IN-SITU MANAGEMENT OF RICE AND WHEAT STUBBLE THROUGH APPLICATION OF BIO-DECOMPOSER

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DEDICATION

Dedicated to Noor of 2023, who almost gave up but didn't.

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LIST OF ABBREVIATIONS

CFU	Colony Forming Unit
DMRT	Duncan's Multiple Range Test
FPU	Filter Paper Unit
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
XRD	X-Ray Diffraction

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ABSTRACT

The deterioration of air quality due to stubble burning poses a significant threat to health, especially in South Asia. In Pakistan, an agriculture-based economy, crop residue burning, particularly in the rice-wheat belt, exacerbates air pollution. Annually, Pakistan produces 69 million tons of crop residue, with 32 million tons burnt, escalating pollution. Despite laws and penalties, farmers continue this practice for its cost-effectiveness in land preparation. However, it harms soil health and air quality long-term, necessitating sustainable alternatives. This research proposes biodegrading agricultural residue using a ready-to-use inoculum based on a microbial consortium of two bacterial strains, *Bacillus pumillus* and *Pseudomonas aeruginosa*, and a fungus, *Trichoderma spp*., native to Pakistan's soil. Results show the microbial consortia achieved the highest FPase activity (2.674 U/ml for rice straw and 3.188 U/ml for wheat straw) over 7 days and the highest decomposition rates (57.70% for rice straw and 49.40% for wheat straw) by day 21. Among individual microbes, *Pseudomonas aeruginosa* exhibited the highest decomposition rates (49.33% for rice straw and 52.90% for wheat straw) and significant FPase activity (1.044 U/ml for rice straw and 1.526 U/ml for wheat straw). *Trichoderma spp.* and *Bacillus pumillus* also showed notable decomposition rates and FPase activity, though to a lesser extent. The findings highlight the potential of microbial applications for accelerating stubble decomposition, offering a sustainable solution for *in-situ* crop residue management in Pakistan. This research lays a foundation for future studies to maximize the efficiency of different microbial consortia under various conditions, promoting healthier agricultural practices and improved air quality.

CHAPTER 1

INTRODUCTION

Stubble burning, also known as crop residue burning, involves intentionally setting fire to remaining plant material such as straw, stumps, and post-harvest residues. The practice is widespread in various agricultural regions around the world, especially in areas with large-scale mechanized agriculture. Although stubble burning has benefits such as rapid field clearing, pest control, and nutrient recycling, it has become a significant environmental problem due to its significant adverse effects.

Stubble burning, also referred to as crop residue burning, is the intentional practice of setting fire to the remaining plant materials such as straw, stubble, and residues after crop harvest. This practice is widespread in various agricultural regions globally, particularly in areas with large-scale mechanized farming. While stubble burning has its uses, including quick field clearance, pest control, and nutrient recycling, it has become a major environmental concern due to its significant negative impacts.

1.1. Environmental Consequences

The adverse environmental consequences of stubble burning are consistent across regions including following impacts.

Table 1.1. Environmental consequences of stubble burning

Stubble Burning		
Sr.no.	**Consequences**	**Description**
1.	Air Pollution	Stubble burning releases a variety of harmful pollutants into the atmosphere, including particulate matter, carbon dioxide, carbon monoxide, and volatile organic

		compounds. These pollutants worsen air quality and cause respiratory diseases, cardiovascular diseases, and other health problems. Harmful gases released when burning crop residues also contribute to global warming and climate change. It is estimated that about 20% of total annual agricultural carbon dioxide emissions in the United States come from burning crop residues.
2.	Ozone Depletion	The pollutants released during stubble burning can contribute to ozone layer depletion in the stratosphere, which protects the Earth from harmful ultraviolet radiation.
3.	Soil Degradation	Burning stubble removes organic material from the soil, which is essential for soil structure, nutrient cycling, and water storage. Reduced organic matter levels can lead to soil erosion, reduced fertility, and a decline in the overall health of the soil.
4.	Loss of Biodiversity	Stubble burning negatively impacts soil microorganisms, insects, and other organisms that are essential for maintaining ecosystem balance. This disruption can lead to biodiversity loss and ecosystem imbalance.
5.	Deterioration of Water Quality	Ash and residue particles carried by wind or rain runoff can contaminate water bodies, causing nutrient imbalances, algae blooms, and reduced water quality.

6.	Acid Rain	Emissions of sulfur dioxide and nitrogen oxides during burning can contribute to the formation of acid rain, which can damage ecosystems and infrastructure.
7.	Regional Climate Effects	Stubble burning contributes to haze and smog formation, affecting regional climate patterns, visibility, and potentially impacting tourism and transportation.

1.2. Drivers of Stubble Burning

The studies reveal that the expenses involved in preparing wheat fields for planting after rice harvest push farmers to choose the practice of burning crop residue. As a result, only a small fraction of farmers opts for incorporating residue into the soil, even though doing so has beneficial effects on the soil's biological, chemical, and physical properties. Some agricultural studies have explored that factors like time constraints, household off-farm income, output prices, salinity issues, perceptions of long-term profitability, scale of operation and various land related aspects determine farmers' adoption of crop residue management strategies (Ahmed et al., 2015; Gupta, 2012).

Shifting from burning to alternatives requires a comprehensive approach that includes education, incentives, access to machinery, and policy support to promote sustainable residue management practices.

1.3. Management Strategies

Stubble burning, a widespread agricultural practice, has led to the implementation of various management strategies aimed at reducing its negative environmental effects.

These strategies vary from global to regional levels. Globally, techniques such as conservation tillage, mulching, cover cropping, regulatory measures, and financial incentives have been adopted. In South Asia, including countries like India and Pakistan, there are specific challenges due to the high rates of stubble burning. In these regions, approaches include subsidies for machinery, awareness campaigns, bioenergy initiatives, and training programs for farmers (Sarkar, 2020). In Pakistan, particularly in Punjab, there has been a noticeable shift from burning practices to more sustainable alternatives. Incentives for machinery usage are encouraging this change, while policy enforcement aims to discourage burning. Innovative solutions, such as bio-decomposer technology, are being used to facilitate the decomposition of crop residues, reducing the need for burning (Zahid et al, 2020). Education is a key factor in ensuring that farmers are aware of and effectively implement these alternatives.

Overall, these strategies focus on education, accessibility to equipment, economic incentives, and the adoption of new technologies to reduce stubble burning. The goal is to promote sustainable residue management techniques that enhance soil health, reduce air pollution, preserve soil carbon, and protect public health. By adopting these alternatives, countries strive to balance agricultural productivity with environmental conservation, working towards a healthier and more sustainable agricultural future.

Currently, crop residues are managed through either of the ways:

a) *Ex situ* management

b) *In situ* management

1.3.1. *Ex situ* management

Managing crop residues outside of the fields is a crucial strategy for sustainable farming and addressing environmental issues. This approach involves repurposing agricultural by-products for various practical applications. Several methods contribute to effective

ex-situ management:

- **Mulching:** This involves spreading crop residues over the soil surface to create a protective layer. It helps retain soil moisture, prevent erosion, and improve soil fertility (Reddy et al., 2023) .
- **Composting**: Crop residues are subjected to composting, where organic matter is broken down into nutrient-rich humus through microbial action. The resulting compost serves as an organic fertilizer, enhancing soil structure and fertility (Bisen & Rahangdale, 2017; Chaudhry et al., 2019).
- **Bioenergy Production**: Crop residues are used as raw materials for bioenergy production, using technologies like anaerobic digestion or biomass-to-energy processes to convert residues into valuable energy sources such as biofuels or biogas(Bisen & Rahangdale, 2017; Reddy et al., 2023).
- **Livestock Feed:** Processed crop residues can be utilized as feed for livestock, supporting sustainable agricultural practices(Mohan et al., 2016).
- **Industrial Uses:** Certain crop residues are used as raw materials in various industries. For instance, bagasse from sugarcane processing can be used in the paper and pulp industry.
- **Biomass Briquetting**: Crop residues like straw or husks can be compressed into biomass briquettes, providing a clean and efficient fuel source for heating or cooking
- **Fiber Production:** Specific crop residues, such as cotton stalks or jute remnants, can be used for fiber production, supplying valuable inputs for industries like textiles and packaging(Das et al., 2016).
- **Landfill Cover**: In some instances, crop residues can serve as cover material in

landfills, reducing environmental impact and enhancing landfill stability.

The choice of ex-situ management practices depends on factors such as the type of crop residue, local environmental conditions, and the availability of relevant technologies. These practices collectively support sustainable agriculture, address environmental challenges, and align with the principles of the circular economy.

1.3.2. *In situ* management

Crop residue management is essential for sustainable agriculture, and in-situ management methods are increasingly recognized for their beneficial environmental impact. These methods involve handling crop residues directly in the fields where they originate, without removing them. The goal is to enhance soil health, improve nutrient cycling, and increase the resilience of the overall ecosystem. Two key approaches within in-situ management include the utilization of cellulase enzyme-producing microbes and bio-decomposers.

a) *In situ* Management via Cellulase Enzyme Production

Cellulase enzymes, produced by various microbes such as bacteria, fungi, and actinomycetes, break down cellulose - a significant component of plant cell walls - into simpler sugars that microorganisms can use as an energy source. This enzymatic action is vital for the effective decomposition of crop residues. The process is further enhanced by the enzymatic synergy of microbial consortia, including species like *Bacillus*, *Trichoderma*, and Actinobacteria, which help accelerate the decomposition of crop residues (Borah et al., 2016; Chaudhry et al., 2019).

The breakdown of crop residues by cellulase enzymes offers several benefits:

- **Nutrient Cycling**: As cellulose is decomposed, the nutrients stored in crop residues are released into the environment, contributing to nutrient cycling and making these

nutrients available for plant uptake and microbial use.

- **Soil Health Improvement**: The decomposition of crop residues, facilitated by cellulase enzymes, increases soil organic matter content. This process enhances soil structure, water retention, and microbial activity, ultimately improving overall soil health.
- **Carbon Sequestration**: Instead of releasing carbon into the atmosphere through burning, the microbial breakdown of cellulose results in the incorporation of organic carbon into the soil, aiding in carbon sequestration.
- **Biocontrol and Disease Suppression**: Certain cellulase-producing microbes, like *Trichoderma*, possess biocontrol properties that can suppress plant pathogens, contributing to improved crop health.

b) Bio-decomposers

Bio-decomposers are formulations containing a mixture of beneficial microorganisms, including cellulase producers. These formulations are designed to speed up the natural decomposition of organic materials, such as crop residues. They contain various microorganisms that produce cellulase and other enzymes like ligninases, which break down complex organic compounds in crop residues into simpler substances such as carbon dioxide, water, and nutrients. This process enhances soil organic matter content and soil structure, promoting water retention and aeration. However, the effectiveness of bio-decomposers depends on factors such as the composition of the microbial consortium, environmental conditions, and types of residues. Following recommended application guidelines is essential for optimal results.

Incorporating cellulase-producing microbes and bio-decomposers into crop residue management practices offers a sustainable solution to stubble burning (Shinde et al, 2022). These methods enable efficient decomposition, nutrient cycling, and soil health

enhancement while mitigating the negative environmental impacts associated with burning. Customizing these strategies to local soil and climate conditions is vital for successful implementation.

Addressing crop residue burning requires action on global, regional, and local scales. By adopting a hierarchy of management strategies ranging from regulatory policies to localized education and partnership initiatives, the harmful impact of stubble burning can be mitigated. This multi-tiered approach emphasizes innovation, collaboration, and awareness to foster more sustainable agricultural practices, ensuring environmental and human well-being.

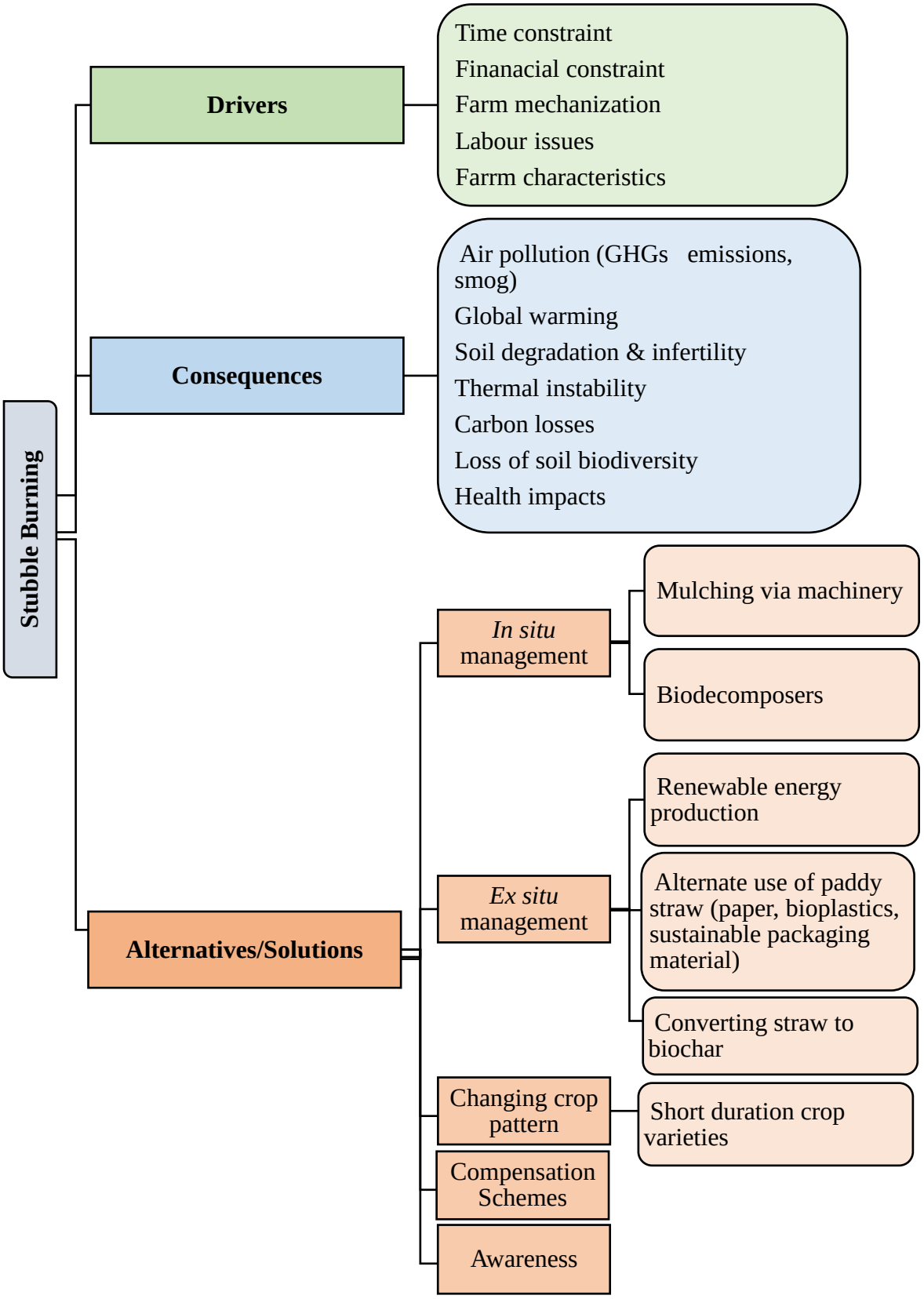

Figure 1.1: Stubble burning: drivers, consequences and alternatives/solutions

1.4. Significance of the Study

This study is significant as it offers a practical solution to the severe air pollution caused by stubble burning, a major environmental issue in the region. The use of bio-decomposers enhances soil fertility by efficiently breaking down crop residues, addressing the soil degradation that threatens Pakistan's agriculture-based economy. This method provides economic benefits to farmers by improving crop yields and reducing reliance on chemical fertilizers, thus promoting economic stability. Furthermore, the study supports sustainable agricultural practices, aligning with regional efforts to minimize environmental impact. The findings can inform policymakers about the feasibility of bio-decomposers, potentially leading to supportive policies and incentives for their adoption.

1.5. Objectives of the Study

The study was conducted to achieve the following objectives:

1. Comparative analysis of fungal (*Trichoderma spp*.), bacterial (*Bacillus pumillus, Pseudomonas aeruginosa*) species and their consortia activity in decomposition of stubble residue.
2. Optimization for ready-to-use inoculum based upon fungal and bacterial consortium.

CHAPTER 2

LITERATURE REVIEW

2.1. Overview

Air pollution is a pressing environmental concern in Pakistan, exacerbated by industrial activities, rapid population growth, and heavy dependence on non-renewable energy sources (Zahid et al. 2020). Poor air quality has detrimental effects on human health and the environment, threatening a country already susceptible to the impacts of climate change. This literature review explores the practice of stubble burning, its consequences, and various mitigation strategies, with a particular emphasis on using microbial consortia for enzyme production through submerged fermentation (SmF). The review covers the topic from a global perspective, focusing on South Asia, and specifically Pakistan, to identify research gaps and the need for further studies.

2.2. Global Perspective on Stubble Burning and its Impacts

Direct combustion, the practice of using agricultural waste as fuel, is one of the oldest known biomass conversion techniques and has been used since ancient times. This process involves the rapid chemical reaction between agricultural residues and oxygen, resulting in the release of energy in the form of light and heat, alongside the formation of carbon dioxide and water. Despite the emergence of advanced technologies for biomass energy conversion, combustion remains the prevailing method, accounting for over 95% of current biomass energy utilization (Obi et al., 2016). However, direct biomass combustion has adverse environmental effects, emitting particulate matter, volatile organic compounds, and carbon monoxide, leading to air pollution and health risks. Additionally, it releases short-term carbon dioxide emissions, contributes to deforestation and habitat loss due to unsustainable practices, and raises concerns about

land use competition and proper ash disposal. Burning crop straw in fields and the subsequent decomposition of straw are practices that are not only wasteful of valuable resources but also lead to environmental degradation and human health concerns (Aruya et al., 2016).

Biomass burning is a complex process with spatial and temporal variations. Biomass burning releases gases and aerosols that impact territorial air quality, visibility, atmospheric chemistry, biogeochemical cycles, Earth's radiative budget, and climate. Emissions like CO, CH_4, and VOCs affect tropospheric oxidation, leading to ozone and photo-oxidant formation. CH_3Br emissions can degrade stratospheric ozone. Particulate matter (PM) contributes to cloud acidification, alters radiation balance, and influences cloud formation. Smoke, a prominent product, contains constituents like BC, brown carbon, OC, and mineral dust, affecting both regional air quality and global climate. Forest burning disrupts CO_2 sinks and oxygen sources, while enhancing greenhouse gas production like nitrous oxide and methane. Forest fires can significantly impact local air quality and ozone levels, with immediate consequences for populations and ecosystems (Sidar, 2018; Tripathi et al., 2024).

Biomass emissions primarily originate from various sources such as wood burning for household cooking, open field incineration of municipal waste, and wildfires. However, it is noteworthy that in Asian countries such as China, a significant 60% of biomass emissions stem from stubble burning (Zhang et al., 2015). Zooming out to a global perspective, it becomes evident that stubble burning contributes significantly, comprising approximately one-fourth of all biomass burning emissions, encompassing events like forest fires (Tripathi et al., 2024; Zhang et al., 2016).

Crop residue burning offers farmers advantages like disease control and time savings, but its detrimental effects are often underestimated. These impacts include degraded air

quality from smoke particles and the loss of essential organic materials for soil enrichment. Agricultural burning releases smoke directly into the air, comprising particles like soot and ashes. These tiny particles can deeply enter the lungs and bloodstream, particularly harming those with respiratory issues. For instance, paddy growers burn wheat stubble to reduce pests, but this emits pollutants like particulate matter, nitrogen dioxide, and volatile compounds, posing health risks, particularly for individuals with respiratory diseases like asthma (Lal, 2008).

The environmental burden of straw field burning extends both regionally and globally (Long, 2016). Historical data reveals the prevalence of this practice in various regions, such as the United Kingdom (UK), where approximately 600,000 hectares of crop straw were annually burnt in the 1980s (Prew, 1995; Prew et al., 1995). Similarly, it was a common practice in the Great Plains of the United States of America (USA) during the same period, as reported by (Jenkins, 1992). In the southeastern USA, crop straw burning occurred annually from 2000 to 2004, contributing significantly to fire activities, as noted by (McCarty, 2007). Australia saw the burning of rice straw to prepare for winter rice production, as highlighted by (Vagg, 2015). Additionally, field burning of rice straw in eastern Spain led to an increase in PM10 concentrations, as reported by (Viana, 2008). In countries like China, Thailand, and northern India, straw burning remains a common practice on farms, driven by the consistent increase in straw production over the years. It was estimated that China burned approximately 140 million tons of straw annually, contributing significantly to atmospheric pollution, accounting for 86.02–97.58%, as indicated by (Zhou, 2017).

2.3. Stubble Burning in South Asia

The Indo-Gangetic Plain (IGP) in South Asia relies heavily on the rice-wheat rotation system for agriculture (Raza et al. 2022). Covering around 20% of the land in India,

Pakistan, Bangladesh, and Nepal, this region is known for its fertile farmlands and rich ecosystems (Sahoo et al., 2019). Farmers in these countries commonly use combine harvesters for planting and harvesting crops. While these machines are efficient in performing tasks such as reaping, threshing, and winnowing simultaneously, they generate a substantial amount of stubble. This stubble consists of stalks approximately 15 cm tall, which are difficult to incorporate back into the soil (Chawala and Sandhu, 2020; Abdurrahman et al., 2020; Singh et al., 2020).

Stubble burning in this region has a significant impact on air quality, especially during winter months when temperature inversion traps smoke, leading to the formation of smog and various health hazards (Quencheng et al. 2024). Studies have shown that burning rice stubble has a more significant impact on air quality than burning wheat stubble due to its higher ash content. Recent analyses have demonstrated that pollutants can persist in the atmosphere long after the burning period has ended, highlighting the extended environmental impact (Chawala and Sandhu, 2020).

In Pakistan, where the economy is largely based on agriculture, the burning of crop residues, particularly in the rice-wheat belt, is a major issue (Rafiq et al, 2019). The country produces 69 million tons of crop residue annually, with 32 million tons burned, significantly contributing to air pollution. Despite the existence of laws and penalties, farmers continue to burn crop residues because it is a cost-effective and efficient method of preparing land for the next sowing season. However, this practice has detrimental long-term effects on soil health and air quality, making the search for sustainable alternatives essential (Azhar, 2019; Zahid et al. 2020).

Table 2.1: Region-wise annual crop residue burning, the primary crop residues burnt and the resulting emissions

Region	Annual Crop Residue Burning (Tons)	Primary Crop Residues Burnt	Major Emissions	References
India (Northwestern States)	92 million	Rice, Wheat	$PM_{2.5}$, CO, NOx, VOCs, SO_2	Kumar & Reddy, 2017 ; Shyamsundar et al.,2019 Banerjee & Srivastava,
Pakistan (Punjab Region)	32 million	Rice, Wheat	CO_2, NOx, PM, VOCs, Black Carbon	Azhar, 2019; Sharma et al., 2023
China (Northeastern Region)	140 million	Rice, Wheat, Maize	CO_2, CH_4, PM, NOx	Zhang et al., 2015; Chen et al., 2017; Zhou, 2017; Li et al., 2023
United States (Southeast and Midwest)	15 million	Corn, Wheat, Soybeans	CO_2, PM, NOx, VOCs	McCarty et al., 2007; Lin et al., 2023
Brazil (Amazon Region)	20 million	Sugarcane, Corn	CO_2, Black Carbon,	Perillo et al., 2022

			NOx	
Australia (Victoria Region)	2 million	Rice, Wheat, Barley	PM_{10}, CO, VOCs	Vagg et al., 2015; Smith et al., 2023
Spain (Eastern Region)	5 million	Rice	PM_{10}, CO, VOCs, NOx	Viana et al., 2008; Santiago et al., 2018
Thailand (Northern Region)	8 million	Rice, Corn	CO_2, $PM_{2.5}$, NOx, CH_4	Phairuang, 2021
Russia (Siberian Region)	7 million	Wheat, Barley	CO_2, Black Carbon, PM	Soja et al., 2007; Nizhelskiy, 2024
Ukraine (Eastern Europe)	10 million tons	Wheat, Sunflower	CO_2, NOx, VOCs, PM	Hall et al., 2021
Indonesia (Sumatra)	5 million	Palm Oil, Rice	CO_2, $PM_{2.5}$, VOCs	Andini et al., 2018
Mexico (Yucatán)	3 million	Sugarcane, Corn	CO_2, CH_4, $PM_{2.5}$	Yokelson et al., 2011; Santiago et al., 2018

This table highlights how various regions contribute to crop residue burning and their associated emissions. India and China are among the largest contributors globally, with rice and wheat crops significantly affected. These regions emit high levels of carbon dioxide (CO_2), particulate matter ($PM_{2.5}$ and PM_{10}), and other pollutants, aggravating air pollution and health concerns.

To address the adverse effects of stubble burning, several strategies have been suggested and implemented worldwide. One promising approach is the use of microbial consortia to biodegrade agricultural residues through submerged fermentation (SmF). This method utilizes the enzymatic activities of specific bacteria and fungi to effectively decompose crop residues, offering a potential sustainable solution to the problem.

2.4. Microbial Consortia and Enzyme Production via SmF

Microbial consortia utilize the combined action of different microorganisms to break down lignocellulosic biomass. Research has shown that bacterial consortia are effective in producing cellulase enzymes and degrading residues. For instance, a study by Aruna et al. (2023) examined the breakdown of banana peel through submerged fermentation using a mixed bacterial culture, demonstrating significant potential for generating biofuels from lignocellulosic waste. Similarly, Singh and Dutta (2024) employed microbial consortia for the biodegradation of kitchen waste, illustrating the efficiency of these consortia in managing waste.

In another study, Bhattacharjya et al. (2021) explored the use of lignocellulolytic microbial consortia to decompose crop residues in the field. Their findings indicated that in-situ decomposition led to better crop yields compared to burning or removing residues. The process effectively reduced the lignin and cellulose content in rice, wheat residues, and sugarcane trash, underscoring its environmentally friendly benefits. Jiang et al. (2023) demonstrated that using a bacterial consortium consisting of *Bacillus subtilis* and *Pseudomonas putida* significantly improved the breakdown of rice straw, reducing its lignin content by 45% within 45 days. Wang et al. (2023) found that combining bacterial and fungal species for rice residue decomposition resulted in a 30% faster breakdown than using bacteria alone. Zhang et al. (2024) showed that fungal consortia using *Trichoderma reesei* produced higher concentrations of ligninase,

leading to improved biofuel production from sugarcane waste.

Table 2.2: Recent studies on microbial consortia and enzyme production via submerged fermentation (SmF)

Microbial Consortium Type	Crop Residue Targeted	Enzyme(s) Produced	Research Findings	References
Compost-based microbial consortia	Kitchen and agricultural waste	Cellulase, Protease	Effective biodegradation of waste, with high enzyme yields, promoting waste-to-energy solutions.	Singh & Dutta, 2024
Mixed fungal-bacterial consortia	Rice husk, Corn cob residues	Cellulase, Hemicellulase	Increased enzyme yield by 40% in bioethanol production from rice husk and corn cob residues.	Sharma & Gupta, 2024

Mixed bacterial culture	Banana peel and lignocellulosic waste	Cellulase, Xylanase	Efficient degradation of banana peel biomass, yielding high cellulase and xylanase enzymes, enhancing biofuel production.	Aruna et al., 2023
Thermophilic microbial consortia	Rice husk	Hemicellulase, Cellulase	Higher efficiency in converting rice husk into fermentable sugars for biofuel production.	Singh et al., 2019
Alkaliphilic microbial strains	Cotton and jute residues	Lignocellulase, Xylanase	Efficient degradation under extreme pH, yielding high enzyme	Shinde et al., 2022

			activity.	
Marine-derived fungal consortia	Marine biomass	Cellulase, Xylanase	High efficiency in decomposing marine biomass, offering potential for marine biofuel production.	Liu et al., 2023
Fungal-bacterial consortia	Wheat straw	Cellulase, Amylase	Enhanced glucose and reducing sugar yields for bioethanol production.	Chen et al., 2022
Thermophilic-acidophilic bacteria	Rice straw	Cellulase, Pectinase	Rapid composting with improved enzyme activity and nutrient recovery.	Chukwuma et al., 2021
Lignocellulolytic microbial consortia	Rice straw, Sugarcane trash	Cellulase, Laccase	Significant improvement in nutrient	Bhattacharjya et al., 2021

			recycling and crop yield due to effective residue decomposition.	
Bacillus sp. and Aspergillus sp.	Corn stover, Wheat bran	Amylase, Cellulase	Effective degradation for bioethanol production using a synergistic microbial approach.	Sarkar et al., 2021

2.5. Research Gaps and Limitations

Despite these promising outcomes, there are several gaps and limitations in the current research on microbial consortia and stubble management. One major gap is the scarcity of regional studies, as most research has been conducted outside Pakistan. There is a pressing need for studies specific to the region to understand how these consortia perform under local climatic and soil conditions. Additionally, the microbial consortia often include non-native microbes, pointing to the necessity of identifying and using microbes indigenous to Pakistani soil to boost cellulase enzyme production and improve stubble degradation efficiency. Another significant limitation is the lack of research on the effects of microbial consortia-based stubble management on soil health and crop productivity, which is crucial for validating the sustainability of these

practices. Moreover, there is limited research on the economic viability of using microbial consortia for large-scale stubble management. Conducting cost-benefit analyses is essential to encourage farmers to adopt these technologies.

In conclusion, while the integration of microbial consortia for stubble residue management via submerged fermentation offers a sustainable and eco-friendly alternative to traditional stubble-burning practices, further research is needed to address these existing gaps, particularly focusing on the selection of native microbial species.

CHAPTER 3

MATERIALS AND METHODS

For this research, rice and wheat straw were used as substrates to determine the production of cellulase enzyme by fungus *Trichoderma spp.* and two strains of bacteria i.e., *P. aeruginosa and Bacillus pumillus* native to regional soil for degradation of agricultural waste. The experiment was carried out with both treated and untreated substrates to determine if impurities have any impact on microbial activity under different conditions. Three replicates for each microbial species were taken to extract crude enzyme over a time period of 1 week.

The pre-treatment of substrates and cellulase enzyme assay was performed following the same methodology for both fungal and bacterial species. However, the steps in between were different for both *Trichoderma spp.* and bacteria and were carried out accordingly. The methodology adopted for this research comprised of the following steps:

3.1. *Trichoderma* spp.

3.1.1. Pre-treatment of substrates (rice straw and wheat straw)

The substrates were treated with 1% (w/v) NaOH for 1 hour in 1:10 (substrate:solution). They were brought to neutral pH by washing thoroughly with distilled water and then were allowed to cool at room temperature before autoclaving at 121℃ for 1 hour.

3.1.2. Medium preparation

Potato Dextrose Broth (PDB) was used as a medium for SmF prepared by mixing 12g of PDB in 500ml of distilled water. It was then autoclaved at 121℃ for 1hr 30 min to remove any microbial activities.

3.1.3. Inoculum preparation

A loop of *Trichoderma* spp. was shifted from slant to Erlenmeyer's flask containing 100mL of media in sterilized conditions. The flask was kept in a shaker at 200rpm at 30℃ for 3 days before using it for the fermentation process.

3.1.4. Submerged fermentation (SmF)

SmF was carried out in 250mL Erlenmeyer flasks containing 100 mL of fermentation medium. The composition of fermentation medium in g/L of distilled water is as follows:

- L-Glutamic acid – 0.3g
- NH_4NO_2 – 1.4g
- K_2HPO_4 – 2.0g
- $CaCl_2$ – 2.0g
- $MgSO_4$ – 0.3g
- $FeSO_4$ – 5.0g
- $MnSO_4$ – 1.6g
- $ZnSO_4$ – 1.4g
- Protease peptone – 7.5g
- Tween80 – 20% (v/v)
- Substrate – 30g

The fermentation medium was autoclaved at 121℃ for 15 min. Each flask was inoculated with 1mL of prepared inoculum and the cultures were incubated in a rotary shaker at 120rpm at 30℃ for incubation time of T_1 = 3 days; T_2 = 5 days; and T_3 = 7 days.

3.1.5. Enzyme extraction

The culture broth from SmF was centrifuged at 6000rpm for 15 min. The clear

supernatant thus obtained was the extracellular crude enzyme source.

3.1.6. Enzyme assay

The cellulase activity was measured according to the Laboratory Analytical Procedure (LAP) provided by the National Renewable Energy Laboratory (NREL).

3.2. Bacteria (*P. aeruginosa* and *Bacillus pumillus*)

3.2.1. Pre-treatment of substrates (rice straw and wheat straw)

The substrates were treated with 1% (w/v) NaOH for 1 hour in 1:10 (substrate:solution). They were brought to neutral pH by washing thoroughly with distilled water and then were allowed to cool at room temperature before autoclaving at 121℃ for 1 hour.

3.2.2. Medium preparation

Nutrient Broth was used as a media for bacterial SmF prepared by mixing 13g of nutrient broth with 1L of distilled water. It was then autoclaved at 121℃ for 15 min to remove any impurities.

3.2.3. Inoculum preparation

The glass vials containing 5ml of autoclaved broth were inoculated with bacterial strains. The vials were kept for 24 hours in an incubator at 37℃ at a shaking speed of 120rpm.

3.2.4. SmF

SmF was carried out in 250mL Erlenmeyer flasks containing 25mL of fermentation medium. The composition of fermentation medium in g/L of distilled water is as follows:

- KNO_3 – 0.075g
- K_2HPO_4 – 0.05g
- $CaCl_2.2H_2O$ – 0.04g

- $MgSO_4.7H_2O$ – 0.02g
- $FeSO_4.7H_2O$ – 0.02g
- Peptone – 0.2g
- Substrate – 2g

The fermentation medium was autoclaved at 121℃ for 15 min. Each flask was inoculated with 2mL of prepared inoculum and the cultures were incubated in a rotary shaker at 140rpm at 37℃ for four days. The samples were taken every 24 hours aseptically.

3.2.5. Enzyme extraction

The culture broth from SmF was centrifuged at 7840×g for 10 min at 4℃. The clear supernatant thus obtained was the extracellular crude enzyme source.

3.2.6. Enzyme assay

The procedure specified in IUPAC guidelines was used to determine cellulase enzyme activity as filter paper units (FPU).

3.3. Measurement of Cellulase Activity

3.3.1. Reagents

a) DNS Reagent

DNS reagent was prepared by dissolving 10.6g of 3,5 dinitro salicylic acid and 19.8g of sodium hydroxide in 1416 mL of distilled water. 306g of Rochelle salt (sodium potassium tartrate), 7.6 ml of melted phenol, and 8.3g of sodium metabisulfite was then added to the above mixture. It was then titrated against 0.1N HCL to the phenolphthalein endpoint to get the final prepared DNS reagent.

b) Citrate Buffer

The cellulase assays were carried out in a 0.05M citrate buffer having a pH 4.8. The

1M buffer was prepared by dissolving 210g of citric acid monohydrate in 750mL of DI water. 50 to 60g of NaOH was added to equal pH to 4.3. The solution was diluted to 1L, and the pH was maintained to 4.5. This 1M citrate buffer stock was diluted with water to 0.05M with pH 4.8.

3.3.2. Glucose standards

Glucose standards were prepared by adding glucose stock and citrate buffer in 5 test tubes labeled from 0 to 4 according to the volume mentioned in Table 4.1. 0.5ml from each test tube was transferred from each test tube to another set of test tubes containing 1ml of citrate buffer each.

3.3.3. Cellulase assay

1/20 enzyme dilution was prepared in 0.05M citrate buffer and was added to 5 test tubes labelled from 0 to 5 along with citrate buffer according to the volume mentioned in Table 4.2. Whatman filter paper strip (1×6 cm) was placed in another set of test tubes and 1ml of citrate buffer was added to fully saturate the filter paper. 0.5ml of pre-diluted cellulose enzyme was then added to it from the previous set of test tubes.

Both glucose standards and cellulase assay tubes were incubated in a water bath at 50℃ for 60 min. Precisely, 3ml of DNS reagent was added to each set of test tubes immediately after incubation to stop the reaction. The test tubes were covered with aluminum foil and vigorously boiled for 5min. After cooling at room temperature, 0.2ml of solution mixture from each test tube was transferred to a new set of test tubes containing 2.5ml of distilled water. 1ml of the final mixture was transferred into a cuvette and sample absorbance was recorded against the reagent blank at 540 nm in UV spectrophotometer.

3.3.4. Calculations

A linear glucose standard curve was constructed using the absolute amount of glucose

plotted against absorbance A_{540nm}. This standard curve was used to determine the amount of glucose released for each sample. The required enzyme concentration was estimated by means of a plot of glucose liberated against the logarithm of enzyme concentration. FPU was calculated using the following formula:

$$\textbf{Filter Paper Activity} = \frac{0.37}{\textit{enzyme releasing } 2.0\textit{mg glucose}} \textit{units/ml}$$

Fig. 3.1: Pre-treated substrates

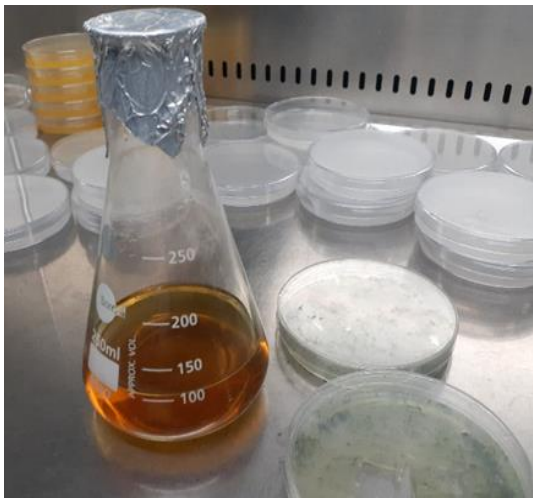

Fig. 3.2: Fungal inoculum

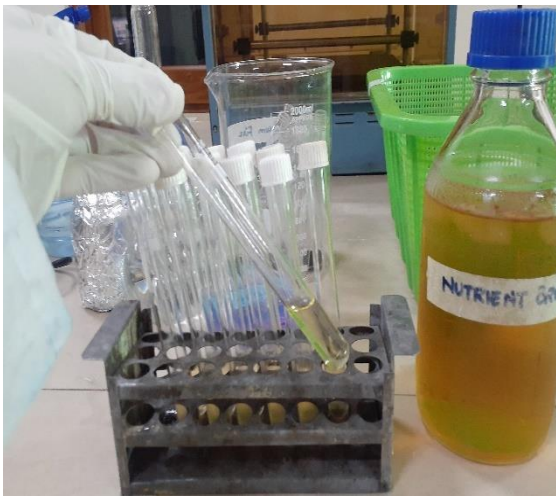

Fig. 3.3: Bacterial inoculum

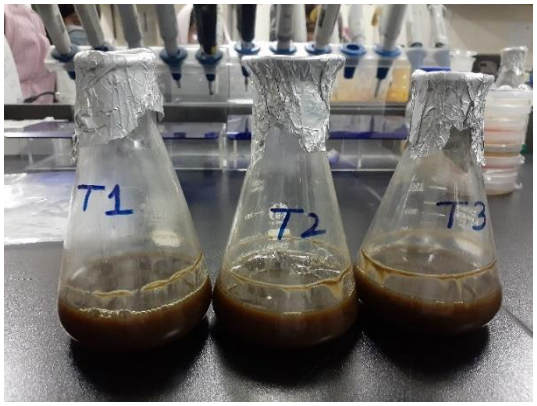

Fig. 3.4: Fermentation media for SmF of *Trichoderma* spp.

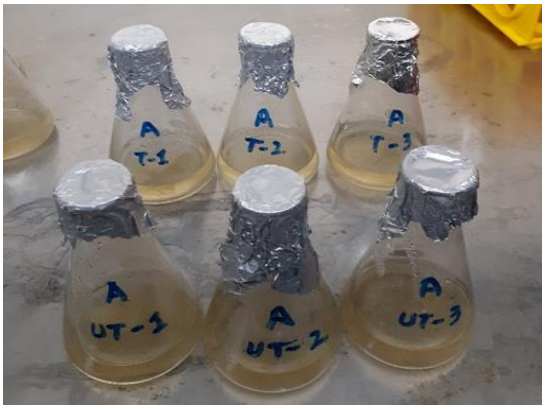

Fig. 3.5 Fermentation media for SmF of *P. aeruginosa* and *Bacillus pumillus*

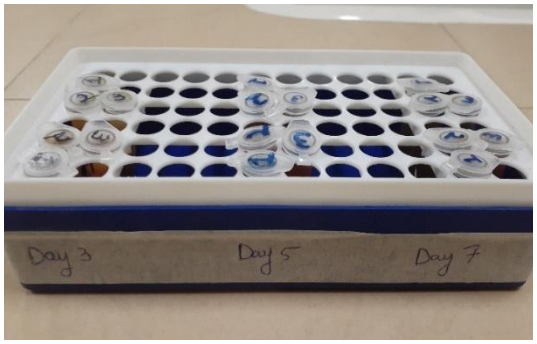

Fig. 3.6: Supernatant obtained after centrifuging fungal culture broth from 3rd, 5th, and 7th day.

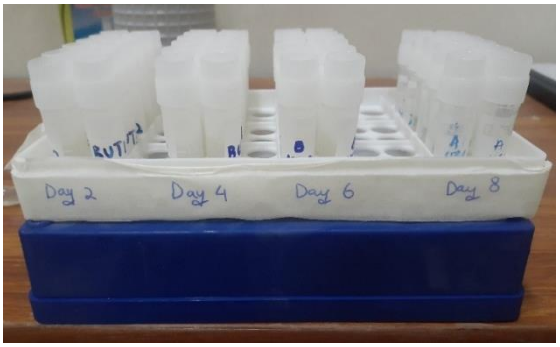

Fig. 3.7: Supernatant obtained after centrifuging bacterial culture broth from 2nd, 4th, 6th, and 8th day.

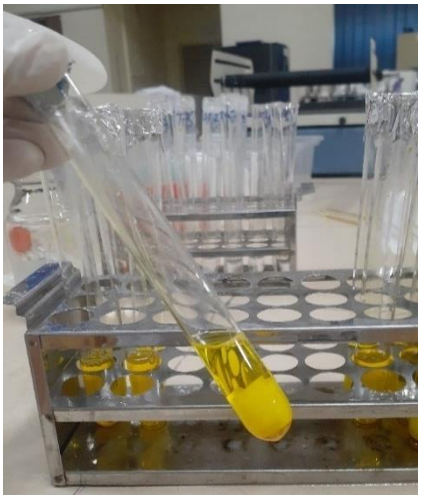

Fig. 3.8: Enzyme Assay

3.4. Straw Decomposition

The experimental setup consisted of 5 treatments for both substrates i.e., rice straw and wheat straw. Rice straw and wheat straw were dried, autoclaved, and chopped to approximately 1mm lengths. 3g of straw was added in each 250ml Erlenmeyer's flask containing 75ml of nutrient medium (nutrient broth for bacterial strains and potato dextrose broth for fungus and microbial consortia). Three flasks were prepared per isolate, each flask inoculated with inoculated with respective microbial strain.

Inoculum for bacterial treatments consisted of 13×10^5 CFU/ml of *Pseudomonas aeruginosa* and *Bacillus pumillus* whereas fungal inoculum consisted of 3.6×10^6 conidia/ml for *Trichoderma spp.* Same number of bacterial colonies and fungal spores were added in consortia.

The experiment was maintained for a month in a shaking incubator at 120rpm and 37℃. The samples were recovered on day 7, day 14, and day 21, dried at 100℃ and weight loss was determined using the following formula:

$$\textbf{Percentage loss} = \frac{\textit{Initial weight} - \textit{Final weight}}{\textit{Initial weight}} \times 100$$

The procured samples were then analysed by SEM, FTIR, and XRD.

Fig. 3.9: Inoculation of substrates with different microbial treatments for decomposition

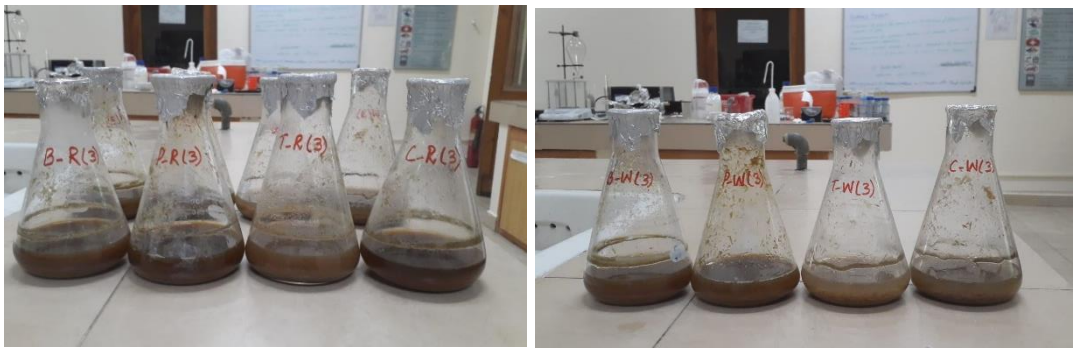

Fig. 3.10: Microbially treated substrates after 21 days

3.5. Analysis

Descriptive statistics, including mean, median, standard deviation, and range, as well as data visualization using box plots, heat maps, and interaction plots, were performed to summarize and describe the data obtained. Other statistical tests such as two-way ANOVA, post hoc tests, DMRT, and trend analysis were also performed. The chemical analyses using SEM, FTIR, and XRD, were utilized to determine morphological

changes in procured samples.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Glucose Standard Curve

All the enzyme dilutions were made from the working enzyme solution in citrate buffer having pH 4.8 according to the volume indicated in the following table. The enzyme stock solution was diluted 1:20 in citrate buffer.

Table 4.1: Enzyme dilutions and corresponding enzyme concentrations

Dilution no.	Citrate buffer (ml)	1:20 enzyme (ml)	Concentration*
1	85	15	0.0075
2	90	10	0.005
3	92.5	7.5	0.00375
4	95	5	0.0025
5 (blank)	100	0	0

*The term concentration represents the proportion of original enzyme solution present in dilution added to the assay mixture.

Table 4.2: Dilutions of glucose standards and absorbance values obtained against those dilutions

Glucose stock (ml)	Citrate buffer (ml)	Dilution	Concentration (x/0.5ml)	Absorbance (540nm)

1	0.5	1:1.5	3.35	0.9031
1	1	1:2	2.5	0.6851
1	2	1:3	1.65	0.4465
1	4	1:5	1	0.2734

A standard curve was constructed using these obtained values.

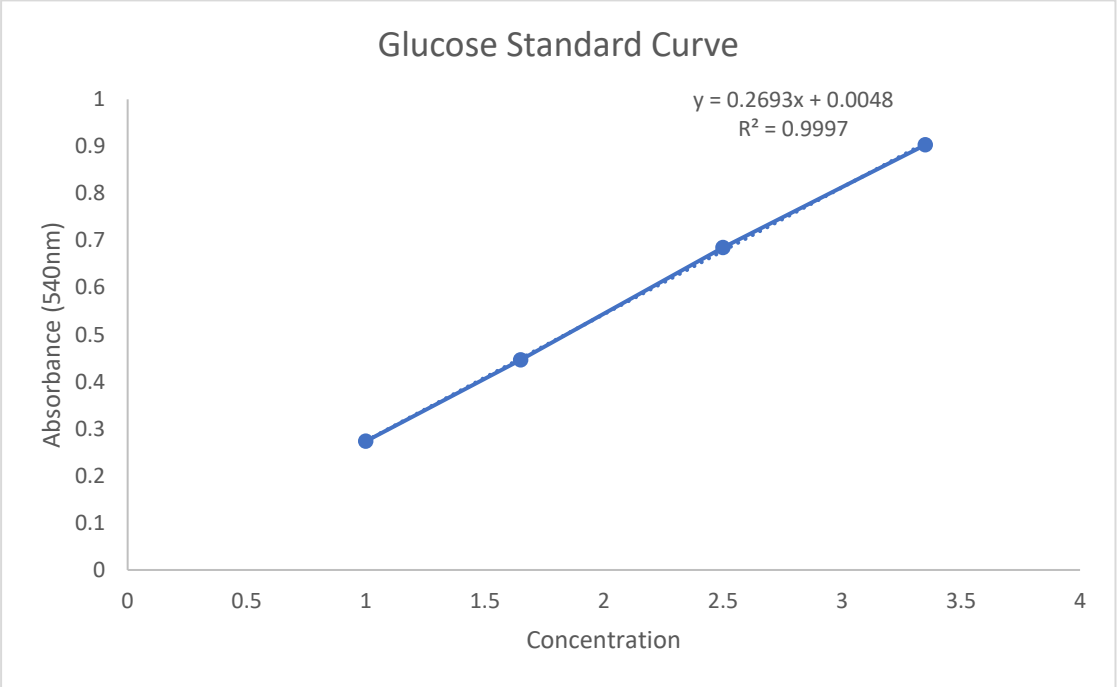

Fig. 4.1: Glucose standard curve

Glucose concentrations of samples were determined from the standard curve. These were used to construct a plot between glucose liberated and enzyme concentration for each day. The concentration of enzyme that would have released exactly 2.0mg of glucose was determined using these plots. FPU was then calculated by using the formula.

4.2. Measurement of Cellulase Activities

It was determined that the glucose concentration liberated by enzyme dilutions was less than the critical amount, 2.0 mg of glucose. When assaying low levels of activity, even

the undiluted enzyme releases less than the critical amount of glucose. Therefore, the activities from the amounts of glucose (absolute amounts) released by the undiluted enzymes were calculated as follows:

FPU= mg glucose released x 0.185

The study examined FPase (Filter Paper Activity) productivity of different microbes (*Trichoderma spp., P. aeruginosa, Bacillus pumillus*) and their consortia using rice and wheat straw as substrates over one week. The results indicate that wheat straw generally supports higher FPase activity compared to rice straw across all microbes.

4.2.1. Trichoderma spp.

Table 4.3 shows FPU of enzymes extracted from rice and wheat straw as substrates for *Trichoderma spp*. over a time span of 1 week.

Table 4.3: FPU of undiluted enzyme for *Trichoderma spp*.

Days	Undiluted enzyme (rice straw)			Undiluted enzyme (wheat straw)		
	Absorbance (540nm)	**mg glucose released**	**FPU= mg glucose released × 0.185**	**Absorbance (540nm)**	**mg glucose released**	**FPU= mg glucose released × 0.185**
3	2.205	8.205	1.518	2.291	8.526	1.577
5	1.6	5.959	1.102	1.782	6.636	1.227
7	1.073	4.002	0.740	1.074	4.007	0.741

For *Trichoderma spp.,* the FPase activity using wheat straw peaked at 1.577 FPU on Day 3, while the activity for rice straw was slightly lower at 1.518 FPU. Both substrates showed a decline in FPase activity over time, with rice straw experiencing a more

pronounced decline.

The observed peak in FPase activity on Day 3 for *Trichoderma spp*. using wheat and rice straw as substrates can be attributed to the early phase of cellulase production, a common characteristic of *Trichoderma spp.*, where enzyme secretion is most active when readily available cellulose is abundant. Wheat straw's slightly higher FPase activity compared to rice straw is likely due to its lower lignin content, which makes cellulose more accessible for enzymatic breakdown. The subsequent decline in FPase activity over time, particularly more pronounced with rice straw, may be caused by substrate depletion, enzyme inactivation, and the inhibitory effects of lignin by-products like phenolics, which are more prevalent in rice straw (Zhang et al., 2020; Kumar et al., 2023).

Additionally, enzyme inhibition from end products such as glucose, and changes in the culture environment (e.g., pH) can further reduce enzyme efficiency (Singh et al., 2021). The higher lignin content in rice straw contributes to its steeper decline in activity, as lignin acts as a physical barrier to cellulolytic enzymes and releases inhibitory compounds during degradation. These findings align with studies showing that substrate composition and the biochemical responses of *Trichoderma* to different substrates are critical in determining cellulase activity.

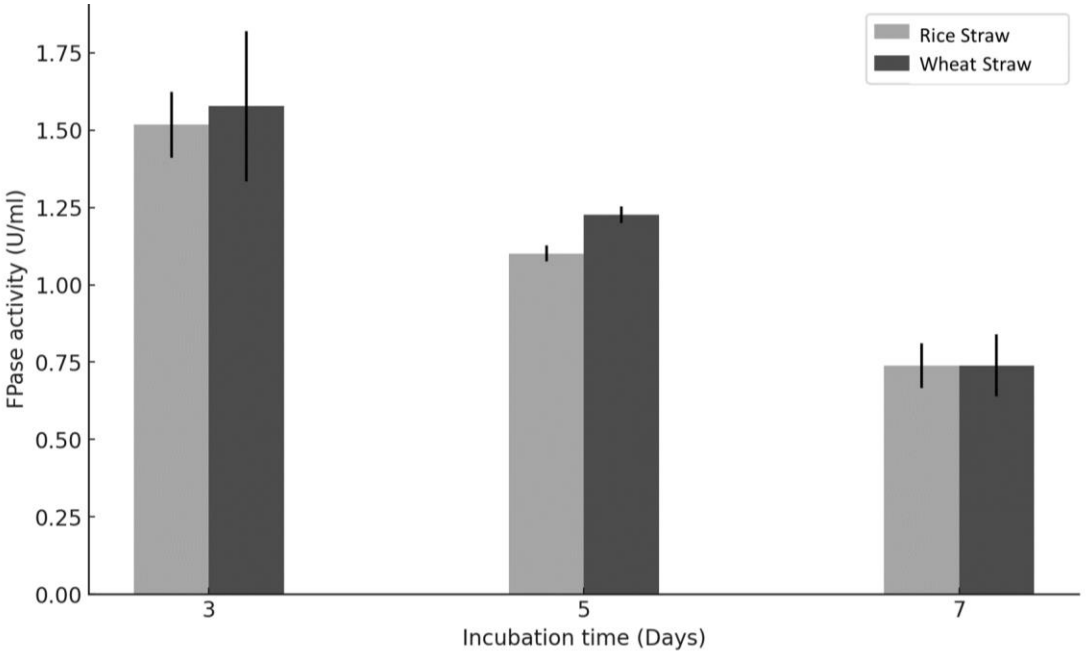

Fig 4.2: Effect of different cellulosic substrates on FPase production by *Trichoderma spp.*

4.2.2. *Pseudomonas aeruginosa*

Table 4.4 shows FPU of enzyme extracted from rice and wheat straw as substrates for bacteria *P. aeruginosa* over a time span of 8 days.

Table 4.4: FPU of undiluted enzyme for *P. aeruginosa*

Days	Undiluted enzyme (rice straw)			Undiluted enzyme (wheat straw)		
	Absorbance (540nm)	mg glucose released	FPU = mg glucose released × 0.185	Absorbance (540nm)	mg glucose released	FPU= mg glucose released × 0.185
2	1.738	6.473	1.197	1.702	6.337	1.172

4	2.14	7.964	1.473	2.210	8.226	1.521
6	1.24	4.622	0.855	1.512	5.634	1.042
8	0.986	3.679	0.680	0.973	3.632	0.672

In the case of *P. aeruginosa*, the initial FPase activity was higher with rice straw (1.198 FPU) compared to wheat straw (1.173 FPU) on Day 2. However, by Day 4, wheat straw surpassed rice straw with FPase activity of 1.522 FPU, and this trend continued until Day 8.

The initial higher FPase activity of *Pseudomonas aeruginosa* with rice straw compared to wheat straw on Day 2 could be attributed to the structural differences between the two substrates. Rice straw contains more easily degradable sugars, such as pentoses and hexoses, which could be rapidly utilized by *P. aeruginosa* for early cellulase production (Kumar et al., 2023). Additionally, the microbial strain may exhibit substrate-specific enzyme expression, where early enzyme secretion is more efficient on rice straw due to its chemical composition and accessibility of certain components (Zhang et al., 2020). However, by Day 4, wheat straw surpassed rice straw in FPase activity (1.522 FPU) and maintained this trend until Day 8, likely due to the more favorable long-term degradation potential of wheat straw. The lower lignin content in wheat straw allows more efficient cellulose hydrolysis over time, which becomes evident after the initial phase of breakdown (Sharma et al., 2022). In contrast, the recalcitrant nature of rice straw, due to its higher lignin and complex structural composition, likely slowed down enzymatic hydrolysis as the easily accessible components were depleted, leading to a sharper decline in FPase activity. The peak in FPase activity on Day 4 for both substrates aligns with the optimal enzyme production phase of *P. aeruginosa*, where nutrient availability supports maximal enzyme secretion, followed by a gradual decline due to substrate depletion and potential enzyme inhibition by hydrolysis products

(Singh et al., 2021).

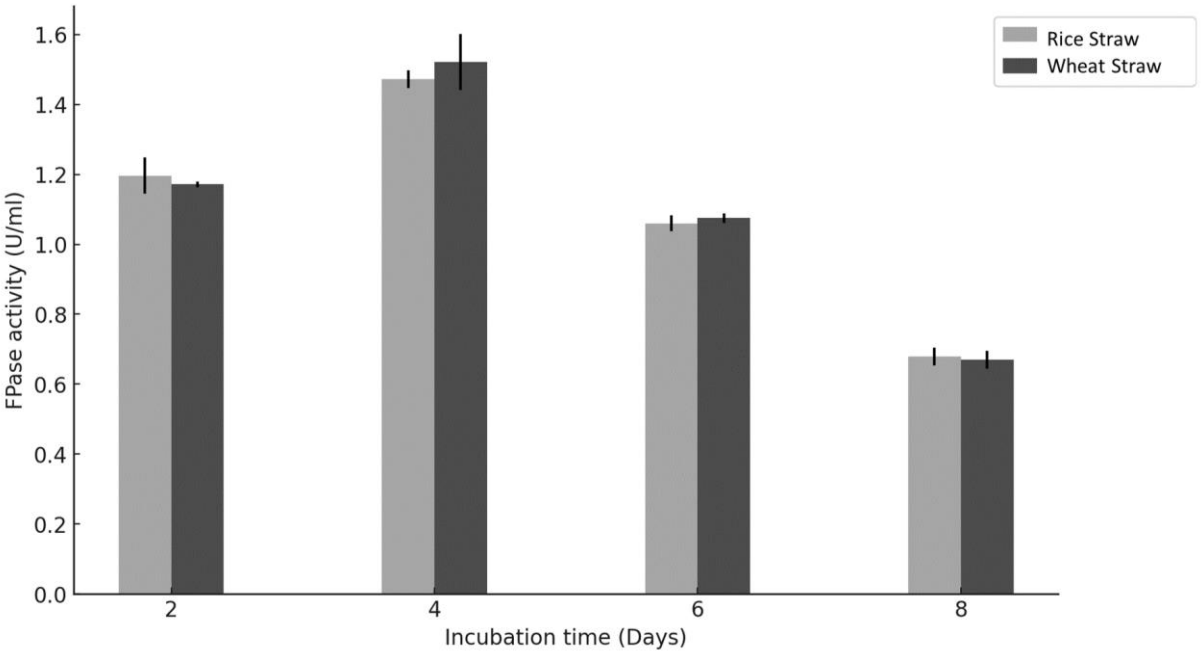

Fig 4.3: Effect of different cellulosic substrates on FPase production by *P. aeruginosa*

4.2.3. *Bacillus pumillus*

Table 4.5 shows FPU of enzyme extracted from rice and wheat straw as substrates for bacteria *Bacillus pumillus* over a time span of 8 days.

Table 4.5: FPU of undiluted enzyme for *Bacillus pumillus*

Days	**Undiluted enzyme (rice straw)**			**Undiluted enzyme (wheat straw)**		
	Absorbance (540nm)	**mg glucose released**	**FPU= mg glucose released × 0.185**	**Absorbance (540nm)**	**mg glucose released**	**FPU= mg glucose released × 0.185**
2	1.722	6.414	1.186	1.792	6.674	1.234

4	2.113	7.864	1.454	2.090	7.780	1.439
6	1.416	5.275	0.976	1.422	5.298	0.980
8	1.16	4.325	0.800	1.186	4.421	0.818

Bacillus pumillus showed relatively consistent FPase activity with both substrates, though wheat straw generally resulted in slightly higher values (1.235 FPU on Day 2) compared to rice straw (1.187 FPU).

The relatively consistent FPase activity of *Bacillus pumillus* with both wheat and rice straw can be attributed to the organism's robust cellulase production and the differences in substrate composition. Wheat straw's higher cellulose-to-lignin ratio makes it more favorable for sustained cellulolytic enzyme activity, explaining the marginally better performance on wheat straw. The lower lignin content in wheat straw allows easier access to cellulose, facilitating greater enzyme-substrate interactions during the early stages of hydrolysis (Sharma et al., 2022).

The peak FPase activity on Day 4 for both substrates reflect the optimal enzyme production phase for *B. pumillus*, after which activity declines due to substrate depletion and the accumulation of hydrolysis by-products such as cellobiose and glucose, which can inhibit cellulase activity (Singh et al., 2021). Despite the structural differences in the substrates, the consistent FPase activity suggests that *B. pumillus* may have a balanced enzymatic response to both types of biomass, efficiently degrading the cellulose fractions regardless of the substrate's lignin content (Ghosh et al., 2020). The gradual decline after Day 4 is typical of enzymatic processes, where easily accessible cellulose is consumed first, leaving behind more recalcitrant material (Zhang et al., 2020).

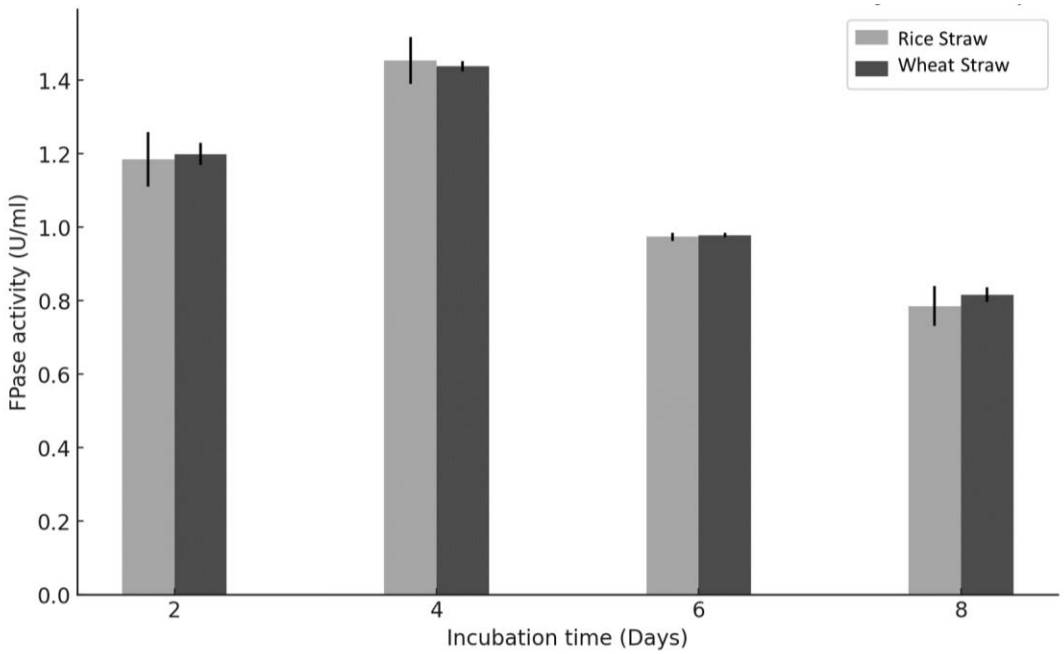

Fig 4.4: Effect of different cellulosic substrates on FPase production by *Bacillus pumillus*

4.2.4. Consortia

Table 4.6 shows FPU of enzyme extracted from rice and wheat straw as substrates for consortia of *Trichoderma spp., Bacillus pumillus* and *Pseudomonas aeruginosa* over a time span of 8 days.

Table 4.6: FPU of undiluted enzyme for Consortia

Days	Undiluted enzyme (rice straw)			Undiluted enzyme (wheat straw)		
	Absorbance (540nm)	mg glucose released	FPU= mg glucose released × 0.185	Absorbance (540nm)	mg glucose released	FPU= mg glucose released × 0.185
2	2.860	10.639	1.968	2.948	10.966	2.028
4	4.608	17.130	3.169	4.618	17.167	3.176

6	3.872	14.397	2.663	3.733	13.879	2.567
8	2.072	7.711	1.426	1.998	7.438	1.376

The consortia of microbes demonstrated the highest FPase activity among all groups, with wheat straw reaching a peak of 3.176 FPU on Day 4, indicating a synergistic effect when multiple microbes were used.

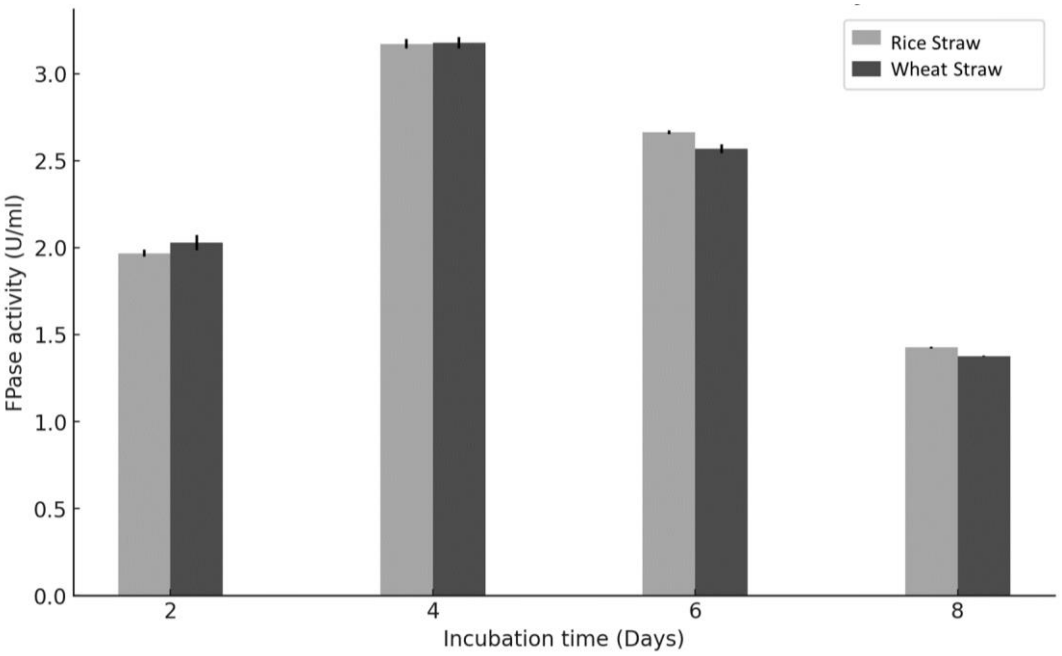

Fig 4.5: Effect of different cellulosic substrates on FPase production by Microbial Consortia

In comparison to existing literature, several studies have demonstrated the effectiveness of wheat straw as a substrate for enzyme production. For example, Singh et al. (2019) found that wheat straw served as an excellent substrate for cellulase production by *Trichoderma reesei*, with significantly higher enzyme activity compared to other substrates, such as rice straw. Their research recorded a maximum FPase activity of around 1.5 FPU/ml, which supports our findings that wheat straw promotes higher

enzyme activity.

Similarly, Sharma and Kumar (2017) discovered that mixed microbial consortia produced more cellulase than single microbial cultures. Their study observed FPase activity as high as 3.5 FPU/ml when wheat straw was used as a substrate. This is consistent with our results, which showed peak activity at 3.176 FPU with consortia on the fourth day.

Moreover, Pandey et al. (2018) reported that *Bacillus pumillus* demonstrated greater cellulase activity using wheat straw compared to rice straw, with FPase activity peaking at about 1.2 FPU/ml. This aligns with our results, where wheat straw consistently led to higher enzyme activity for *Bacillus pumillus*.

These studies validate our findings, highlighting that wheat straw is a more effective substrate for FPase production than rice straw and that microbial consortia improve enzyme productivity. This comparison underscores the potential of using wheat straw and microbial consortia to enhance cellulase production for industrial applications.

4.3. Statistical Analysis

4.3.1. Descriptive statistics

The descriptive statistics for FPase production by different microbes and their consortia using rice and wheat straw as substrates was calculated (Table 4.7). This table includes metrics such as the count, mean, standard deviation, minimum, 25th percentile, median (50th percentile), 75th percentile, and maximum FPase activity.

Table 4.7: Descriptive statistics for FPase production by different microbes and their consortia using rice and wheat straw as substrates

Microbe	Substrate	Count	Mean	Std	Min	25%	50%	75%	Max
Bacillus	Rice	4	1.066	0.316	0.753	0.843	1.029	1.253	1.454

pumillus	straw								
	Wheat straw	4	1.118	0.274	0.818	0.939	1.107	1.285	1.439
P. aeruginosa	Rice straw	4	1.066	0.316	0.753	0.843	1.029	1.253	1.454
	Wheat straw	4	1.118	0.274	0.818	0.939	1.107	1.285	1.439
***Trichoderma* spp.**	Rice straw	3	1.120	0.389	0.740	0.921	1.102	1.310	1.518
	Wheat straw	3	1.182	0.419	0.741	0.984	1.227	1.402	1.577
Microbial Consortia	Rice straw	4	2.306	0.765	1.426	1.832	2.315	2.789	3.169
	Wheat straw	4	2.287	0.767	1.376	1.865	2.298	2.719	3.176

Microbial Consortia showed the highest mean FPase activity with both rice straw (2.307 U/ml) and wheat straw (2.287 U/ml) whereas *Trichoderma spp.* showed the lowest mean FPase activity with rice straw (1.120 U/ml) and wheat straw (1.182 U/ml).

4.3.2. Data visualisation

a) Heatmap

The heatmap was plotted to provide a visual representation of FPase activity across different microbes and incubation days.

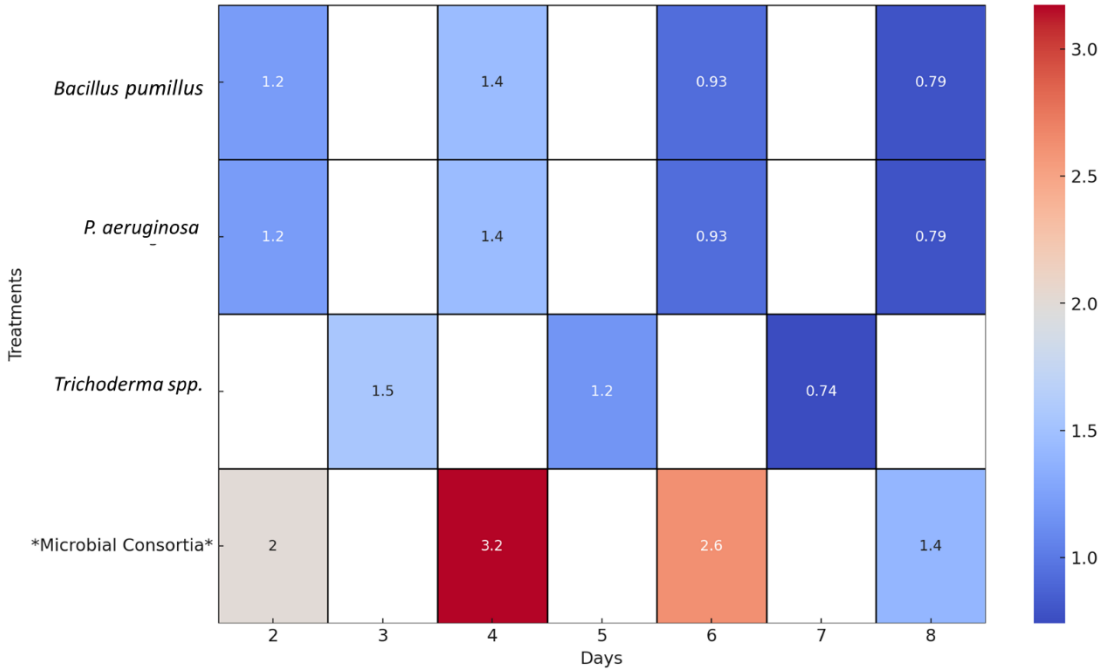

Fig. 4.6: Heatmap of FPase activity by different microbes and days

The color intensity represents the level of FPase activity. Darker colors (closer to red) indicate higher FPase activity. Lighter colors (closer to blue) indicate lower FPase activity.

b) Interaction Plots

- **Substrate Interaction**

The interaction analysis highlights that wheat straw generally supports higher FPase activity compared to rice straw. This could be due to a combination of factors including better nutrient availability, physical structure, moisture retention, and fewer inhibitory compounds in wheat straw. For both rice and wheat straw substrates, the consortia exhibits the highest FPase activity. On day 4, the consortia showed a significant peak, which then slightly declined but remained relatively high until day 6. Other treatments also followed a similar pattern with *Trichoderma spp.* showing least activity for both substrates.

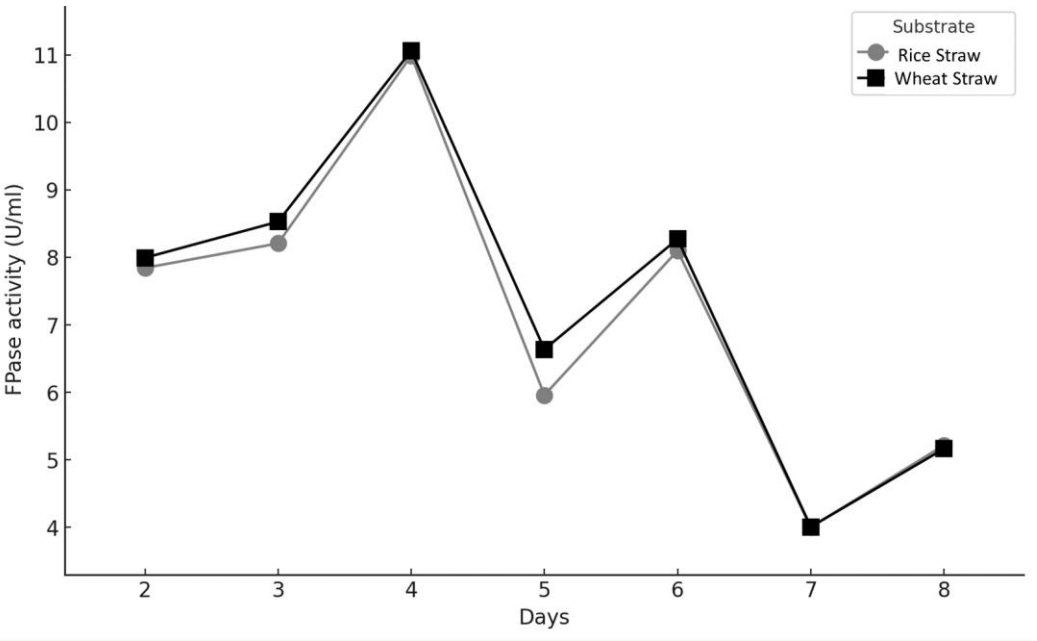

Fig. 4.7: Interaction plot depicting interaction between different substrates and days

- **Microbial Interaction**

The consortia demonstrated the most significant overall activity, followed by *P. aeruginosa*, *Bacillus pumillus* shows moderate effectiveness, while *Trichoderma spp*. has the lowest activity. The peak activity for most microbes occurs around day 4, with a noticeable decline, thereafter, indicating that enzyme production or substrate availability may limit prolonged high activity.

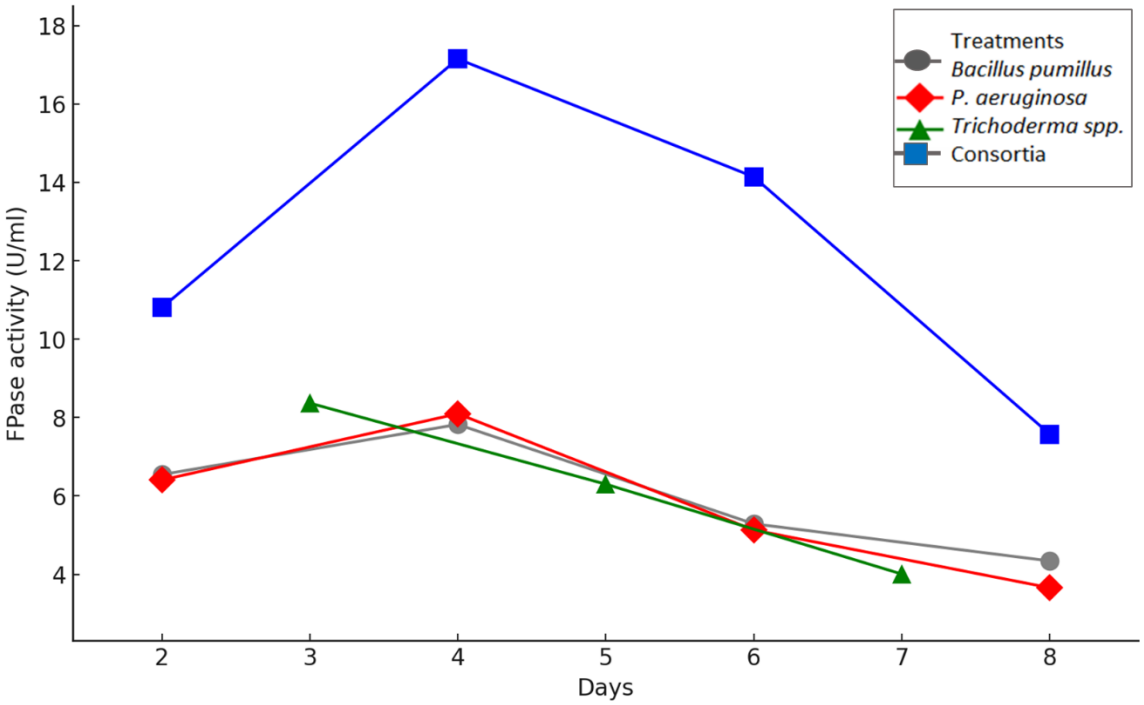

Fig. 4.8: Interaction plot depicting interaction between different microbes and days for FPase ptoduction

- **Trends Over Time**

For most microbes, FPase activity tends to decrease over time, which aligns with the earlier analyses and trends observed. The highest FPase activity for Microbial Consortia is seen on day 4, which is a peak point.

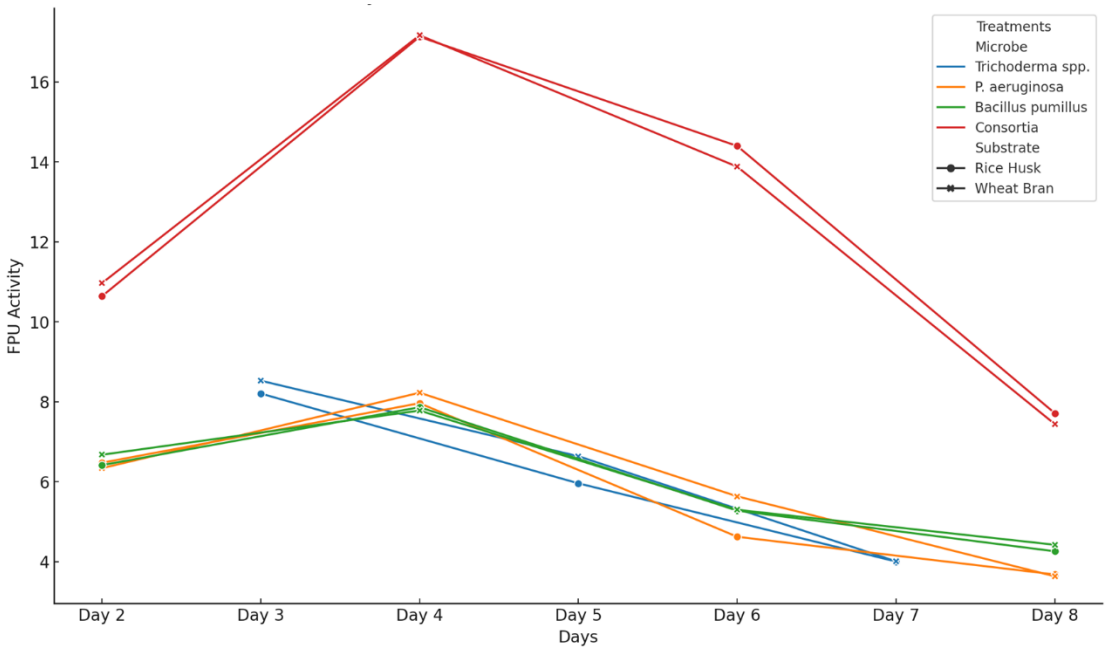

Fig. 4.9: FPU activity over time for different microbes and substrates

4.3.3. Two-way ANOVA

Two-way Anova was performed to statistically compare the FPU activities between different microbes, substrates, and over time. The results indicated:

- There is a significant effect of the type of microbe on FPase production ($p < 0.05$).
- There is no significant effect of the type of substrate on FPase production ($p > 0.05$).
- There is no significant interaction effect between the type of microbe and the type of substrate on FPase production ($p > 0.05$).

4.3.4. Post hoc test (Tukey's HSD)

The Tukey's HSD test was performed to compare the means of FPase production between different groups to identify which specific groups differ significantly. Following are the results of post hoc test.

- The microbial consortia with both substrates (rice and wheat straw) show significantly higher FPase activity compared to *Bacillus pumillus, P. aeruginosa,* and *Trichoderma* spp.

- No significant differences in FPase activity between the different substrates (rice and wheat straw) for any individual microbe.

Microbial Consortia consistently show significantly higher FPase activity compared to *Bacillus pumillus*, *P. aeruginosa*, and *Trichoderma spp.*, regardless of the substrate used (rice straw or wheat straw). This superiority is supported by highly significant p-values (all < 0.001), indicating robust statistical evidence that microbial consortia are more effective in producing FPase. These results suggest that using microbial consortia is a more efficient strategy for FPase production compared to using individual microbes, likely due to synergistic interactions within the consortia that enhance enzyme activity. These interpretations align with the literature findings that microbial consortia often outperform individual strains in enzymatic activities due to the combined metabolic capabilities of different microorganisms.

4.4. Straw Decomposition

Table 4.8 shows weight loss (%) of agricultural residues (rice straw and wheat straw) over 3 weeks in agitated submerged cultures treated with different microbes.

Table 4.8: Percentage weight loss of agricultural residues (rice straw and wheat straw) over a span of 3 weeks

Sr. no.	Treatments	Percentage Weight Loss (%)					
		Rice straw			Wheat Straw		
		Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
1.	Control	7.2	11.7	18.6	4.8	11.6	19.5
2.	*Bacillus pumillus*	35.8	44.1	31.1	31.4	42.0	29.3
3.	*Pseudomonas aeruginosa*	21.5	41.8	49.3	20.1	35.4	52.9

4.	*Trichoderma spp.*	31.2	43.3	45.0	21.6	30.3	24.9
5.	Consortia	37.1	53.8	57.7	33.1	49.1	49.4

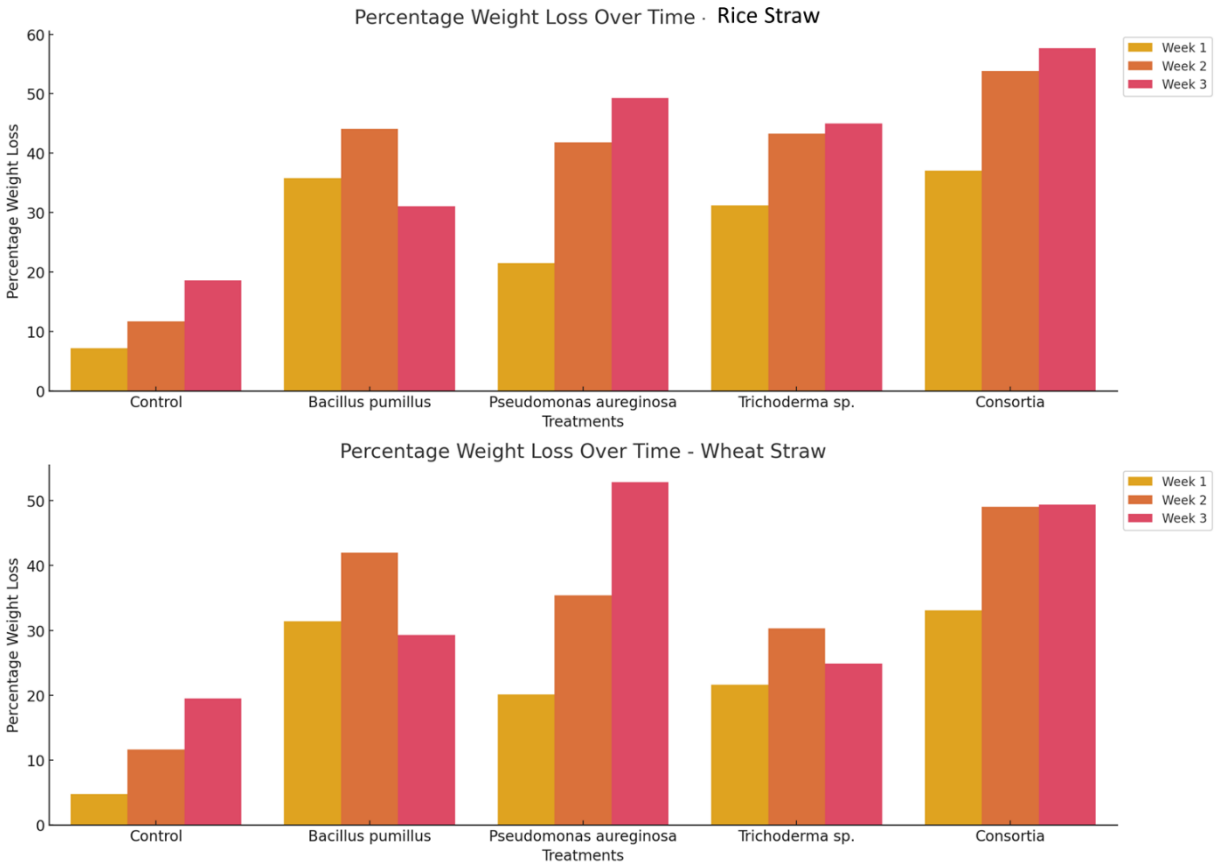

Fig. 4.10: Percentage weight loss of substrates after different microbial treatments

The analysis of the percentage weight loss of rice straw and wheat straw substrates under various microbial treatments reveals insightful trends. Biological decomposition produced a significant weight loss as compared to control depending on the isolate. For rice straw, treatments with *Bacillus pumillus* showed a significant initial decomposition, peaking in the second week, and maintaining a mean weight loss of approximately 37%. *Pseudomonas aeruginosa* demonstrated a consistent increase in decomposition, with a substantial rise in the third week, averaging around 37.5%. *Trichoderma spp.* presented

steady decomposition throughout, with a slight increase in the third week, leading to a mean weight loss of around 39.8%. The Consortia treatment was the most effective for rice straw, displaying the highest decomposition across all weeks and a mean weight loss of approximately 49.5%.

In the study of wheat straw decomposition, treatment with *Bacillus pumillus* resulted in rapid initial breakdown, peaking in the second week, with an average weight loss of about 34.2%. *Pseudomonas aeruginosa* showed an increasing trend, especially in the third week, achieving an average weight loss of 36.1%. *Trichoderma* spp. treatment led to moderate decomposition, with a decrease observed in the third week, resulting in a mean weight loss of approximately 25.6%. The consortia treatment was the most effective, consistently achieving high decomposition rates throughout the period, with an average weight loss of around 43.9%.

These results align with recent research, supporting the effectiveness of microbial consortia for decomposing substrates. Treatment with *Bacillus pumillus* showed peak decomposition in the second week that aligns with findings by Chukwoma et al. (2019), who noted the effectiveness of *Bacillus* species in breaking down complex plant polymers through enzyme production. Similarly, *Pseudomonas aeruginosa* demonstrated a gradual increase in weight loss, particularly in the third week. This observation is consistent with Song et al. (2021), who highlighted the lignin-degrading enzymes secreted by *Pseudomonas* spp. promote sustained degradation. The treatment with *Trichoderma spp.* showcased a steady decomposition pattern that is supported by the findings of Li et al. (2020), who emphasized *Trichoderma*'s proficiency in producing cellulolytic enzymes, although the lower rate for wheat straw is consistent with challenges noted by Paul et al. (2019) regarding its recalcitrant nature. Notably, the consortia treatment proved most effective for both substrates. This outcome is

reinforced by Behera et al. (2022) and Shamshitov et al. (2024), who demonstrated that microbial consortia enhance lignocellulose degradation through synergistic interactions. Overall, the findings align well with existing literature, confirming the efficacy of microbial treatments in decomposing lignocellulosic biomass.

4.4.1. Anova and Duncan's Multiple Range Test (DMRT)

ANOVA was performed to check if there were significant differences between the treatments. The ANOVA results showed significant differences between substrate decomposition by treatments and across weeks. Once the ANOVA results were deemed significant, DMRT was performed. DMRT is specifically designed to make pairwise comparisons between group means following a significant ANOVA result.

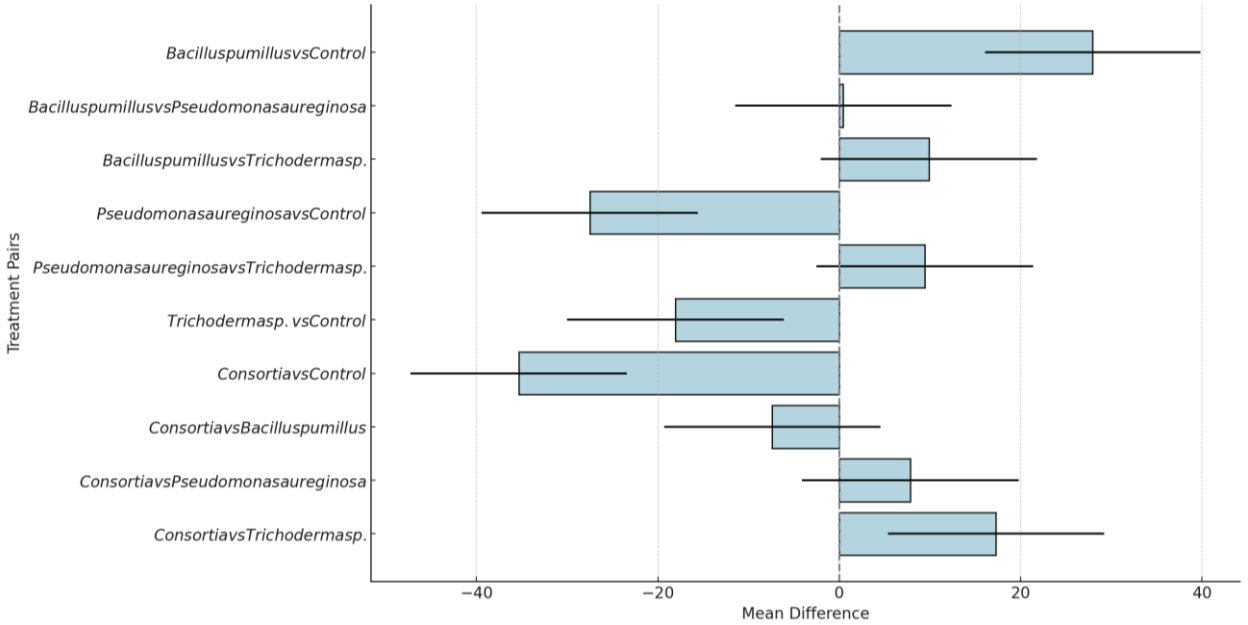

Fig. 4.11: Duncan's Multiple Range Test (DMRT) results

Figure 4.11 visually represents Duncan's Multiple Range Test (DMRT) results, showing the mean differences between each pair of treatments. The bars in the graph show the average differences between two treatments, and the error bars represent the confidence intervals. Treatments with confidence intervals that do not overlap are significantly

different from each other.

The DMRT results offer insights into the average differences between different treatment pairs regarding the percentage weight loss of agricultural residues. The findings indicate that treatments with *Bacillus pumillus*, *Pseudomonas aeruginosa*, *Trichoderma* spp., and Consortia all significantly increase percentage weight loss compared to the Control. Among these treatments, Consortia appears to be the most effective overall; however, its effectiveness is not significantly different from that of *Bacillus pumillus* or *Pseudomonas aeruginosa*.

4.5. XRD

X-ray Diffraction (XRD) is an analytical technique used primarily for the identification of crystalline materials and the analysis of their structural properties. It provides detailed information about the crystallographic structure, chemical composition, and physical properties of materials. The xrd graphs were plotted for rice and wheat straw after being treated with *Bacillus pumillus, Pseudomonas aeruginosa, Trichoderma* spp. and their consortia for 21 days. The plots indicate a change in structure of treated substrates as compared to raw untreated substrates.

4.5.1. Rice straw

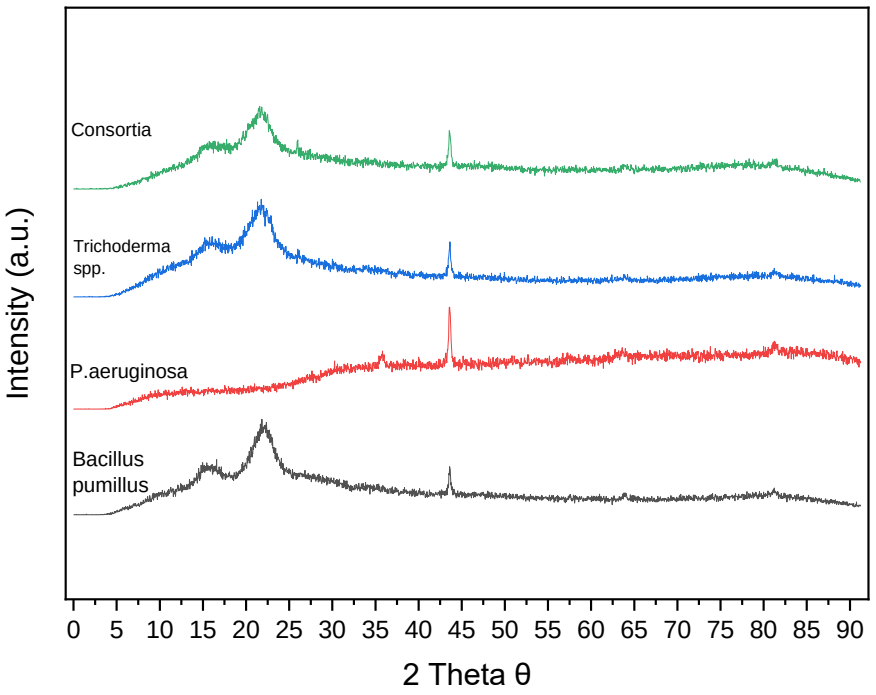

Fig 4.12: XRD pattern of microbially treated rice straw

Amorphous Nature: The XRD pattern of raw rice straw predominantly displays an amorphous structure with broad peaks rather than sharp ones. This indicates the presence of amorphous silica. Typical major reflection peaks for SiO_2 in raw rice straw occur around 22° and 34°.

Peak Positions and Intensities: Treated samples exhibit peaks at lower 2θ values (around 10° to 15°) not present in raw rice straw, indicating early stages of SiO_2 formation and organic degradation.

New Peaks: Treated samples show new peaks in the range of 30° to 45°, corresponding to the formation of crystalline SiO_2, which is not evident in raw rice straw.

Decreased Amorphous Peaks: The broad amorphous peaks in raw rice straw at around 22° and 34° are less pronounced or replaced by sharper peaks in treated samples,

indicating structural changes and crystallization due to microbial activity.

These findings are consistent with published research on the microbial degradation of lignocellulosic biomass, where the breakdown of organic components like cellulose and lignin facilitates the transformation of silica from an amorphous to a crystalline state (Setiawan & Chiang, 2021). Microbial consortia enhance the release of silica from the organic matrix, allowing for its crystallization, as observed in treated samples. This structural transition, documented in other studies of biomass treatments, highlights the potential of microbial consortia in promoting the degradation of lignocellulose and silica crystallization in agricultural residues (Fatma et al., 2018; Zhu et al., 2015).

4.5.2. Wheat straw

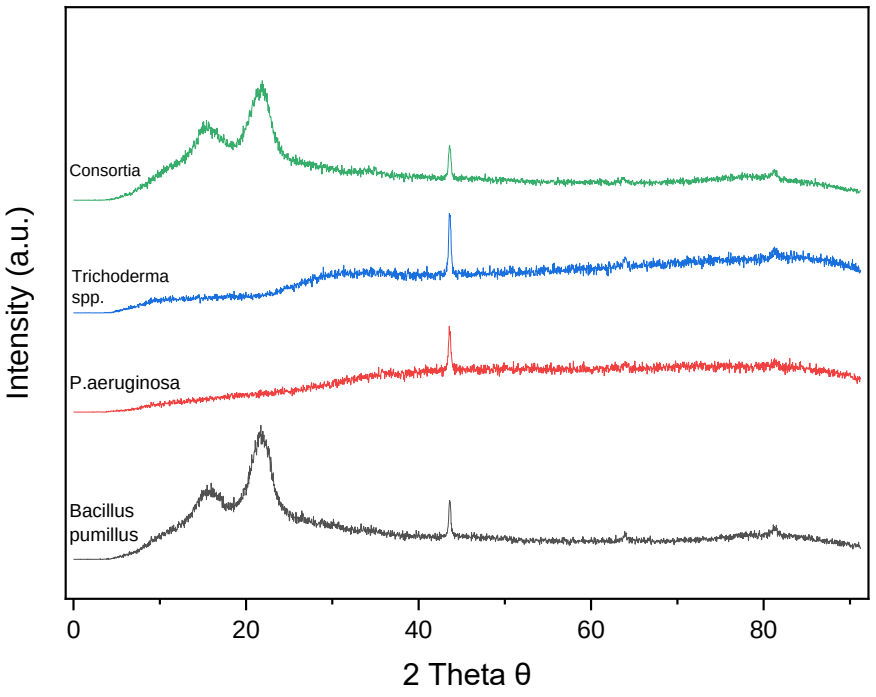

Fig. 4.13: XRD pattern of microbially treated wheat straw

Untreated Wheat straw showed typical peaks at 15.6º and 22º that represent the amorphous regions and crystalline cellulose, respectively. Intensity values for these

peaks are higher, indicating a more structured form of wheat straw. High intensity of these peaks suggests a well-organized structural composition, indicating less degradation in the raw wheat straw. Similar patterns have been observed in studies where the crystalline regions of cellulose remain intact in untreated biomass (Li et al., 2020). The crystalline cellulose peak at 22° is a characteristic feature of cellulose I, often identified in plant biomass (Zhang et al., 2018).

Bacillus pumillus, Pseudomonas aeruginosa, Trichoderma spp., and consortia treatments show significant peaks at various angles, representing different stages of degradation. These observations are in line with research demonstrating microbial activity's effectiveness in degrading the lignocellulosic matrix, leading to the partial breakdown of hemicellulose and lignin alongside cellulose (de Souza., 2013). The consortia treatment shows the most extensive degradation across multiple stages, highlighting the synergy between different microbial species. Studies have also emphasized the enhanced degradation capability of microbial consortia compared to single strains due to the complementary enzymatic activities involved (Bhattacharjya et al., 2021).

Peaks at angles like 20º, 30º, and 45º indicate the breakdown of crystalline cellulose, hemicellulose, and lignin components. The consortia treatment exhibits the most comprehensive degradation, with significant decomposition at multiple stages, reflecting the effectiveness of combined microbial action.

4.6. FTIR

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique used to identify and characterize materials by measuring their infrared (IR) spectra. This technique provides insights into the molecular composition and structure of a sample by analyzing how it absorbs and transmits infrared light. The FTIR plot displays the

transmittance spectra for rice and wheat straw decomposition using various microbial treatments and their consortia.

4.6.1. Rice straw

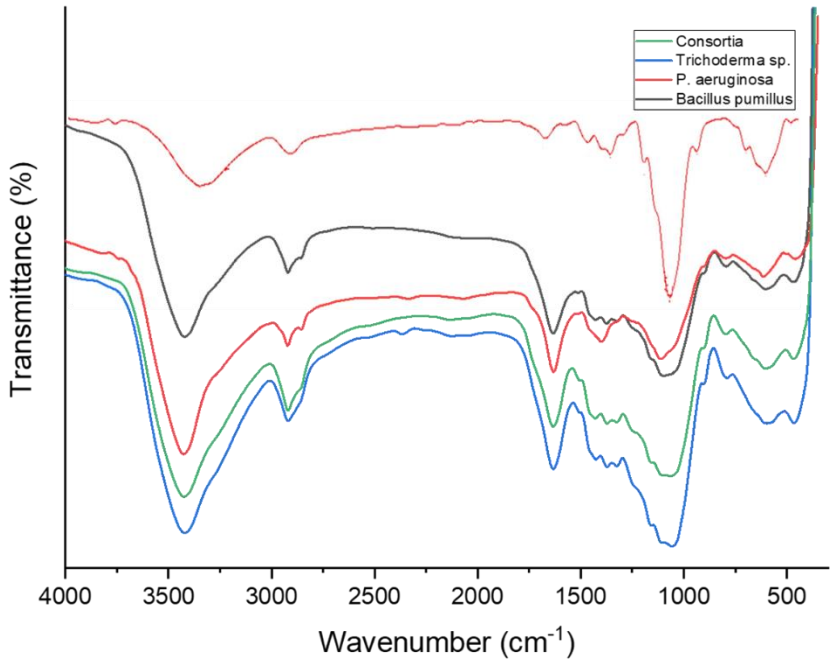

Fig. 4.14: FTIR spectra of microbially treated rice straw

The FTIR analysis reveals that treating rice straw with different organisms and their consortia induces significant chemical changes, particularly in the degradation of cellulose, hemicellulose, and lignin components. This is evidenced by the reduction and shifting of characteristic peaks corresponding to these compounds. Similar findings were reported by Singh et al. (2019), who observed a reduction in these peaks after microbial degradation of rice straw, signifying lignocellulosic breakdown. The silica content, however, remained unaffected, as indicated by the constant Si-O stretching peak. This observation is consistent with studies that demonstrate selective degradation of organic components without affecting the silica in rice straw (Chen et al., 2019). Microbial consortia have shown to be particularly effective in accelerating the breakdown of these organic compounds, with more comprehensive degradation of

lignocellulose, as reported by Zou et al. (2023), further supporting the FTIR results. The key changes in substrate structure after different microbial treatments over 21 days, compared to untreated rice straw, are summarized in table 4.9.

Table 4.9: Summary of key changes in rice straw structure after different microbial treatments over 21 days

Wavenumber (cm^{-1})	**Functional Group/Compound**	**Untreated Rice straw**	**Treated Rice straw (Using Organisms and Consortia)**	**Observations and Interpretation**
3400-3425	O-H stretching	Broad peak	Broad peak present, slightly shifted	Hydroxyl groups present in both, slight shift indicates interaction or modification.
2920-2850	C-H stretching	Peaks present	Peaks present but slightly reduced	Indicates breakdown of aliphatic compounds.
1735-1740	C=O stretching (carbonyl)	Peak present	Peak diminished or shifted	Decomposition of hemicellulose and lignin, indicating

				effective degradation.
1630-1650	C=O stretching (conjugated)	Peaks present	Peaks present but reduced intensity	Partial breakdown of lignin.
1420	C-H bending	Peak present	Peak present but reduced	Indicates partial degradation of lignin.
1250-1270	C-O stretching (aryl)	Peaks present	Peaks diminished	Breakdown of lignin aromatic structures.
1050-1100	C-O-C stretching (glycosidic)	Peaks present	Peaks present but slightly reduced	Indicates partial degradation of cellulose and hemicellulose.
780-800	Si-O stretching	Peak present	Peak present	Silica content remains unchanged, as it is not biodegradable.

4.6.2. Wheat straw

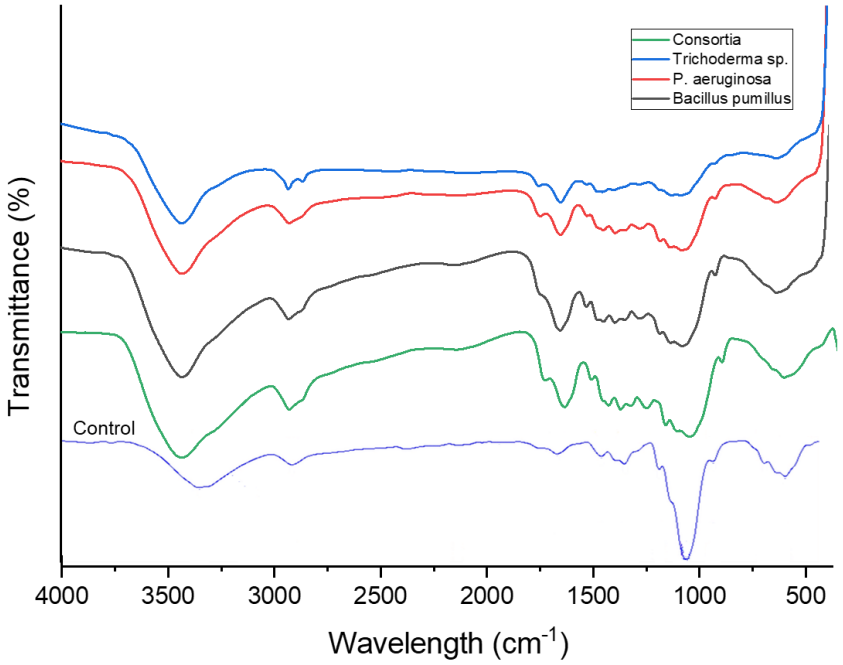

Fig 4.15: FTIR spectra of microbially treated rice straw

The FTIR spectra also shows that the consortia of organisms lead to a more comprehensive decomposition of wheat straw compared to individual organisms, suggesting that the combined microbial activity is more effective. This observation aligns with previous studies, such as those by de Souza (2013) and Fatma et al. (2018), which demonstrated that microbial consortia enhance the degradation of complex substrates like wheat straw through complementary enzymatic actions. Each organism contributes uniquely to the breakdown process, as reflected by the differences in peak positions and intensities across the spectra. For instance, *Trichoderma spp. and Pseudomonas aeruginosa* display similar patterns, with slight variations in peak positions and intensities, highlighting their distinct contributions to decomposition.

Interestingly, *Bacillus pumillus* exhibits an additional peak at 896 cm^{-1}, suggesting a unique decomposition pathway involving C-H bending, in addition to the common

functional groups observed with other organisms. This indicates that *Bacillus pumillus* may contribute to the degradation of more recalcitrant components of wheat straw, a result consistent with previous studies highlighting the unique enzymatic capabilities of *Bacillus* species in lignocellulosic biomass breakdown (Bisen & Rahangdale., 2017). The key changes in substrate structure after different microbial treatments over 21 days and their comparison with untreated wheat straw is demonstrated in Table 4.10.

Table 4.10: Summary of key changes in wheat straw structure after different microbial treatments over 21 days

Wavenumber (cm^{-1})	**Functional Group/Compound**	**Untreated Wheat straw**	**Treated Wheat straw (Using Organisms and Consortia)**	**Observations and Interpretation**
3400-3425	O-H stretching	Broad peak	Broad peak present, slightly shifted	Hydroxyl groups present in both, slight shift indicates interaction or modification.
2920-2850	C-H stretching	Peaks present	Peaks present but slightly reduced	Indicates breakdown of aliphatic compounds.

1630-1650	C=O stretching (conjugated)	Peaks present	Peaks present but reduced intensity	Partial breakdown of lignin.
1400-1420	C-H bending	Peak present	Peak present but reduced	Indicates partial degradation of lignin.
1050-1100	C-O stretching	Peaks present	Peaks present but slightly reduced	Indicates partial degradation of cellulose and hemicellulose.
460-470	Si-O-Si bending	Peak present	Peak present	Silica content remains unchanged, as it is not biodegradable.

4.7. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) provides detailed information about the surface morphology and composition of samples. SEM was performed to determine structural changes in consortia treated rice and wheat straw indicating effectiveness of the different treatments on substrate decomposition.

4.7.1. Rice straw

Scanning Electron Microscopy (SEM) images of rice straws treated with microbial consortia revealed significant changes in structure and surface morphology compared to untreated samples. The treated rice straw displayed signs of surface erosion and degradation, attributed to microbial activity breaking down its lignocellulosic structure.

This breakdown resulted in a more porous surface, with the microbial consortia creating pores and channels by decomposing complex carbohydrates. These observations highlight the effectiveness of microbial consortia in degrading and modifying rice straw structure, which is valuable for applications such as biofuel production, bioremediation, and serving as a substrate for further biochemical processes.

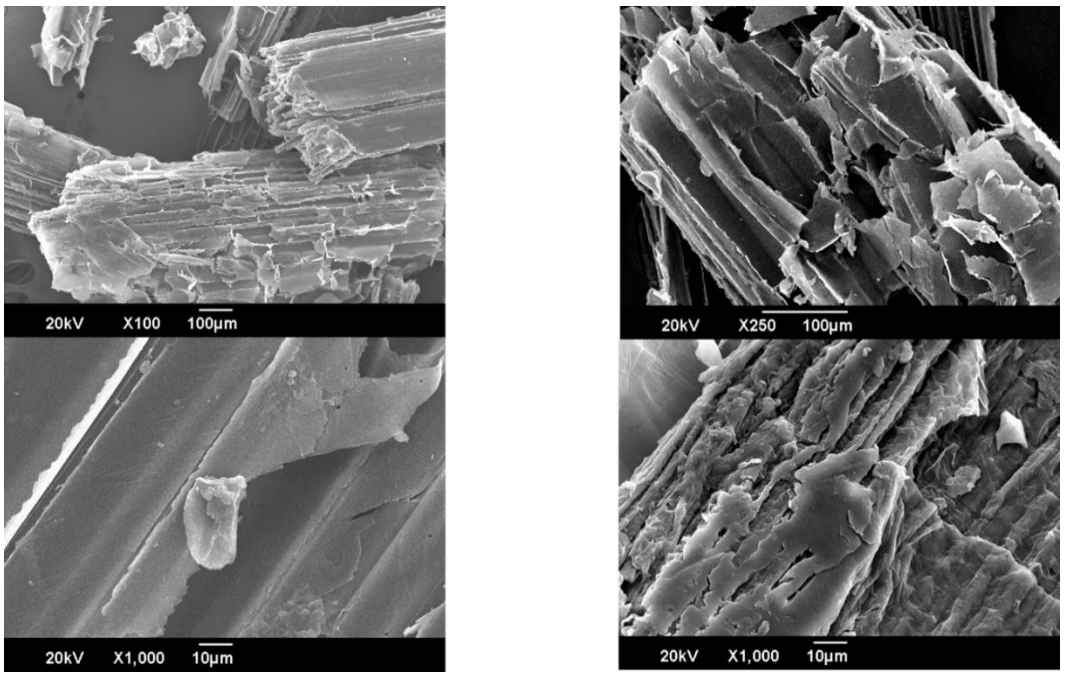

Fig. 4.16: SEM images of untreated rice straw (left) and consortia-treated rice straw (right)

4.7.2. Wheat straw

Similarly, SEM images of wheat straw treated with microbial consortia showed distinct structural and morphological alterations compared to untreated wheat straw. The treated wheat straw exhibited surface degradation and erosion, indicating the breakdown of cellulose, hemicellulose, and lignin by the microbial consortia. Increased surface porosity was prominent, as microbial activity created pores and channels while metabolizing the organic material. SEM images also revealed the formation of cracks and cavities within the straw structure due to the enzymatic activity of the microbes.

Additionally, some areas showed decreased surface roughness, resulting in a smoother appearance as the microbial consortia degraded the outer layers. These structural changes demonstrate the effectiveness of microbial consortia in modifying wheat straw's structure and composition, making it suitable for applications in fermentation, animal feed production, and various bioprocessing industries.

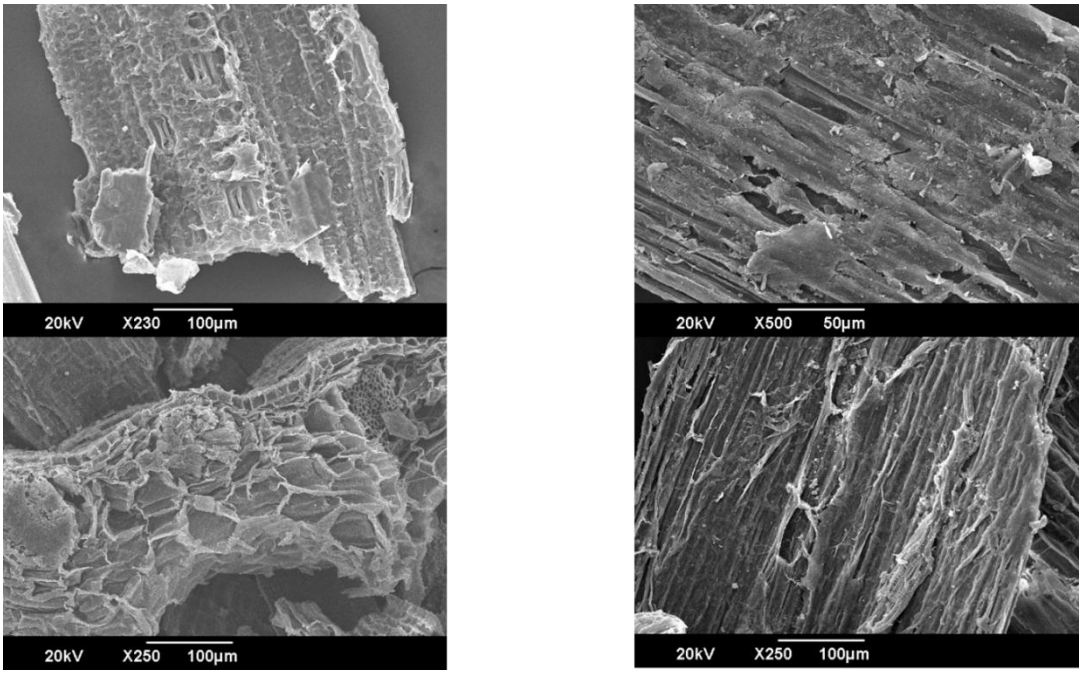

Fig. 4.17: SEM images of untreated wheat straw (left) and consortia-treated wheat straw (right)

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The study demonstrated the effectiveness of various microbial treatments in producing cellulase enzymes (FPase activity) and decomposing substrates. It confirmed that microbial consortia exhibited the highest FPase activity and substrate decomposition rates on both rice and wheat straw. This synergistic effect among mixed microbial cultures underscores their potential for rapid and efficient stubble management. The findings advocate for the use of microbial consortia *in situ* for sustainable stubble management, presenting a viable solution.

Key findings of the research include:

1. The consortia exhibited the highest FPase activity (2.674 U/ml for rice straw and 3.188 U/ml for wheat straw) followed by *Trichoderma spp.* (1.518 U/ml for rice straw and 31.577 U/ml for wheat straw), *Pseudomonas aeruginosa* (1.473 U/ml for rice straw and 1.521 U/ml for wheat straw) and *Bacillus pumillus* (1.454 U/ml for rice straw and 1.439 U/ml for wheat straw), respectively. The activity peaked on day 3/4 and then declined gradually.
2. Significant statistical differences ($p < 0.05$) were observed between the cellulase production capabilities of the different microbes and their consortia for both substrates.
3. Biological decomposition led to noteworthy weight loss where consortia depicted the highest weight loss for rice straw (57.7%) and *Pseudomonas aeruginosa* led to the highest weight loss for wheat straw (52.9%). *Trichoderma spp*. treatment proved to be more effective for rice straw as compared to wheat

straw with decomposition rates of 45% and 24.9% respectively. *Bacillus pumillus* also exhibited significant decomposition rates (31.1% for rice straw and 29.3% for wheat straw).

4. Significant structural and chemical changes were observed in rice and wheat straw post-microbial treatment.

5.2. Recommendations

Based on the findings of this study, the following recommendations are given for the *in situ* management of stubble residue within the region:

1. Further research is recommended to optimize consortia ratios and incubation conditions that might yield even higher enzyme activity.
2. Investigate the specific interactions and synergy between different microbial species within the consortia to refine and enhance their decomposing capabilities.
3. Expand studies to include other agricultural residues and industrial by-products to assess the general applicability of these microbial treatments.
4. Explore the use of these treatments in composting and soil health improvement practices.
5. Conduct further research to optimize the formulations and application methods of bio-decomposers to maximize efficiency and cost-effectiveness.
6. Advocate for policy support and incentives for farmers adopting bio-decomposer technologies. This can include subsidies, financial assistance, or technical support to encourage the transition from stubble burning to sustainable residue management practices.

REFERENCES

Abdurrahman, M.I., Chaki, S., Saini, G., 2020. Stubble burning: Effects on health & environment, regulations and management practices. Environ. Adv. 2, 100011. https://doi.org/10.1016/j.envadv.2020.100011.

Akhtar, K., Wang, W., Ren, G., Khan, A., Feng, Y., Yang, G., 2018. Changes in soil enzymes, soil properties, and maize crop productivity under wheat straw mulching in Guanzhong, China. Soil Tillage Res. 182, 94-102. https://doi.org/10.1016/j.still.2018.05.007.

Andini, A., Bonnet, S., Rousset, P. and Hasanudin, U., 2018. Impact of open burning of crop residues on air pollution and climate change in Indonesia. *Current Science*, *115*(12), pp.2259-2266. https://agritrop.cirad.fr/590428/

Aruya, E.I., Yusuf, R.O., Yusuf, Y.O., 2016. An assessment of crop residue characteristics and factors militating against efficient management in the Ikara local government area of Kaduna state, Nigeria. Waste Manag. Environ. 333-344. https://doi.org/10.2495/WM160291.

Azhar, R., Zeeshan, M., Fatima, K., 2019. Crop residue open field burning in Pakistan; multi-year high spatial resolution emission inventory for 2000–2014. Atmos. Environ. 208, 20-33. https://doi.org/10.1016/j.atmosenv.2019.03.031.

Banerjee, T., Srivastava, R.K., 2012. Analysis of ambient concentration of criteria pollutants during post monsoon crop residue burning episodes in the Indo-Gangetic Plains of India. Environ. Pollut. 166, 204-213. https://doi.org/10.1016/j.envpol.2012.03.007.

Bansal, R.C., Goyal, P., Kumar, A., 2019. Stubble burning: A menace for the environment and solutions. J. Soil Water Conserv. 18(2), 100-106.

Behera, L., Datta, D., Kumar, S., Kumar, S., Sravani, B. and Chandra, R., 2022. Role of microbial consortia in remediation of soil, water and environmental pollution caused by

indiscriminate use of chemicals in agriculture: Opportunities and challenges. *New and Future Developments in Microbial Biotechnology and Bioengineering*, pp.399-418. https://doi.org/10.1016/B978-0-323-85577-8.00019-6

Bhatia, A., Jain, N., Pathak, H., 2013. Methane and nitrous oxide emissions from Indian rice paddies: Potential mitigation strategies. Environ. Sci. Technol. 47(18), 10222-10231. https://doi.org/10.1002/ghg.1339

Bhattacharjya, S., Sahu, A., Phalke, D.H., Manna, M.C., Thakur, J.K., Mandal, A., Patra, A.K., 2021. In situ decomposition of crop residues using lignocellulolytic microbial consortia: A viable alternative to residue burning. Environ. Sci. Pollut. Res. 28, 32416-32433. https://doi.org/10.1007/s11356-021-12611-8

Bhuvaneshwari, S., Hettiarachchi, H. and Meegoda, J.N., 2019. Crop residue burning in India: policy challenges and potential solutions. *International journal of environmental research and public health*, *16*(5), p.832. doi: 10.3390/ijerph16050832

Bisen, N., Rahangdale, C.P., 2017. Crop residues management option for sustainable soil health in rice-wheat system: A review. Int. J. Chem. Stud. 5(4), 1038-1042.

Borah, N., Barua, R., Nath, D., Hazarika, K., Phukon, A., Goswami, K., Barua, D.C., 2016. Low energy rice stubble management through in situ decomposition. Procedia Environ. Sci. 35, 771-780. https://doi.org/10.1016/j.proenv.2016.07.092

Chakraborty, M., Pathak, H., 2016. Greenhouse gas emissions and global warming potential of rice–wheat cropping system: A review. Agric. Ecosyst. Environ. 225, 80-92. https://doi.org/10.1016/j.agee.2012.11.003.

Chaudhary, A., Chhokar, R.S., Yadav, D.B., Sindhu, V.K., Ram, H., Rawal, S., Gill, S.C., 2019. In-situ paddy straw management practices for higher resource use efficiency and crop productivity in Indo-Gangetic Plains (IGP) of India. J. Cereal Res. 11, 172-198. https://doi.org/10.25174/2249-4065/2019/96323

Chawala, P., Sandhu, H.A.S., 2020. Stubble burn area estimation and its impact on ambient air quality of Patiala & Ludhiana district, Punjab, India. Heliyon 6(1), e03228. https://doi.org/10.1016/j.heliyon.2019.e03095

Chen, J., Gong, Y., Wang, S., Guan, B., Balkovic, J., Kraxner, F., 2019. To burn or retain crop residues on croplands? An integrated analysis of burning impacts on soil organic carbon and crop yield. Agric. Ecosyst. Environ. 272, 89-99. https://doi.org/10.1016/j.scitotenv.2019.01.150

Chukwuma, O.B., Rafatullah, M., Tajarudin, H.A. and Ismail, N., 2021. A review on bacterial contribution to lignocellulose breakdown into useful bio-products. *International journal of environmental research and public health*, *18*(11), p.6001. https://doi.org/10.3390/ijerph18116001

Das, S., Singh, S.K., Dutta, S., Dey, S., 2016. The impact of post-harvest crop residue burning on atmospheric pollution and measures for mitigation. J. Agric. Sci. 8(1), 123-134. https://doi.org/10.5539/jas.v8n1p123 .

de Souza, W.R., 2013. Microbial degradation of lignocellulosic biomass. Sustainable degradation of lignocellulosic biomass-techniques, applications and commercialization, 15, pp.207-247. https://dx.doi.org/10.5772/54325

Fatma, S., Hameed, A., Noman, M., Ahmed, T., Shahid, M., Tariq, M., Sohail, I. and Tabassum, R., 2018. Lignocellulosic biomass: a sustainable bioenergy source for the future. Protein and peptide letters, 25(2), pp.148-163. https://doi.org/10.2174/0929866525666180122144504

Ghosh, M., Chakraborty, S., Dey, S., 2020. Crop residue burning: Issues, challenges, and mitigation strategies in the Indian context. Energy Procedia 158, 5069-5074.

Gupta, P., Sahai, S., Singh, N., Dixit, C.K., Singh, D.P., Sharma, C., Tiwari, M.K., Gupta, R.K., Garg, S.C., Mitra, A.P., 2004. Residue burning in rice–wheat cropping system: Causes

and implications. Curr. Sci. 87(12), 1713-1717.

Gupta, R.K., Ritzema, H.P., 2016. Sustainability of post-Green Revolution agriculture: The rice-wheat cropping systems of the Indo-Gangetic Plains and China. Rev. Agric. Ecosyst. Environ. 216, 14-22. https://doi.org/10.1016/j.agee.2015.09.001.

Hall, J.V., Zibtsev, S.V., Giglio, L., Skakun, S., Myroniuk, V., Zhuravel, O., Goldammer, J.G. and Kussul, N., 2021. Environmental and political implications of underestimated cropland burning in Ukraine. *Environmental Research Letters*, *16*(6), p.064019. https://doi.org/10.1088/1748-9326/abfc04

Kumar, P., Kumar, S. and Joshi, L., 2015. Socioeconomic and environmental implications of agricultural residue burning: A case study of Punjab, India (p. 144). Springer Nature. https://doi.org/10.1007/978-81-322-2014-5

Kumar, V., Garg, N., Singh, P., 2021. An assessment of the impact of crop residue burning on the environment in Punjab. Sustainability 13(2), 631. https://doi.org/10.3390/su13020631.

Kumar, V., Reddy, K.V., 2017. A review of the impact of crop residue burning on air quality in India. Atmos. Environ. 164, 175-183. https://doi.org/10.1016/j.atmosenv.2017.06.035.

Lal, M.M., 2008. An overview to agricultural waste burning. *Indian Journal of Air Pollution Control*, *8*(1), pp.48-50.

Li, J.X., Zhang, F., Jiang, D.D., Li, J., Wang, F.L., Zhang, Z., Wang, W. and Zhao, X.Q., 2020. Diversity of cellulase-producing filamentous fungi from tibet and transcriptomic analysis of a superior cellulase producer *Trichoderma* harzianum LZ117. *Frontiers in microbiology*, *11*, p.1617. doi: 10.3389/fmicb.2020.01617

Liu, J., Xu, B., Xu, Z., Yang, L., Li, D., 2017. Crop residue management: Potential and constraints in China. Agric. Ecosyst. Environ. 250, 25-33.

https://doi.org/10.1016/j.agee.2017.03.007.

Liu, Y., Zhang, Y., Zhang, L., Wei, Z., 2020. Health impacts of crop residue burning in rural China: A review. Int. J. Environ. Res. Public Health 17(2), 470. https://doi.org/10.3390/ijerph17020470.

Mohan, D., Kumar, S., Singh, S., 2016. Management of crop residues for sustainable agriculture in the Indo-Gangetic Plains of India. Renew. Sust. Energ. Rev. 58, 1106-1116. https://doi.org/10.1016/j.rser.2015.12.126.

Nizhelskiy, M.S., Kazeev, K.S., Vilkova, V.V., Fedorenko, A.N., Sushkova, S.N. and Kolesnikov, S.I., 2024. Continuous and Periodical Effects of Smoke from Crop Residue Combustion on Soil Enzymatic Activity. *Eurasian Soil Science*, *57*(4), pp.623-634. https://doi.org/10.1134/S1064229323603256

Obi, F.O., Ugwuishiwu, B.O. and Nwakaire, J.N., 2016. Agricultural waste concept, generation, utilization and management. *Nigerian Journal of Technology*, *35*(4), pp.957-964.

Paul, S., Sarkar, D., Rajput, R.S., Singh, S., Parihar, M., Parewa, H.P., Pal, S., Singh, H.B. and Rakshit, A., 2019. *Trichoderma*: a part of possible answer towards crop residue disposal. *Journal of Applied and Natural Science*, *11*(2), pp.516-523. https://doi.org/10.31018/jans.v11i2.2090

Perillo, L.I., de Oliveira Bordonal, R., de Figueiredo, E.B., Moitinho, M.R., Aguiar, D.A., Rudorff, B.F.T., Panosso, A.R. and La Scala, N., 2022. Avoiding burning practice and its consequences on the greenhouse gas emission in sugarcane areas southern Brazil. *Environmental Science and Pollution Research*, *29*, pp.719-730. https://doi.org/10.1007/s11356-021-15318-y

Phairuang, W., 2021. Biomass burning and their impacts on air quality in Thailand. In *Biomass Burning in South and Southeast Asia* (pp. 21-38). CRC Press.

Prew, R.D., Ashby, J.E., Bacon, E.T.G., Christian, D.G., Gutteridge, R.J., Jenkyn, J.F., Powell, W. and Todd, A.D., 1995. Effects of incorporating or burning straw, and of different cultivation systems, on winter wheat grown on two soil types, 1985–91. *The Journal of Agricultural Science*, *124*(2), pp.173-194. https://doi.org/10.1017/S0021859600072853

Quencheng., Yang, Z., Chen, Z., Li, M., Gao, B., Yang, J., Chen, X. and Xu, B., 2024. Crop residue burning in China (2019–2021): Spatiotemporal patterns, environmental impact, and emission dynamics. *Environmental Science and Ecotechnology*, *21*, p.100394. https://doi.org/10.1016/j.ese.2024.100394

Rafiq, M., Ahmad, F. and Atiq, M., 2019. The determinants of the crop residue management in Pakistan: an environmental appraisal. *Business & Economic Review*, *11*(4), pp.179-200. https://dx.doi.org/10.22547/BER/11.4.8

Ravindra, K., Singh, T. and Mor, S., 2019. Emissions of air pollutants from primary crop residue burning in India and their mitigation strategies for cleaner emissions. *Journal of cleaner production*, *208*, pp.261-273. https://doi.org/10.1016/j.jclepro.2018.10.031

Raza, M.H., Abid, M., Faisal, M., Yan, T., Akhtar, S. and Adnan, K.M., 2022. Environmental and health impacts of crop residue burning: Scope of sustainable crop residue management practices. *International Journal of Environmental Research and Public Health*, *19*(8), p.4753. https://doi.org/10.3390/ijerph19084753

Reddy, K.S., Triveni, S., Pratibha, G., Jayasree, G. and Ravali, C., 2022. Impact of In-situ Paddy Straw Burning on Soil Enzyme Activity, Soil Microbial Population and Green House Gas Emissions in Sandy Loam Soil. International Journal of Environment and Climate Change, 12(11), pp.3633-3640. https://doi.org/10.9734/ijecc/2022/v12i111411

Sahoo, R., Singh, S., Pradhan, M., 2019. Crop residue burning in the Indo-Gangetic Plains: Causes, impacts, and strategies for management. Sustainability 11(22), 6173.

https://doi.org/10.3390/su11226173.

Santiago-De La Rosa, N., González-Cardoso, G., Figueroa-Lara, J.D.J., Gutiérrez-Arzaluz, M., Octaviano-Villasana, C., Ramírez-Hernández, I.F. and Mugica-Álvarez, V., 2018. Emission factors of atmospheric and climatic pollutants from crop residues burning. *Journal of the Air & Waste Management Association*, *68*(8), pp.849-865. https://doi.org/10.1080/10962247.2018.1459326

Sarkar, S., Skalicky, M., Hossain, A., Brestic, M., Saha, S., Garai, S., Ray, K. and Brahmachari, K., 2020. Management of crop residues for improving input use efficiency and agricultural sustainability. *Sustainability*, *12*(23), p.9808. https://doi.org/10.3390/su12239808

Setiawan, W.K. and Chiang, K.Y., 2021. Crop residues as potential sustainable precursors for developing silica materials: a review. Waste and Biomass Valorization, 12, pp.2207-2236. https://doi.org/10.1007/s12649-020-01126-x

Shamshitov, A., Kadžienė, G. and Supronienė, S., 2024. The Role of Soil Microbial Consortia in Sustainable Cereal Crop Residue Management. *Plants*, *13*(6), p.766. https://doi.org/10.3390/plants13060766

Sharma, S., Singh, N., Kumar, A., 2020. Crop residue management: An overview of sustainable practices in India. Agric. Sci. 11(5), 685-696. https://doi.org/10.4236/as.2020.115048.

Shinde, R., Shahi, D.K., Mahapatra, P., Naik, S.K., Thombare, N. and Singh, A.K., 2022. Potential of lignocellulose degrading microorganisms for agricultural residue decomposition in soil: A review. *Journal of Environmental Management*, *320*, p.115843. https://doi.org/10.1016/j.jenvman.2022.115843

Sidar, R.S., 2018. Crop residue burning in India: potential solutions. *Journal of Pharmacognosy and Phytochemistry*, *7*(5), pp.3451-3455.

Singh, R., Choudhary, S., Sharma, M., 2018. The effects of crop residue burning on

environmental health: A review. Environmental Monitoring and Assessment 190(11), 645. https://doi.org/10.1007/s10661-018-7083-2.

Singh, S., Jaiswal, D.K., Sivakumar, N. and Verma, J.P., 2019. Developing efficient thermophilic cellulose degrading consortium for glucose production from different agro-residues. Frontiers in Energy Research, 7, p.61. https://doi.org/10.3389/fenrg.2019.00061

Song, Y.J., Zhao, N.L., Dai, D.R. and Bao, R., 2024. Prospects of Pseudomonas in Microbial Fuel, Bioremediation, and Sustainability. *ChemSusChem*, p.e202401324. https://doi.org/10.1002/cssc.202401324

Tripathi, S., Yadav, S. and Sharma, K., 2024. Air pollution from biomass burning in India. Environmental Research Letters. https://doi.org/10.1088/1748-9326/ad4a90

Vagg, A., 2015. Rice straw utilisation: value adding and alternative uses for the Australian Rice Industry. *Nuffield Australia Project No1314*.

Yadav, I.C. and Devi, N.L., 2019. Biomass burning, regional air quality, and climate change. *Encyclopedia of Environmental Health*, *2*. https://doi.org/10.1016/B978-0-12-409548-9.11022-X

Yadav, S., Gupta, R., Bharti, A., 2021. Crop residue burning in India: A review of management practices and environmental implications. Int. J. Agron. 2021, 1-12. https://doi.org/10.1155/2021/9998657.

Yokelson, R.J., Burling, I.R., Urbanski, S.P., Atlas, E.L., Adachi, K., Buseck, P.R., Wiedinmyer, C., Akagi, S.K., Toohey, D.W. and Wold, C.E., 2011. Trace gas and particle emissions from open biomass burning in Mexico. Atmospheric Chemistry and Physics, 11(14), pp.6787-6808. https://doi.org/10.5194/acp-11-6787-2011

Zahid, A., Ali, S., Ahmed, M. and Iqbal, N., 2020. Improvement of soil health through residue management and conservation tillage in rice-wheat cropping system of Punjab,

Pakistan. *Agronomy*, *10*(12), p.1844. https://doi.org/10.3390/agronomy10121844

Zhang, G., Liu, Y., Wang, J., 2019. An assessment of the impact of crop residue burning on the environment in China. Int. J. Environ. Res. Public Health 16(5), 777.

Zhang, W., Zhao, L., Liu, Z., 2020. Crop residue management: A sustainable approach to improve soil quality and productivity. Renew. Agric. Food Syst. 35(3), 297-310.

Zhang, X., Zhu, A., Xin, X., Yang, W., Zhang, J. and Ding, S., 2018. Tillage and residue management for long-term wheat-maize cropping in the North China Plain: I. Crop yield and integrated soil fertility index. *Field Crops Research*, *221*, pp.157-165.

Zhou, Y., Chen, H., Liu, Q., 2018. Air pollution from crop residue burning: Health impacts and mitigation measures. Environ. Pollut. 243, 125-134. https://doi.org/10.1016/j.envpol.2018.08.079.

Zhou, Y., Zhang, L., Wei, C., 2022. Strategies for reducing air pollution from crop residue burning in rural areas of China. Atmospheric Environment 275, 118870.

Zou, C., Xu, J., Hu, W., 2023. The role of microbial consortia in enhancing crop residue decomposition: A review. Front. Microbiol. 14, 1122345.https://doi.org/10.3389/fmicb.2023.1122345.

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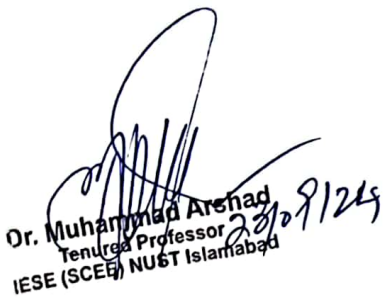

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