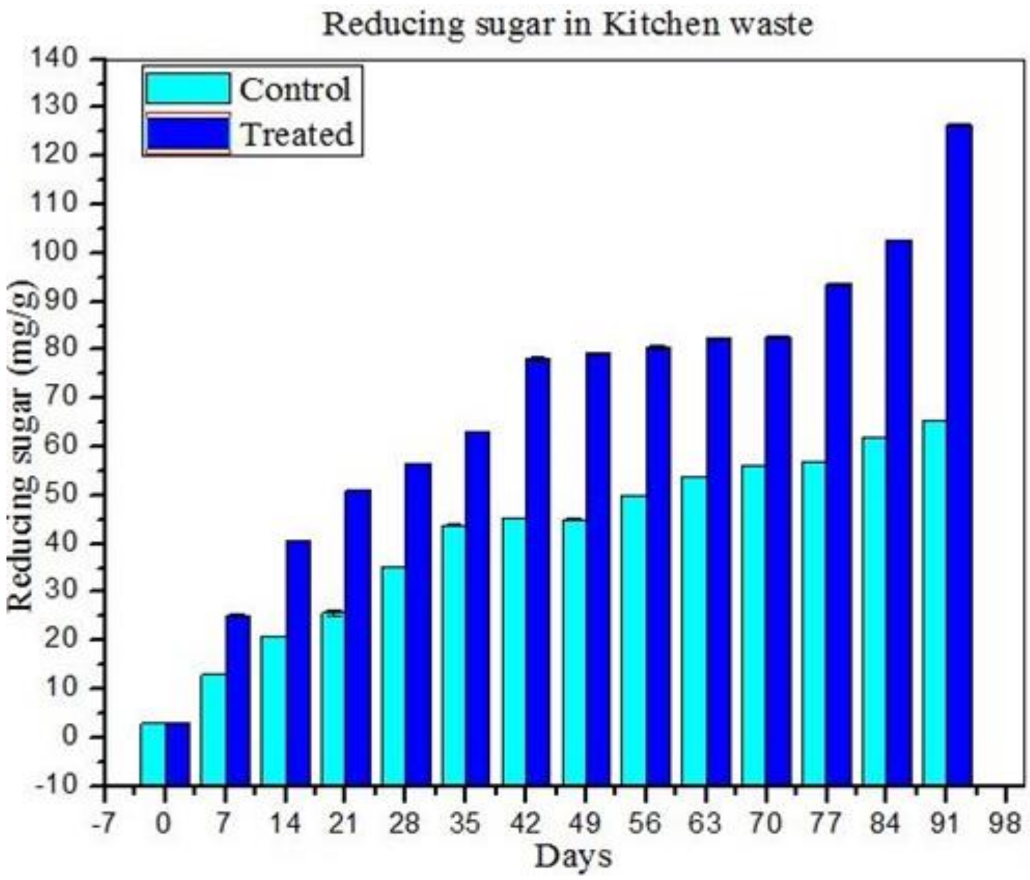

Fig. 21: Reducing sugar at different intervals in treated and untreated Kitchen waste

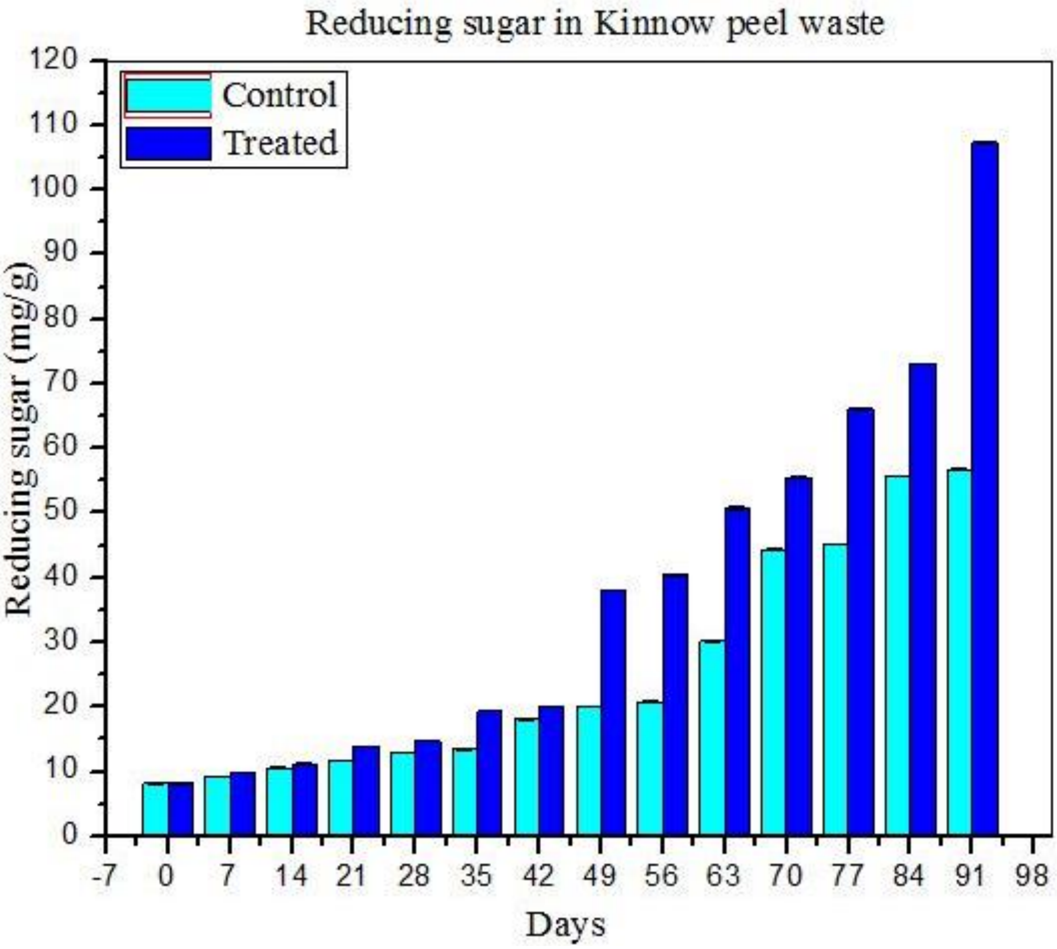

Fig. 22: Reducing sugar at different intervals in treated and untreated Kinnow peel waste

4.10 Ash content

Increasing trend was observed in case of ash content. Initially the ash content on day 0 was observed to be 7.17% and towards the end of 90 days of composting it increased by 54.54% in control while in treated compost it was observed to increase by 68.84% (Table 16). Similar pattern was observed in kinnow peels as in case of control the ash content increased by 55.21% and in case of treated there was an increase by 65.01% (Fig. 23 & Fig 24). Similar increasing trend was observed by Zhang and Cai, 2008, where the ash content increased after the treatment with NaOH.

Table 16: Ash content at different intervals in treated and untreated Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	7.17±0.04	7.17±0.04	0.10±0.02	0.10±0.02
7	11.87±0.15	12.58±0.08	0.13±0.006	0.31±0.03
14	16.26±0.05	17.07±0.02	9.07±0.06	11.50±0.02
21	20.64±0.03	21.43±0.25	11.14±0.01	15.30±0.44
28	21.80±0.02	23.68±0.01	14.44±0.17	16.40±0.4
35	23.36±0.03	25.54±0.03	17.32±0.02	23.30±0.42
42	29.07±0.03	32.04±0.05	18.52±0.02	25.06±0.06
48	33.13±0.02	38.25±0.07	20.04±0.06	32.40±0.1
56	40.76±0.01	46.05±0.04	35.07±0.11	39.53±0.15
63	44.15±0.01	50.15±0.03	38.93±0.04	42.67±0.11
70	45.60±0.1	55.16±0.04	42.30±0.35	57.98±0.07
77	52.65±0.05	56.96±0.02	48.18±0.11	58.38±0.16
84	58.13±0.02	63.39±0.02	52.13±0.35	63.72±0.04
91	61.73±0.04	76.07±0.06	55.35±0.06	65.14±0.05

± Values of Standard Deviation

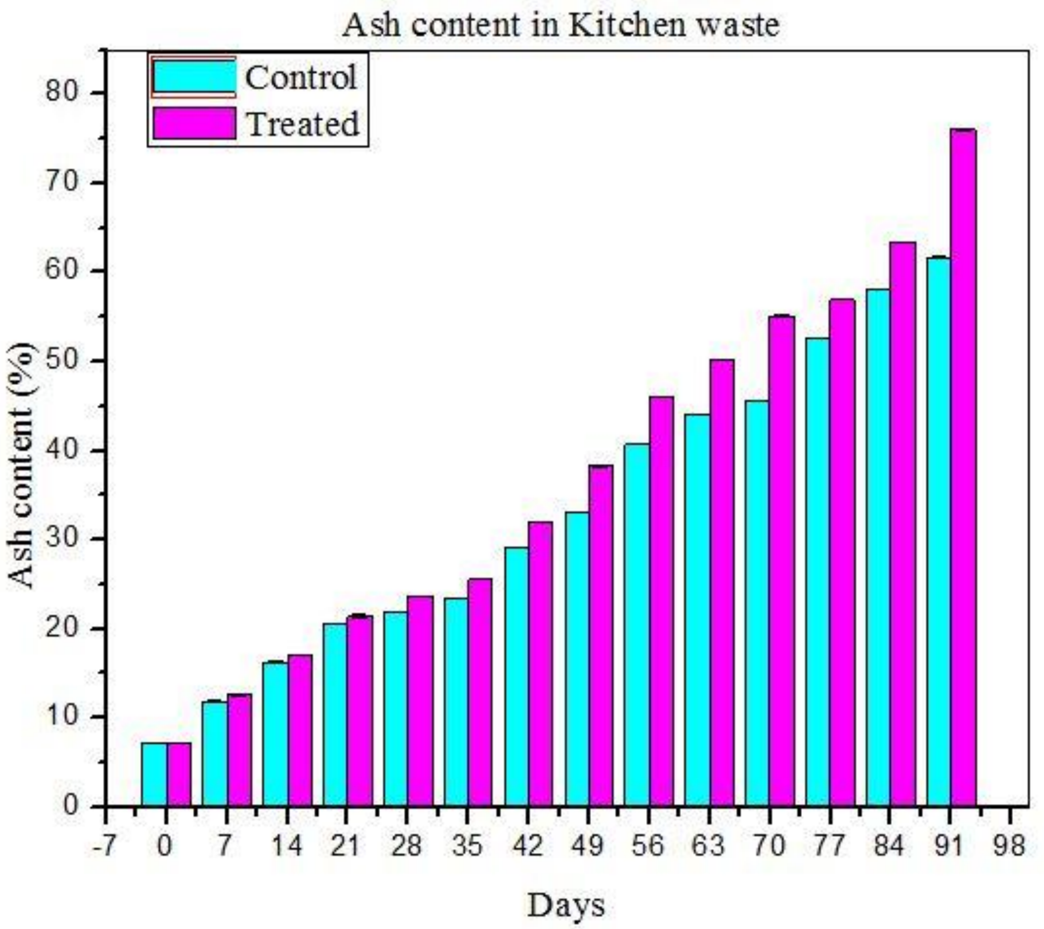

Fig. 23: Ash content at different intervals in treated and untreated Kitchen waste

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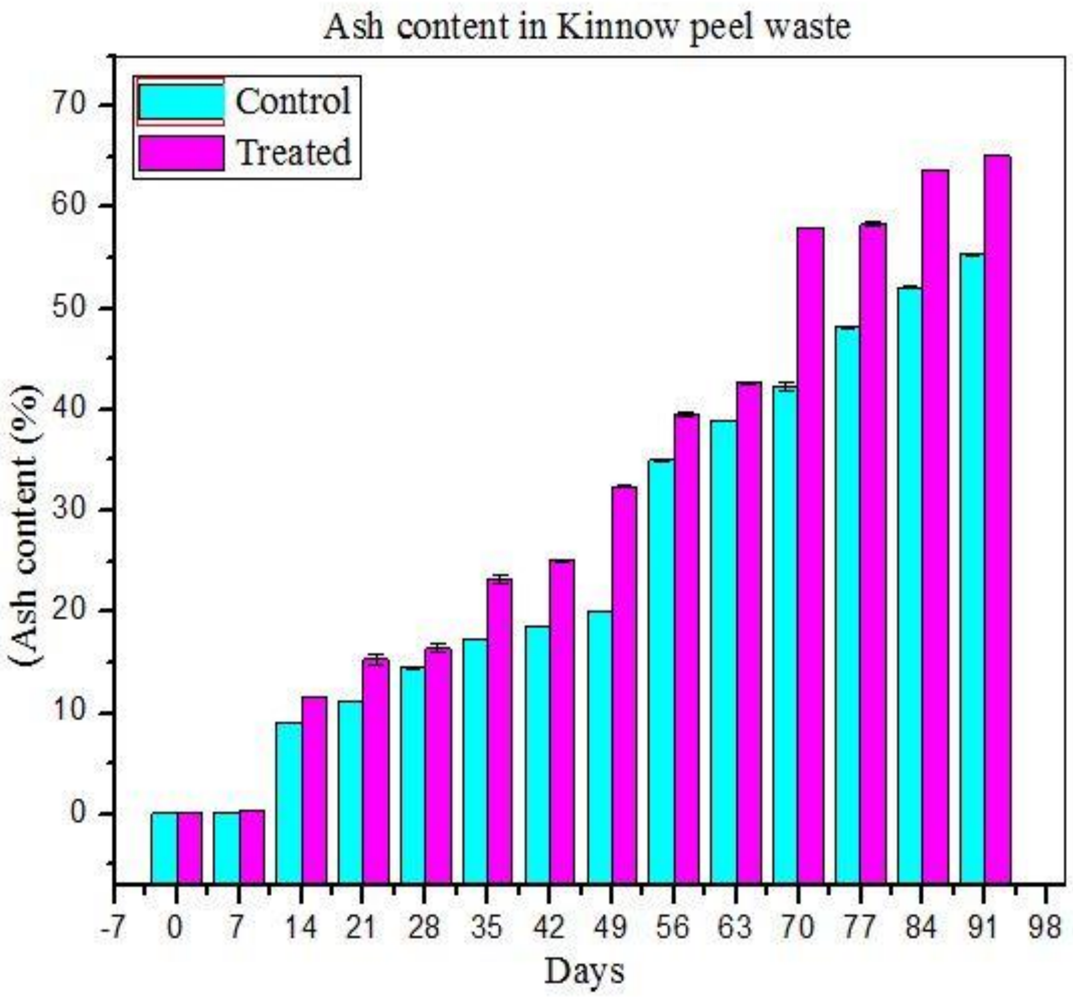


Fig. 24: Ash content at different intervals in treated and untreated Kinnow peel waste

4.11 Volatile solids

Volatile solids provide a measure of ease with which biomass can be ignited and oxidised, depending on how the biomass is to be utilized as an energy source. (McKendry., 2002). In case of kitchen waste treated with consortia, volatile matter reduced to 15.19% while in control it was 19.04% (Table 17). In kinnow peel waste reduction in volatile matter was by 39.97% in control and 43.14% in treated as in Fig 25 & Fig 26. Biomass from *Areca catechu*, *Ziziphus rugosa* and *Albizaia lucida* also showed volatile matter of 82.4, 78.3 and 75 % respectively (Sasmal et al., 2012).

Table 17: Volatile solids at different intervals in treated and untreated Kitchen waste and Kinnow peel waste

Days	Kitchen waste Control	Kitchen waste Treated	Kinnow peels Control	Kinnow peels Treated
0	85.33±4.50	86.67±0.94	58.17±0.12	58.17±0.12
7	75.33±0.47	63.70±0.28	50.17±0.09	49.20±0.42
14	61.40±0.29	62.37±0.12	47.43±0.09	46.34±0.13
21	58.83±0.05	50.33±0.47	42.43±0.09	40.58±0.04
28	54.87±0.09	46.37±0.26	40.67±0.05	38.37±0.05
35	50.67±0.47	40.53±0.05	35.23±0.09	32.55±0.04
42	46.2±0.14	39.27±0.05	33.17±0.09	30.50±0.71
48	41.4±0.16	32.27±0.21	32.33±0.21	29.67±0.05
56	32.27±0.21	29.60±0.08	29.13±0.12	25.20±0.08
63	30.30±0.16	25.60±0.08	27.10±0.14	23.77±0.09
70	29.17±0.09	21.43±0.09	25.04±0.02	20.57±0.05
77	25.47±0.09	19.53±0.05	20.3±0.16	19.53±0.31
84	20.54±0.04	17.57±0.09	18.87±0.26	16.17±0.09
91	19.04±0.02	15.19±0.01	18.20±0.22	15.03±0.02

± Values of Standard Deviation

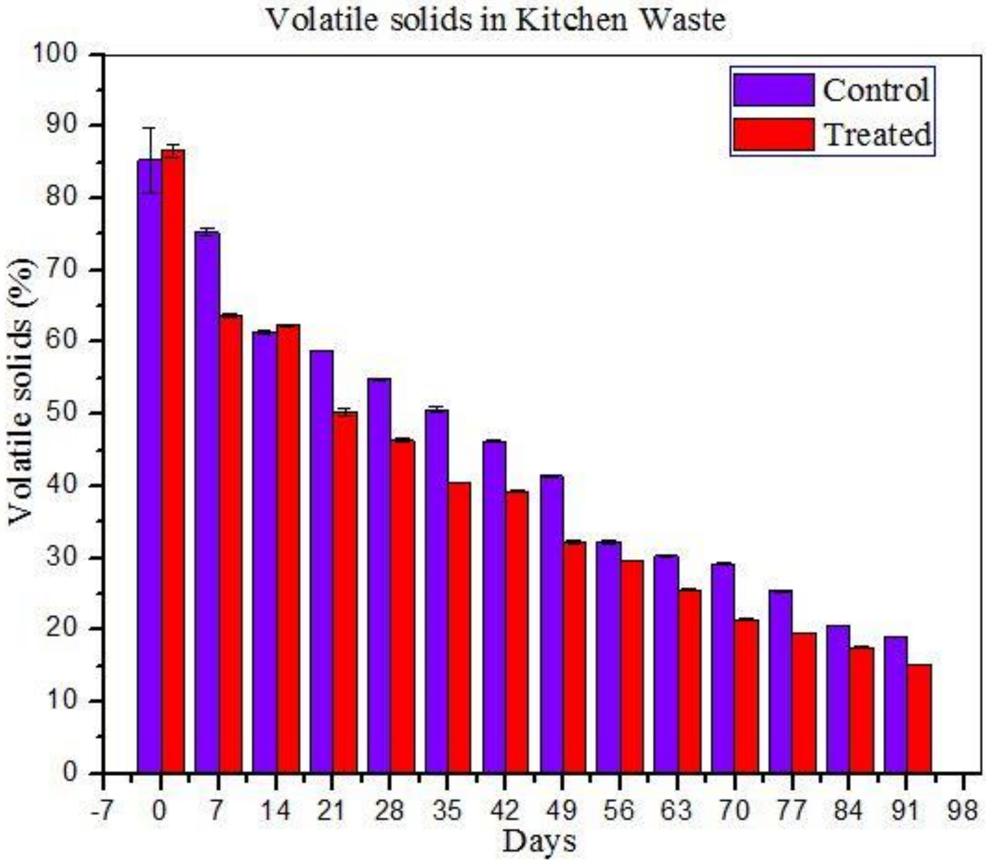


Fig.25: Volatile solids at different intervals in treated and untreated Kitchen waste

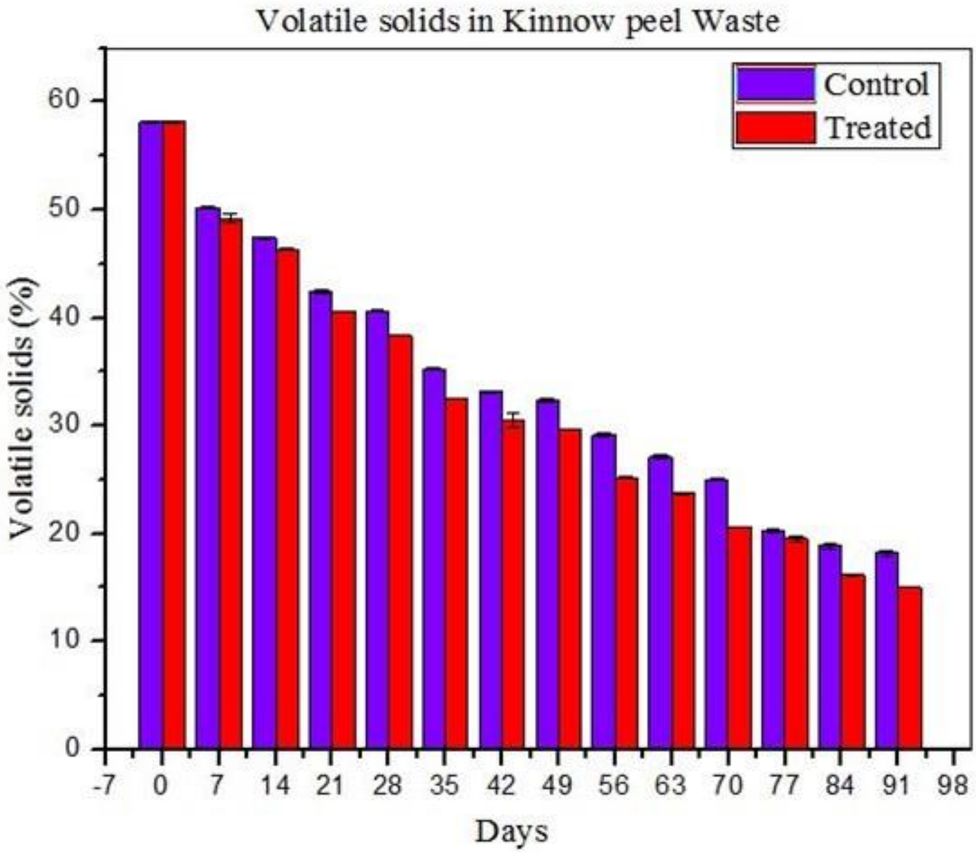
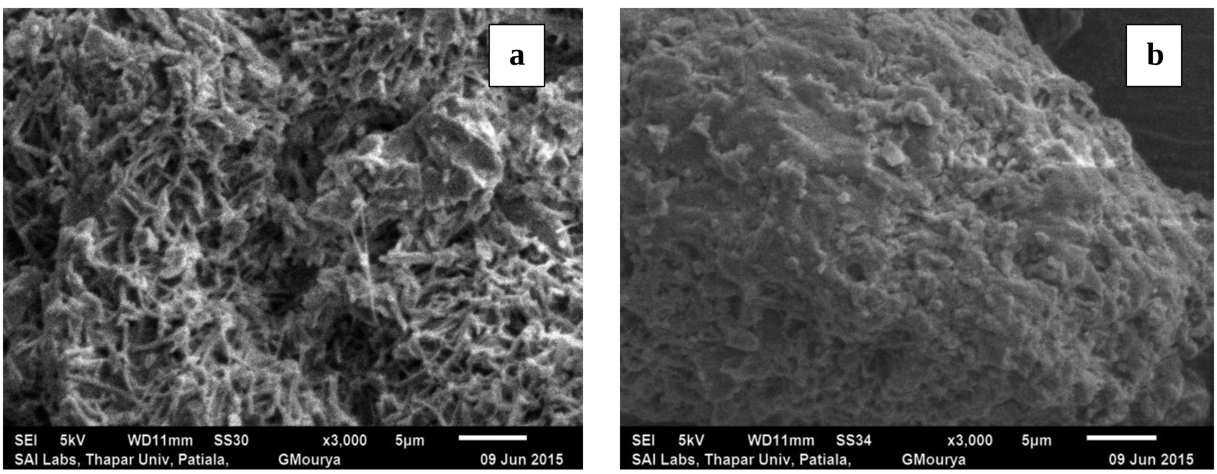


Fig. 26: Volatile solids at different intervals in treated and untreated Kinnow peel waste.

4.12 Scanning Electron microscopy

Physical appearance of control and treated biomass samples (0.5 mm) was observed using SEM at different magnifications for best results at day 0, day 35 and day 90 was observed.



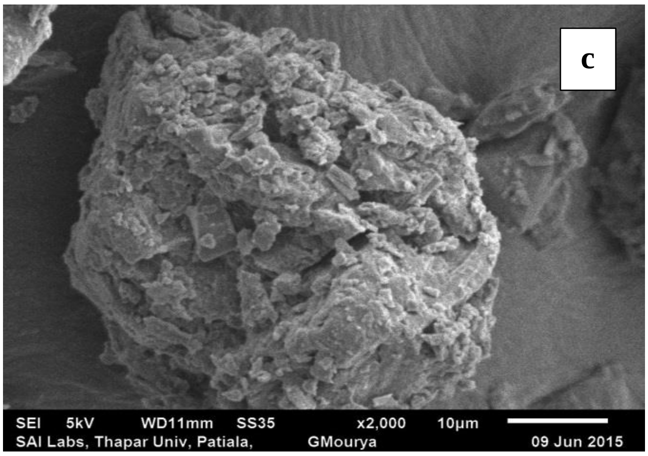


Fig. 27: Scanning electron microscopy of biomass samples
(a) day-0 (b) day-35 and (c) day-90

Fibre breakage was much more in case of treated biomass sample as compared to control. Thus, from Fig.27 we can conclude that the microbial degradation was more in case on biomass sample of day-90 as compared to day-0.

4.13 X-ray diffraction analysis (XRD)

XRD analysis of control and treated sample are presented in Fig. 28. Crystallinity index (*CrI*) of these samples along with amorphous and crystalline.

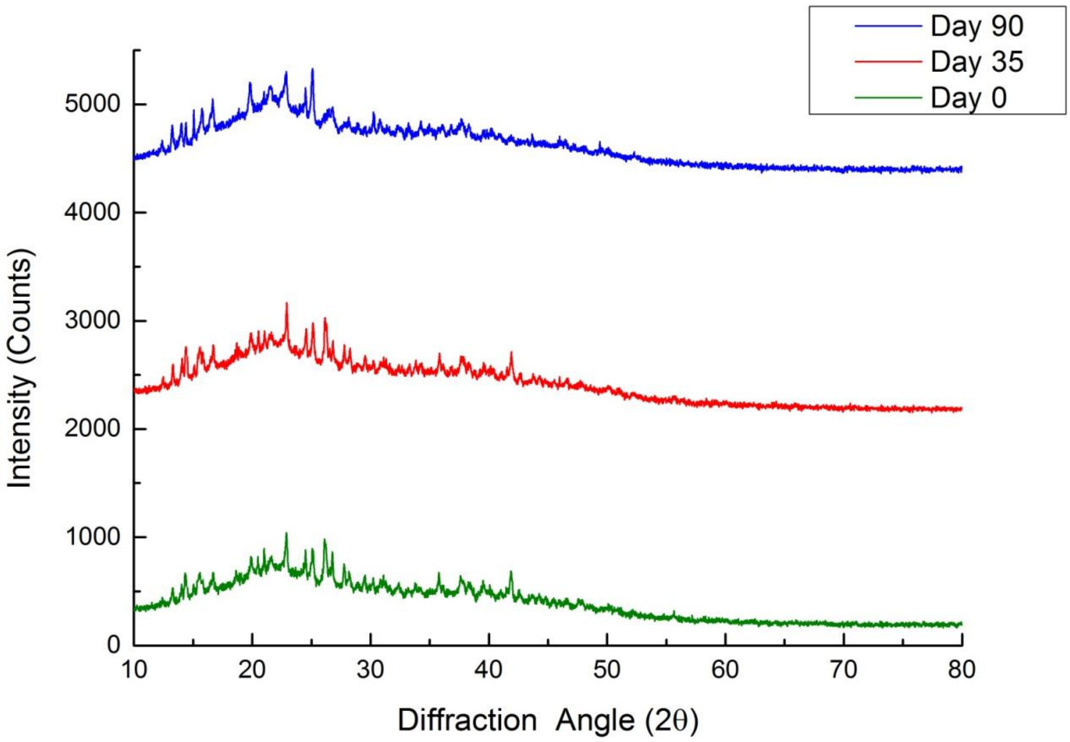


Fig. 28: XRD graph for biomass samples

Table 18: Peak at crystalline and amorphous region and CrI of biomass of Kinnow peel waste at different intervals

Biomass	Crystalline portion peak (I_{002})	Amorphous portion peak (I_{am})	*CrI (%)*
Day-0	21.82	18.52	20.90
Day-35	22.19	18.47	23.15
Day90	22.27	18.07	39.20

The biomass at different time intervals showed peak of crystalline region at 2θ angle range of 21.82-22.27, whereas amorphous portion peak range of 18.07-18.52 (Table 18). According to Kiran and Srikantaswamy, 2014 from the overall data obtained it is clear that the particle size is decreasing during the degradation process of solid waste. So, from the above increasing *CrI* value we can conclude that the degradation process led to increase in crystalline peak from day-0 to day 90.

4.14 FTIR Analysis

Fourier transform infrared (FTIR) spectroscopy was carried out to detect changes in functional groups of the biomass the obtained spectrum is represented in Fig 29.

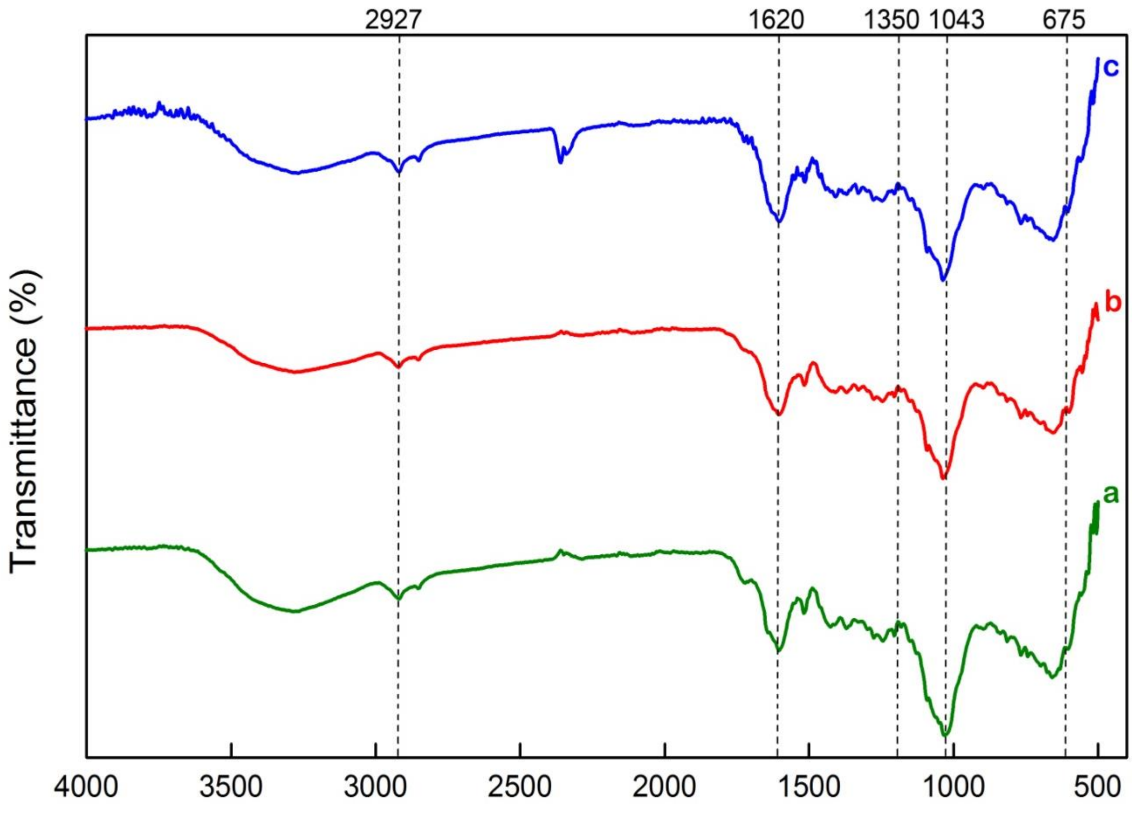


Fig. 29: FTIR spectra of biomass sample at (a) Day-0 (b) Day-35 and (c) Day-90

The main characteristics were attributed to different chemical groups present in biomass the peaks are based on the literature values (Chefetz et al., 1998; Smidt et al., 2002; Reveille et al., 2003) which are summarized in Table 19. Most prominent bands were 2927, 1620, 1350, 1043 and 675 cm^{-1} (Fig.29).

Table 19: Assignment of FTIR bands of functional groups in biomass samples

Name of characteristic group	Wavenumber (cm^{-1})
Aliphatic methylene group these bands are found in all waste samples and assigned to fats and lipids	2925
Amide I (H-bonded C=O carbonyl stretch). A band of protein origin	1620
Nitrate band, Sharp, stable, reproducible. Appears towards the end of the process when the material is well composted	1350
C–O stretching of polysaccharides or polysaccharide-like substances. Si–O asymmetric stretch of silicate impurities	1043
=C-H strong bonding in biomass	675

The most intensive broad absorption band appears in the characteristic carbohydrate region with maximums at 1043 cm^{-1} assigned to vibrations of C-3-H–O-3-H and C-6-H2–O-6-H. Pronounced peak at 1043 cm^{-1} is assigned to the vibration where carbohydrate content is more in case of day-0 as compared to day-90. Thus, it is evident that the carbohydrate content decreased as the degradation increased (Table 19). Symmetric and asymmetric stretching of bands appeared in region of 2925 cm^{-1}. The band at 1350 cm^{-1} depicted the nitrate band in biomass which is the significant vibration to analyse the end of the process of the well composted material as reported by (Chefetz et al., 1998; Smidt et al., 2002; Reveille et al., 2003). The band vibration at 675 cm^{-1} depicts strong C-H bonding mostly reported in case of fruits and plants (Smidt et al., 2002). The vibration change at 1620 cm^{-1} depicts the change in amide group preferably of the protein origin (Filip and Bielek, 2002).

4.15 Bio-efficacy trials

The compost prepared from kitchen waste and kinnow peels was applied to brinjal and lady finger respectively. The growth of these plants was monitored for 30 days. And the comparison was done with the control in which no compost was added. The soil physic-chemical properties were studied before and after the compost application. Moreover the growth of crop in case of pots with compost was more as compared to control (Fig 29).

Fig 30: Growth of (A) Brinjal and (B) ladyfinger in compost and control

Table 20: Physico-chemical analysis of soil

a. Initial Soil analysis (Day-0)

Parameter	Brinjal		Ladyfinger	
	Control	Treated	Control	Treated
pH	8.1±0.002	7.4±0.001	8.1±0.002	7.2±0.001
Organic carbon (%)	50.16	45.16	51.56	46.07
Total nitrogen (%)	0.78	0.94	0.84	0.98
C: N ratio	64.3:1	48: 1	61.38:1	47:1

b. Final Soil analysis (Day-30)

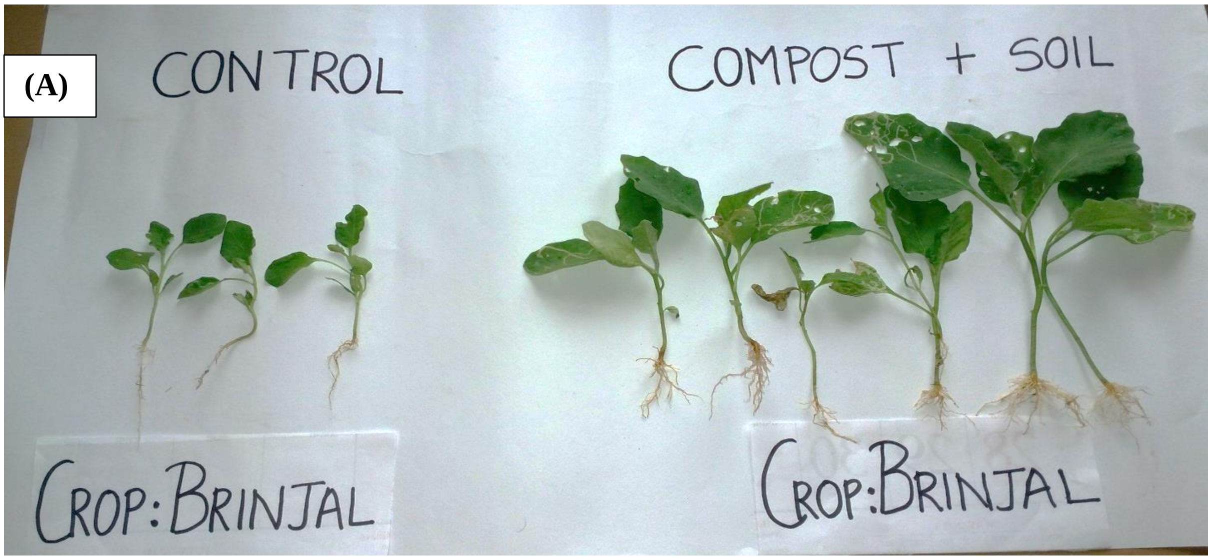
Parameter	Brinjal		Ladyfinger	
	Control	Treated	Control	Treated
pH	7.8±0.001	**7.**2±0.004	7.6±0.023	7.1±0.002
Organic carbon (%)	46.25	35.03	48.56	36.17
Total Nitrogen (%)	0.84	1.352	0.96	1.23
C: N ratio	55:1	25.94: 1	50.58:1	29.40:1

4.15.1 Destructive analysis of plants grown in compost

The crops grown in soil and compost in the ratio 3:1 were analyzed and compared to the plants grown in control. The parameters like pH, root length, shoot length and biomass weight were analyzed (Table 21). Extensive rooting system was developed in case of brinjal within 30 days. The germination index was comparatively more, than observed in control.

Table 21: Biometric parameters of plants with and without compost

Parameters	Brinjal		Lady finger	
	Control	Treated	Control	Treated
Root length (cm)	4..3	5.37	3.8	5
Shoot length (cm)	6.9	15.5	14.5	16.7
Biomass weight (g)	3.12	9.40	3.08	10.54
Germination Index (%)	30	70	16.6	66



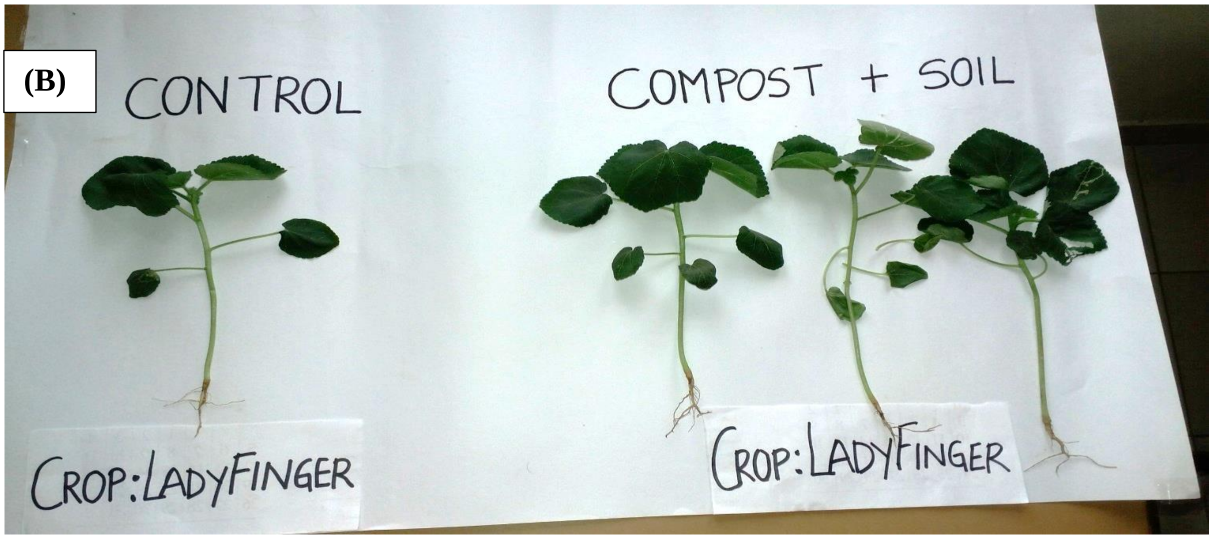


Fig 31: Destructive analysis of compost treated crops (A) Brinjal and (B) Ladyfinger

Conclusions

1. Present research work was aimed at developing a system for recycling and utilization of compost derived from kitchen wastes for soil amendment to improve plant growth. Treatment of Kitchen waste and Kinnow peel waste with a consortium of fast degrading microbes was done.
2. Various parameters such as pH, E.C, Organic carbon were monitored at different intervals for 90 days. The carbon content was reduced to 36.31% from 69.03% in 56 days of composting while in case of kinnow peels it reduced to 48%.
3. There was a drop in C: N ratio, optimum C: N ratio was obtained in just 56 days while in the case of kinnow peels the C: N ratio dropped to optimum range of 27.09 in 42 days. Loss in moisture content was monitored in kitchen waste, which reduced from 55.5% to 21.1% over the period of 90 days. On the other hand moisture content in kinnow peels dropped to 18.5%.
4. Reducing sugar yield was observed to have an increasing trend to 65.4 mg/mL from 3 mg/mL while in case of kinnow peels it increased to 107.3 mg/mL from 8 mg/mL.
5. The total nitrogen values tend to increase from 0.063% on day 0 to 2.15% in 90 days of composting. Also the kinnow peels showed the trend, where the nitrogen content increased from 0.057% to 3.66%.
6. Structural and chemical changes in the degraded biomass after 90 days were compared with untreated raw waste. Scanning electron microscopy (SEM) images showed the degradation pattern of the untreated and microbially treated biomass. XRD results evaluated the results by destruction of biomass *CrI*. FTIR results were also supported by changes in functional groups and hence, the degradation process was understood based on the changes in functional groups.
7. Bio-efficacy trials of the compost prepared from kitchen waste and kinnow peels was checked for its effect on plant growth in pots using brinjal and ladyfinger.
8. The Germination index in brinjal with compost was 70 % and in ladyfinger it was 66%. The biomass produced in case of soil amended with compost was 9.40 g and 10.54 g in case of brinjal and ladyfinger respectively, while in case of control it was 3.12 g and 3.08 g in brinjal and ladyfinger respectively.

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Appendix-I

A. Standard curve of glucose

Reducing sugar concentration of control and treated biomass was measured by Dinitrosalicylic acid (DNS) method (Miller, 1959) using dextrose as the standard.

Materials:

a. Stock: 2mg/mL glucose

b. DNS reagent

Distilled water	1415 mL
3, 5- Dinitrosalicylic acid	10.5 g
NaOH	19.8 g
Dissolve the above and then add	
Rochelle salts (Na-K tartarate)	306g
Phenol (melt at 50 °C)	7.6 mL
Sodium meta-bisulphite	8.3 g

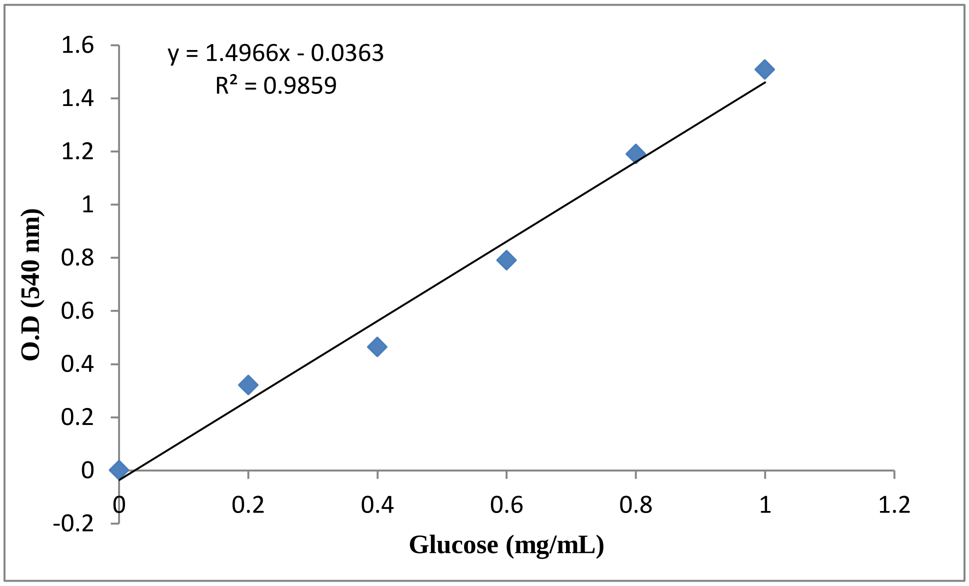

Fig 32: Standard curve for glucose.

Appendix-II

A. Nutrient Broth (NB)

Ingredients	Quantity (g/L)
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5

B. Potato Dextrose Broth (PDB)

Ingredients	Quantity (g/L)
Potato extract	4
Dextrose	20

C. Yeast Extract Peptone Dextrose (YEPD)

Ingredients	Quantity (g/L)
Peptic digest of animal tissue	20
Yeast extract	10
Dextrose	20

Appendix-III

Reagents for Carbon estimation

Potassium dichromate (1N): Prepared by dissolving 49.04g of $K_2Cr_2O_7$ in distilled water and diluted to 1 L.

Ferrous ammonium sulphate (0.5 N): Prepared by dissolving 198g of salt in 1 L distilled water and also 20 mL of conc. Sulphuric acid was added.

Diphenylamine indicator: Prepared by adding 0.5 g of diphenylamine in a mixture of 20 mL distilled water and 100 mL concentrated sulphuric acid.