Evaluation of microbial consortium for rapid composting of rice straw

A

DISSERTATION REPORT

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In

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Submitted by:

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CERTIFICATE

Certified that the thesis "**Evaluation of microbial consortium for rapid composting of rice straw**" submitted by Ms. **Tamanraj Kaur** (301601028) in partial fulfilment of the requirement for the award of the degree of **Masters of Science** in Biotechnology in the Department of Biotechnology, Thapar Institute of Engineering and Technology, 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 "**Evaluation of microbial consortium for rapid composting of rice straw**" submitted by me for the award of the degree of Masters in Science in the Department of Biotechnology, Thapar institute of engineering and technology, Patiala, is true and original record of my own independent and original research work carried out under the supervision of **Dr. Dinesh Goyal**, Professor, Department of Biotechnology, Thapar institute of engineering and technology, 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.

Tamanraj Kaur

Place: Patiala (Tamanraj Kaur)

Date: 15-6-18

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Life itself is a unique project, the objectives of which can never be accomplished alone despite putting our best endeavor's 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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Tamanraj Kaur

Date: 15-6-18

Place: Patiala

Dedicated

To

My beloved

Parents

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List of Symbols

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

List of Abbreviations

CH_4	Methane
CO_2	Carbon dioxide
$CuSO_4$	Copper sulphate
DNS	Dinitro-salicylic acid
DPA	Diphenylamine
et al	And others
etc	And other things
FAS	Ferrous ammonium sulphate
g	Grams
H	Hour
H_2SO_4	Sulphuric acid
HgO	Mercuric Oxide
$K_2Cr_2O_7$	Potassium dichromate
K_2SO_4	Potassium sulfate
Kg	Kilograms
M	Molarity
mg	Milligrams
min	Minutes
MSW	Municipal solid waste
MTCC	Microbial type culture collection

NCIM	National collection of industrial microorganism
NH_3	Ammonia
s	Second
sp.	Species
w/v	weight by volume

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Abstract

Present research work was aimed at developing a system for rapid composting of agriculture wastes such as rice straw for its applications as soil amendment to improve plant growth. Out of 19 bacterial isolates from the rhizospheric soil of rice straw, seven bacterial isolates were found to be Gram positive, four were positive for methyl red test, 17 were starch hydrolysis positive, only two were Voges-Proskauer positive. Nine bacterial isolates were found to be cellulose degrading bacteria. Composting of rice straw with a consortium of fast degrading microbes was also done. Rice straw was collected from Devigarh, Patiala. Composting of rice straw was done in plastic beakers using a consortium of microbes, comprising *Bacillus subtilis* (NA 15), *Paenibacillus polymyxa*, *Saccharomyces cerevisiae* and *Trichoderma reesei* (MTCC 164) with and without fertilizer (DAP- diammonium phosphate) treatment. Various parameters such as pH, temperature, organic carbon, nitrogen, reducing sugar and cellulose content were monitored at different intervals for 60 days. Organic carbon content for rice straw was recorded as 69.1% in case of control and it dropped to 40%, In case of consortium it was recorded as 69.1% and it dropped to 30.2%, In case of consortium + fertilizer it was recorded as 69.1% and it dropped to 25% and in case of fertilizer only it was recorded as 69.1% and it dropped to 29% from 0 day to 60th day of observation.The nitrogen content showed increasing trend from 0.063% on 0 day to 2.0% on 60th day when treatment with microbial consortia was given to the rice straw, When consortia + fertilizer treatment was given it the increase was observed from 0.063% to 2.3%, In case of fertilizer only the increase in nitrogen was observed from 0.063% to 2.1% while in case of control it was increased from 0.063% to 1.42%. Simultaneously, there was a drop in C: N ratio was observed from 1096.8 to 28.23 in case of control, When treatment with microbial consortia was given to rice straw it got reduced from 1096.8 to 14.51, In case of consortium + fertilizer it reduced from 1096.8 to 10.91 and in fertilizer only it reduced from 1096.8 to 13.85 from 0 day to 60th day of observation.Loss in moisture content was from 60.2% to 41.1% over the period of 60 days. Reducing sugar yield was observed to have an increasing trend from

0.91 mg/ml on 7th day to 2.0 mg/ml on 60thday when treatment with microbial consortia was given to the rice straw, when consortia + fertilizer treatment was given it the increase was observed from 1.06 mg/ml to 2.3 mg/ml, in case of only fertilizer the increase in reducing sugar was observed from 0.91 mg/ml to 1.9 mg/ml while in case of control the reducing sugar increased from 0.3 mg/ml to 1.11 mg/ml. The total weight of biomass got reduced as a result of microbial degradation. There was gradual decline in cellulose content however rapid decline was observed in rice straw treated with consortia and fertilizer. Treatment with consortium + fertilizer was the best among all that could reduce the period of composting almost from 60 days to 30 days. This treatment can be used for large scale recycling of rice straw.

Chapter 1

Introduction

Agricultural wastes hasbecome a major problem for the survival of living organisms. Agricultural waste management is an important component for improving soil fertility. These mainly include crop residues in the form of straw, husk, animal wastes like dung, urine, bones, fish processing wastes and human wastes like garbage, sewage and sludge and similar wastes. These are burnt in many states of India including Punjab, Haryana and even in some parts of Uttar Pradesh. It is estimated that around 78 to 85 million tons of rice husk, straw and wheat straw is generated in India each year out of which nearly 17 to19 million tons is burnt in the field itself, which causes serious problem to the environment and is a threat to public health (Kausar *et al*., 2011).Waste volume continues to increase, which leads to loss of resources and increased environmental risks, generation of harmful gaseslike carbon dioxide and polluted water resources, thusaffects soil fertility resulting in loss of productivity(Pan *et al.*, 2012).

Rice straw disposal is a major problem because its decomposition is slow,which causes environmental contamination, diseases and it cannot be used asa feed for animals due to its low digestibility. Rice straw is a renewable carbon resource for improving poor soil conditions. It is rich in carbon source consisting of cellulose (41.3%), hemicellulose (20.4%) and lignin (12.1%) (Akhtar *et al.*, 2015). Rice straw can be degraded by cellulolytic microbes in paddy fields thus reducing the use of urea fertilizer (Linda *et al.,* 2017).Many microorganisms are present in the environment which are used for biodegradation of wastes (Pan *et al.*, 2012).

Composting is a method where organic wastes areconverted into organic fertilizers. Composting process involves microorganisms such as bacteria (*Bacillus subtilis*), fungi (*Trichoderma reesei*) and yeast(*Saccharomyces cerevisiae*) converting organic waste to humus (Haseen*et al*., 2001). Composting is cheap, ecofriendly and convenient method. Evaluation of composting is done by judging various parameters such as temperature, C:N ratio, pH, moisture content, oxygen(%), ash content etc(Steger *et al.*2007). 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 usage efficiency of nitrogen as organic fertilizer (Amlinger *et al.*, 2003).Application of compost to agricultural land is required to be carried out in such a manner that it ensures sustainable development.

The present study was focused on the evaluation of the degradation pattern of rice straw using consortium of micro-organisms i.e*Bacillus subtilis,Bacillus polymyxa*, *Trichoderma reesei* and*Saccharomyces cerevisiae*. Screening and isolation of cellulose degrading bacteria from rhizospheric soil of rice straw was another objective of the present investigation.

Chapter- 2

Review of Literature

Rice (*Oryza sativa*) is the main staple food for a large part of the world's population. In India, tons of straw is generated each year from the fields of paddy. It is a renewable carbon resource andafter decomposition cancontribute towards improving soil conditions. It is a rich carbon source for soil microorganisms including fungi, bacteria and yeast and microbial composting of rice straw can also help to increase soil fertility.

After harvesting the straw/stalk left upon combine harvesting is considered as waste. In many countries, the disposal of huge amount of straw is done through field burning (Fig.1) and landfills. Accumulation of wastes on the field side as landfills can contribute to the problems like unpleasant odour, breeding home for mosquitoes and harmful microorganisms, occupying large areacreating disposal problems.It is estimated that in Asia, the amount of biomass burnt is approx 730 Tg (Teragram =10^{12} grams), out of which 250 Tg comes through agricultural waste burning. Open field burning is a process of combustion during whichcarbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxides (NO_X), unburnt carbon and sulphur dioxide (SO_2) is released into the atmosphere,effecting human health (Gadde *et al*.,2009).

Figure 1. a) Burning of rice straw inpaddy fields b) View of paddy field after burning (Gadde *et al*., 2009)

Composting is a method of conversion of theend products of agriculture into an organic fertilizer. With the increase in fertilizer costs, fluctuating product prices and decrease in soil productivity, the farmers are shifting towards the use of organic material as nutrient source. However, the availability of organic matter and its appropriate recycling is an equally important factor. The utilization of biodegradable organic fraction of urban wastes, cattle waste and crop residues are a rich source of plant nutrient (Game *etal*.,2017). Plant and agricultural waste, animal waste and other waste like city garbage etc (Table.1) are the waste material that can be used for composting (Chatterjee *et al*., 2017).

Table 1. Materials that can be used for composting

Wastes	Their Types	Sources
Plant and agricultural waste	Crop residues	Field crop residues and biomass
	Kitchen wastes	Daily kitchen wastes
	Green market wastes	Fruits and vegetable market wastes
	Coconut waste	By products
	Forest biomass	Natural forest biomass.
	Road side vegetation	Weeds and other unwanted plant biomass
	Aquatic plant biomass	Biomass of aquatic plants
Animal waste	Dung and urine	Domestic animals & dairies
	Poultry excreta	Poultry droppings of layering farm
	Fish wastes	Fish meal
Other wastes	City garbage	City garbage and municipal solid wastes
	Biogas slurry	By products
	Sewage and sludge	Industrial and municipal waste water treatment plants

2.1The Composting Process

The process of composting is that where degradation of raw materials is done by microorganisms like bacteria, fungi and yeast under controlled conditions. During the composting process, microorganisms consume the oxygen when they feed on the raw material, which later on generates heat, carbon dioxide and also release vapours into the environment (Fig. 2). Composting reduces the weight and amount of raw material while converting them into a valuable end product which help in conditioning the soil (William, 2000).

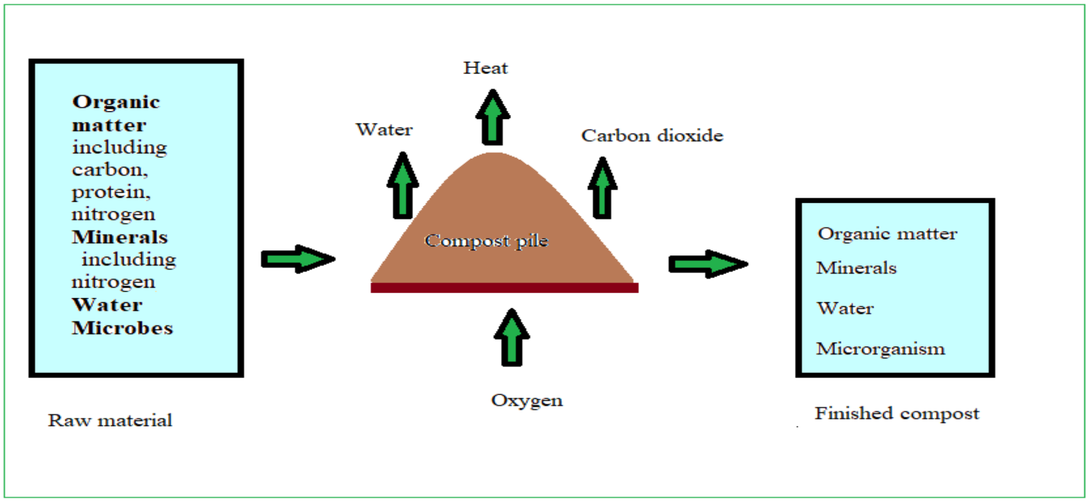

Figure 2. Processof composting (William, 2000)

2.2 Types of composting

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

2.2.1 Aerobic composting

Composting refers to the break-down of raw material or waste in the presence of oxygen (air). Products evolved include $CO_2$from O_2, $NH_3$from N_2, heat and water. Thus, appropriate blend of ingredients and environmental conditions complete the composting process successfully. The parameters like moisture content (60-70%) and carbon to nitrogen ratios (C: N) of 30:1 are desirable. Any type of change can lead to inhibition of the process of degradation. Sufficient amount of oxygen is made to circulate in the organic waste through ventilation or forced and passive aeration(Tweib*et al*., 2011).

2.2.2 Anaerobic composting

Anaerobic composting without oxygen releases byproducts 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 waste (Tweib*et al,* 2011).

Table 2. Types of composting(Neal *et al*., 1991)

Aerobic composting	**Anaerobic composting**
Oxygen present	Oxygen absent or in limited supply
Release more energy	Release less energy
Glucose completely broken down	Glucose not completely broken down
Toxicity decreases	Toxicity increases
Odor decreases	Odor increases

2.3 Phases of composting

The time–temperature course of the composting process can be divided into different phases as discussed below

2.3.1 Mesophilic phase: Proliferation of the distinct population of mesophilic bacteria and fungi takes place in this phase and the carbon dioxide concentration increases along with temperature. The reduction in substrate is noticed due to degradation of sugar and proteins. This whole degradation is done by the activity of mesophilic organisms, thereby increasing the temperature to about 45°C. The temperature of new compost is suitable for mesophiles to degrade the substrate. When the substrate is broken down the temperature of pile increases, becomes unfavorable for the mesophiles (*Pseudomonas, Staphylococcus aaureus, Streptococcus pyrogenes)* (Pan *et al*., 2012).

2.3.2 Thermophilic phase: During second phase there is an increase in temperature from 45 °C to 70°C in the piles of compost. At this stage, mesophiles species are replaced by the thermophiles species of bacteria, actinomycetes and fungi. Thermophiles at their high temperature destroy all unwanted microorganisms or pathogens. For ex. *Chloroflexusaurantiacus, Thermusaquaticus*(Pan *et al*., 2012).

2.3.3 Stationary phase: It is regarded as the third phase, there is no 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 (Pan *et al*., 2012).

2.3.4 Maturing phase: This phase leads to the decrease in temperature in the compost pile.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 (Pan *et al*., 2012).

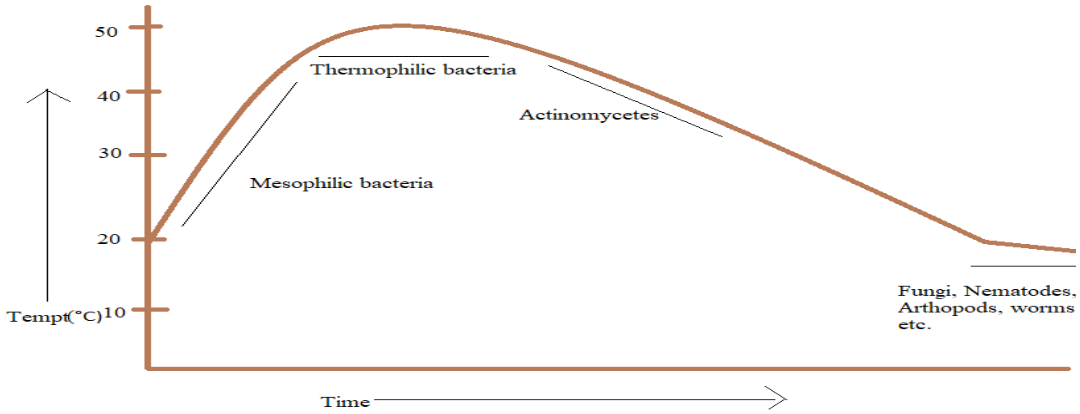

Figure 3. Phases of composting (Bernal *et al.*, 2017)

Composting is majorly done to replenish the soil nutrients. Composting has many beneficial properties. 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**.** Composting is best soil conditioner. It improves soil fertility and texture. It is cheap method which generate healthier plants.Compost is easy to handle and can be store easily as there are no problems like foul smell or fly problem. Composting is ecofriendly (pollution free) method.

Benefits of composting (William, 2000) are, a) it is practical and convenient method, b) Cost effective method, c) Ecofriendly, d) Improves soil texture and fertility, e) control diseases and insects, f) Healthier plants and increased crop productivity (William, 2000).

2.4 Methods of composting

2.4.1 Aerated static pile: In this method the pile of biomass is loaded over the air source with aeration cones, plastic pipes or floor (perforated),or by forcing air into the pile. There are equipments to monitorthe time, duration and direction of air flow. Air flow requirements will depend on the type of biomass to be decomposed, the size of pile, and the compost age. The top of the pile after addition of biomass is covered with the insulation sheet and left for composting process(William, 2000).

2.4.2 Turned windrow: In this method the compost production is done in windrow by the usage of mechanical aeration. Windrow turner aerated the mixture of compost, further it can be self-powered or powered by a farm tractor. Turned windrow composting is a process which shows low technology with uniform production of compost and require medium labour(William, 2000).

2.4.3 In-vessel composting: It refers to degradation of biomass within a vessel. In-vessel methods depend upon a variety of forced aeration and mechanical turning techniques to brush up the composting process. There are different combinations of vessels, aeration devices, and turning mechanisms. This method of composting requires regular level of management to make it a cost efficient method (William, 2000).

2.4.4 Bin composting: It is some-what similar to in-vessel composting. The compost production in this method done in the bin. It is done by natural aeration, turning of waste or compost in the bin at fixed intervals by tractor front-end loader. Bin composting isless convenient with medium quality production and requireless labour(William, 2000).

Table 3. Various methods of composting using different raw material

S.No	**Raw material**	**Method of composting**	**Influencing factor**	**References**
1	Sewage sludge	Static pile system	Organic carbon concentration, C:N ratio, nitrogen content	Bernal *et al.*, 1998
2	Amended wheat straw	Tunnel reactor composting system.	Degradation of biomolecules	Veeken*et al.*, 2001
3	Poultry waste and olive-mill waste	Turned windrows	Exposure to thermophilic phase, manure toxicity	Hachicha*et al.*, 2006
4	Mixed food waste	Turned windrow and passively aerated windrow	Compost Stability Interpretation. Efficacy of Food Waste Composting Methods	Matteson *et al.*, 2006

5	Rice straw	Static pile	Effective microbes	Jusoh*et al*., 2013
6	Municipal solid waste	In vessel method	bulking agent of different types and size for reducing N-losses	Rich *et al*., 2014

2.5 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 are: a) pH, b) temperature, c) moisture content, d) C: N ratio.

2.5.1 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)

2.5.2 Temperature

Heat generated by the microbes in the pile increase the temperature of the compost 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).

2.5.3Moisture 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).

2.5.4 C: N ratio

The carbon source present in biomass is the real energy booster for the micro-organisms. Moreover, a very small amount of carbon gets incorporated into microorganisms. Meanwhile, nitrogen is essential for growth of microbes. If there is deficiency of nitrogen, microbial population will remain lesser and available carbon decomposition rates will be lower. According to Golueke (1992),entire and rapidhumification of substrates by the microorganisms primarily depends on C: N ratiobetween 25 and 35.

Several authors have concluded that amalgamation of various parameters is usually required instead of using single parameter (Table 2). These include temperature, moisture content %, C: N ratio, pH etc. these are the parameters used to determine the degradation of raw material in the process of composting. There are some standardized range for composting is set which are shown in the following table (Dickson *et al*., 1991).

Rich *et al*., (2014) explained the concepts of composting in municipal solid waste (MSW) by studying temperature, pH, moisture content, aeration rate, C: N ratio, micro-organisms (Table. 4). pH range between 6.2 - 9 supported microbial activity. Pineapple leaves and chicken manure was degraded to obtain high quality organic fertilizer. Chang *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.

Table 4. Parameters of composting and their optimum range (Dickson *et al*., 1991)

Parameter	Standardized 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

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 moves towards maturity, there is a gradual change in C: N ratio as it decreases from 30:1 to 15:1 which is perfect for the finished compost. According to Chen *et al*., 2011 this occurs every time raw material or substrate is consumed by micro-organisms, 2/3 of the carbon is converted and given off as carbon dioxide. 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.

2.6 Compost – Habitat of effective microbes

2.6.1 Source: Micro-organisms which enhance the biodegradation process are found all around the environment. In order to maintain microbial population during the composting process various parameters such as change in pH, shift in temperature, and C: N ratio are regularly monitored (Waksman *et al*., 1940).

2.6.2 Micro-organism:The micro flora of the compost comprises various bacteria, yeast and fungi. The majority of microorganisms which are responsible for the compost production are aerobes. Inmoist environment, microbes takes place because as they livedin water films. The moisture content of 50-60% is considered optimal for the process (Madigan *et al*., 2017).

2.6.3 Bacteria: The most dominant entity of compost is bacteria. Actinomycetes and fungi are usually proliferate later on stages. The inevitable role played by bacteria basically lies in considerable degradation of cellulose based organic wastes (Table 5). The available substrate enable the proliferation of fast-growing microorganism which is bacteria. Initial degradation is dominant by the mesophilic bacteria. These bacteria release heat by the breakdown of huge amount of substrate which is easily degraded (Asha *et al.*, 2012).

2.6.3 Fungi: Fungi are responsible for degradation process of biomass. The hyphae (single cell into long filament) play significant role in degradation process of biomass. Fungi also play an important role in the breakdown of dead plant materials. Commonly highlighted studies are on fungi of class soradiomycetes, belonging to *Trichoderma sp*. namely *Trichoderma viride* and *Trichoderma reesei*(Table 6).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 (Kumar *et al.* 2008)

Table 5. Cellulose degrading bacteria

Bacteria	Optimum pH	Temperature Range (°C)	Reference
Acidothermuscellulolyticus	5	37-65	Mohagheghi*et al.*, 1986
Pseudomonas fluorescens	10	30-35	Bakare *et al.*, 2005
Bacillus pumilus EB3	6	30-70	Affrin*et al*.,2006
Sinorhizobium fredii	7	30-45	Chen *et al*., 2011
Cellulomonas spp.	7.5	40-60	Irfan *et al*., 2012
Bacillus coagulans Co4	7.5	35-75	Adeleke*et al.*,2012
Paenibacillus barcinonensis	7	30-65	Asha *et al.*, 2012
Bacillus subtilis AS3	9.2	>60	Deka*et a*l., 2013

Table 6. Cellulose degrading fungi

Fungus	**Raw material**	**Reference**
Phanerochaetechrysosporium	Wheat straw	Kuhar*et al* (2008)
Trichoderma reesei	Cellulosic biomass	Kumar *et al (*2008)
Phlesbiafascicularia	Wheat straw	Arora *et al* (2009)
Aspergillusawamori	Rice straw	Goyal*et al* (2011)
Aspergillus fumigates	Paddy straw	Viji*et al (*2014)
Rhizopusoryzae	Paddy straw	Viji*et al* (2014)

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 (Pace *et al.*, 1995).

2.7 Agricultural waste biomass

Compost provide a source of carbon and nitrogen for microorganisms in the soil, improve its texture, fertility, structure, reduce soil erosion and increase its water holding capacity (Adebayo *et al.*, 2011). Rice straw has not been used optimally as it is renewable carbon resource and serve as raw material for decomposition process thus improving of poor soil condition. It rich in carbon sources consisting of cellulose

(41.3%), hemicelluloses (20.4%) and lignin (12.1%). Composition of some lignocellulosic and organic waste by several reporters (Table. 7).

Table 7. Composition of some lignocellulosic and organic waste

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Rice husk	31	24	14	Raveendran *et al*.,1996
Wheat straw	35	29	21	Naik *et al.,* 2010
Sugarcane bagasse	35	24	22	Rezende *et al*., 2011
Rice straw	39	24	14	Chandra *et al.,* 2012
Corn stove	43	24	11	Zeng *et al*., 2014

Some agricultural waste biomass such as rice straw used for composting combination with other substrates were discussed in table. 8

Table 8. Agricultural waste biomass with other substrates used for composting

S.no.	Raw Material	Influencing Factors	Results	References
1	Rice straw	Sewage sludge	The optimum results was obtained at lower C:N value (29:1)	Iranzo*et al*., 2004
2	Rice straw with poultry Manure	Poultry manure and oilseed rape cake	After 90 days C:N ratio and GI(glycemic index) reach to 13.3–8.9 and 71.1–81.6%	Abdelhamid*et al.,* 2004
3	Cattle manure with rice straw	Influences of temperature	Thermophilic condition is more significant on enhancement of composting	Tang *et al*., 2007

4	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
5	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
6	Rice straw	Lignocellulolytic fungal consortium	A consortium of *A. niger* (F44) and *T. viride* (F26), gave a partial compatible interaction	Kausar *et al*., 2011
7	Rice straw	Effective microbes	Table of content of treated pile decreased to 36.3% from initial value of 47.6%	Jusoh*et al.*, 2013

Table 9. Various method of composting using various microorganisms for degradation of raw material

Raw material	Method of composting	Organism	Reference
Mixed organic waste-chlorinated and aeromatic hydrocarbons	Consortia with TCE degrade capacity – inocula culture SRP isolate	Microbial consortium	Phelps *et al*., 1991
Sewage waste or gray water	Sewage – inoculated – continuous flow bioreactor	Microbial consortium from sewage	Konopka *et al*., 1996
Cowdung and weeds	Vermicomposting	*Eudriluseugeniae*	Jeyabal *et al.*, 2001
Urban municipal solid waste	Soil sample – characterize (M.C%, pH,C,K,P)-isolate bacteria – screening-combine – inoculation	*Bacillus spp, Clostridium,Pseudomonas*	Sarkar *et al*., 2003
Cattle dung, agriculture residue	Enzyme mediated transformation (fats,NA,proteins), hydrolysis, acidogenesis, methane fermentation	*Bactericides ,Clostridia spp.,Streptococci spp.*	Yadvika *et al*., 2007
Municipal solid waste, Biomass, Fruit & vegetable waste, Manures	Anaerobic digestion – mixing – feedstock-co-digestion-pretreatments (alkali,thermal,ultrasonic,particle size, cell lysate, metals)-Monitoring (GC,spectro)-control systems	*Methanobacterium ,Methanococcus*	Ward *et al.*, 2008
Municipal solid waste	Fungi & bacteria – isolate from MSW waste – incubate Assay of pectinolytic & cellulolytic enzyme	Microbial consortium	Gautam *et al*., 2010

Sugarcane waste or by-product of sugar industry	Pre-decomposed inoculation(30 days) followed by vermicomposting	*Trichoderma viridae,Aspergillusniger, Pseudomonas straitum*	Kumar *et al*., 2010
Food waste, Agricultural waste, Organic fraction of municipal solid waste	Inoculation – SSAD reactor (solid contents,tempt,C/N ratio)	*Methanobacterium ,Methanococcus*	Li *et al*., 2011
Fruit waste,vegetable waste, leaves, newspaper,wheat straw, rice straw	Samples – inoculated – monitor for upto 6 months	*Bacillus subtilis,* *Pseudomonas*	Pan *et al*., 2012
Micro-algal biomass	Anaerobic digestion for methane generation,Dark fermentative,hydrogen production,ethanol fermentation	*Cynobacterium ,Arthrospira maxima, Ulva lactuca,Chlorella kessleri,Chlorella vulgaris,Chlamydomonas*	Lakaniemi *et al*., 2013
Biomass (lignocellulosic)	Isolation of hydrolic bacterium,determination of optimal culture & screening for cellulolytic activity of bacteria	Microbial consortium	Poszytek *et al*., 2016
Rural and urban waste	Pit – 1st – test consortium 2nd- commercial consortium 3rd-control Test perform after 7 days -tempt, pH,N,C,P,K,Moisture,C/N Ratio	*Bacillus spp, Aspergillusterreus, Streptomyces spp.*	Game *et al*., 2017

Chapter 3

Materials and Method

3.1 Sample collection and isolation of bacteria from rhizospheric soil

Soil sample from roots of rice straw (Fig. 4) were collected from Devigarh, Patiala. 1 g of sample was weighed and inoculated in 50 ml nutrient broth (peptone 5g/L, beef extract 3g/L, sodium chloride 5g/L) for the growth and further isolation of bacterial strains and was incubated at 37ºC for 24 hours under shaking conditions (120 rpm).

Figure 4. Soil sample from roots of rice straw

3.2 Morphological and biochemical characterization of bacterial isolates

The morphological and biochemical characterization of all bacterial strains was done by conventional biochemical and physiological tests (Cappuccino and Sherman, 1999).

3.2.1 Gram staining

The bacterial smear was made and fixed by slight heating.Slide was flooded with primary stain crystal violet for 1 min followed by Gram's iodine for 1 min, decolourisingagent for 30-40 s andthen counter stain, safranin, was added for 1 min and washed under tap water to remove excess stain. The slide was air dried and observed under microscope.

3.2.2 Catalase test

A drop of hydrogen peroxide (3%) was taken at the centre of slide. A drop of culture was taken aseptically in laminar air flow chamber onto the drop of hydrogen peroxide on the slide and observe the results.

Observations:

1. Bubbling or foaming occurs in the drop – catalase positive
2. Bubbling or foaming does not occurs in the drop – catalase negative

3.2.3 Nitrate reduction test

Nitrate broth medium (Appendix-I) was used to test the ability of bacteria to convert nitrates to nitrites as they can produce the enzyme nitrate reductase. The main component of nitrate broth medium is nitrate. The nitrate broth medium was prepared and pH was adjusted to 7.2. The broth is distributed into test tubes (approx.10ml) and broth tubes were autoclaved at 121°C at 15 psi for 15 min. the tubes were allowed to cool at room temperature. The test bacteria were inoculated aseptically in broth tubes and incubated at 37ºC for 24 to 48 hours in an incubator. After that, 0.5 ml each nitrate reagent solution A and nitrate reagent solution B were added into each test tube and shaken well and color changes was observed after 15 minutes.

Observations:

1. Red color produced: Nitrate reduction positive
2. Red color not produced: Nitrate reduction negative

3.2.4 Methyl red test

MR test was done to check the ability of bacteria to form acid like lactic acid,acetic acid or formic acid by utilizing any monosaccharide sugar. MR broth medium

(Appendix-I) ingredients were weighed and dissolved in 100 ml distilled water and pH was adjusted to 6.9. The broth was distributed into test tubes and autoclaved at 121 ºC at 15 psi pressure for 15 minutes. The test bacteria was inoculated aseptically in broth tubes under laminar and incubated at 37 ºC for 24 to 48 hours in an incubator. Alcoholic solution (Appendix-I) of methyl red was dropped into each test tubes.

Observations:

1. Red color produced: MR positive
2. Red color not produced: MR negative

3.2.5 Voges-Proskauer test

Voges-Proskauer test was done to test the ability of bacteria to convert glucose into acetyl-methylcarbinol (acetoin). The ingredients of VP broth media (Appendix-I) were weighed and dissolved in 100 ml distilled water and pH was adjusted to 6.9. The broth is distributed into test tubes and autoclaved at 121ºC at 15 psi pressure for 15 minutes. The test bacteria was inoculated aseptically in broth tubes under laminar and incubated at 37ºC for 72 hours in an incubator. The VP solution 1 (Appendix-I) was dropped into each test tube (0.2 ml). Further, VP solution 2 (Appendix-I) was dropped into each test tube (0.6 ml) and mixed gently and allowed to stand for 30 minutes to 2 hour.

Observations:

1. Rose Pink color produced: VP positive
2. Rose Pink color not produced: VP negative

3.2.6 Carbohydrate fermentation test

The test was done to test the ability of bacteria to ferment carbohydrates mainly sugars. The ingredients of carbohydrate broth media (Appendix-I) were weighed and dissolved in 100 ml distilled water and pH was adjusted to 6.8. The broth is distributed into test tubes. Durham tubes was put in inverted position into test tubes. The test tubes were autoclaved at 121 °C at 15 psi pressure for 15 minutes. The test bacteria was inoculated aseptically in broth tubes under laminar and incubated at 37 °C for 24 hours in an incubator.

Observations:

1. If color of broth changes to yellow and gas accumulates in Durham tube then bacteria is fermentative for carbohydrate and aerogenic.
2. If color of broth changes to yellow but no gas accumulates in Durham tube then bacteria is fermentative for carbohydrate and an-aerogenic.
3. If color of broth does not changes, then bacteria is Non-Fermentative for carbohydrate.

3.2.7 Starch hydrolysis test

This test was done to check the ability of bacteria to hydrolyze starch by producing enzyme 'α- amylase'. The components of starch agar medium (Appendix-I) were weighed and dissolved in 100 ml distilled water and pH was adjusted to 7.2. The flask containing media was autoclaved at 121 ^{o}C at 15 psi pressure for 15 minutes. In warm molten condition, starch agar was poured aseptically into petri dishes and allowed to cool at 37 ºC for about 1 hour. The inoculation of the test bacteria was done aseptically. The inoculated plates were incubated at 37 ºC for 24 to 48 hours till the colonies of bacteria were become visible. The plates were flooded with lugol's iodine (Appendix-I) solution.

Observations:

1. Transparent clear zones formed around bacterial colony : Positive
2. No transparent clear zones around bacterial colony : Negative

3.3 Screening of cellulose degrading bacteria

All the isolates was inoculated on the petri plates containing nutrient agar supplemented with 0.5% (w/v) carboxymethyl cellulose (CMC) and incubated at 37ºC for 24 h. The culture plates were stained with 0.1% (w/v) congo red for 15 min followed by destainingwith 1M NaCl for 15 min (Teather and Wood, 1982). Clear zone around the colonies confirms positive test.

3.4 Micro-organisms

Bacteria

Bacillus subtilis (NA 15)

Paenibacillus polymyxa (MTCC 3088)

Yeast

Saccharomyces cerevisiae (NCIM 3215)

Fungi

Trichoderma reesei (MTCC 164)

Bacterial inoculum

The strains of *Bacillus subtilis*, *Paenibacillus polymyxa* were grown on nutrient broth and later on used for the biodegradation of biomass. 1% inoculum was added and incubated at 37°C for 24 h at 120 rpm.

Yeast inoculum

The strain of *Saccharomyces cerevisiae* was grown in YEPD. YEPD was inoculated with 1% inoculum and kept at 28 °C for 48 h at 120 rpm.

Fungal inoculum

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

3.5 Collection of rice straw

Rice straw was collected from Devigarh, Patiala. This biomass consisted majorly straw which was left after harvesting of paddy. This straw was collected, dried and shredded and 1kg of the same was used.

3.6 Experimental set up

3.6.1 Processing and pulverization of biomass

Dried sample was shredded into small size and stored for further study.

Figure 5. a) Dried sample of rice straw and b) shredded rice straw

Shredded rice straw was taken in the 1000 litre plastic beakers. The total rice straw collected was 1 Kg (approximately). The rice straw was treated with different treatments given below:-

a) Control (No microorganisms)

b) Consortium (*Bacillus subtilis*+ *Paenibacillus polymyxa*+ *Saccharomyces cerevisiae*+ *Trichoderma reesei*)

c) Consortium + Fertilizer (*Bacillus subtilis*+ *Paenibacillus polymyxa*+ *Saccharomyces cerevisiae*+ *Trichoderma reesei*+ di-ammonium phosphate)

d) Fertilizer (di-ammonium phosphate)

3.6.2 Inoculation of microbial consortium

Jaggery based media was used to collectively grow cultures of bacteria, fungi and yeast which was made uptovolume of 500 ml and was maintained at 28°C room temperature on a rotary shaker at 120 rpm for 3 days. After 3 days this consortia namely, *Bacillus subtilis* (NA 15), *Paenibacillus polymyxa* (MTCC 3088) *Saccharomyces cerevisiae* (NCIM 3215) and *Trichoderma reesei* (MTCC 164) were added to the beakers containing rice straw and biodegradation process was checked after every 7 days followed by turning of the waste in the beakers at regular intervals of 3 days.

3.7 Measurement of pH(Lewis, 1984)

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 some time using a glass rod.

d) Allowed the suspension to settle down.

e) Meanwhile pH electrode was washed with distilled water and wiped with tissue paper carefully.

f)pH was measured by immersing the electrode in the supernatant of the suspension.

g) The stabilized readings shown by the pH meter were recorded.

3.8 Measurement of temperature

Temperature was recorded every day using digital thermometer.

3.9 Determination of moisture content (Prihar, 1968)

a) 1g 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.

c) After cooling the dry weight was noted.

d) The percentage difference of weight was expressed as the moisture content in the sample.

MC (%) = (Wt of container & sample - Wt of container & sample after drying)×100

(Wtof container and sample after drying - Wt of empty container)

3.10 Estimation of Organic carbon

Percent organic carbon was measured bythe method given 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, without sample.

Calculation: **%Oxidisable organic carbon = 10 (T-B) × 0.003 × 100/B × g of sample**

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

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

3.11 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.

$$\text{Total Nitrogen \%} = \frac{(T - B) \times 0.0014 \times 100}{\text{Weight of sample}}$$

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

3.12 Determination of reducing sugar

Reducing sugar in rice straw 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.

3.13 Gravimetric analysis

Weight of all the samples were checked after every 7 days. Change in weight was observed and recorded.

3.14 Estimation of cellulose

Estimation of cellulose in rice straw were estimated usinganthrone assaymethod as**Hedge and Hofreiter, 1962**

a) Add 3 mL acetic/nitric reagent (150 mL of 80% acetic acid and 15 mL of concentrated acetic acid) to a 1 g of the sample and the mixture was kept in a water bath at 100 °C for 30 min.

b) Cool and then centrifuge the contents at 8000 rpm for 15-20 min, discard the supernatant.

c) The residue was washed twice with distilled water.

d) Add 10 mL of 67% $H_2SO_4$and allow it to stand for 1 h.

e) Dilute 1 mL of the above solution to 100 mL, to 1 mL of this diluted solution, add 10 mL of anthrone reagent (dissolve 200 mg anthrone in 100 mL concentrated sulphuric acid) and mix well.

f) Heat the tubes in a boiling water bath for 10 min. Cool and measure the color at 630 nm.

g) Set a blank with anthrone reagent and distilled water.

h) Take 100 mg cellulose in a test tube and proceed from step no. d for standard. Instead of just taking 1 mL of the diluted solution (step e) take a series of volumes (say 0.4 to 2 mL corresponding to 40-200 micro gram of cellulose) and develop the color.

Chapter- 4

Results and discussions

4.1 Isolation and screening of bacteria from rhizospheric soil

Soil sample from roots of rice straw was serially diluted and plated on NA plates and incubated at 37ºC for 24 hours. After 24 hours the bacterial colonies were observed (Fig.6).

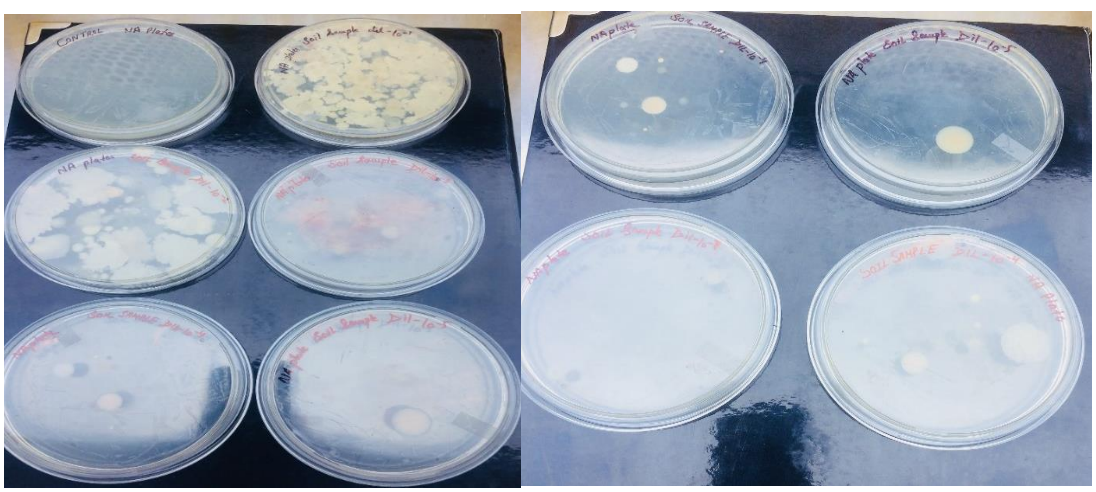

Figure 6. Bacterial colonies isolated from rhizospheric soil

4.2 Morphological and biochemical characterization of bacterial isolates

Out of 19 isolates from the rhizospheric soil of rice straw, seven bacterial isolates were found to be Gram positive, rest were gram negative (Table.10). Only four were found to be positive for methyl red test i.e this bacterial isolates utilized glucose and converted it to stable acid like lactic acid, acetic acid through mixed acid pathway. All bacterial isolates were found to be non-fermentative i.e they did not have the ability to ferment sugars. For catalase test, all were found to be catalase negative i.e they did not have ability to degrade hydrogen peroxide and convert it into water with the evolution of oxygen gas with the help of enzyme 'catalase'. For starch hydrolysis test, 17 bacterial isolates were found to be starch hydrolysis positive i.e they have ability to hydrolyse starch produce by enzyme 'α- amylase'.For VP (Voges-Proskauer) test, only two were found to be VP positive as they utilized glucose and convert it into neutral end product, acetoin, via the 'butylene glycol pathway'. 10 isolates have 'nitrate reductase' enzyme which reduced nitrate to nitrite (Table. 10).

4.3 Screening of cellulose degrading bacteria

Bacteria grown in minimal media enriched with (0.5%, w/v) carboxymethyl cellulose (CMC) were isolated and further screened from the rhizospheric soil of rice straw for production of cellulase on nutrient agar plate supplementary with (0.5%, w/v) CMC. After 24 h of incubation at 37ºC, out of 19 bacterial isolates, nine cellulose degrading bacteria were screened by congo red staining on the basis of clear zone of hydrolysis around colonies.

4.4 Collection of organic wastes and its recycling

Rice straw was collected from Devigarh, Patiala. The biomass was collected, dried, shredded and treated in plastic beakers (Fig.7). In triplicates 12 beakers were divided into different treatments, control treated with tap water, consortium treated with specially designed microbial consortium containing *Bacillus subtilis* (NA 15), *Paenibacillus polymyxa*, *Saccharomyces cerevisiae* and *Trichoderma reesei*, consortium + fertilizer were treated with consortium and fertilizer i.e DAP (Diammonium phosphate) both and fertilizer were treated with fertilizer i.e DAP

(Diammonium phosphate) only. 50 % moisture content was maintained in beakers and kept under dark conditions. The biomass was mixed thoroughly after every 3 days with equivalent aeration.

Table 10. Biochemical characterization of bacterial isolates

Test / sample	**Carbohydrate Fermentation**	**Catalase**	**Starch hydrolysis**	**Gram staining**	**Voges - proskauer**	**Nitrate reductase**	**Methyl red**
Colony 1	Non – fermentative	—	+	—	—	+	—
2	Non – fermentative	—	+	+	—	+	—
3	Non – fermentative	—	+	+	—	—	—
4	Non – fermentative	—	—	+	—	—	+
5	Non – fermentative	—	+	+	—	+	—
6	Non – fermentative	—	+	—	—	+	—
7	Non – fermentative	—	+	—	—	+	—
8	Non – fermentative	—	+	—	—	+	—
9	Non – fermentative	—	+	+	—	+	—
10	Non – fermentative	—	+	—	+	—	—
11	Non – fermentative	—	+	—	+	—	+
12	Non – fermentative	—	—	—	—	—	—
13	Non – fermentative	—	+	+	—	—	—
14	Non – fermentative	—	+	—	—	—	—

15	Non - fermentative	—	+	—	—	+	+
16	Non - fermentative	—	+	+	—	+	—
17	Non - fermentative	—	+	—	—	+	—
18	Non - fermentative	—	+	—	—	—	+
19	Non - fermentative	—	+	—	—	—	—

The effect of microbial consortium, fertilizers was justified by studying various parameters such as pH, moisture content was monitored during 60 days and the degradation pattern was observed after every 7 days.

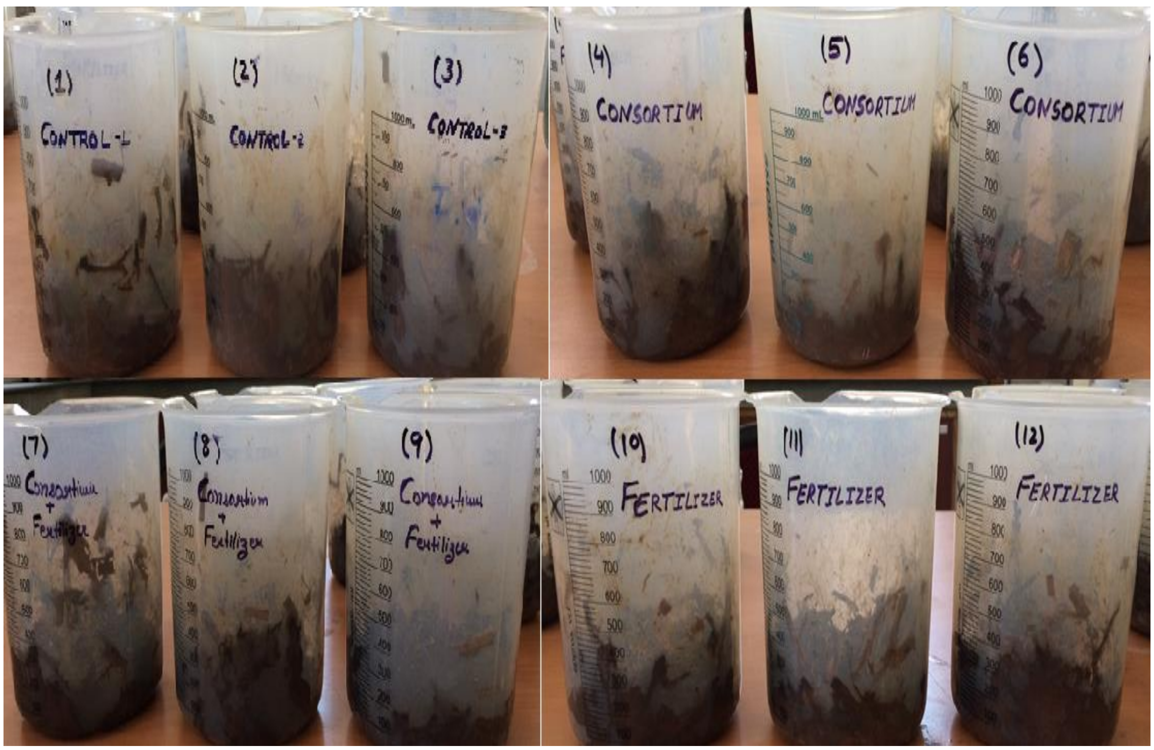

Figure 7. Experimental set up for composting of rice straw

4.5 pH

pH plays an important role in microbial degradation. pH of the rice straw was recorded to be 6.75 in the initial stages, after 42 days the pH reach around 8.0 and at last of composting on 63th day the pH was recorded to be 7.35. Also as observed, the pH of the mature compost was typically 7.0 ± 0.2(Pan *et al.,* 2012). According to Frederickson *et al.*, 1997, the pH of fresh green leaves waste was 7.6.

4.6 Temperature

During the composting of rice straw, the fluctuation was seen in the temperature pattern.Temperature during dark incubation varied from 26 to 32ºC, which showed increase during the day time as compared to morning and evening. This temperature range is ideal for mesophilic microbial growth.Temperature is a vital parameter in determining the success of a composting processHubbe*et al.* (2010).

Table 11.Temperature in morning, afternoon and evening during composting

Days	Morning (°C)	Afternoon (°C)	Evening (°C)
1	26.8	27.8	28.2
2	26.0	27.0	26.4
3	26.1	27.3	27.6
4	29.1	29.9	29.8
5	26.0	26.8	26.8
6	26.8	27.0	27.3
7	26.0	27.0	26.8
8	27.0	27.8	27.3
9	30.0	30.2	26.4
10	30.3	33.6	29.0
11	30.0	32.2	29.9
12	30.3	33.9	33.0
13	30.0	32.6	30.0
14	30.3	33.3	31.2
15	29.1	30.1	29.2
16	26.1	29.6	29.0
17	29.1	30.8	29.5
18	26.8	33.6	31.0

19	27.0	30.2	29.4
20	30.3	33.6	31.2
21	30.0	33.4	32.1
22	29.1	32.4	31.2
23	30.5	32.0	29.0
24	32.1	32.4	29.0
25	32.1	33.5	29.9
26	32.9	33.2	29.9
27	30.3	33.9	33.0
28	30.0	32.6	30.0
29	30.3	33.3	31.2
30	29.1	30.1	29.2
31	26.1	29.6	29.0
32	29.1	30.8	29.5
33	26.8	33.6	31.0
34	27.0	30.2	29.4
35	30.3	33.6	31.2
36	27.0	30.2	29.4
37	30.3	33.6	31.2
38	30.0	33.4	32.1
39	29.1	32.4	31.2
40	30.5	32.0	29.9
8	32.1	32.4	29.0
42	32.1	33.5	29.9
43	32.9	33.2	29.9
44	30.5	32.0	29.0
45	32.1	32.4	29.0
46	32.1	33.5	29.9
47	32.9	33.2	29.9
48	30.5	32.0	29.0
49	32.1	32.4	29.0
50	32.1	33.5	29.9
51	32.9	33.2	30.0
52	32.1	33.5	29.9
53	32.9	33.2	30.0
54	30.5	32.0	29.0
55	32.1	32.4	31.0
56	32.1	33.5	31.9
57	32.9	33.2	30.9
58	32.1	33.5	30.9

59	32.9	33.2	30.0
60	30.5	32.0	30.1

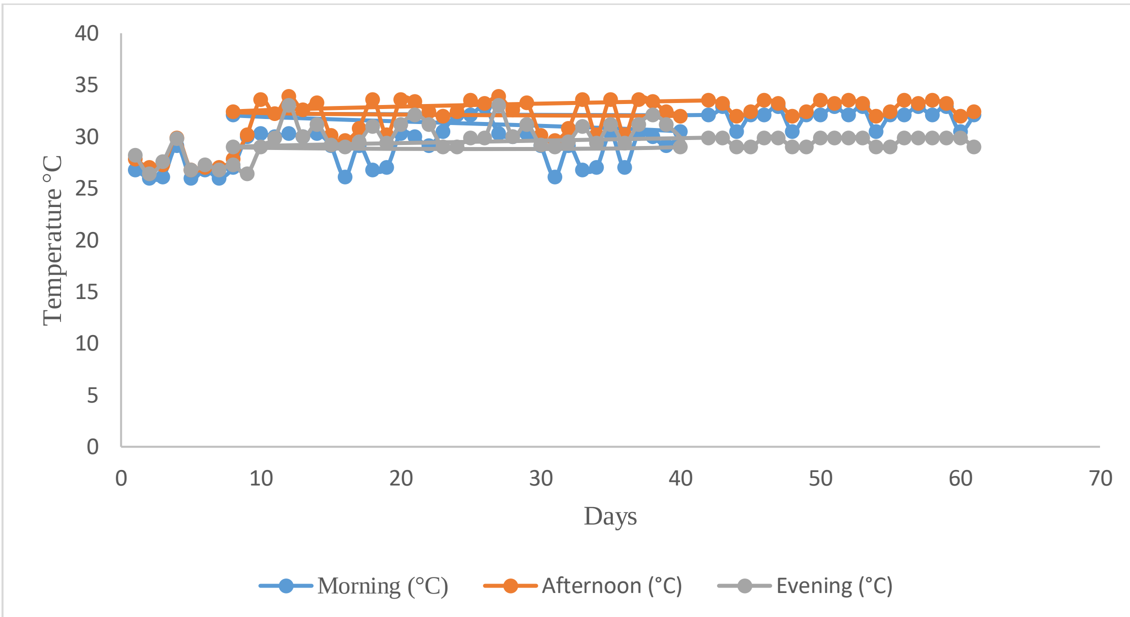

Figure 8. Temperature in morning, afternoon and evening during composting

4.7 Moisture content

Moisture is significant for the metabolic activities of the microbial community in compost. For the activity of microorganisms the balanced moisture is required which positively effect the microorganism growth which can breakdown organic matter into humus (McLaurin and Wade, 2012). Moisture content varied from 40-60% during the composting in all different treatments, which was maintained equally in all treatments. There was decline in moisture content from 58% to almost 41%. Environmental factors also effect the changes in microbial activities and the composting process, mainly moisture content, since water availability is directly related to oxygen supply (Tiquia*et al.*, 1996). Moisture content decreased in later stages of composting. Moisture content for rice straw waste was recorded as 57.6% in case of control and it dropped to 41.5%, in case of consortium it was recorded as 59% and it dropped to 41.5%, in case of consortium + fertilizer it was recorded as 58.6% and it dropped to 41.4% and in case of fertilizer it was recorded as 58.6% and it was dropped to 41% from 7th day to 60th day of observation (Table 12). 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). Moisture content generally decreases as composting proceeds; hence the need to add additional water to the compost (Pace et al., 1995).

Table 12. Moisture content (%) at different intervals during composting of rice straw

Days	Rice straw Control	Rice straw Consortium	Rice straw Consortium + fertilizer	Rice straw Fertilizer
7	57.6 ± 1.2	59 ± 1.7	58.6 ± 2.3	58.6 ± 0.8
14	60.2 ± 5.2	60.6 ± 2.3	55.6 ± 3.3	59.3 ± 1.7
21	57.6 ± 1.4	57.6 ± 1.2	56.7 ± 2.2	58.1 ± 1.2
28	51 ± 0.6	58 ± 3.2	58.0 ± 2.0	56.3 ± 1.3
35	51 ± 0.3	51 ± 0.6	50.3 ± 0.5	51.7 ± 1.6
42	50.2 ± 1.6	48.3 ± 0.6	46.5 ± 1.2	48.7 ± 2.4
49	49 ± 0.5	43.3 ± 0.3	44.6 ± 0.3	45 ± 1.0
56	43.4 ± 1.5	46.6 ± 1.1	42.3 ± 2.03	42.1 ± 1.0
63	41.5 ± 1.3	41.5 ± 0.9	41.4 ± 1.0	41 ± 2.08

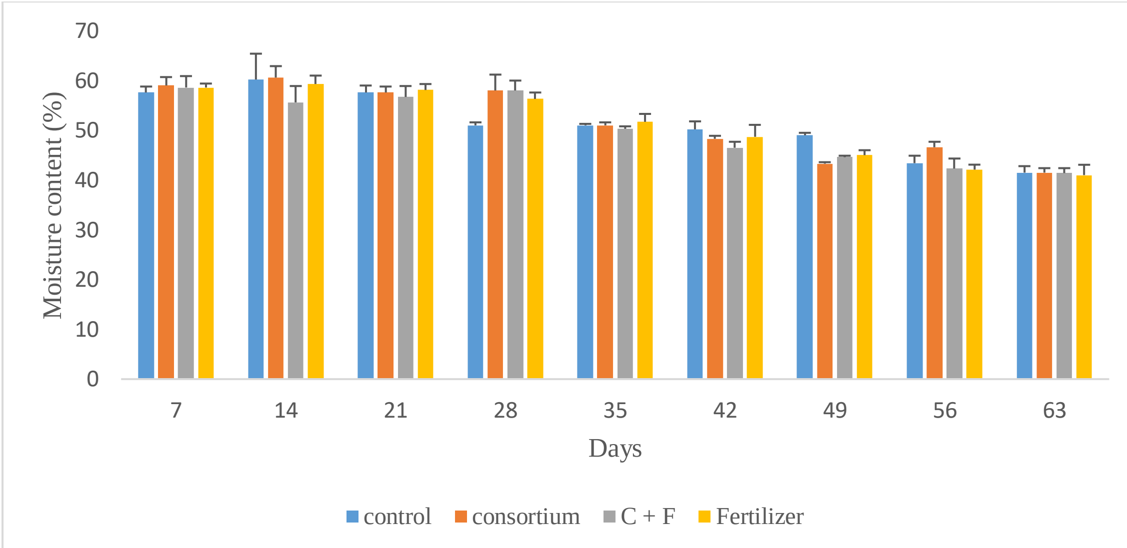

Figure 9. Moisture content (%)at different intervalsduring composting of rice straw

4.8 Organic carbon content

The organic carbon content reduced as the decomposition progressed. Organic carbon content for rice straw was recorded as 69.1% in case of control and it dropped to 40%, In case of consortium it was recorded as 69.1% and it dropped to 30.2%, In case of consortium + fertilizer it was recorded as 69.1% and it dropped to 25.1% and in case of fertilizer only it was recorded as 69.1% and it dropped to 29.1% (Table 13) from 0 day to 60th day of observation. The carbon content was observed to be 25.1 for green leaves waste (Frederickson *et al.*, 1997). According to Chatterjee *et al.*, 2017 the carbon content for different treatments of substrate was observed to be between 25-35%.

Table 13. Organic carbon content (%)at different intervals during composting of rice straw

Days	Rice straw Control	Rice straw Consortium	Rice straw Consortium + fertilizer	Rice straw Fertilizer
0	69.1 ± 0.1	69.1 ± 0.1	69.1 ± 0.1	69.1 ± 0.1

7	67.06 ± 6.0	66.09 ± 6.0	65.09 ± 1.0	66.1 ± 3.0
14	65.6 ± 4.0	63.5 ± 1.0	61.1 ± 1.0	62.8 ± 0.5
21	62.66 ± 1.0	60.7 ± 1.0	59.1 ± 6.0	60.2 ± 1.0
28	58.4 ± 1.0	55.56 ± 3.0	52.4 ± 3.0	56.2 ± 8.0
35	52.447 ± 1.0	50.2 ± 1.0	47.6 ± 6.0	51.1 ± 5.0
42	50.68 ± 4.0	46.7 ± 8.0	41.9 ± 4.0	45.4 ± 7.0
49	48.667 ± 1.0	40.66 ± 1.0	36.88 ± 1.0	41.2 ± 4.0
56	44.367 ± 1.0	35.77 ± 1.0	30.22 ± 4.0	36.1 ± 5.0
63	40.1 ± 1.0	30.2 ± 1.0	25.1 ± 1.0	29.1 ± 1.0

A good decreasing trend in organic carbon was observed over 60 days thus faster rate of degradation was observed in case of (Figure 10). 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.

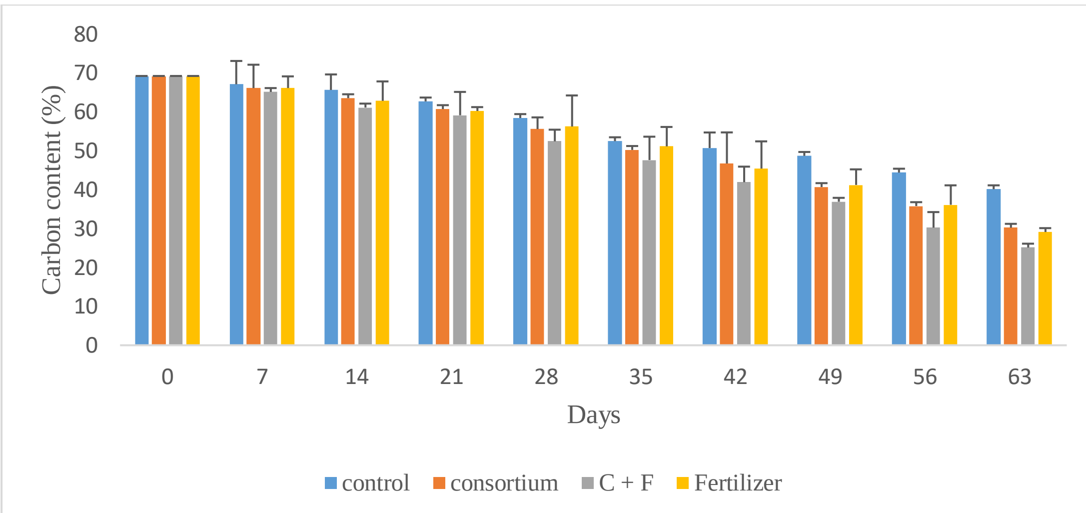

Figure 10. Carbon content (%)at different intervals during composting of rice straw

4.9 Total Nitrogen content

The Nitrogen content increased as the degradation proceeded (Figure 11). The nitrogen content showed increasing trend from 0.063% on 0 day to 2.0% on 60th day when treatment with microbial consortia was given to the rice straw, When consortia + fertilizer treatment was given it the increase was observed from 0.063% to 2.3%, In case of fertilizer only the increase in nitrogen was observed from 0.063% to 2.1%

while in case of control it was increased from 0.063% to 1.42% (Table 16). In composting, total N in the literature may vary from 0.8% to 3% (Iglesias-Jimenez & Alvarez, 1993; Wolkowski, 2003; Zmora-Nahum *et al*., 2007). Accordingly Chatterjee *et al.*, 2017 the total nitrogen content was observed to be around 0.2% to 2% for different substrates. According to Game *et al*., 2017 the total nitrogen content of rural and urban waste is observed to be nearby 1%.

Table 14. Total Nitrogen (%)at different intervals during composting of rice straw

Days	Rice straw Control	Rice straw Consortium	Rice straw Consortium + fertilizer	Rice straw Fertilizer
0	0.063 ± 0.1	0.063 ± 0.1	0.063 ± 0.1	0.063 ± 0.1
7	0.7 ± 0.1	0.78 ± 0.1	0.84 ± 0.07	0.78 ± 0.07
14	0.78 ± 0.1	0.84 ± 0.07	0.99 ± 0.08	0.84 ± 0.07
21	0.84 ± 0.002	0.99 ± 0.04	1.12 ± 0.04	0.99 ± 0.1
28	0.99 ± 0.002	1.12 ± 0.07	1.36 ± 0.04	1.12 ± 0.1
35	1.06 ± 0.002	1.24 ± 0.14	1.56 ± 0.07	1.36 ± 0.07
42	1.11 ± 0.002	1.36 ± 0.1	1.62 ± 0.1	1.56 ± 0.03
49	1.15 ± 0.002	1.56 ± 0.15	2.08 ± 0.04	1.62 ± 0.12
56	1.21 ± 0.002	1.62 ± 0.13	2.1 ± 0.15	1.8 ± 0.05
63	1.42 ± 0.08	2.08 ± 0.02	2.3 ± 0.06	2.1 ± 0.11

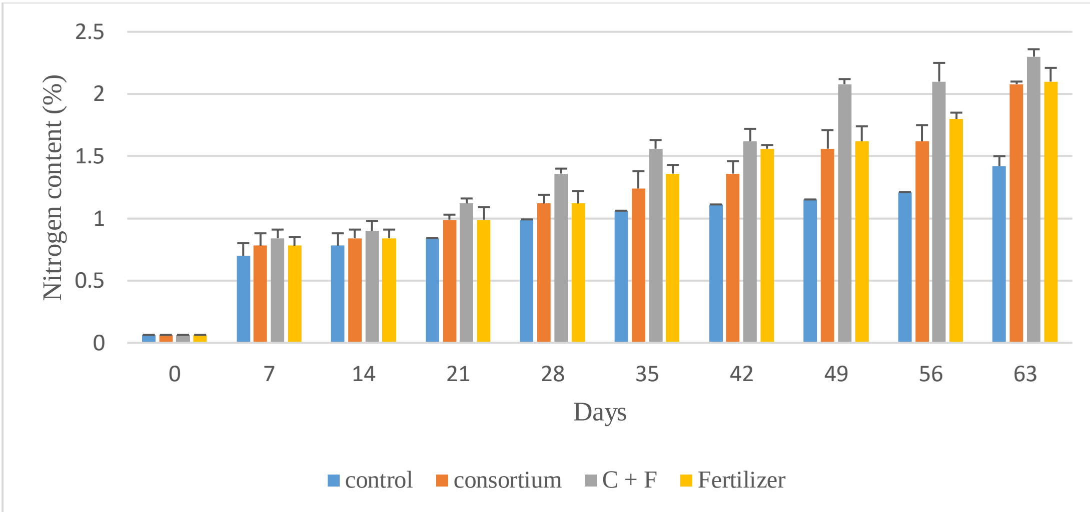

Figure 11. Total Nitrogen (%) of composting of rice straw at different intervals

4.10 Reducing sugar

Estimation of reducing sugar showed increasing trend from 0.91 mg/ml on 7th day to 2.0 mg/ml on 60th day when treatment with microbial consortia was given to the rice straw; When consortia + fertilizer treatment was given it the increase was observed from 1.06 mg/ml to 2.3 mg/ml, In case of only fertilizer the increase in reducing sugar was observed from 0.91 mg/ml to 1.9 mg/ml while in case of control the reducing sugar increased from 0.03 mg/ml to 1.11 mg/ml (Table 15). Increase in reducing sugar was also reported by Oberoi*et al*., 2012.

Table 15. Reducing sugar at different intervalsduring composting of rice straw

Days	Rice straw control	Rice straw consortium	Rice straw Consortium+fertilizer	Rice straw fertilizer
7	0.3 ± 0.005	0.91 ± 0.027	1.06 ± 0.011	0.91 ± 0.021
14	0.37 ± 0.010	0.95 ± 0.003	1.05 ± 0.005	1.02 ± 0.026
21	0.5 ± 0.012	1.06 ± 0.007	1.17 ± 0.021	1.09 ± 0.006
28	0.55 ± 0.002	1.1 ± 0.008	1.41 ± 0.032	1.13 ± 0.002
35	0.065 ± 0.001	1.18 ± 0.023	1.59 ± 0.002	1.43 ± 0.123
42	0.77 ±0 .019	1.41 ± 0.001	1.73 ± 0.057	1.42 ± 0.038
49	0.92 ± 0.008	1.64 ± 0.045	1.92 ± 0.006	1.58 ± 0.11
56	1.0 ± 0.012	1.68 ± 0.026	2 ± 0.008	1.7 ± 0.160

63	1.11 ± 0.010	2 ± 0.010	2.3 ± 0.019	1.9 ± 0.361

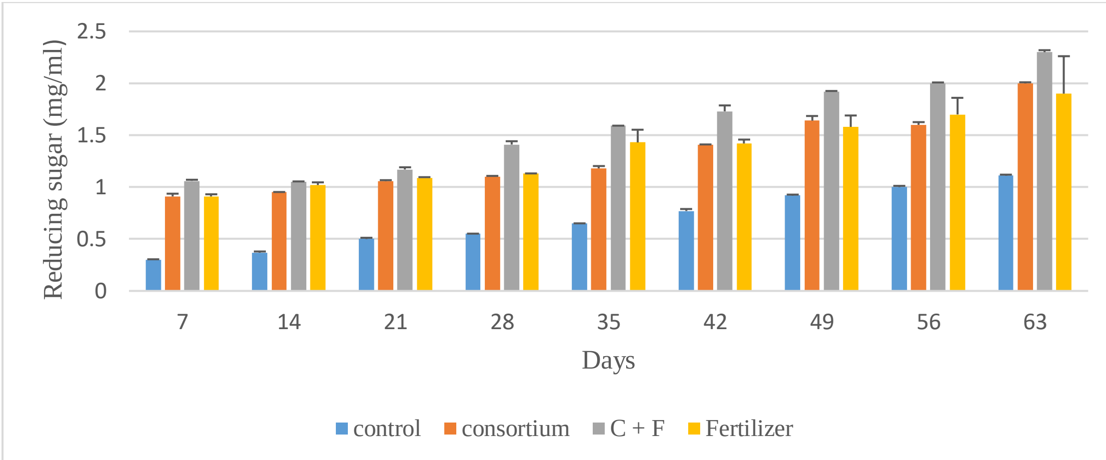

Figure 12. Reducing sugar at different intervals during composting of rice straw

4.11 Gravimetric analysis

Gravimetric analysiswas determined through the measurement of mass. Declining trend was observed from 548 g to 390 g in case of control, when treatment with microbial consortia was given to rice straw it was reduced from 520 g to 320 g, In case of consortia + fertilizer it was reduced from 490 g to 254 g and in case of fertilizer only it was reduced from 493g to 290g (Table 16) from 7th day to 60th day of observation. Maximum reduction in mass was observed in consortium + fertilizer treatment which was 254 g. The microorganisms degraded more than 40% of rice straw within 60 days (Haruta*et al.*, 2002).

Table 16. Gravimetric analysis at different intervals during composting of rice straw

Days	Rice straw Control	Rice straw Consortium	Rice straw Consortium + fertilizer	Rice straw Fertilizer
7	548±4.4	520±3.3	490±5.7	493±8.8
14	540±5.7	500±5.7	486±8.8	486±3.3
21	513±8.8	480±5.7	450±5.7	460±3.3

28	496±3.3	460±3.3	400±5.7	410±5.7
35	480±5.7	446±6.7	380±3.3	390±3.3
42	470±14.5	420±8.8	350±5.7	360±5.7
49	450±16.0	400±16.6	283.4±16.6	316.7±16.6
56	400±16.6	380±16.6	260±10	300±8.8
63	390±15.2	320±16.6	254±26.03	290±3.3

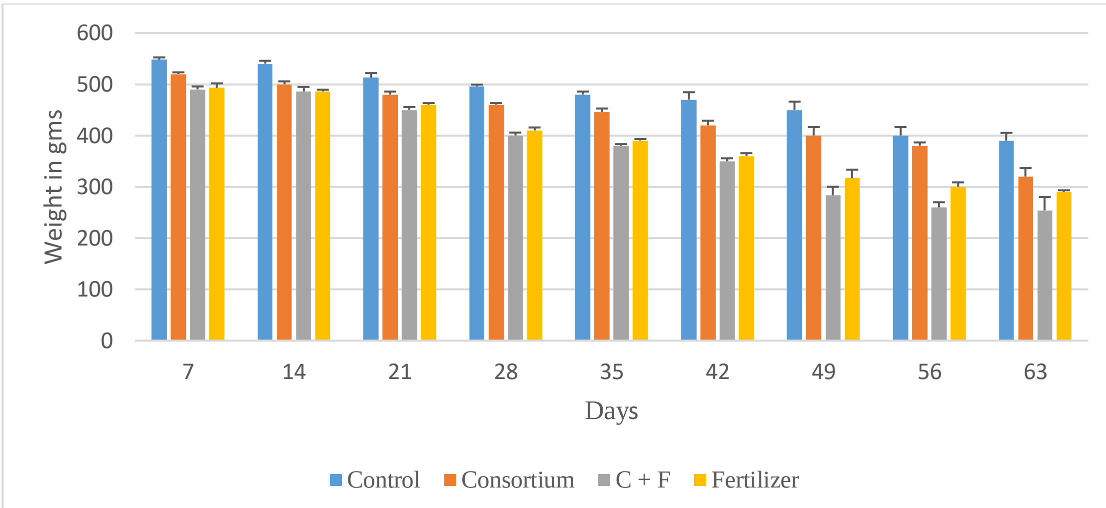

Figure 13. Gravimetric analysis at different intervals during composting of rice straw

4.12 Cellulose estimation

Estimation of cellulose showed decreasing trend from 16.66 mg/ml on 14th day to 16.25 mg/ml on 60th day when treatment with microbial consortia was given to the rice straw; When consortia + fertilizer treatment was given it the decrease was observed from 16.63 mg/ml to 16.0 mg/ml, In case of only fertilizer the decrease in cellulose was observed from 16.64 mg/ml to 16.26 mg/ml while in case of control the reducing sugar increased from 16.66 mg/ml to 16.35 mg/ml (Table 17). Consortium + fertilizer treatment shows maximum decline in shortest time. In case of control, maximum reduction was 16.35 mg/ml on 63rd day while in case of consortium + fertilizer, it was observed on 35th day i.e in half the time.

Degradation of cellulose indicates maturity in composting (Linda *et al*., 2017). According to Frederickson *et al.*, 1997, the cellulose content of fresh green waste was 18.0%. The cellulose content in rice straw, wheat straw, corn, husks was observed to be around 35-45% (Chandra *et al.*, 2012).

Table 17. Cellulose estimation at different intervals during composting of rice straw

Days	Rice straw Control	Rice straw Consortium	Rice straw Consortium + fertilizer	Rice straw Fertilizer
14	16.66 ±0.002	16.66 ±0.008	16.63 ±0.004	16.64 ±0.004
21	16.59 ±0.005	16.60 ±0.007	16.55 ±0.008	16.63 ±0.005
28	16.55 ±0.004	16.50 ±0.004	16.50 ±0.003	16.50 ±0.003
35	16.50 ±0.003	16.46 ±0.002	**16.40 ± 0.0008**	16.47 ±0.002
42	16.48 ±0.003	16.40 ±0.004	16.32 ±0.008	16.40 ±0.0008
49	16.44 ±0.004	16.32 ±0.003	16.28 ±0.004	16.34 ±0.008
56	**16.40 ± 0.002**	16.29 ±0.001	16.20 ±0.0006	16.29 ±0.005
63	16.35 ±0.004	16.25 ±0.002	16.0 ±0.003	16.26 ±0.007

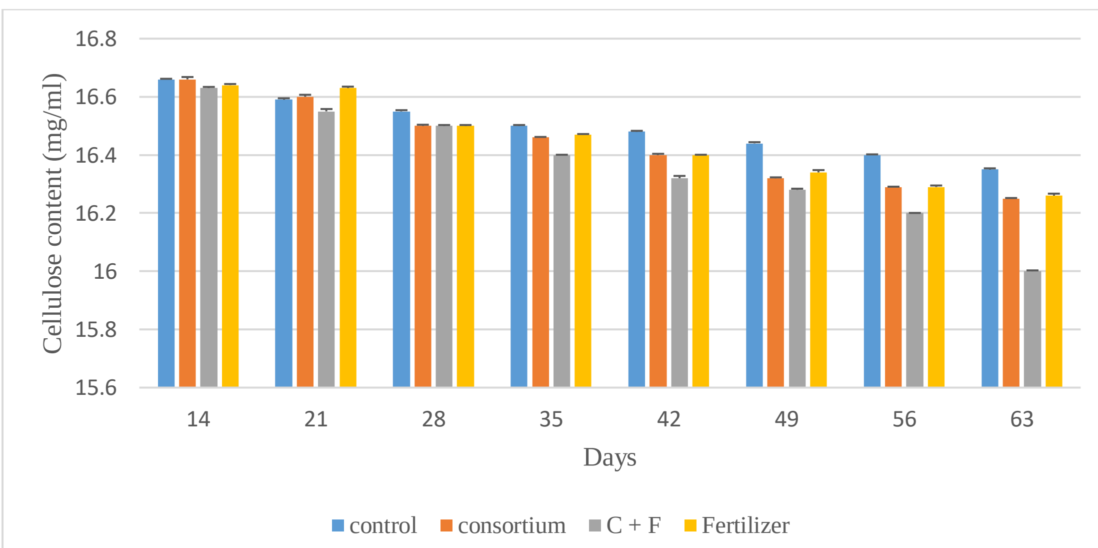

Figure 14. Cellulose estimation at different intervals during compostingof rice straw

4.13 Carbon: Nitrogen ratio (C: N ratio)

C: N ratio is most important parameter which can 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 rice straw treated with consortia on day 0 was noted to be which showed the decreasing trend and reached till the end of 60 days. A low C: N ratio in ecosystem increased the microorganism growth (Begone*et al.*, 1997) and therefore the O_2 uptake is increased. The C:N ratio for each substrate reduced to 25-30:1 within 120 days and after that it remain constant (Pan *et al.*, 2012). According to Frederickson *et al.*, 1997, the C: N ratio of fresh green waste was 25:1. According to Chatterjee *et al*., 2017 the C: N ratio for different treatments of substrate was observed to be in between 14 to 38. The C: N ratio of the waste was around 14(Game *et al.*, 2017).

Declining trend was observed from 1096.8 to 28.23 in case of control, When treatment with microbial consortia was given to rice straw it reduced from 1096.8 to 14.51, In case of consortium + fertilizer it reduced from 1096.8 to 10.91 and in fertilizer only it reduced from 1096.8 to 13.85(Table18) from 0 day to 60th day of observation. Maximum decline was observed in case of consortium + fertilizer treatment.

Table 18. C: N ratio at different intervals during composting of rice straw

Days	Rice straw Control	Rice straw Consortium	Rice straw Consortium + fertilizer	Rice straw Fertilizer
0	1096.8 ± 111	1096.8 ± 111	1096.8 ± 111	1096.8 ± 111
7	95.8 ± 6.0	84.73 ± 6.0	77.48 ± 1.0	84.74 ± 3.0
14	84.10 ± 0.1	75.59 ± 0.1	67.88 ± 1.0	74.76 ± 5.0
21	74.59 ± 0.1	61.31 ± 0.1	52.76 ± 6.0	60.80 ± 1.0
28	58.98 ± 0.1	49.60 ± 3.0	38.52 ± 3.0	50.17 ± 0.8
35	49.47 ± 0.1	40.48 ± 0.1	30.51 ± 0.6	37.57 ± 0.5
42	45.65 ± 0.1	34.33 ± 0.8	25.86 ± 4.0	29.10 ± 0.7
49	42.31 ± 0.1	26.06 ± 0.1	17.73 ± 1.0	25.43 ± 0.4
56	36.6 ± 0.2	22.08 ± 1.0	14.39 ± 4.0	20.05 ± 0.5

63	28.23 ± 0.1	14.51 ± 1.0	10.91 ± 1.0	13.85 ± 1.0

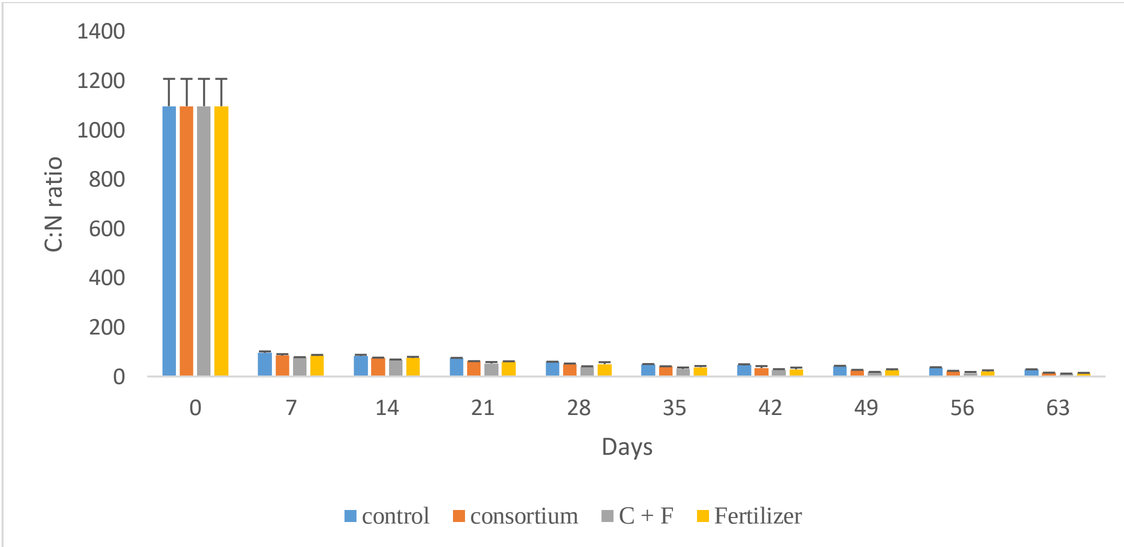

Figure 15. C: N ratio at different interval duringcomposting of rice straw

Conclusions

1. Out of 19 isolates from the rhizospheric soil of rice straw, seven bacterial isolates were found to be Gram positive, four were positive for methyl red test, 17 were starch hydrolysis positive, only two were VP positive. Nine isolates were cellulose degrading bacteria.
2. Treatment of rice straw with a consortium of fast degrading microbes was done. Various parameters such as pH, temperature, Organic carbon were monitored at different intervals for 60 days. Organic carbon content for rice straw was recorded as 69.1% in case of control and it dropped to 40%, In case of consortium it was recorded as 69.1% and it dropped to 30.2%, In case of consortium + fertilizer it was recorded as 69.1% and it dropped to 25.1% and

in case of fertilizer only it was recorded as 69.1% and it dropped to 29.1% from 0 day to 60th day of observation.

3. There was a drop in C: N ratio was observed from 1096.8 to 28.23 in case of control, When treatment with microbial consortia was given to rice straw it got reduced from 1096.8 to 14.51, In case of consortium + fertilizer it reduced from 1096.8 to 10.91 and in fertilizer only it reduced from 1096.8 to 13.85 from 0 day to 60th day of observation. Loss in moisture content was from 60.2% to 41.1% over the period of 60 days.
4. Reducing sugar yield was observed to have an increasing trend from 0.91 mg/ml on 7th day to 2.0 mg/ml on 60th day when treatment with microbial consortia was given to the rice straw, when consortia + fertilizer treatment was given it the increase was observed from 1.06 mg/ml to 2.3 mg/ml, in case of only fertilizer the increase in reducing sugar was observed from 0.91 mg/ml to 1.9 mg/ml while in case of control the reducing sugar increased from 0.3 mg/ml to 1.11 mg/ml.
5. The total weight of biomass got reduced as a result of microbial degradation. There was gradual decline in cellulose content however rapid decline was observed in rice straw treated with consortia and fertilizer.
6. Treatment with consortium + fertilizer was the best among all that could reduce the period of composting almost from 60 days to 30 days.

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Appendix- I

Nitrate broth medium (pH-7.2)

Composition	Quantity (g/l)
Peptone	5
Beef extract	3
Potassium nitrate	5
Sodium chloride	5
Distilled water	1000

MR-VP broth (pH-6.9)

Composition	Quantity (g/l)
Peptone	7
Dextrose	5
Di -potassium orthophosphate	5
Distilled water	1000

Methyl red solution

Composition	Quantity (g/l)
Methyl red	0.2
Ethyl alcohol	600
Distilled water	400

VP-sol 2

Composition	Quantity (g/l)
Napthol	50
Ethanol	950

VP-sol 2

Composition	Quantity (g/l)
Potassium hydroxide	400
Distilled water	950

Carbohydrate fermentation test (pH-6.8)

Composition	Quantity (g/l)
Peptone	10
Sodium chloride	3
Xylose	10
Bromocresol purple	0.016

Distilled water	1000

Starch hydrolysis test (pH-7.2)

Composition	Quantity (g/l)
Peptone	5
Beef extract	3
Sodium chloride	5
Starch	2
Agar	20
Distilled water	1000

Lugol'siodine solution

Composition	Quantity (g/l)
Iodine	10
Distilled water	1000

Appendix- II

A. Standard curve for 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

Composition	Quantity g/l
Distilled water	1415 ml
3, 5- Dinitrosalicylic acid	10.5
NaOH	19.8
Rochelle salts (Na-K tartarate)	306
Phenol (melt at 50 °C)	7.6ml
Sodium meta-bisulphite	8.3

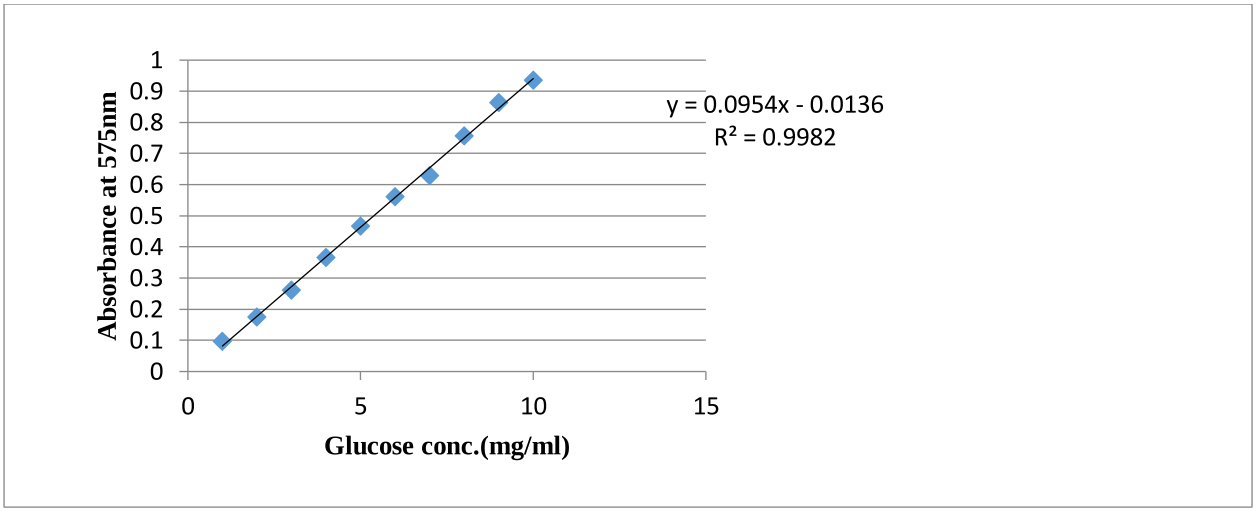

Fig 16. Standard curve for glucose.

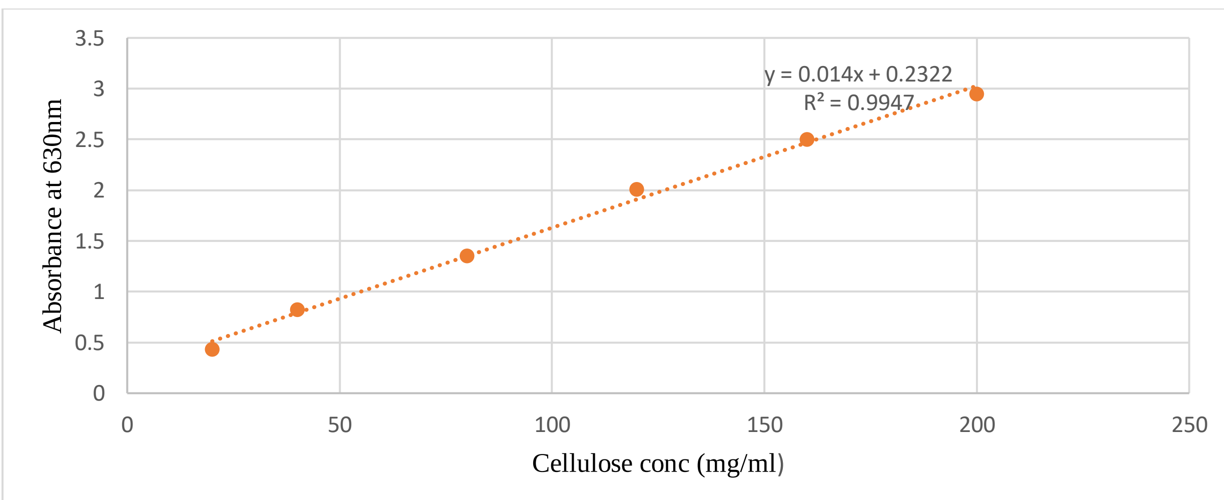

Figure 17. Standard curve for cellulose

Appendix-III

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	Quantity(g/L)
Potato extract	4
Dextrose	20

C. Yeast Extract Peptone Dextrose (YEPD)

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

Appendix-IV

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.