



**ICAR-INDIAN AGRICULTURAL RESEARCH
INSTITUTE**

NEW DELHI-110012

. . .प. -

प 110012

**Title of the study: *Mass Production of Manure
/Fertilizer from Agricultural Biomass***

Final Draft Report

Period of Study: May 2021 to March 2022

Submitted to

National Institution for Transforming India (NITI), Aayog,

**Governance and Research Vertical,
Sansad Marg New**

Delhi-110 001

Project Research Team

Project Investigator-

1. Dr. K Annapurna, Head, Division of Microbiology, ICAR-IARI, New Delhi

Co-Investigator-

1. Dr. Livleen Shukla, Principal Scientist, Division of Microbiology, ICAR-IARI, New Delhi
2. Dr. Satish Lande, Senior Scientist, Division of Agricultural engineering, ICAR-IARI, New Delhi
3. Dr. Lata, Principal Scientist, Division of Microbiology, ICAR-IARI, New Delhi
4. Ms. Anju Arora, Scientist (SG), Division of Microbiology, ICAR-IARI, New Delhi

Objectives / TORs

- To develop a technology to convert crop bio waste (particularly paddy) into farm compost in less than six months period with economically efficient methods
- To convert bio waste into wealth and offer economically viable alternative to prevent burning of crop residues, stubble etc.
- Create possibility of giving an added value to the agricultural activity through the availability of an additional source of income for managing the treatment and selling resultant compost.
- Availability of a new material to improve the soil fertility with the application of compost (in substitution of chemical fertilizers).

CONTENT

Sl. No.	Particulars	Page No.
	Project Title and objectives	1-2
	Research team	2
	Content	3
	List of Table	4
	List of figures	5
Chapter 1	Introduction	6-8
Chapter 2	Review of literature	9-29
Chapter 3	Data and Methodology	30-34
Chapter 4	Results and discussion	35-56
Chapter 5	Conclusions and Limitations	57-58
	Bibliography	59-78

LIST OF TABLES

TABLE NO.	TITLE OF TABLES	PAGE NO.
1.	Various enzymes involved in lignin degradation	15
2.	Standards of compost as described in Fertilizer Control Order (1985)	33
3.	Comparative cost of different paddy straw management systems	37
4.	Changes in various enzyme activities during composting of paddy straw	41
5.	Evaluation of Mesophilic and thermophilic bacterial and fungal Population	41
6.	List of Punjab and Haryana Farmers for <i>ex-situ</i> paddy straw decomposition in year 2021-2022	39
7	List of Punjab Farmers for <i>in situ</i> paddy straw decomposition	50
8.	Range of percent increase in Soil OC, available N, Soil dehydrogenase activity and Microbial Biomass C in field samples where PD was applied after 25 DAS.	55
9.	Changes in NPK content during <i>in-situ</i> decomposition at farmer's field	49

LIST OF FIGURES

FIGURE NO.	TILTLE OF FIGURE	Page No.
1.	Disposal of paddy straw by burning in field	6
2.	Lignocellulolytic material (Plant derived)	10
3.	Chemical structure of lignin	17
4.	Village Kattiyanwali, Shri Muktsar sahib	34
5.	Location map of Punjab districts where Pusa Decomposer was applied by the research team in farmers' fields	51
6.	Application of Pusa Decomposer in farmers field	52
7.	Field demonstration of PUSA Decomposer and farmer workshops in Punjab State	53
8.	Soil biological parameters over a period of time in PD treated and untreated fields	54
9.	CO ₂ emissions in PD treated and untreated plots	55

Chapter 1

1.1 Introduction:

India generates 686.0 MT dry biomass annually from various crops of which, 234.5 MT is considered as surplus crop residue (Cardoen et al., 2015). Rice residues alone contribute 34% of the total crop residues in India. A major share of farmers from various parts of India, utilize rice residue for livestock feed, soil mulching and composting. They also use rice residue as a substrate for mushroom cultivation and energy production. However, disposal of crop residues is still a challenging mission since a large quantity of these are left unutilized in the fields. In India, paddy stubble burning is predominant in northern states like Punjab, Haryana and Uttar Pradesh and other states like West Bengal (Prasad et al., 2012; DAC, 2014; Kumar et al., 2016). Where the subsequent crop is grown in a short period. Straw burning (**Fig. 1**) contributes to the release of trace gases such as CO₂, CH₄, CO, N₂O, SO₂ and vast amounts of particulates, causing harmful effects on human health. India is expected to emit 144719 mg of total particulate matter yearly from the open field burning of rice straw (Kumar et al., 2015). When it comes to soil-related loss, crop residue burning leads to the loss of beneficial microbes as well as nutrients in the soil like C, N, P, K (DAC, 2014; Prasad et al., 2020).



Fig. 1. Disposal of paddy straw by burning in field

Rice straw contains approximately 40% cellulose, 20% hemicellulose and 12% lignin (Juliano, 1985). Microorganisms quickly degrade cellulose and hemicellulose. However, lignin, a chemically complex aromatic biopolymer, makes a covalent bond with cellulose and makes itself resistant to degradation.

Lignin is a complex polymer of three different phenylpropanoid alcohols, viz., coniferyl, coumaryl, and sinapyl alcohols. Other than these three components, feed stocks are also made up of small quantity of pectin, mineral residues and some nitrogenous compounds (Pollegioni, et al. 2015). Degradation of lignin is slower and complicated due to its structural complexity and macromolecular features. Since a large part of the organic carbon in lignin and in other compounds is shielded by lignin, research on lignin biodegradation is important and essential. Over the past few decades, research on microbial degradation of lignin has gained momentum to

break the accessibility barrier and use the carbohydrates released.

Fungal species capable of decomposing lignin can be divided into three major groups based on their morphology *viz*; brown rot fungi, white rot fungi and soft rot fungi. Further, there are some groups of fungi that are dung-dwelling (coprophilic) fungi, and litter- decomposing fungi also can degrade lignin effectively (Blanchette 1995; Liers et al. 2011).

South-east Asia is the principle niche of rice crop, and in this subcontinent rice and wheat occupy nearly 59.16 and 42.55 million ha, respectively, and annual grain output is around 181.35 and 109.07 million tonnes, respectively (RWC-CIMMYT,2003). The common farming system in the Indo-Gangetic Plain (IGP) is the rice-wheat rotation system. With the introduction of combine harvesters, more than 75% of the rice area is harvested mechanically in north-western parts of the Indo-Gangetic Plains. Most of the farmers remove wheat straw for feeding the animals. However, management of the rice straw is a major challenge as it is considered to be a poor feed for the animals owing to high silica content. Although direct transformation of rice straw as mulch in fields is an alternative for its cost-effective utilization, soil application of large doses of undecomposed rice straw can lead to unfavourable effects on successive plant growth and crop yields due to production of certain phytotoxic allelochemicals (Chung et al., 2001; Inderjit et al., 2004; Lee et al., 1999).

Under these circumstances it is imperative to develop technologies which are eco-friendly, economically viable and easy to use. The technologies must be demonstrated under farmer's field conditions for them to adopt. A fundamental shift in farmer's behaviour is possible at large scale, if actionable and affordable solutions are made available to them on time. Other than machinery, biological interventions are feasible.

Development and application of effective microbial products for accelerated decomposition of paddy residue is one such solution. In the three Indo-Gangetic States, viz: Punjab, Haryana and UP, this practice of burning has become more prevalent in recent years, as for the next crop to be sown in time, the window for field preparation is very small and the farmer does not have any other option than burning. This leads to increase in air pollution due to GHG emissions, loss in soil nutrients leading to reduced fertility, deterioration of soil health and loss of microbial diversity. It has become a major concern and efforts by the Agriculture Ministry, GOI, ICAR and IARI are being made to come up with a multiple solutions/strategies to curtail burning and make the farmers aware of the hazards of the same.

Chapter 2

2. Review of literature

Rice (*Oryza sativa*) occupies a pivotal place in Indian agriculture and is the staple food for more than 70% of population. Rice is grown in almost all the states covering an area of 44.6 m ha with annual production of 87 million tonnes. Straw is a major by-product of rice cultivation with an annual production of 120 million tonnes. With the emergence of intensive dairying, the focus shifted from rice straw as fodder, as it possesses lesser nutritive value in comparison to feed concentrates, contains excessive amounts of silica (11-15%) and has very less digestibility (Juliano, 1985). In the changing scenario rice straw started being considered as a waste product to be disposed by burning (Plate 1).

The presence of lignin-cellulose complex in straw makes degradation process by microorganisms arduous. Hence, pre-treatment of lignocellulosic substrates is a prerequisite for the enhancement of their susceptibility to hydrolytic agents. Pretreatment involves partial delignification by protein enrichment of crop residue and requires alkali and urea treatment, but still exhibits only 55-60 per cent digestibility (Singh and Shiere, 1993). In contrast, composting of crop residue through the action of lignocellulolytic microorganisms is easier to manage.

2.1 Lignocellulose degrading microorganisms

The organic substrate, bulking agents and the amendments used in composting are mostly derived from plant material. Lignocellulose, the composite of the predominant polymers of vascular plant biomass, is composed of polysaccharides like cellulose and hemicellulose and the phenolic polymer lignin (**Fig.2**). Hence, the capacity of microorganisms to assimilate organic matter depends on their ability to produce the enzymes needed for degradation of the substrate components i.e., cellulose, hemicellulose and lignin. The more complex the substrate, the more extensive and comprehensive is the enzyme system required. Through the synergistic action of microorganisms, complex organic compounds are degraded to smaller molecules, which can then be utilized by microbial cells (Golueke, 1991, Shukla et al., 2014, 2016).

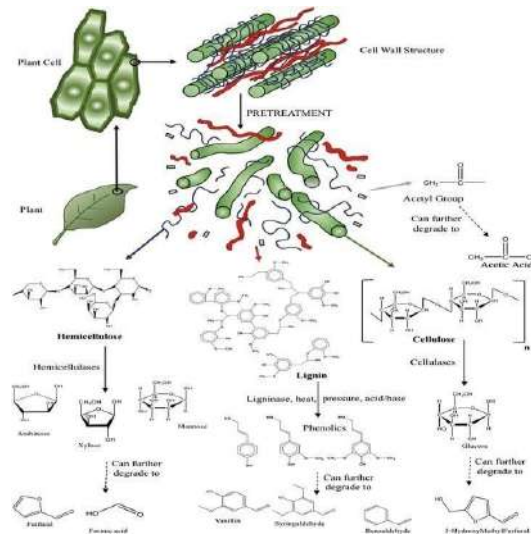


Fig 2. Lignocellulolytic material (Plant derived)

Fungi: Hundreds of species of fungi are able to degrade lignocellulose. There are mainly three types of fungi living on dead wood that preferentially degrade one or more wood components viz. soft rot fungi, brown rot fungi and white rot fungi (Kirk, 1983). Soft rot fungi (Ascomycetes and fungi imperfecti) can efficiently decompose cellulose but are reported to degrade lignin slowly and incompletely. The brown rot fungi (Basidiomycetes) generally exhibit preference for the carbohydrate components of wood (Janshekar and Friecher, 1983; Kirk, 1983) with activity towards lignin largely confined to demethylation (Kirk, 1983). White rot fungi are capable of degrading both lignin and cellulose. The most extensively studied lignocellulolytic fungi are *Trichoderma* and *Phanerochaete*. Lignolytic and cellulolytic Lynch et al. (1981) observed that *Cladosporium sp.*, *Alternaria sp.* and *Fusarium sp.* were more active decomposers than *Phoma sp.* Nigam and Parvu (1985) reported the cellulolytic activity of Basidiomycetes sp., *Pleurotus ostreatus* and *Polyporus versicolor*.

Bacteria

Cellulolytic bacteria are ubiquitous in nature. Under appropriate conditions bacteria produce cellulase and hence many bacterial strains are known to solubilize and modify the lignocellulosic structures extensively. But their ability to mineralize lignin is limited (Ball et al., 1989; Eriksson et al., 1990; Godden et al., 1992). *Cellulomonas* and *Cytophaga* are the aerobic mesophilic bacteria able to produce cellulose degrading enzymes (Thayer et al., 1984; Rajoka and Malik, 1986). More than one-half of the *Bacillus sp.* examined to date produces extracellular cellulases. Mesophilic, aerobic and anaerobic forms of *Bacillus*, *B. subtilis*, *B. polymyxa*, *B. licheniformis*, *B. pumilus*, *B. brevis*, *B. firmus*, *B. circulans*, *B. megaterium* and *B. cereus* are known to be cellulose and hemicellulose degraders. Thermophilic cellulolytic *B. stearothermophilus*, *B. brevis*, *B. sphaericus*, *B. subtilis* and two other species of *Bacillus* were isolated by Strom (1985) from soil waste composter. Ray et al. (2007) isolated two bacteria viz. *Bacillus subtilis* CY5 and *Bacillus circulans* TP3 from fish gastrointestinal tracts which were able to produce cellulase optimally at 40 °C. Walker et al. (2006) isolated an alkaliphilic cellulase producing bacteria *Nocardiopsis sp.* from an indoor contaminated agar plate during a screening program which showed prominent clear hydrolysis of the CM cellulose even at pH 10.

Actinomycetes

Actinomycetes isolated from soil and related substances show primary biodegradative activity, secreting a range of extracellular enzymes and exhibiting the capacity to metabolize recalcitrant molecules. In neutral and alkaline environment, *Streptomyces viridosporus* is likely to be dominant over fungi as a decomposer of lignin and cellulose (Pometto and Crawford, 1986). Thermophilic cellulase producing *Thermoactinomyces*, *Streptomyces* and *Thermomonospora* were found to be present in dry, warm land and also where salt concentrations are too high and soil pH is

Alkaline (Stutzenberger, 1972). From Indian desert soil of Jodhpur, Rao and Venkateswaralu (1983) isolated *Streptomyces*, *Micromonospora* and *Thermoactinomyces*. These organisms were found to depolymerize crystalline celluloses by two cellulase enzyme systems and glucosidase. Strom (1985) isolated thermophilic and highly cellulolytic *Streptomyces*, *Thermoactinomyces sp.* from solid waste composter. Jang and Chen (2003) isolated eighteen strains of actinomycetes from the compost of agricultural wastes (vegetable residues supplemented with corncob, straw and rice hull) and cultivated them at 50 °C for the thermostable cellulase production. Chellapandi and Himanshu (2008) isolated two cellulolytic *Streptomyces sp.* from garden soil and they were found to be good producer of endoglucanase under SmF.

2.2 Degradative enzymes

Cellulose degrading enzymes: In nature, cellulose components of biomass are hydrolysed to sugars by a complex enzyme system called 'Cellulase'. Cellulase is a hydrolytic enzyme complex containing chiefly endo- and exo- β -glucanases and cellobiase. Reese (1956) concluded that a true cellulolytic microbes possess two enzymes termed C1 and Cx. C1 was postulated to attack crystalline cellulose, either producing short chains or decrystallising glucan chains so that the cellulose was then susceptible to attack by hydrolytic Cx enzymes.

A name of 'Cellulase complex' has been proposed and it refers to a system of three different enzymes whose combined action leads to the efficient degradation of cellulose, endo- β -1, 4 glucanase, exo- β -1, 4 glucanase and β -glucosidase (Ladisch et al., 1983). β -Glucosidases, appear to be exclusively cell-bound (Choi et al., 1978; Stoppok et al., 1982). The presence of cellulose in growth media usually gives the greatest yield of enzyme, however, in some species the enzymes are always present regardless of the carbon source (Prasertsan and Doelle (1987). All the three distinct enzymatic activities are required for the hydrolysis of cellulose to glucose.

Hemicellulose degrading enzymes: Hemicellulose component of a biomass can be degraded by different kinds of enzymes viz. (i) endoenzymes - which randomly cleave the bonds between the building blocks of a polymer (ii) exoenzymes - which cleave either a single dimer or monomer from the end of the polysaccharide chain and (iii) glycosidases- hydrolyse the oligomers or disaccharides of hemicellulose polymers. According to Dekker and Richards (1976) hemicellulase can be classified into L-arabinase, D-galactanase, D-mannanase and D- xylanase. Of the four types of hemicellulases, D-xylanase is seen in many types of microorganisms. These bring about the hydrolysis at β -D-(1-3) linkages of xylan. Xylanolytic enzymes include endo-1-4- β -xylanases (EC 3.2.1.8) and β -xylosidase (EC 3.2.1.37) which are produced by many microorganisms including fungi *A. niger* (Conrad, 1981; Gokhale et al., 1986), *Aspergillus* sp. and *T. reesei* (Dekker, 1983; Chahal, 1985). Of the thermophiles, *Thermomyces lanuginosus* RM-B performed best producing 154 U xylanase. The enzyme was reported to have optimum temperature 60-70°C at neutral pH (Bakalova et al., 2002).

Lignin degrading enzyme system: Enzymes involved in lignin degradation can be grouped into two categories. First group of enzyme called phenol oxidases. Mutants of *Sporotrichum pulverulentum* lacking phenol oxidase could not degrade lignin while its revertant degraded all wood components. Another enzyme ‘cellobiase oxidoreductase’ from *Sporotrichum pulverulentum* and *Polyporus versicolor* was involved in lignin degradation. The investigation on *Pleurotus ostreatus* wild type with a cellulase less mutant of *S. pulverulentum* suggested that the phenol oxidase activity was not necessary for lignin degradation (Liwicki et al., 1985). Laccase is another kind of phenol oxidase that has been widely associated with lignin degradation. Laccase causes free radical formation of cinnamyl alcohol and this non- enzymatic polymerization may induce cleavage of bonds between aromatic rings and propane side chain as well as the formation of the carboxyl group in the side chain, which enhance the rate of lignin degradation. Green (1977) observed associated enzyme laccase glucose:quinone oxidoreductase in lignin degradation. Ishihara (1980) observed demethylation activity of laccase in both lignin and lignin model compounds and concluded that depolymerization activity of laccase could be the first step in lignin degradation.

Peroxidase may contribute its hydroxyl ion (- OH) formation, which has been suspected in lignin biodegradation (Dordick et al., 1986). However, the exact role of peroxidase in lignin degradation is yet to be established. Meanwhile, hydrogen peroxide dependent oxygenase enzyme has been identified and proved to be involved in the initial depolymerisation of lignin degradation. Hydrogen peroxide dependent oxygenase enzyme from lignolytic cultures of wood degrading *Phanerochaete chrysosporium* formed radical oxygen by the generation of ethylene from 2-keto-3- thiomethyl butyric acid (KTBA).

The lignin degradation is not catalyzed by any particular enzyme but concerted action of oxidative coupling of phenol oxidase, peroxidase, glucose oxidase and ligninase. Ligninase catalyze breakdown of the ether linkages with glucose oxidase providing necessary co-substrate peroxide, then peroxidase is involved in radical formation, phenoloxidases polymerize and depolymerize phenols often initial degradation depends on the level of glucose oxidases. **Table 1** gives the lignin degrading enzyme system details.

Table 1: Various enzymes involved in lignin degradation (Janusz et al., 2017)

Enzyme	EC Number	Compounds oxidized	Commonly produced Organisms
Lignin peroxidase	EC 1.11.1.14	Phenolic aromatic compounds and non-phenolic lignin model compounds	<i>Phanerochaete</i> , <i>Chrysosporium</i> , <i>Trametes versicolor</i> and <i>Phlebia tremellosa</i>
Manganese peroxidase	EC 1.11.1.13	Monomeric phenols and lignin model compounds	<i>P. chrysosporium</i> , <i>Panus tigrinus</i> and <i>Agaricus bisporus</i>
Versatile peroxidase	EC 1.11.1.16	Methoxybenzenes and non-phenolic model lignin compounds	<i>Pleurotus eryngii</i> , <i>Pleurotus ostreatus</i> and <i>Bjerkandera adusta</i>
Dye-decolorizing peroxidase	EC 1.11.1.19	Lignin and dyes	<i>Geotrichum candidum</i> , <i>Termitomyces albuminosus</i> and <i>Rhodococcus josti</i>
Laccase	EC 1.10.3.2	Phenolic moieties in lignin, aromatic amine benzenothiols and hydroxyindols	All white rot fungi, <i>Streptomyces griseus</i> and some bacteria
Glyoxal oxidase	EC 1.2.3.5	Glyoxal and methylglyoxal compounds	<i>Phanerochaete chrysosporium</i>
Aryl alcohol oxidase	EC 1.1.3.7	Phenolic and non-phenolic aryl-alcohols	<i>Agaricales</i> species, <i>Aspergillus</i> , and <i>Fusarium</i>
Heme-thiolate haloperoxidases	EC 1.11.1.10	Organic sulfides, olefins and aromatic rings	<i>Caldariomyces fumago</i>

Lignin, a chemically complex aromatic biopolymer (**Fig. 3**), makes a covalent bond with cellulose and makes it resistant to degradation. Cellulose, hemicellulose and lignin are the major constituents of any lignocellulosic raw materials in which, strands of cellulose and hemicellulose are bound together by lignin.

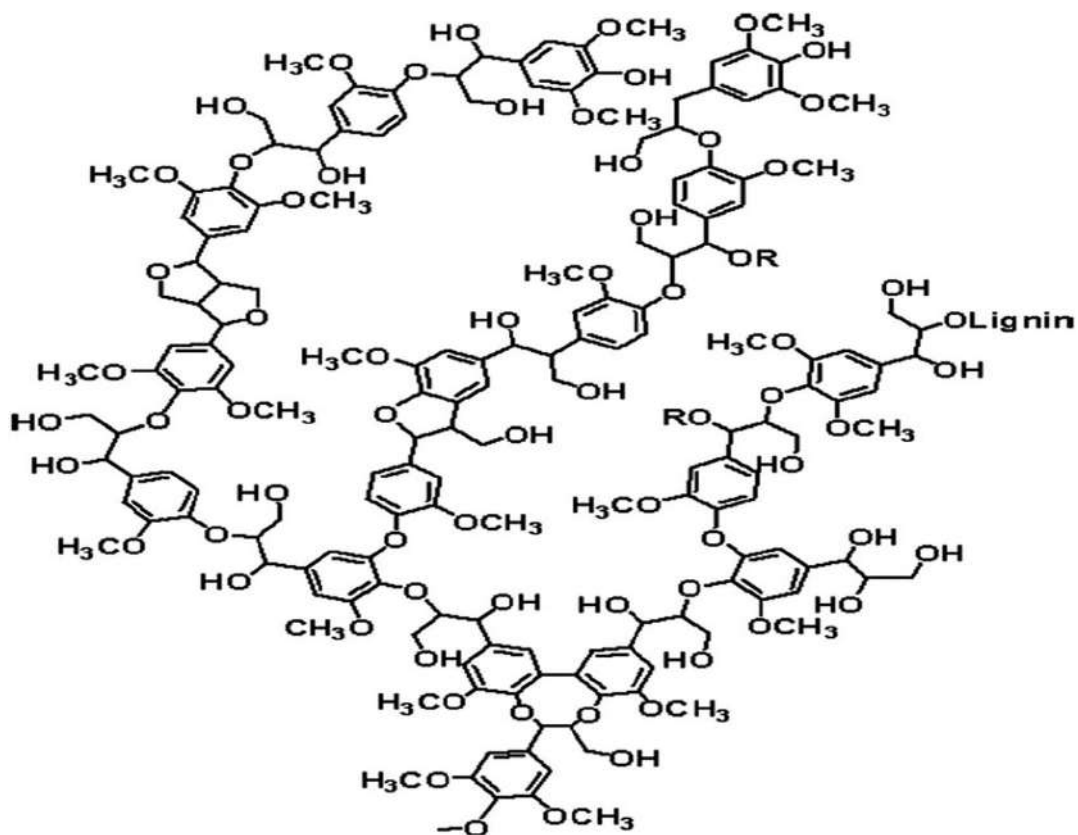


Fig: 3 Chemical structure of lignin

2.3 Lignin degrading microorganisms

Microbial delignification is one of the most efficient mechanisms for natural degradation of lignin. Complete degradation of lignin is a result of cooperative action of a wide variety of microorganisms such as bacteria and fungi (Janshekar and Fiechter, 1983). Degradation of lignin mediated by bacteria is slow and limited, while fungi are more efficient (Sigoillot et al., 2012).

2.3.1 Bacteria

Bacteria that are reported to degrade lignin mainly belong to three classes, α -Proteobacteria, γ – Proteobacteria and actinobacteria (Bugg et al., 2011). Many strains of bacteria belonging to the genera *Ochrobactrum*, *Brucella*, *Sphingobium* and *Sphingomonas* are reported to degrade lignin. *Pseudomonas fluorescens* is known to be most efficient bacterium for lignin peroxidase production (Tian et al., 2014).

So far, numerous filamentous bacterial species capable of lignin degradation have been isolated and identified from *Streptomyces* group, and about ten enzymes that participate in lignin degradation have been characterized till now (Fernandes et al., 2014). Jing and Wang (2012) observed that the actinobacterial species, *Streptomyces cinnamomeus* produces both laccase and lignin peroxidase enzymes. Laccase activity and lignin peroxidase activity were also observed in another strain of *Streptomyces viridosporus* (Bugg et al. 2011; Tian et al. 2014). In another study, Větrovský et al. (2014) isolated *Streptomyces* spp. from forest soil and meadow. These strains were able to solubilize up to 4% of poplar lignin and 64% of catechol. Majumdar et al. (2014) studied the lignin degradation activity of laccases from *Streptomyces lividans* TK24, *Streptomyces coelicolor* A3 (2), *Streptomyces viridosporus* T7A, and *Amycolatopsis* sp. 75iv2 using ethanosolv lignin and some lignin model compounds. All four laccases were capable of effectively degrading various lignin model compounds.

2.3.2 Fungi

There are a large number of fungal species capable of decomposing lignocellulotic substrates. In natural and human- affected forest environments, wood-degrading fungi live mainly as saprotrophs or weak parasites (Couturier et al. 2012). They can be divided into three major groups based on morphology viz; brown rot fungi, white rot fungi and soft rot fungi. Further, there are some groups of fungi that are dung-dwelling (coprophilic) fungi, and litter-decomposing fungi also can degrade lignin effectively (Blanchette 1995; Liers et al. 2011). Many fungi belonging to orders Agaricales and Polyporales viz: *Ganoderma* spp., *Lentinula edodes*, *Phlebia radiata* or *Pleurotus* spp. are known as white rot fungi. However, fungal species such as *Gloeophyllum trabeuma* and *Coniophora puteana* are categorized as brown rot fungi (Blanchette 1995). Suhara et al. (2012) screened 51 fungal strains isolated from bamboo culms for lignin degradation. After 12 weeks, *Punctularia* sp. TUF20056, a white rot fungus and TUF20057 (unidentified fungi) exhibited more than 50% lignin degradation and high lignin/holocellulose loss ratios (>6). Chang et al. (2012) collected fungal samples from woody surfaces and screened for lignolytic property on rice straw. One of the isolates (No 812 identified as *Fusarium moniliforme* exhibited maximum lignin degradation (34.7%). This was much more than the traditional wood degrading fungi *Phanerochaete chrysosporium* (28.3% lignin degradation). Zhang et al. (2012) investigated the lignin removal efficiency of *Phanerochaete chrysosporium* from rice straw. After ten days of solid state fermentation, the lignin removal efficiency was observed to increase sharply to 50.13%. In another study, Liang et al. (2010) observed that lignin degradation by *Phanerochaete chrysosporium* improved by 54% in the presence of 0.007% dirhamnolipid. At the same concentration, dirhamnolipid also increased lignin peroxidase activity of *Phanerochaete chrysosporium* by 86%.

Pildain et al. (2005) reported that several *Eutypella* species produce white rot decay in the late stages of wood decay and soft rot in the early stages. Mustafa et al. (2016) used *Pleurotus ostreatus* and *Trichoderma reesei* for pretreatment of rice straw to enhance methane production. Pretreatment with *P. ostreatus* at 75% moisture content removed 33.4% lignin after 20 days, and *Trichoderma reesei* removed 23.6% lignin at same conditions.

2.5 Enzymes involved in lignin degradation

Lignin degrading enzymes are broadly classified into two classes, lignin-oxidizing enzymes and lignin-degrading auxiliary enzymes. Commonly known enzymes such as laccases, lignin peroxidase, versatile peroxidases, manganese peroxidases and chloroperoxidases belong to lignin-oxidizing enzymes (Levasseur et al. 2008).

2.5.1 Lignin peroxidase (LiP; EC 1.11.1.14)

Lignin peroxidase (LiP) oxidizes phenolic aromatic substrates and several non-phenolic lignin model compounds non-specifically in the presence of H₂O₂ and is secreted by microorganisms as isozymes, and its relative composition as well as isoelectric points are decided by the nutrient conditions (Santhanam et al. 2012). Sigoillot et al. (2012) stated that the three-dimensional structure of LiP is constituted with two Ca²⁺ binding sites, two glycosylation sites and four disulfide bridges. Isoelectric point of this enzyme varies from 3.1 and 4.7, makes lignin peroxidase capable of oxidizing a wide variety of substrates that are not oxidized by other classes of peroxidases. Kumari et al. (2002) demonstrated secretion of LiP from various fungi such as *Aspergillus terreus*, *Penicillium citrinum* and *Fusarium oxysporum*. They observed that induction of these enzymes was highest in liquid broth in which coir dust was added.

In a recent study, Fan et al. (2019) used lignin peroxidase produced from *Aspergillus oryzae* for degradation of corn stover lignin. The apparent maximum rate of degradation reaction of lignin at an enzyme concentration of 3.75 U/100 mL was found to be 1.68 (mg/mL)/min.

2.5.2 Laccase (EC 1.10.3.2)

Laccase, one of the oldest enzymes, is a multi-copper enzyme that is ubiquitous in white-rot fungi. Laccase, being an essential part of lignolytic system, can have extracellular, intracellular or periplasmic location, depending upon its physiological functions. However, in contrast to the other ligninolytic enzymes, it is mostly reported as extracellular proteins.

Kumar et al. (2016) isolated laccase producing strain of *Aspergillus flavus*. They observed that the yield of laccase is highest with cellulose (8%), peptone (2%) and incubation at 35 °C. Ghosh and Ghosh (2017) optimized laccase production from another strain of *Aspergillus flavus* PUF5. They used agro-waste including ribbed gourd peel as a substrate and the fermentation was performed under submerged conditions. At yeast extract concentration of 0.3% and pH 4, laccase production was improved by 4.6-fold (15.96 U/ml). *Aspergillus fumigatus* strain VkJ2.4.5 was a potential source of laccase, when banana peel was used as substrate in solid state fermentation. While growing on banana peel, the fungal strain produced significant amount of laccase ($6281.4 \pm 63.60 \text{ U l}^{-1}$) and notable levels of manganese peroxidase ($1339.0 \pm 131.23 \text{ U l}^{-1}$) (Vivekanand et al., 2011). Jin and Ning (2013) studied laccase production from a different strain of *Aspergillus fumigatus* AF1. The research revealed that optimized conditions for the highest laccase ($142,198 \pm 3586 \text{ U L}^{-1}$) are NaOH at 0.39 mol L^{-1} , pH 3.12 and temperature 25.43 °C.

2.5.3 Manganese peroxidase (EC 1.11.1.13)

Manganese peroxidase is another vital lignin modifying enzyme secreted in multiple isoforms and detected in species such as *Panus tigrinus*, *Lenzites betulinus*, *Nematoloma frowardii*, *Bacillus pumilus* and *Azospirillum brasilense* (Janusz et al., 2017). The catalytic mechanisms of manganese peroxidase are similar to LiP, but utilize Mn (II) as their reducing substrate and generate Mn (III) (Martinez et al., 2005).

2.6 Degradation of rice residue

Rice straw decomposition depends on certain microbes that can attack and degrade its different components, including cellulose, hemicellulose and lignin (Coronel et al., 1991). Kumar et al. (2008) applied a consortium of three fungi, namely, *Cytalidium thermophilum* (Th5), *Aspergillus nidulans* (Th4) and *Humicola* sp. (Th10) to degrade a mixture of paddy straw and soybean trash. The C: N ratio of the material was reduced to 9.5:1 after composting for three months. Pandey et al., (2009) studied the effect of a hyperlignolytic fungal consortium containing *Aspergillus awamori* F-18, *Aspergillus nidulans* ITCC 2011, *Trichoderma viride* ITCC 2211 and *Phanerochaete chrysosporium* NCIM 1073 on rice straw degradation. Native microbial community with externally applied consortium accelerated the degradation of paddy straw and reduced its C: N ratio to an acceptable level within one month. Moreover, supplementation of poultry manure improved the degradation of paddy straw.

Kausar et al. (2010) also developed a lignocellulolytic fungal consortium targeting rapid composting of paddy straw. Two promising cultures identified as, *Aspergillus niger* (F44) and *Trichoderma viride* (F26) were tested for rice straw degradation *in-vitro*. The results revealed that cellulose, hemicellulose, lignin, and total carbon were significantly decomposed by the fungal consortium over control. In the three weeks of the decomposition processes, the C/N ratio was decreased to 19.5 from an initial value of 29.3, thus demonstrating the potential of this method for use in large-scale rice straw composting.

Wei et al. (2019) augmented various lignocellulosic materials, including rice straw and wheat straw with thermophilic actinomycetes. The results showed that inoculation of actinomycetes not only altered the composition of the actinomycetes and bacterial community but also enhanced the

Degradation of cellulose, hemicellulose and lignin and intensified the activities of key enzymes, including xylanase, CMCase, lignin peroxidase, manganese peroxidase and laccase, primarily from rice straw and wheat straw throughout composting process. Finally, the study concluded that inoculation of actinomycetes enhanced lignocellulose degradation by 34.3 per cent and enzyme activity by 8.3 per cent.

2.7 Factors affecting degradation of lignocellulose

Degradation of lignocellulosic biomass is associated with several factors which influence the process. In order to incorporate into the soil or boost the decomposition rate, straw chopping is required as a pretreatment. It decreases the straw length, preventing long pieces of material from fouling on cultivation. It also simplifies the process of soil mixing and increases the successful biological breakdown of the straw (Muzamil et al., 2015). Silva et al. (2012) studied the effect of grinding on enzymatic degradation of wheat straw. Various grinding size (ranges between 50 μm to 800 μm) of wheat straw was investigated using *Trichoderma reesei* enzymatic cocktail. The results revealed that the degradability of wheat straw was increased up to 100 μm size reduction. Dai et al. (2019) also studied the effect of rice straw particle size on its degradation and methane production. Different particle sizes (20, 1, 0.15, and 0.075 mm) were studied, and methane yield was observed to increase as the size reduced (from 107 mL g^{-1} VS to 197 mL g^{-1} VS). The rate of degradation of cellulose was also increased from 27% to 93%. These results indicated that particle size reduction in rice straw could boost the methane yield in anaerobic digestion processes in conjunction with optimized microbial growth. The large particle size restricts the entry of fungi into the biomass and inhibits water, air and intermediate metabolites from spreading into the particles. Whereas, tiny particles reduce the size of the inter-specific channel, which would adversely affect the circulation of the inter-particle gas. Therefore, for successful biological pretreatment, ideal particle size has to be used (Sindhu et al., 2016).

The composition of microbial communities and enzymatic activities vary with nutrient availability and environmental factors like temperature and moisture. It is known that, nitrogen influence rate of decomposition. Recently, a detailed litter bag study was conducted to estimate rice straw decomposition and microbial community dynamics under different levels of nitrogen. L- leucine aminopeptidase and N- acetyl-glucosamidase activities associated with rice straw were maximum

at 180 kg N ha⁻¹ and 270 kg N ha⁻¹ with higher activities at early stages of decomposition. The straw associated actinobacterial gene (GH48) and cellulolytic fungi gene (cbh1) abundance also varied with the stage of decomposition in such a manner that, during the middle stage of decomposition, higher abundance of these genes was recorded. Fungi and actinobacteria played a key role in the degradation of recalcitrant compounds at the later stage of decomposition (Guo et al., 2018).

C/N ratio is another important factor for lignocellulose degradation. Several studies had been conducted to know the extent to which C/N ratio influence the microbial decomposition of rice stubble. Asgher et al. (2016) reported that production of various lignolytic enzymes such as manganese peroxidase, laccase and lignin peroxidase from a white-rot fungus *Schizophyllum commune* IBL-06 produced maximally, when the C:N ratio of rice straw was adjusted to 20:1. The resulting crude ligninolytic extract was used to delignify various agro-industrial residues. The enzyme extract induced lignin removal from the banana stalk, sugarcane bagasse, corn cobs and wheat straw by 61.7%, 72.3 %, 47.5 % and 67.2 %, respectively. Yan et al. (2015) studied the effect of C: N ratio on anaerobic digestion of rice straw. The study revealed that maximum biogas production happens when the C:N ratio was 29.6:1. Moreover, significant interactive effect of temperature, initial substrate concentration and C/N ratio was found on the biogas production from rice straw. Khudzari et al. (2016) checked the effect of different C/N ratios on power generation in compost microbial fuel cells. They used vegetable fruit mix and soil with different C/N ratios. The results indicated that lower C/N ratio (C/N ratio 24) had a higher power output with a maximum power density, signifying a more favourable microbial growth condition.

For successful establishment of microbial growth in the biomass, the initial moisture content is important. The initial moisture content profoundly influences the development of fungal growth as well as enzyme production and directly improves the degradation of lignin (Sindhu et al., 2016). The initial moisture content of lignocellulosic biomass has an important impact on the rate of microbial degradation. Lignocellulosic substrate at moisture level 12% and 30% were degraded under solid-state fermentation conditions after steam explosion. The results indicated that higher moisture content enhanced lignin removal and sugar recovery from biomass (Cullis et al., 2004). Reid (1989) reported that several white-rot fungi degrade lignin optimally at moisture content ranges from 70% to 80%. Later, Fujian et al. (2001) stated that Manganese peroxidase and lignin peroxidase enzymes production is favored by lower solid liquid ratio. Shi et al. (2008) pretreated cotton stalks using *Penicillium chrysogenum* at different moisture levels. The results indicated that

Moisture content between 75-80% resulted in more lignin degradation than moisture at a low level (65%). However, optimum moisture content for degradation depends on the species of microorganism and type of biomass.

Concentration and the type of inoculum influence rate of decomposition considerably. This is mainly because inoculum size can influence the time required by the organism to colonize the substrate. Generally, spores are used as inoculum and large size of inoculum will shorten the time required for colonization on substrates (Sindhu et al., 2016). Recently, Li et al. (2018a) carried out solid-state anaerobic digestion of tomato residues along with corn stover and dairy manure. They reported that anaerobic digestion was quickly initiated at a substrate inoculum ratio of 6. Maximum production of methane was observed when a substrate inoculum ratio of 2 was applied. Li et al. (2018b) studied the effect of moisture and inoculum size on delignification and subsequent saccharification of switch grass after solid state fermentation with *Pleurotus ostreatus*. After 80 days, highest degradation of lignin (52%) and maximum ethanol yield (31%) were recorded in treatment containing 75% moisture content and 5 mL inoculum.

Optimum incubation temperature is important during biological pretreatment of lignocellulosic residues. Though the optimum temperature is different for each fungus, majority of ascomycetes white-rot fungi grow optimally around 39°C, and white-rot fungi belonging to basidiomycetes grow optimally between 25 and 30°C. Fungi generate a considerable amount of heat during metabolism and develop temperature gradients in solid-state media. Fungal physiology, fungal strain, and type of substrate are responsible for the differences in optimal temperature for biological degradation of biomass.

2.8 *In situ* degradation of rice stubble

There are scanty publications related to *in situ* degradation of rice stubble using bio-inoculants. Earlier, Darmwal and Gaur (1988) studied the effect of cellulolytic fungi *Aspergillus awamori* and *A. niger* along with nitrogen fixer *Azospirillum lipoferum* in wheat soil supplemented with rice straw. The soil inoculated with microorganism's recorded highest yield and fixed nitrogen. Among the inoculants, *Aspergillus awamori* recorded promising results followed by *A. niger* and *lipoferum*. Gai and Nain (2007), incorporated paddy straw in field and applied *Aspergillus awamori* F18 (phosphate dissolving and cellulolytic) and *Trichoderma reesei* MTCC164. The study finalized that, *in situ* incorporation of paddy straw combined with *T. reesei* and N60P60 was effective for proper disposal of paddy straw as well as to improve soil health.

Recently, Borah et al. (2018), compared degradation in two different varieties of rice namely, *Mahsuri* and *Ranjit*. The stubbles of the two varieties after harvest were sprayed with laboratory culture of cellulose degrading microorganism (CDM) or commercial yoghurt or mixture of CDM and yoghurt with glyphosate. The variety *Mahsuri* showed significant decreases in dry biomass (61.1%) and per cent organic carbon (45.3%) than *Ranjit* (47.1% and 46.4%) with stubbles, following treatment with CDM culture or yoghurt with glyphosate solution.

Patel et al. (2016) conducted a field experiment to study effects of *in situ* decompositions of paddy straw inoculated with composting culture in an onion field. They observed an overall significant change in properties such as pH and EC as well as mineral composition of soil after harvest on onion crop. In another study, Bhattacharjee et al. (2013) evaluated the changes in the nitrogen profile during *in situ* management of rice stubble. Various treatments such as lignocellulolytic fungal inoculated stubble, phosphocompost blended uninoculated stubble and uninoculated stubble were evaluated. They observed that stubble blended with phosphocompost along with nitrogen fertilizer recorded highest total, nitrate and ammonical nitrogen as well as biomass carbon (MBC). The urease activity in wheat rhizosphere soil was also recorded the most elevated in phosphocompost blended rice stubble. Meena et al. (2016) conducted an open pit field

experiment using ICAR-IARI compost inoculants contain four hyper lignocellulolytic fungi namely, *Trichoderma viride*, *Aspergillus nidulans*, *Phanerochaete chrysosporium* and *Aspergillus awamori* for degradation of rice straw. As compared to control, a treatment containing compost inoculants along with molasses 5% spray recorded the lowest value of C/N ratio. This may be due to rapid multiplication of applied compost inoculants in the presence of 5% molasses.

3.0 Alternate approaches of managing agri-residue

The Govt. of India has attempted to manage this problem, through numerous measures and campaigns designed to promote sustainable management methods such as converting crop residue into energy. Government has already taken steps to encourage production of cellulosic ethanol from agricultural wastes and residues that would otherwise be burnt. The mandate for National Biofuel Policy (NBP) is to produce 10 million litres of E10 biofuel which would further save Rs 28 crore in forex and around 20,000 tonnes of carbon dioxide emissions. However, biomass removal put additional burden to replenish soil with nutrients. Conversion of agro-waste to organic fertilizer is extremely important into the framework of circular economy compared to other approaches as it addresses ground level issues on agriculture, environment and socio economy of Indian farmers.

Composting, biochar production, conservation agriculture, etc. are a few effective sustainable techniques that can help to curtail the issue while recycle and retain the nutrients present in the crop residue and improving soil health.

Composting technologies: *Ex-situ* management

Phospho-Sulpho-Nitro Compost Technology

Indian soils are widely deficient in phosphorus a major soil nutrient responsible for crop productivity. On the other hand, rock phosphate (containing 11-32 % P_2O_5) deposits are present in different parts of India viz., Udaipur (Rajasthan), Jhabua (Madhya Pradesh), Visakhapatnam (Andhra Pradesh), Purulia (West Bengal), Mussori (Uttaranchal) etc. Low-grade rock phosphate can be used for preparation of nutrient enriched “Phospho-Sulpho-Nitro Compost” and applied as a source of organic matter and phosphorus for crop production. This technology includes the use of rock phosphate, pyrites, and P solubilizing organisms including P solubilizing fungi (*Aspergillus awamori*) and P- solubilizing bacteria (*Bacillus polymyxa*, *Pseudomonas striata*) as bioinoculum for solubilizing P from rock phosphate. ***P-S-N compost can be prepared by pit and heap methods.*** To avoid the leaching of nutrients, the floor should be cemented for heap method. In pit (10 Ft length x 5 ft width x 3 ft deep) and in heap (7.5 Ft length x 6 ft width x 3 ft height) methods about 500 kg of wastes can be used for decomposition.

Vermicomposting

Vermicomposting is a mesophilic process of composting using epigeic earthworms and differs from ordinary or conventional composting in several ways. It contains cocoons, excreta and undigested feed of earthworms which is highly rich in antibiotics, vitamins and enzymes like cellulase, protease, amylase, chitinase and lipase. These enzymes continue disintegration of organic matter after excretion from the worms as casts. Though nutrient value of vermicompost and vermicast is always lower than any standard chemical fertilizer but the nutrient value in this compost is better than conventional compost. Advances in vermiculture technology have recently led to novel products like vermiwash. This product has now not only caught the attention of commercial vermiculturists but also the farmers. Farmers in their own way have started collecting vermiwash for foliar application.

Vermicompost Preparation by Pit and Heap Methods

- Open permanent pits of 10 feet length 3 feet width 2 feet deep are constructed under the tree shade, which is about 2 feet above ground to avoid entry of rainwater into the pits.
- Brick walls are constructed above the pit floor and perforated into 10 cm diameter 5-6 holes in the pit wall for aeration. The holes in the wall are blocked with nylon screen (100 mesh) so that earthworms may not escape from the pits.
- Partially decomposed dung (dung about 2 months old) is spread on the bottom of the pits to a thickness of about 3-4 cm. This was followed by addition of layer of litter/residue and dung in the ratio of 1:1 (w/w).
- A second layer of dung is then applied followed by another layer of litter/crop residue in the same ratio up to a height of 2 feet.
- Three species of epigeic earthworm's viz., *Eisenia foetida*, *Eudrillus eugineae* and *Perionyx excavatus* are inoculated in the pit.
- Moisture content is maintained at 60-70% throughout the decomposition period.
- Jute bag (gunny bags) are spread uniformly on the surface of the materials to facilitate maintenance of suitable moisture regime and temperature conditions.
- Watering by sprinkler is often done.

The materials are allowed to decompose for 15-20 days to stabilize the temperature because to reach the mesophilic stage, the process has to pass the thermophilic stage, which comes in about 3 weeks. Earthworms are inoculated in the pit or heap with 10 adult earthworms per kg of waste material and a total of 500 worms are added to each pit or heap. The materials are allowed to decompose for 110

days. The forest litter was decomposed little earlier (75 to 85 days) than farm residue (105 days). About 450-500 kg of vermicast can be harvested from one ton of residue mixture within 3 months.

Table 2. Standards of compost as described in Fertilizer Control Order (1985)

Parameter	Compost
Moisture percent by weight	15.0-25.0
Colour	Dark brown to black
Odour	Absence of foul odour
Particle size	Minimum 90% material should pass through 4.0mm IS sieve
Bulk density (g/cm ³)	<1.0
Total organic carbon, percent by weight, minimum	16.0
Total nitrogen (as N), percent by weight, minimum	0.5
Total phosphates (as P ₂ O ₅), percent by weight, minimum	0.5
Total potash (as K ₂ O), percent by weight, minimum	1.0
C:N ratio	20:1 or less
pH	6.5-7.5
Conductivity (as dsm ⁻¹), not more than	4.0
Pathogens	Nil
Arsenic (as As ₂ O ₃)	10.0
Cadmium (as Cd)	5.0
Chromium (as Cr)	50.0
Copper (as Cu)	300.0
Mercury (as Hg)	0.15
Nickel (as Ni)	50.0
Lead (as Pb)	100.0
Zinc (as Zn)	1000.0

Chapter 3

Methodology

3.1 Development of microbial consortia

Pusa Decomposer: A consortium of seven hypercellulolytic fungal cultures, namely *A. awamori* ITCC 8945; *A. clavatus* ITCC 8306; *T. harzanium* ITCC 8946; *A. niger* ITCC 7790; *T. asperellum* ITCC 7793; *P. oxalicum* ITCC 6587 and *Ch. globosum* ITCC 3680 was optimized for compost production on the basis of their lignocellulolytic enzyme production potential. The consortium has been effectively used for composting of diverse agricultural wastes such as paddy straw, fruit waste, vegetable waste and garden waste.

3.2 Qualitative screening of cellulolytic isolates: The cellulolytic ability of the fungal isolates was assessed by Congo Red test on the basis of zone of clearing on Carboxy methyl cellulose (CMC) agar plates.

Congo Red Test (Teather and Wood, 1982): The CMC agar plates were prepared by adding 1% carboxymethyl cellulose (low viscosity) and 2% agar to the following basal medium ($(\text{NH}_4)_2\text{SO}_4$: 0.5 g; KH_2PO_4 : 1.5 g; K_2HPO_4 : 5.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.1 g; Yeast extract: 0.1 g; NaCl: 0.2 g). The final volume of medium was made to 1 liter with distilled water. Medium was autoclaved at 15 psi for 15 minutes. The organisms were point inoculated on the CMC agar plates and incubated at 30°C. After five days, the plates were flooded with Congo Red solution (1 mg/ml in distilled water). The dye was decanted after 20 minutes and the plates were flooded with 5M NaCl. After 20-30 minutes, the NaCl was decanted and CMC case producing colonies were seen to be surrounded by a pale orange to clear zone against an otherwise red background.

3.2 Quantitative screening of cultures : For this purpose, the cultures were grown in Reese's mineral medium with chopped paddy straw as sole carbon source.

Preparation of fungal inoculum: A bit of fungal mycelium was aseptically transferred to a petri dish having 30 ml of potato dextrose agar medium. After 48 hours of incubation at 30°C, one agar

plug (6 mm) from the growing edge of the colony was scooped out using a sterilized cork borer and used as an inoculum.

Production of crude enzyme: Reese's (1956) mineral medium (100 ml) supplemented with rice straw (1%) as carbon source was sterilized in 250 ml Erlenmeyer flask at 15 psi for 30 minutes and used for submerged fermentation (SmF). For fungi, each flask was inoculated with one agar plug of diameter 6.0 mm taken from the edge of 48 hours old fungal colonies and incubated at 30°C on a rotary shaker. Two sets of flasks were used for each fungus and one set was withdrawn after 7 days and other set after 15 days and filtered through Whatman filter paper No. 1 to collect the filtrate for estimating enzymatic activity. The filtrate was stored at 4°C until use for estimating the activity of extracellular lignocellulolytic enzymes.

Determination of Carboxymethyl Cellulase (CMCase) activity: The carboxymethyl cellulase (Endo- β -1-4 glucanase) activity of cell free culture filtrates was estimated by the method described by Ghose et al. (1983). An aliquot of 0.5 ml of enzyme filtrate was incubated with 2% carboxymethylcellulose (0.5 ml) at 50°C for 30 minutes. The reducing sugar was estimated as described for the FPase activity. An enzyme blank was prepared similarly without the substrate. The enzymatic activity of filtrate was expressed as unit per ml (U/ml) which is defined as the amount of enzyme, which liberates one μ g of reducing sugars per minute under assay conditions.

Determination of cellobiase activity: The Cellobiase (β -glucosidase) activity of cell free culture filtrates was estimated by the method described by Wood and Bhat (1988). To 0.5 ml of culture

filtrate in test tubes, 0.5 ml of substrate solution was added. Enzyme blanks were also prepared similarly without the substrate. All the tubes were incubated at 50°C for 30 minutes. After incubation, 1.5 ml of Glycine buffer was added to each tube and the absorbance was taken at 430 nm. The amount of p-nitrophenol produced was determined spectrophotometrically at 430 nm from a standard curve of p-nitrophenol (20-200 μ g). One unit of cellobiase enzyme is defined as the amount of enzyme required to release one μ g of p-nitrophenol per ml per minute.

Determination of xylanase activity: The xylanase activity of cell free culture filtrates was estimated by the method described by Ghose and Bisaria (1987).

3.5 Estimation of total Nitrogen (AOAC) in soil:

Nitrogen content in the sample was determined by micro-kjeldahl method. Sample (100mg) was weighed and taken in a digestion flask. Concentrated sulfuric acid (5ml) and catalyst mixture

(100mg) were added to the flask and digestion process were performed until the solution became colourless. The content of flask was transferred from digestion flask and made up the volume to 25ml. Distillation was performed in distillation unit with 5 ml digested sample and 10 ml of 40% NaOH solution. Further, ammonia released were trapped in 25 ml of 1% boric acid solution containing mixed indicator. Once the ammonia was trapped, this was back titrated with 0.01N hydrochloric acid solution. A blank was maintained only with distilled water

N% = ml of HCl in sample solution- ml of HCl in blank sample /weight of sample (100mg)

X Normality of HCl X 14

3.6 Estimation of soil dehydrogenase activity (Casida et al. 1964)

Air dried soil (20g) was mixed with 0.2g CaCO₃. Distributed 6g of this mixed soil in screw cap tube, added 1 ml of 3% TTC aqueous solution and 2.5ml distilled water. The contents were mixed thoroughly with glass rods. Tubes were covered properly and incubated at 37°C for 24 hours. The cover was removed after 24 hours and added 10 ml methanol. The mixture was filtered after vortexing the mixture for 1 minute. Again added 10ml methanol and repeated the same step. Absorbance was recorded at 485nm. Concentration of TPF released

3.7 Determination of soil alkaline phosphatase activity was done following the method of Tabatabai and Bremner (1969). The concentration of PNP released was calculated by referring standard curve prepared with different concentrations of p-nitrophenol. Alkaline phosphatase activity was expressed as µg PNP released g⁻¹ hr⁻¹.

3.8 Enumeration of total fungi and Bacteria in the field

The soil samples were serially diluted and plated on Nutrient agar and Potato dextrose agar for enumeration of total bacterial and total fungal count respectively.

Compatibility test: Compatibility test was performed by the modified method of Tehrani et al. (2001). Selected fungi showing the maximum enzymatic activity were tested for their compatibility to grow together, by point inoculation on single PDA plate. The plates were incubated at 30°C for 4 days in a BOD incubator.

Pusa Decomposer (PD); A microbial consortium has been developed for rapid decomposition of paddy straw, both for *in-situ* and *ex-situ* decomposition. All the selected fungi were produced in mass using modified jaggery medium and packed in the form of capsules. Liquid consortium was provided for demonstrations at farmer's field.

Four capsules of this product can be scaled up to 25L liquid formulation which can be applied *in-situ* to 1.0 ha of combine fitted with SMS harvested rice field having 5-6tonnes of paddy straw. It accelerates process of paddy straw decomposition and field ready for potato, peas and wheat sowing in 20-25 days following conventional tilling (CT) practices. CT is one of the major activities in the Govt. funded CRM scheme. Its use enriches the soil with organic carbon (OC) and nutrients while improving the soil biological properties. Pusa Decomposer is a long term sustainable solution for management of paddy straw in conjunction with CT, Happy Seeder and Super Seeder options. We worked out a tentative comparative cost of PDSM systems as below.

Table 3. Comparative cost of different paddy straw management systems

S.No.	Operations	Cost (Rs./acre) of different options			
		Happy Seeder	Super Seeder	Conventional Tillage (CT)	CT with PD
1.	Happy Seeding	1300	-	-	-
2.	Super seeding	-	2000	-	-
3.	Chopper	-	-	1200	1200
4.	Rotavator (mixing residue)	-	-	1000	1000
5.	Irrigation	400	400	400	400
6.	Rotavator (before sowing)	-	-	1000	1000
7.	Seed cum Ferti-drill	-	-	600	600
8.	Cost of PD and application	-	-	-	300
	Total	1700	2400	4200	4500

3.9 Selection of study area:

In situ locations: Five districts of Punjab viz: Gurdaspur, Mukerian, Amritsar, Srimuktsar sahib and Fazalika and one of Haryana which was Village Anwal, Rohtak.

Ex –situ locations:

Twelve districts viz: VPO- Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab; VPO- Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab; VPO- Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab; Village-Machhiwala, Near Ramdas, Teh. Ajnala; Village-Machhiwala, Near Ramdas, Teh. Ajnala; Vill. Tuto Mazara, Mahilpur, Distt. Hoshiarpur; Vill. Hayatpur Teh. Mukerian Distt. Hoshiarpur; Vill. Kingra, Distt. Malout Balim, Gurdaspur; Vill. Anwal, Distt. Rohtak, Haryana; Bhavdin Kheda Sadh. Trial was also carried out at Sonipat, village Badwasini and Sirsa, Sardulgarh village.

Soil samples were collected from each study field from 5 points 100g each, packed in polythene bags and brought to the laboratory where composite was made. The soil was homogenized, sieved and then analyzed for different parameters.

Field application methodology: A standard protocol was followed for the *in situ* straw degradation using Pusa Decomposer. Pusa Decomposer in 10 L was mixed in 200L of water and was applied/sprayed on one acre field having approximately 2-2.5 ton straw. The SOPs followed was to first spray by tractor driven, followed by incorporation using Rotavator and the irrigating the field to ensure moisture. This procedure allowed the degradation to take place in the shortest possible time and enabled the farmer to go for his wheat sowing. Below are the farmers field photos (Fig 4).



Fig 4. Village Kattiyawali, ShriMuktsar

Chapter 4

Result and Discussion

Our major focus was on agri-residue management using Pusa Decomposer under *in situ* farmer field conditions. However, as an in-house research program studies on *ex-situ* management using compost technologies using pit and windrow method and developing machinery for agri-residue management were carried out.

- All the staff was recruited after advertisement in newspaper and IARI web site on time, most of the chemicals, glassware were procured on time and for travel to villages the vehicle was hired from IARI rate contract travel agency.

4.1 Laboratory studies

- Paddy straw collection was done from the IARI experimental fields. Paddy straw of eight different varieties namely Pusa Basmati 1, Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 1637, Basmati 1407 and Tarawari Basmati 1 was collected after harvesting. Unchopped paddy straw of each variety was inoculated with liquid inoculum of Pusa decomposer @ 5 litres per tonne. The material was mixed properly along with cowdung and then kept in polythene bags and kept for decomposition. After 15 days the material in the bags was mixed properly and kept further for decomposition. The straw of all the varieties degraded within 25-30 days of inoculation and the decomposed material was collected in trays. This was a preliminary laboratory study to ascertain the degradation potential of Pusa Decomposer, a microbial consortium developed at Division of Microbiology, IARI, on different varieties of paddy. Phytotoxicity tests have shown the compost to be of good quality.

4.2 Rapid decomposition of paddy straw using pit method

- The crop residues have been traditionally used for preparing compost. For this, crop residues are used as animal bedding and are then heaped in dung pits. In the animal shed each kilogram of straw absorbs about 2-3 kg of urine, which enriches it with N. The residues of rice crop from one hectare land, on composting, give about 3 tons of manure as rich in nutrients as farmyard manure (FYM). Indian Agricultural Research Institute (IARI), New Delhi, has successfully

developed a biomass-compost unit for making of good quality compost. This mechanized unit efficiently uses waste biomass and crop residues generated in the IARI farm. The decomposition process, which is hastened by a consortium of microorganisms, takes 75-90 days. During the year 2019-20, the unit prepared about 5000 tons of compost.

- **For pit method:** 100 kilograms of compostable material for each treatment T1 and T2 was thoroughly mixed and moisture was adjusted to 80% water holding capacity (WHC). The material was put in cemented pits at Division of Microbiology, IARI and allowed to decompose. The contents were turned after 15, 30, 60 and 75 days and samples were drawn at each interval and analysed for mesophilic and thermophilic bacterial and fungal population, multifunctional bacterial population mainly P solubilisers, N fixers, lipolytic, amylolytic, Gram positive, and Gram negative bacterial population. Different enzyme activities cellulases, xylanases and proteases were also estimated. The cellulases activity increased during decomposition and was found maximum at 30 days and declined at 60 days (Table 4) for uninoculated treatment whereas a decline in cellulose activity was observed after 60 days in inoculated treatment and it was found higher than control due to the inoculation of fungal consortium. Similarly, the initial xylanase and protease activity increased in inoculated treatment as compared to uninoculated treatment.
- Population of mesophilic bacteria and fungi was found highest at 30 days in inoculated treatment (Table 5) while bacterial population declined after 30 days but fungal population showed an increase till 60 days and declined after 60 days whereas thermophilic bacteria and fungi were found maximum at 15 days and bacterial population declined at 30 days but fungal population increased at 60 days and declined at 75 days. Among bacterial population, gram positive bacteria predominated the population as compared to Gram negative population.

Table 4: Changes in various enzyme activities during composting of paddy straw

Treatment	Cellulase activity(mg reducing sugar kg ⁻¹ dry matter h ⁻¹)				Xylanase activity (mg reducing sugar kg ⁻¹ dry matter h ⁻¹)				Protease activity (mg tyrosine kg ⁻¹ dry matter h ⁻¹)			
	15	30	60	75	15	30	60	75	15	30	60	75
T1 Paddy straw + Cow dung	26	81	51	36	58	65	53	30	309	530	954	797
T2 Paddy straw + Cow dung +Pusa decomposer	41	197	277	63	71	108	127	83	552	1240	1520	907

Initial cellulase activity : 8 mg reducing sugar kg⁻¹ dry matter h⁻¹ ; Initial xylanase activity: 22 mg reducing sugar kg⁻¹ dry matter h⁻¹ ; Initial Protease activity: 159 mg tyrosine kg⁻¹ dry matterh-1

Table 5: Evaluation of Mesophilic and thermophilic bacterial and fungal population

Treatment	Mesophilic bacteria X 10 ⁹ g ⁻¹			Thermophilic bacteria X 10 ⁵ g ⁻¹			Mesophilic fungi X 10 ⁸ g ⁻¹			Thermophilic fungi X 10 ⁵ g ⁻¹		
	0	30	60	0	30	60	0	30	60	0	30	60
Paddy straw + Cow dung	30	164	75	0	10	5	2	14	17	0	4	6
Paddy straw + Cow dung + Pusa decomposer	30	207	114	0	24	20	5	25	32	0	21	38

The range of initial pH in pits was from 4.5 to 5.5 and it started dropping till 7 days and reached less than

4.0 within 7 days and after 7 days again it started increasing and a maximum of 7.9 was observed after 30 days. Electric conductivity: The EC range was 3 - 6 U siemens / cm was observed in finished product while it fluctuated when thermophilic phase was observed. The initial moisture content was recorded 60.8% and after 15 days it increased to 68.9 % and later in final product it decreased to 45 .2% in all the treatments. The result of germination test with *Lepidum sativum* seeds showed that all the samples taken at the maturation period had GI values of greater than 50%, which indicates a phytotoxin-free compost product. All the germination rates treated with 1% extraction of composting products were above 95%, indicating the compounds present in the raw wastes or produced during the first days of composting as intermediate products of microbial metabolism, were degraded within 30 days of composting.

Development of Compost Turner cum Mixer: A powerful technology to convert the biomass into nutrient rich compost by windrow / pit method using microbial culture and mechanical intervention. Suitable for thorough turning and mixing of cow dung, farm residues and biomass for compost preparation.



- ***In-situ* demonstrations**

Under the Niti Aayog banner a slogan was prepared “Jalao Nahi Galao” and as the harvesting season of paddy started many trials at IARI and farmers field were organized in various villages of Punjab. The trial was initiated at IARI field where after harvesting of paddy, Pusa Decomposer was sprayed @ 10litre/acre and whole straw was turned in the soil using Rotavator and the field was irrigated. Soil samples were collected periodically from initial till 25 days to estimate the various soil health parameters and study the degradation of straw. Simultaneously tours were also conducted to various villages of Punjab to demonstrate the application of Pusa Decomposer at farmer’s field with the aim to make them aware about ill-effects of burning and how they can benefit by decomposing the straw into nutrient rich manure by use of Pusa Decomposer. The villages covered were from district Gurdaspur, Mukerian, Amritsar, Moga, Srimuktsar sahib and Fazalika (Fig. 5). All progressive farmers (Table 4) were provided with Pusa Decomposer capsules as well as liquid solution for spraying in field (Fig 6). Soil samples were collected. The role of Niti Aayog was also highlighted in all the farmers meet. We organized three workshops with Punjab farmers in the fields by close interactions (Fig.7).

- ***Ex-situ* Demonstrations**

We conducted *Ex-situ* management of paddy straw in different villages of Haryana and Punjab at **twelve sites** at farmer’s field. At each site a good number of farmers were present having interest in composting and they were explained the benefits of Pusa Decomposer for *ex-situ* management of paddy straw along with cow dung. All the three methods of composting namely pit, heap, and windrow were explained in detail and farmers were

demonstrated pit as well as heap method. The farmers were highly satisfied with the technology as a low cost economically viable as the only output needed was Pusa Decomposer and rest of the things like Paddy straw and cow dung were already available with them.

Windrow method

In village Anwal, District Rohtak, Haryana, windrow method of composting was demonstrated by the team on Shri. R. N. Arichwal farm where he used machines for making windrows and sprayed Pusa Decomposer and applied the ready compost for vegetables. In the village Kingra a good gathering of farmers was observed and both methods: heap and pit were demonstrated. Farmers themselves suggested to use tractor operated machines to mix the straw and cow dung along with decomposer and prepared a small pile and used another small machine to prepare the heap to convert the rice straw into compost. Therefore, all methods of composting was demonstrated and farmers were advised to give turning after every fifteen days. The evaluation of complete decomposition was also provided to farmers in detail. The farmers reciprocated the team efforts and wanted continuous monitoring by the team.

Table 6: List of Punjab and Haryana Farmers for *ex-situ* paddy straw decomposition in year 2021-2022

S.No	Name	Mobile number	Address
1.	Gurmeet Singh	9872397000	VPO- Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab
2.	Ranjeet Singh	8847454682	VPO- Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab
3.	Manpreet Singh	7307200077	VPO- Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab
4.	Jasmittarjit Singh	9855461914	Village-Machhiwala, Near Ramdas, Teh. Ajnala
5.	Sukhwant Singh		Village-Machhiwala, Near Ramdas, Teh. Ajnala
6.	Sukhwinder Singh S/o Balwant Singh	9888972473	Vill. Tuto Mazara, Mahilpur, Distt. Hoshiarpur
7	Onkar Singh S/O Harbinder Singh	9914503010	Vill. Hayatpur Teh. Mukerian Distt. Hoshiarpur
8	Lakhwinder Singh	6284531232	Vill. Kingra, Distt. Malout
9	Cap. Kashmir Singh	8054013648	Balim, Gurdaspur
10	R. N. Arichwal	9711190973 44	Vill. Anwal, Distt. Rohtak, Haryana

11	Baljeet	9813080997	Bhavdin
12	Rajesh	9991648719	Kheda Sadh



Village: Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab



Village: Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab



VPO- Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab



Village: Hayatpur Teh. Mukerian Distt. Hoshiarpur



Village-Machhiwala, Near Ramdas, Teh. Ajnala



Village: Tuto Mazara, Mahilpur, Distt. Hoshiarpur



Village- Anwal, Distt. Rohtak, Haryana



- **Field Day**

A field day was organized on 12th March, 2022 at village Anwal, Distt. Rohtak, Haryana. A progressive farmer Sh. R. N. Arichwal is using Pusa Decomposers technology for both *in-situ* and *ex-situ* management of paddy straw. A few farmers from Haryana, Punjab also accompanied IARI team consisting of Nodal Officer Dr. I M Mishra, Head, Microbiology Division Dr. Sunil Pabbi, Principal Scientist Dr. Livleen Shukla and Senior Scientist Dr. Satish Lande and Supporting staff Sh. Goverdhan Thakur and SRF & YP II. During the interactive discussions farmers were apprised about the advantage of applying Pusa

Decomposer for faster degradation of agri residue, explained the *in situ* and *ex-situ* methods as well as SOPs. All were aware of ill-effects of paddy straw burning and appreciated the work done by Sh. Ram Niwas.

- **Compost**

Sh. Ram Niwas ji prepared compost from paddy straw using heap and windrow method applying Pusa Decomposer and suggested other farmers to do the composting using Pusa Decomposer. Quality compost shall be a revenue generating means to the farmer.



Village- Anwal, Distt. Rohtak, Haryana

Success Story of Farmers:

1. **Gurmeet Singh:** Sh. Gurmeet Singh is the resident of village Kattianwali, Teh. Malout, Distt-Sri Muktsar Sahib Punjab. He has total 16 acre land out of which 4 acre land was totally barren, only wild sarkanda was growing, then after interaction with pusa scientists, he start using bio fertilizers, decomposers such as Azotobacter, VAM and phosphate solubilizing bacteria and Pusa Decomposers since 2016. He used *in-situ* method of decomposing of paddy straw and produce 2.5 quintal per acre which was not possible before. He also suggested other farmers of village to follow this practice and helped them getting benefit of this technology. More than 200 farmers adopted *in-situ* Pusa Decomposer technology and incorporated residue in more than 1000 acres in village kattianwali out of total 2285

acres (43.8%).







2. **R. N. Arichwal:** Sh. Arichwal is the resident of village Anwal, Distt-Rohtak, Haryana. He told to IARI team that he was not getting proper crop even after giving proper manure, IARI team suggest him to use Pusa Decomposer and told him every method of making compost like, pit, heap and windrow, since then Sh. R. N. Arichwal is using Pusa Decomposers technology for both *in-situ* and *ex-situ* management of paddy straw. He used windrow and heap method for producing compost using Pusa Decomposer. His land had high pH, high salinity and low N, P, K content but after using Pusa Decomposer and other product like VAM, phosphate solubilizing bacteria, potash solubilizing bacteria, *Azotobacter* his field quality was improved. In the end he told that, I am grateful to using Pusa Decomposers and other products, which decrease the pH, salinity and increase the porosity of soil, however

improved the overall yield of field. He also produced 10 acre seedling of sugarcane using compost prepared from Pusa Decomposer.



- Rajesh Saini:** Sh. Rajesh Saini is the resident of Village: Meerpur, Block-Mukerian, Distt. Hoshiarpur, Punjab. He said management of paddy residue is of utmost important and also discussed other problems related to low yield in his 6 acres of land. He was advised to use of Pusa Decomposer, explained and demonstrated the protocols of composting through pit, heap and windrow methods. Understanding the benefits he started spraying the Pusa Decomposer on his paddy residue. He observed fast degradation in the field with in 25 to 30 days. He is now regularly using the decomposer since 2019. He reports an increase in crop yields, better tillering and reduced chemical inputs. For his low cost and innovative

- **Table 7: List of Punjab Farmers for *in situ* paddy straw decomposition**

S.No	Name	Mobile number	Address
1.	Tarsem Singh	9914609544	Village, Bhagwa, Kalanaur Amritsar
2.	Gurmeet Singh Sandhu	9872397000	Sri Mukstar Saheb, Punjab
3.	Sukhdeep Hayer	9404600003	Sri Mukstar Saheb, Punjab
4.	Rajesh Saini	9815169598	Village:kotli khas, hoshiarpur
5.	Capt. Kashmir Singh	8054013648	Gurdaspur, Punjab
6.	Balwinder Singh	9569236000	Fazilka, Punjab
7	Tarsem Singh Saran	9463073787	Moga, Punjab

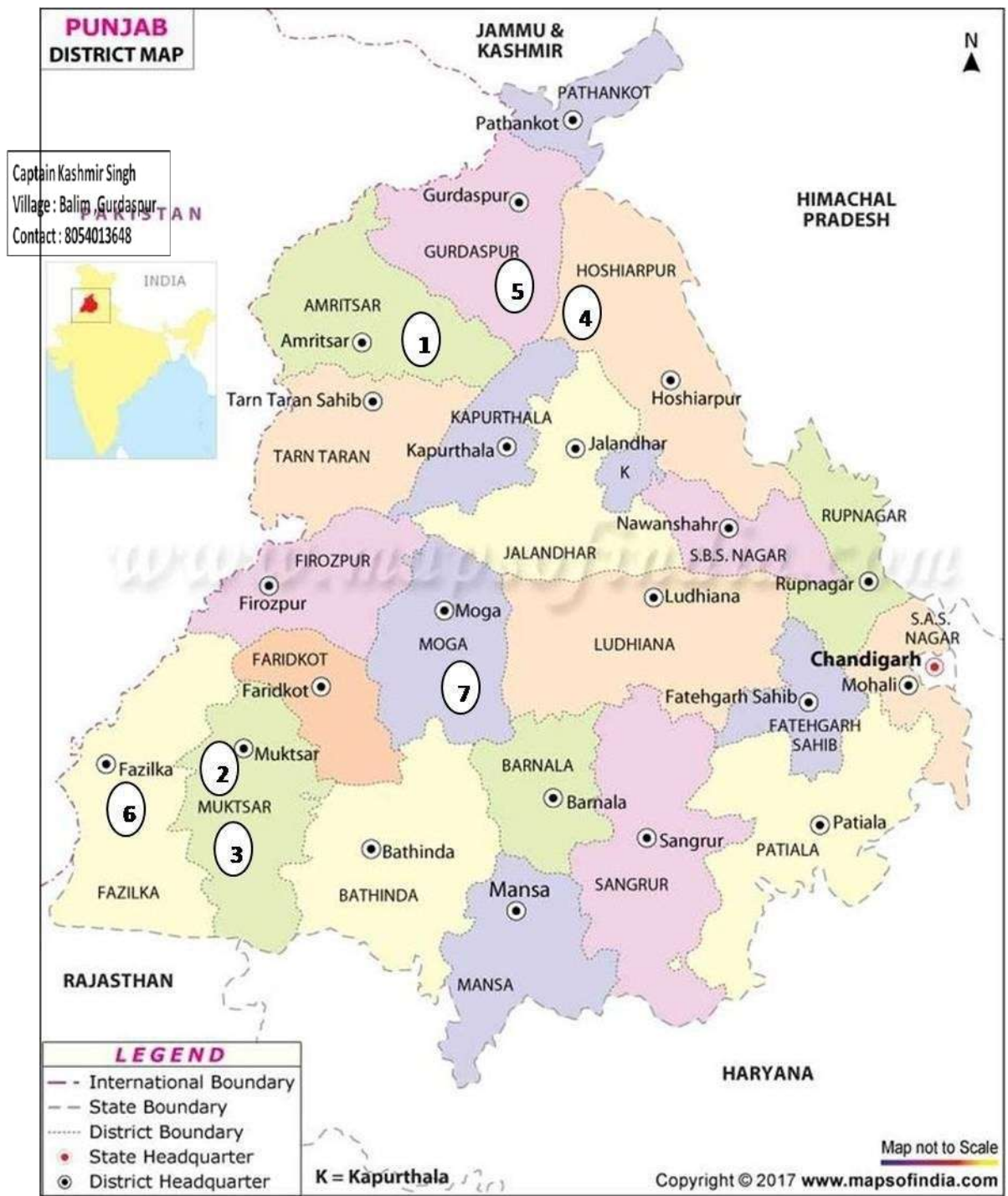


Fig 5. Location map of Punjab districts where Pusa Decomposer was applied by the researchteam in farmers fields (*in-situ*)

Spraying: 17/10/2020



After 25 days



**Germination- 23/11/2020 (Seed was broadcasted on
11/11/2020)**

Fig 6. Application of Pusa Decomposer in farmers field





नीति आयोग

फसल अवशेष प्रबंधन : सूक्ष्मजीवी एवं यांत्रिकी समाधान






प्रयोजक : नीति आयोग (भारत सरकार)

आयोजक : आई. सी. ए. आर. : भारतीय कृषि अनुसंधान संस्थान
नई दिल्ली-110012



Village: Balim (Gurdaspur)



Village: Salina, Moga



Village : Kohali, Amritsar



Village : Kattianwali, Shri Muktsar Sahib

Fig.7. Field demonstration of PUSA Decomposer and farmer workshops in Punjab State

- Soil health parameters like microbial population, soil enzymes and soil OC and available N were estimated (Fig 8 and Table 7, 8). The soil dehydrogenase activity improved from $13\mu\text{g TPF g}^{-1}/24\text{ hr}$ at 0 days to $22\mu\text{g TPF g}^{-1}/24\text{ hrs}$ after 25 days of application of Pusa Decomposer. Similarly the bacterial and fungal count improved from 25×10^8 to 200×10^8 cfu/g soil and 10×10^4 cfu/g soil to 30×10^4 cfu/g soil respectively from 0 days to 25 days of application of Pusa Decomposer. (Fig 8)

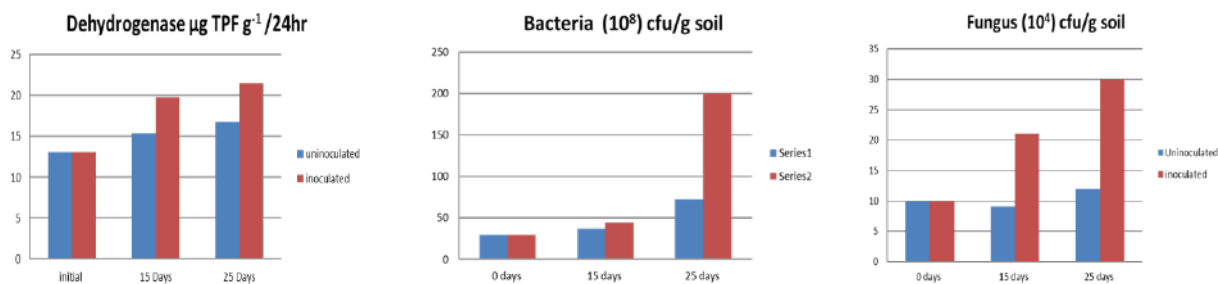


Fig. 8: Soil biological parameters over a period of time in PD treated and untreated fields.

- Soil samples were analysed for organic C (%) and available N (kg/ha) at zero day and after 25 days of Pusa Decomposer application. Increase in the above parameters was found in samples collected after 25 days of Pusa Decomposer application. Soils collected from Punjab and IARI fields, showed an increase in microbial biomass carbon (MBC). Wheat was the rabi season crop in all Punjab farmers' fields where Pusa Decomposer was applied. The farmers reported wheat yield 3226 variety 26q /acre on an average where Pusa Decomposer was sprayed in Kattianwali, Shri Muktsar Sahib Kohali and Amristar .

- **Table 8:** Range of percent increase in Soil OC, available N, Soil dehydrogenase activity and Microbial Biomass C in field samples where PD was applied after 25 DAS.

Villages	Samples	Organic C (%) incr	Available N (kg/ha) Incr	DHA $\mu\text{g TPF g}^{-1}$ soil 24hrs^{-1} incr	MBC $\mu\text{gm of biomass/gm of soil 0D - 25D}$
Punjab	10	6 - 15%	05 - 20%	2 - 5 μg	49 - 149
IARI	10	3 - 5%	8.7 - 21.8%	1 - 3 μg	35 - 192

- At IARI fields, effect of Pusa Decomposer on CH_4 , CO_2 and N_2O emissions for the first 12 days was measured. It was found that CO_2 emissions increased in Pusa Decomposer treated plots compared to non-treated plots (Fig. 9) indicating the microbial activity. This is an indication that degradation is being carried out by the microbial consortium. N_2O emissions were found negligible.

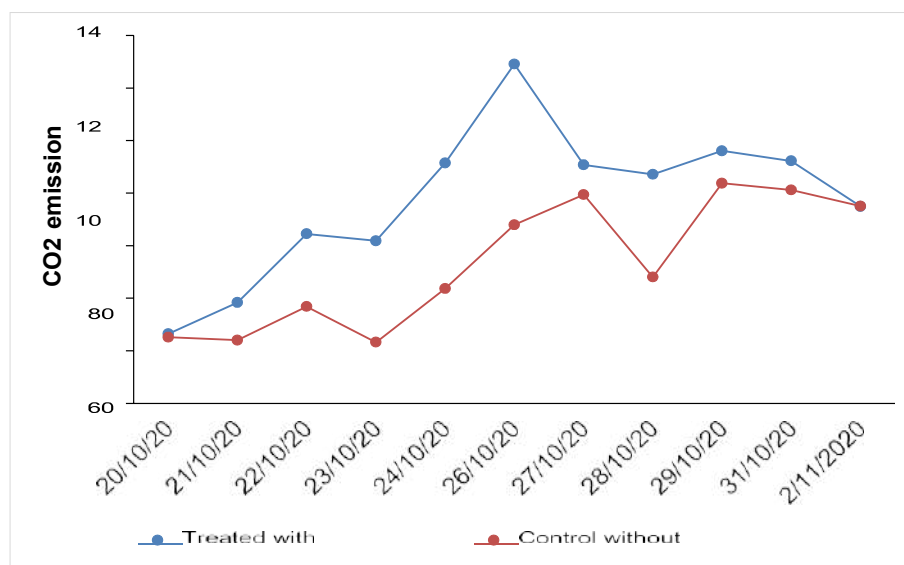


Fig 9: CO₂ emissions in PD treated and untreated plots

Table 9: Changes in NPK content during *in-situ* decomposition at farmer's field

Field Name	N (kg/ha)		P (kg/ha)		K (kg/ha)	
	0 days	25 days	0 days	25 days	0 days	25 days
BALIM 1	209.663	229.927	10.325	12.043	116.683	149.840
Control						
BALIM 2	209.067	250.870	10.971	12.450	116.550	151.280
BALIM 3	214.677	271.680	10.383	12.540	116.700	151.290
BALIM 4	198.160	213.243	10.370	12.610	116.800	151.227
BALIM 5	183.600	209.067	10.370	12.627	116.697	151.257
KOHALI 1	206.607	223.600	20.150	21.560	268.867	324.700
Control						
KOHALI 2	229.957	271.000	20.233	21.787	268.800	330.267
KOHALI 3	250.067	334.000	20.200	20.820	268.800	331.400
KOHALI 4	271.000	292.000	20.187	21.820	268.710	331.893
KOHALI 5	239.000	251.000	20.243	21.600	268.800	330.267
Katianwali	157.333	157.000	60.000	90.900	642.000	825.000
Mukerian	209.000	209.000	24.450	22.700	105.323	127.000
CD @ 5%	4.044	4.833	2.97	2.509	4.321	5.372
S.Em	5.759	8.226	3.105	2.217	6.576	10.160

Chapter 5

Executive Summary

TOR I: To develop a technology to convert crop bio waste (particularly paddy) into farm compost in less than six months period with economically efficient method

- A microbial solution known as Pusa Decomposer has been developed by the scientists of Division of Microbiology, Indian Agricultural Research Institute, New Delhi, to convert paddy residue into manure in 20-25 days.
- It is an Economically Efficient Technology. Using cheap source of C like jaggery, 25L of the microbial solution can be developed. 50g of besan (Rs 6.0) and 750g of jaggery is needed for 25L preparation @40/- per kg. Total cost for preparation of one ha solution of Pusa Decomposer is approx. Rs. 86.0 (Rs. 50 for 4 capsules, Rs.30 jaggery, Rs. 6.0 besan).
- Along with Pusa Decomposer a standard operating protocol (SOPs) has also been developed for *In-situ* management of agri-residue using Pusa Decomposer. The protocol involves application of the Pusa Decomposer spray, followed by rotavator for proper mixing of the spray with the straw and light irrigation to ensure moisture in the field.
- Pusa Decomposer is a promising low cost technology for accelerated degradation of paddy straw in the field. The study proved the effectiveness of Pusa Decomposer for accelerated degradation of the paddy residue in Punjab. However, for successful and wide scale benefit of the impact of this technology, Punjab State Govt. must adopt it in a bigger scale. It should be made an integral part of the agriculture programs in the State.

TOR II: To convert bio waste into wealth and offer economically viable alternative to prevent burning of crop residues, stubble etc

- One ton of paddy residue burning releases 1391.9 Kg of carbon dioxide (CO₂), over 82.62 Kg of carbon monoxide (CO), 0.36 Kg oxides of sulphur (SOX), 0.06 Kg of N₂O, and 4.21 Kg of particulate matter. Our preliminary studies have shown that application of Pusa Decomposer had shown only 0.222 g/t of biomass of CO₂ and 4.6 mg/t of biomass of N₂O evolution.
- The farmers who had used Pusa Decomposer observed the benefits in terms of higher yields and better tillering in wheat which was the next crop. This is an economically viable alternative to prevent burning. Farmers could save one bag of urea. This approach for at least three years should help the farmers in the ease of the burden, discourage them from burning and convince them of the benefits of the application in terms of saving inputs as well as soil health and fertility.

- We have a 300 odd farmers in our group who are in constant touch with us for updates on PD and other aspects. Regular interactions with farmers telling them about the pollution hazards due to burning and saving on the chemicals for crop productivity for sustainable cultivation has made them understand the problem and acceptance of the technology.

TOR III: Create possibility of giving an added value to the agricultural activity through the availability of an additional source of income for managing the treatment and selling resultant compost.

- Under *ex-situ* conditions, the farm residue can be decomposed using Pusa Decomposer and nutrient enriched compost which is generated can be used by the farmer for his own requirements. However, if this activity is carried out as a community venture, it has the potential to generate self-sustainability to village youth and generate income, besides managing the agri residue effectively.
- The *ex-situ* demonstrations were carried out at 12 different sites, viz: Kattianwali (3), Machiwala, TutoMazara (2), Hayatpur, Kingra, Balim, Anwal, Bhavdin, and Kheda Sahib. Pit method was common to all sites, at some locations windrow and heap methods were also demonstrated. Ex-situ is an alternative method of residue management by which farmers can prepare quality compost which can become as a source of income by the sale of the same. At each demonstration, farmers from the village and nearby villages gathered.
- The selling rate of compost by farmers is @Rs 13-15 per kg of the compost. The direct sale would be an additional source of income to the farmer.

TOR IV: Availability of a new material to improve the soil fertility with the application of compost (insubstitution of chemical fertilizers).

- Pusa Decomposer mediated compost/manure from agri-residue can be applied for improving the soil fertility & health. Many farmers have reported to have cut down on their use of urea by one bag.
- The application of Pusa decomposer for *in-situ* decomposition of paddy straw enhanced the fertility of soil. Organic C increased in the range of 6-15% and available N in the range of 5-20%.
- Soil health parameters like microbial population, soil enzymes and soil OC and available N were estimated from the soils in which Pusa Decomposer was sprayed, results indicate improvement of all the parameters.
- The soil dehydrogenase activity improved from 13 $\mu\text{g TPF g}^{-1} / 24 \text{ hr}$ at 0 days to 22 $\mu\text{g TPF g}^{-1} / 24 \text{ hrs}$ after 25 days of application of Pusa Decomposer approx. 69% increase.
- Similarly, the bacterial and fungal count improved from 25 x 10⁸ to 200 x 10⁸ cfu/g soil and 10 x 10⁴ cfu/g soil to 30 x 10⁴ cfu/g soil respectively from 0 days to 25 days of application of Pusa Decomposer.

- Soil samples were analysed for organic C (%) and available N (kg/ha) at zero day and after 25 days of Pusa Decomposer application. Increase in the above parameters was found in samples collected after 25 days of Pusa Decomposer application.

BIBLIOGRAPHY

- Bakalova, N., Petrova, S., Atev, A., Bhat, M. and Kolev, D. (2002). Biochemical and catalytic properties of endo-1, 4- β -xylanase from *Thermomyces lanuginosus* (Wild and mutant strains). *Biotechnology Letters* 24: 1167-1172.
- Ball, A.S., Betts, W.B., McCarthy, A.J. (1989). Degradation of ligninrelated compounds by actinomycetes. *Applied Environmental Microbiology* 55, 1642-1644.
- Bhattacharjee, B., Saha, N., Debnath, A., Sen, S., Roy, S. S. and Mukherjee, D. (2013). In situ Management of Rice Stubble in Relation to soil Nitrogen Status Vis-à-Vis Performance of Wheat Crop in an Entisol. *American-Eurasian Journal of Agriculture & Environmental Sciences*, 13(7): 943-956.
- Bisen, P.S., Ghosh, K. and Agarwal, G.P. (1982). Induction and inhibition of cellulase complex in *Fusarium solani*. *Biochemie und Physiologie der Pflanzen*, 177(7), pp.593-599.
- Biswas, S.R., Jana, S.C., Mishra, A.K. and Nanda, G. (1990). Production, purification, and characterization of xylanase from a hyperxylanolytic mutant of *Aspergillus ochraceus*. *Biotechnology and Bioengineering*, 35(3), pp.244-251.
- Blanchette, R. A. (1995). Degradation of the lignocellulose complex in wood. *Canadian Journal of Botany*, 73: 999–1010
- Bonilla, P. S. (2001). Variation in the cellulose, lignin and silica contents of various parts of different rice (*Oryza sativa* L.) cultivars. *Philippine Agricultural Scientist*, 84(2): 126-137.
- Borah, N., Pathak, P. K., Barua, R., Hazarika, K., Phukon, A. and Bezbaruah, K. P. (2018).

Stubble decomposition (in situ) of two rice varieties through microbial inoculation. *In Utilization and Management of Bioresources*, 65-76.

Bugg, T. D. H., Ahmad, M., Hardiman, E. M., Rahmanpour, R. (2011). Pathways for degradation of lignin in bacteria and fungi. *National production reports*, 28(12):1883e96

Cardoen, D., Joshi, P., Diels, L., Sarma, P. M. and Pant, D. (2015). Agriculture biomass in India: Part 1. Estimation and characterization. *Resources, Conservation and Recycling*, 102: 39-48.

Casida Jr, L.E., Klein, D.A. and Santoro, T. (1964). Soil dehydrogenase activity. *Soil science*, 98(6), pp.371-376.

Chahal, D.S. (1985). Solid-state fermentation with *Trichoderma reesei* for cellulase production. *Applied and Environmental Microbiology*, 49(1), pp.205-210.

Chang, A. J., Fan, J. and Wen, X. (2012). Screening of fungi capable of highly selective degradation of lignin in rice straw. *International Biodeterioration & Biodegradation*, 72: 26- 30.

Chellapandi, P. and Himanshu, J.M. (2008). Production of endoglucanase by the native strains of *Streptomyces* isolates in submerged fermentation. *Brazilian Journal of Microbiology*, 39(1), pp.122-127.

Choi, B.H., Lapham, L.W., Amin-Zaki, L. and Saleem, T. (1978). Abnormal neuronal migration, deranged cerebral cortical organization, and diffuse white matter astrocytosis of human fetal brain: a major effect of methylmercury poisoning in utero. *Journal of Neuropathology & Experimental Neurology*, 37(6), pp.719-733.

- Chung, I.M., Ahn, J.K. Yun, S.J. (2001) Identification of allelopathic compounds from rice (*Oryza sativa* L.) straw and their biological activity. *Canadian Journal of Plant Science* 81:815–819.
- Conrad, D. (1981). Enzymatic hydrolysis of xylans I. A high xylanase and β -xylosidase producing strain of aspergillus niger. *Biotechnology Letters*, 3(7), pp.345-350.
- Coronel, L. M., Joson, L. M. and Mesina, O. G. (1991). Isolation and screening of thermophilic fungi for cellulose production. *Philippine Journal of Science* 120: 379-389.
- Couturier, M., Navarro, D., Olive, C., Chevret, D., Haon, M., Favel, A., Lesage-Meessen. L., Henrissat, B., Coutinho, P. M., Berrin, J. (2012). Post-genomic analyses of fungal lignocellulosic biomass degradation reveal the unexpected potential of the plant pathogen *Ustilago maydis*. *BMC Genomics*, 13:57.
- Cullis, I. F., Saddler, J. N. and Mansfield, S. D. (2004). Effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. *Biotechnology and Bioengineering*, 85(4): 413-421.
- DAC, 2014. National Policy for Management of Crop Residues (NPMCR). Department of Agriculture & Cooperation, Ministry of Agriculture, Government of India, New Delhi. http://agricoop.nic.in/sites/default/files/NPMCR_1.pdf [Accessed on 1-07-2020].
- Dai, X., Hua, Y., Dai, L. and Cai, C. (2019). Particle size reduction of rice straw enhances methane production under anaerobic digestion. *Bioresource Technology*, 293: 122043.
- Darmwal, N. S. and Gaur, A. C. (1988). Associative effect of cellulolytic fungi and *Azospirillum lipoferum* on yield and nitrogen uptake by wheat. *Plant and Soil*, 107(2): 211-218.
- Dekker, R.F. and Richards, G.N. (1976). Hemicellulases: their occurrence, purification, properties, and mode of action. *Advances in Carbohydrate Chemistry and Biochemistry*, 32, pp.277-352.
- Dekker, R.F. (1983). Bioconversion of hemicellulose: aspects of hemicellulase production by

Trichoderma reesei QM 9414 and enzymic saccharification of hemicellulose. *Biotechnology and Bioengineering*, 25(4), pp.1127-1146.

Dordick, J.S., Klibano, A.M. and Marletta, M.A. (1986). Horse radish peroxidase catalysed hydroxylation: mechanistics studies. *Biochemistry* 25: 2946-2951.

Eriksson, K. E. L., Blanchette, R. A. and Ander, P. (1990). *Microbial and Enzymatic Degradation of Wood and Wood Components*. Springer, Berlin, Germany

Fan, Y., Zhang, Z., Wang, F., Li, J., Hu, K. and Du, Z. (2019). Lignin degradation in corn stover catalyzed by lignin peroxidase from *Aspergillus oryzae* broth: Effects of conditions on the kinetics. *Renewable Energy*, 130: 32-40.

Fernandes, T. A. R., da Silveira, W. B., Passos, F. M. L. and Zucchi, T. D. (2014). Laccases from Actinobacteria- What we have and what to expect. *Advances in Microbiology*, 4: 285–96.

Fujian, X., Hongzhang, C. and Zuohu, L. (2001). Solid-state production of lignin peroxidase (LiP) and manganese peroxidase (MnP) by *Phanerochaete chrysosporium* using steam- exploded straw as substrate. *Bioresource Technology*, 80(2): 149-151.

Gaind, S. and Nain, L. (2007). Chemical and biological properties of wheat soil in response to

paddy straw incorporation and its biodegradation by fungal inoculants. *Biodegradation*, 18(4):495-503.

Ghose T. K., Bisaria, V. S. (1987). Measurement of hemicellulase activities Part 1: xylanases.

Pure and Applied Chemistry, 59: 1739-1752.

Ghosh, P. and Ghosh, U. (2017). Statistical optimization of laccase production by *Aspergillus flavus* PUF5 through submerged fermentation using agro-waste as cheap substrate, *Acta Biologica Szegediensis*, 61(1): 25-33.

Godden, B., Ball, A.S., Helvenstein, P., McCarthy, A.J., Penninckx, M.J. (1992). Towards elucidation of the lignin degradation pathway in actinomycetes. *Journal of General Microbiology*. 138, 2441-2448.

Gokhale, D.V., Puntambekar, U.S. and Deobagkar, D.N. (1986). Xylanase and β -xylosidase production by *Aspergillus niger* NCIM 1207. *Biotechnology Letters*, 8(2), pp.137-138.

- Golueke, C.G. (1991). Understanding the process. In: Staff of BioCycle (Eds.). The BioCycle Guide to the Art and Science of Composting. The JG Press, Inc., Emmaus, Pennsylvania, USA. pp. 14-27.
- Government of India. (2019). Pocket book of agricultural statistics. Directorate of Economics & Statistics, Government of India, pp.26.
- Green, T.R. (1977). Significance of glucose oxidase in lignin degradation. *Nature*. 268: 7-80.
- Guo, T., Zhang, Q., Ai, C., Liang, G., He, P. and Zhou, W. (2018). Nitrogen enrichment regulates straw decomposition and its associated microbial community in a double-rice cropping system. *Scientific Reports*, 8(1): 1847.
- Inderjit, S., Rawat, D., Foy, C.L. (2004) Multifaceted approach to determine rice straw phytotoxicity. *Canadian Journal of Botany* 82:168–176.
- Ishihara, T. (1980). The role of laccase in lignin biodegradation. In: Lignin Biodegradation Microbiology, Chemistry and Potential Applications, Vol. 2 (Eds. Kirk, J.K., Highuchi, T. and Chang, H.N.). pp. 1-16, C.R.C. Press Inc., Florida.
- Jang, H.D. and Chen, K.S. (2003). Production and characterization of thermostable cellulases from *Streptomyces* transformant T3-1. *World Journal of Microbiology and Biotechnology*, 19(3), pp.263-268.
- Janshekar, H. and Feichter, A. (1983). Lignin: Biosynthesis, application and biodegradation. *Advances in Biochemical Engineering/Biotechnology*, 27: 119-178.
- Janusz, G., Pawlik, A., Sulej, J., Świdarska-Burek, U., Jarosz-Wilkołazka, A. and Paszczyński, A. (2017). Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. *FEMS Microbiology Reviews*, 41(6): 941-962.

Jing, X. and Ning, Y. (2013). Laccase production optimization by response surface methodology with *Aspergillus fumigatus* AF1 in unique inexpensive medium and decolorization of different dyes with the crude enzyme or fungal pellets. *Journal of Hazardous Materials*, 262: 870-877.

Jing, D. and Wang, J. (2012). Controlling the simultaneous production of laccase and lignin peroxidase from *Streptomyces cinnamomensis* by medium formulation. *Biotechnology for Biofuels*, 5:15.

Juliano, B.O. (1985). Rice hull and rice straw. In: Rice Chemistry and Technology, American Association of Cereal Chemistry, Minnesota, 2nd Edition, pp: 689-775.

Kausar, H., Sariah, M., Saud, H. M., Alam, M. Z. and Ismail, M. R. (2010). Development of compatible lignocellulolytic fungal consortium for rapid composting of rice straw. *International Biodeterioration & Biodegradation*, 64(7): 594-600.

Khudzari, J. M., Tartakovsky, B. and Raghavan, G. V. (2016). Effect of C/N ratio and salinity on power generation in compost microbial fuel cells. *Waste Management*, 48: 135-142.

Kirk T, K. (1984). Degradation of lignin. In: Microbial degradation of organic compounds. (Gibson, D. T., ed.) Marcel Dekker, New York, pp. 399-437

Kumar, A., Gaiind, S. and Nain, L. (2008). Evaluation of thermophilic fungal consortium for paddy straw composting. *Biodegradation*, 19(3): 395-402.

Kumar, P., Kumar, S., Joshi, L. (2015). The extent and management of crop stubble In Socio economic and Environmental Implications of Agricultural Residue Burning, Springer Briefs in Environmental Sciences, 13-34.

Kumar, R., Kaur, J., Jain, S. and Kumar, A. (2016). Optimization of laccase production from *Aspergillus flavus* by design of experiment technique: Partial purification and characterization. *Journal of Genetic Engineering and Biotechnology*, 14(1): 125-131.

Kumari, M., Yadav, R. S. S. and Yadav, K. D. S. (2002). Secretion of ligninperoxidase by *Penicillium citrinum*, *Fusarium oxysporum* and *Aspergillus terreus*. *Indian Journal of Experimental Biology*, 40: 802-806.

Ladisch, M. R., Lin, K. W., Voloch. M. and Tsao, G. T. (1983). Process considerations in the enzymatic hydrolysis of biomass. *Enzyme Microb. Tecthnol.* 5, 8-160.

Lee, C.W., Yoneyama, K., Takeuchi, Y., Konnai, M., Tamogami, S. and Kodama O (1999) Momilactones A and B in rice straw harvested at different growth stages. *Biosci BiotechnolBiochem* 63:1318–13

Levasseur, A., Piumi, F., Coutinho, P. M., Rancurel, C., Asther, M., Delattre, M., Henrissat, B., Pontarotti, P., Asther, M. and Record, E. (2008). FOLy: an integrated database for the classification and functional annotation of fungal oxidoreductases potentially involved in the degradation of lignin and related aromatic compounds. *Fungal Genetics and Biology*, 45(5): 638–645.

Li, M., Marek, S. M., Peng, J., Liu, Z. and Wilkins, M. R. (2018a). Effect of moisture content and inoculum size on cell wall composition and ethanol yield from switchgrass after solid- state *Pleurotus ostreatus* treatment. *Transactions of the ASABE*, 61(6): 1997-2006.

Li, Y., Wang, Y., Yu, Z., Lu, J., Li, D., Wang, G., Li, Y., Wu, Y., Li, S., Xu, F. and Li, G., (2018b). Effect of inoculum and substrate/inoculum ratio on the performance and methanogenic archaeal community structure in solid state anaerobic co-digestion of tomato residues with dairy manure and corn stover. *Waste Management*, 81, pp.117-127.

Liang, Y. S., Yuan, X. Z., Zeng, G. M., Hu, C. L., Zhong, H., Huang, D. L., Tang, L. and Zhao, J. J. (2010). Biodelignification of rice straw by *Phanerochaete chrysosporium* in the presence of dirhamnolipid. *Biodegradation*, 21(4): 615-624.

Liers, C., Arnstadt, T., Ullrich, R. and Hofrichter, M. (2011). Patterns of lignin degradation and oxidative enzyme secretion by different wood- and litter-colonizing basidiomycetes and ascomycetes grown on beech-wood. *FEMS Microbiology Ecology*, 78: 91–102.

Liwichi, R., Peterson, A., Mac Donald, M.J. and Broda. (1985). Phenotypic classes of phenol- oxidase-negative mutants of the lignin degrading fungus *Phanerochaete chrysosporium*. *Journal of Bacteriology* 162: 641-644.

- Lynch, L. M., Slater, J. H., Bennet, J. A. and Harper, S. H. T. (1981). Cellulase activities of some aerobic microorganisms isolated from soil. *J. Gen. Microbiol.* 127: 237-239.
- Majumdar, S., Lukk, T., Solbiati, J. O., Bauer, S., Nair, S. K., Cronan, J. E. and Gerlt, J. A. (2014). Roles of small laccases from *Streptomyces* in lignin degradation. *Biochemistry*, 53(24):4047-4058.
- Martinez, A. T., Speranza, M., Ruiz-Duenas, F. J., Ferreira, P., Camarero, S., Guillén, F., Martínez, M. J., Gutiérrez, A. and del Río, J. C. (2005). Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International Microbiology*, 8: 195–204.
- Meena, R. K., Arya, M. P. S., Meena, A. L., Singh, Y. V., Shukla, L. and Meena, H. S. (2016). Effect of compost inoculants, temperature and indigenous technical knowledge on decomposition process of rice crop residue. *Annual Agricultural Research New Series*, 37 (3): 327-333

Mustafa, A. M., Poulsen, T. G. and Sheng, K. (2016). Fungal pretreatment of rice straw with *Pleurotus ostreatus* and *Trichoderma reesei* to enhance methane production under solid-state anaerobic digestion. *Applied Energy*, 180: 661-671.

Muzamil, M., Mani, I., Kumar, A., Shukla, L., Lande, S. and Kumar Saxena, A. (2015) Optimization of Straw Size, Dose of Compost Inoculant along with Engineering and Microbiological Parameters for Paddy Straw Degradation in Shortest Possible Time. *Journal of Pure and Applied Microbiology*, 9: 1-9.

Nakamura, K.A.T.S.U.M.I. and Kitamura, K.U.M.P.E.I. (1983). Purification and some properties of a cellulase active on crystalline cellulose from *Cellulomonas uda*. *J. Ferment. Technol. ;(Japan)*, 61(4).

Nigam, P. and Prabhu, K.A. (1985). Microbial degradation of bagasse: Isolation and cellulolytic properties of Basidiomycetes Spp. from biomanure from a biogas plant. *Agricultural Wastes*, 12(4), pp.273-285.

Pandey, A. K., Gaiind, S., Ali, A. and Nain, L. (2009). Effect of bioaugmentation and nitrogensupplementation on composting of paddy straw. *Biodegradation*, 20(3): 293-306.

Patel, K. B., Kaswala, A. R., Dubey, P. K. and Patel, K. G. (2016). Effects of in-situ decomposition of paddy straw residues and different organic manures on yield and soil health of onion under organic farming. *Research in Environment and Life Sciences*, 9(10): 1232- 1235

Pildain, M. B, Novas, M. V. and Carmaran, C. C. (2005). Evaluation of anamorphic 'state, wood decay and production of lignin-modifying enzymes for diatrypaceous fungi from Argentina. *Journal of Agricultural Science and Technology*, 1: 81–96.

Pollegioni, L., Tonin, F. and Rosini, E. (2015). Lignin- degrading enzymes. *The FEBS journal*,282(7): 1190-1213.

Pometto, A.L. and Crawford, D.L. (1986). Effects of pH on lignin and cellulose degradation by *Streptomyces viridosporus*. *Applied and Environmental Microbiology*, 52(2), pp.246-250.

Prasad, S., Dhanya, M. S., Gupta, N. and Kumar, A. (2012). Biofuels from biomass: a sustainable alternative to energy and environment. *Biochemical and Cellular Archives*, 12(2): 255-260.

Prasad, S., Singh, A., Korres, N. E., Rathore, D., Sevda, S. and Pant, D. (2020). Sustainable

utilization of crop residues for energy generation: A life cycle assessment (LCA) perspective. *Bioresource Technology*, 303:122964.

Prasertsan, P. and Doelle, H.W. (1987). Nutrient optimization for cellulase biosynthesis by a newly isolated *Cellulomonas* sp. *MIRCEN Journal of Applied Microbiology and Biotechnology*, 3(1), pp.33-43.

Rajoka, M.I. and Malik, K.A. (1986). Comparison of different strains of *Cellulomonas* for production of cellulolytic and xylanolytic enzymes from biomass produced on saline lands. *Biotechnology Letters*, 8 (10), pp.753-756.

Rao, A.V. and Venkateswarlu, B. (1983). Microbial ecology of the soils of Indian desert. *Agriculture, Ecosystems & Environment*, 10(4), pp.361-369.

Ray, A.K., Bairagi, A., Ghosh, K.S. and Sen, S.K. (2007). Optimization of fermentation conditions for cellulase production by *Bacillus subtilis* CY5 and *Bacillus circulans* TP3 isolated from fish gut. *Acta Ichthyologica et Piscatoria*, 1(37), pp.47-53.

- Reese, E.T., 1956. Enzymatic hydrolysis of cellulose. *Applied Microbiology*, 4(1), p.39.
- Reid, I. D. (1989). Solid-state fermentations for biological delignification. *Enzyme and Microbial Technology*, 11(12): 786-803. RWC-CIMMYT. 2003. Addressing resource conservation issues in rice-wheat systems of South Asia: A resource book. New Delhi, India: Rice-Wheat Consortium for the Indo- Gangetic Plains - International Maize and Wheat Improvement Center.
- Santhanam, N., Badri, D.V., Decker, S.R., Manter, D.K., Reardon, K.F. and Vivanco, J.M. (2012). Lignocellulose decomposition by microbial secretions. In *Secretions and exudates in biological systems* (pp. 125-153). Springer, Berlin, Heidelberg.
- Shi, J., Chinn, M. S. and Sharma-Shivappa, R. R. (2008). Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*. *Bioresource Technology*, 99(14): 6556-6564.
- Sigoillot, J.C., Berrin, J.G., Bey, M., Lesage-Meessen, L., Levasseur, A., Lomascolo, A., Record, E. and Uzan-Boukhris, E. (2012). Fungal strategies for lignin degradation. *Advances in Botanical Research*, 61, pp.263-308.
- Singh, K. and Shiere, J.B. (1993). Feeding of ruminants on fibrous crop residues. In Proceedings of an International workshop, NDRI, Karnal, Feb. 4-8, 1991.
- Silva, G. G. D., Couturier, M., Berrin, J. G., Buléon, A. and Rouau, X. (2012). Effects of grinding processes on enzymatic degradation of wheat straw. *Bioresource Technology*, 103(1):192-200.
- Shukla, L., Senapati, A., Tyagi, S. P., and Saxena, A. K. (2014). Economically viable mass production of lignocellulolytic fungal inoculum for rapid degradation of agrowaste. *Current Science*, 1701-1704.
- Shukla, L., D LANDE, S. A. T. I. S. H., ROAF AHMAD PARRAY, A. S., and ANNAPURNA, K. (2019). Recycling flower waste to humus rich compost using effective microbial consortium and mechanical intervention. *Indian Journal of Agricultural Sciences*, 89(7), 140-146.
- Sindhu, R., Binod, P. and Pandey, A. (2016). Biological pretreatment of lignocellulosic biomass—An overview. *Bioresource Technology*, 199: 76-82.

Stoppok, W., Rapp, P. and Wagner, F. (1982). Formation, location, and regulation of endo-1, 4- β -glucanases and β -glucosidases from *Cellulomonas uda*. *Applied and Environmental Microbiology*, 44(1), pp.44-53.

Strom, P.F. (1985). Identification of thermophilic bacteria in solid-waste composting. *Applied and Environmental Microbiology*, 50(4), pp.906-913.

Stutzenberger, F.J. (1972). Cellulolytic activity of *Thermomonospora curvata*: nutritional requirements for cellulase production. *Applied Microbiology*, 24(1), pp.77-82.

Suhara, H., Kodama, S., Kamei, I., Maekawa, N. and Meguro, S. (2012). Screening of selective lignin- degrading basidiomycetes and biological pretreatment for enzymatic hydrolysis of bamboo culms. *International Biodeterioration & Biodegradation*, 75: 176-180.

Tabatabai, M. A. and Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soilphosphatase activity. *Soil Biology and Biochemistry*, 1(4): 301-307.

Teather, R.M. and Wood, P.J. (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied and Environmental Microbiology*, 43(4), pp.777-780.

Tehrani, A.S., Disfani, F. A., Hedjaroud, G. A. and Mohammadi, M. (2001). Antagonistic effects of several bacteria on *Verticillium dahliae* the causal agent of cotton wilt. In: Proceedings of the 53rd International Symposium on Crop Protection, Gent, Belgium, 8 May 2001.

Thayer, D.W., Lowther, S.V. and Phillips, J.G. (1984). Cellulolytic activities of strains of the genus *Cellulomonas*. *International Journal of Systematic and Evolutionary Microbiology*, 34(4), pp.432-438.

Tian, J. H., Pourcher, A. M., Bouchez, T., Gelhaye, E. And Peu. P. (2014). Occurrence of lignin degradation genotypes and phenotypes among prokaryotes. *Applied Microbiology and Biotechnology*, 98: 9527–44.

Větrovský, T., Steffen, K. T. and Baldrian, P. (2014). Potential of cometabolic transformation of polysaccharides and lignin in lignocellulose by soil Actinobacteria. *PloS One*, 9(2): 89108.

Vivekanand, V., Dwivedi, P., Pareek, N. and Singh, R. P. (2011). Banana peel: a potential substrate for laccase production by *Aspergillus fumigatus* VkJ2. 4.5 in solid-state fermentation. *Applied Biochemistry and Biotechnology*, 165(1): 204.

Walker, D., Ledesma, P., Delgado, O.D. and Breccia, J.D. (2006). High endo- β -1, 4-d-glucanase activity in a broad pH range from the alkali-tolerant *Nocardiopsis* sp. SES28. *World Journal of Microbiology and Biotechnology*, 22(7), pp.761-764

Wei, Y., Wu, D., Wei, D., Zhao, Y., Wu, J., Xie, X., Zhang, R. and Wei, Z. (2019). Improved lignocellulose-degrading performance during straw composting from diverse sources with actinomycetes inoculation by regulating the key enzyme activities. *Bioresource Technology*, 271: 66-74.

Yan, Z., Song, Z., Li, D., Yuan, Y., Liu, X. and Zheng, T. (2015). The effects of initial substrate concentration, C/N ratio, and temperature on solid-state anaerobic digestion from composting rice straw. *Bioresource Technology*, 177: 266-273.

Zhang, S., Jiang, M., Zhou, Z., Zhao, M. and Li, Y. (2012). Selective removal of lignin in steam-exploded rice straw by *Phanerochaete chrysosporium*. *International Biodeterioration & Biodegradation*, 75: 89-95.