Anaerobic Digestion of Rice Straw

Thesis submitted in partial fulfilment

for the Award of Degree

Doctor of Philosophy

in

Biochemical Engineering

by

JYOTI RANI

SCHOOL OF BIOCHEMICAL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY

(BANARAS HINDU UNIVERSITY)

VARANASI – 221005, INDIA

Roll No. 17011002 2023

INDIAN INSTITUTE OF TECHNOLOGY (BHU) VARANASI

Varanasi – 221005, India

CERTIFICATE

It is certified that the work contained in the thesis titled "***Anaerobic Digestion of Rice Straw***" by **Jyoti Rani** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree. It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy, and SOTA for the award of Ph.D. Degree.

Signature:

Dr. Abhishek Suresh Dhoble
Assistant Professor
School of Biochemical Engineering
Indian Institute of Technology (BHU)
Varanasi-221005, U.P. INDIA

Dr. Abhishek S. Dhoble

(Supervisor)

School of Biochemical Engineering

Indian Institute of Technology

(Banaras Hindu University)

Varanasi-221005, India

INDIAN INSTITUTE OF TECHNOLOGY (BHU) VARANASI

Varanasi – 221005, India

DECLARATION BY THE CANDIDATE

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Signature of the Student

Place: Varanasi, India

(Jyoti Rani)

CERTIFICATE BY THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of our knowledge.

Dr. Abhishek S. Dhoble

(Supervisor)

School of Biochemical Engineering

Indian Institute of Technology

(Banaras Hindu University)

Varanasi-221005, India

Forwarded.

Prof. Vikash Kumar Dubey

(Co-ordinator)

School of Biochemical Engineering

Indian Institute of Technology

(Banaras Hindu University)

Varanasi-221005, India

INDIAN INSTITUTE OF TECHNOLOGY

(BHU) VARANASI

Varanasi – 221005, India

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Place: Varanasi

Signature of Student

Jyoti Rani

To my dearest husband for constantly being by my side and making everything possible

Acknowledgement

I wish to extend my heartfelt appreciation to my supervisor, ***Dr. Abhishek S. Dhoble***, for his unwavering guidance, support, and motivation. His consistsent encouragement and wise counsel have been a continuous source of motivation and inspiration for me. His strong belief in my abilities has propelled me forward. He consistently promoted me with a profound work ethic during each stage of my research journey, ultimately aiding me in reaching meaningful conclusions. Furthermore, I'd like to acknowledge the contributions of my Research Progress Evaluation Committee (RPEC) members, ***Dr. Pranjal Chandra*** and ***Dr. Sweta,*** for their valuable insights into my work and their dedicated efforts to enhance the overall quality of my outputs.

My special thanks to our Coordinator ***Prof. Vikash Kumar Dubey*** for his continuous hard work to make our school a better and equipped place to work.

Throughout my Ph.D. expedition, I've been fortunate to be surrounded by remarkable seniors, peers from my batch, and juniors. Among my lab companions, I'd like to acknowledge ***Mr. Jeetesh Kushwaha, Ms. Madhumita Priyadarsini, Mr. Kailash Pati Pandey, Mr. Yashpal Sengar,*** and ***Mr. Atith Chitnis*** for their unwavering support, help and enthusiasm during our shared tenure in the JEEWANU Lab. It's important to recognize the contribution of other juniors who conducted their IDD and M.Tech. projects within our lab. ***Mr. Saswata Acharya, Mr. Ankit Dixit,*** and ***Mr. Bhavesh*** deserve mention for their assistance and the ample opportunities they provided for consistent brainstorming. Moreover, I want to offer a distinct token of appreciation to my seniors, ***Dr. Rupika Sinha*** and ***Dr. Sukhendra Singh Raghuvanshi***, whose encouragement was pivotal in the early phases of my PhD expedition.

My deepest gratitude goes out to my perpetually understanding and affectionate husband, ***Mr. Pratyush Shukla***, who always held me strong in all the ups and downs and helped me transform into a better and stronger version of myself. This journey was impossible without his support. I am eternally thankful to my parents, ***Mr. Vichiter Kumar*** & ***Mrs. Luxmi Devi*** who raised me to be what I am today and for teaching me perseverance and compassion. I am extremely grateful to my parents-in-law, ***Mr. Virendra Kumar Shukla*** & ***Mrs. Manju Lata Shukla,*** for believing in me and supporting me throughout this journey**.** I am deeply indebted to my siblings ***Miss. Komal*** and ***Mr. Vivek Jangid*** who were always there to lift my spirits during my lows and my brother-in-law ***Dr. Shashwat Shukla*** whose unwavering backing and wise counsel was indispensable for the successful culmination of my journey. I cannot be thankful enough to my friends ***Mrs. Anviti Pandey*** and ***Mrs. Priya Singh*** for their emotional support and for being my family away from home.

Special recognition is also due to my School's former Coordinators, ***Prof. Subir Kundu*** and ***Prof. Pradeep Srivastava***, other faculty members ***Dr. Sanjay Kumar, Dr. Abha Mishra, Dr. Vishal Mishra, Dr. Sumit Singh, Dr. Prodyut Dhar, Dr. Aditya Padhi,*** and ***Dr. Rajendra Meena,*** alongside the entire non-teaching support staff. Their constant presence and willingness to help whenever needed were truly invaluable.

Jyoti Rani

Table of Contents

List of figures

List of Tables

List of abbreviations

AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model No. 1
ADS	Anaerobic Digester Slurry
ADSIND0	Anaerobic Digester Slurry Inoculum on Day Zero
AIL	Acid Insoluble Lignin
ANOVA	Analysis of Variance
ANN	Artificial Neural Network
ASL	Acid Soluble Lignin
BOD	Biological Oxygen Demand
CD	Cow Dung
CDIND0	Cow Dung Inoculum on Day Zero
CH_4	Methane
CHNS	Carbon Hydrogen Nitrogen Sulphur
CMC	Carboxymethyl Cellulose
CMCase	Carboxymethyl cellulase
C/N	Carbon/Nitrogen
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
DNA	Deoxyribonucleic Acid
DNSA	3,5-dinitro salicylic acid
DT	Decision Tree
XGB	eXtreme Gradient Boosting
F/M ratio	Food to Microorganism ratio

FAS	Ferrous Ammonium Sulphate
FOM	Fermented organic manure
FTIR	Fourier Transform Infrared Spectroscopy
GB	Gradient Boosting
GC	Gas Chromatography
GHG	Green House Gas
HRT	Hydraulic Retention Time
H_2O_2	Hydrogen Peroxide
K	Potassium
KBr	Potassium Bromide
LCB	Lignocellulosic Biomass
LFOM	Liquid fermented organic manure
LiP	Lignin Peroxidase
LR	Linear Regression
MANOVA	Multivariate Analysis of Variance
ML	Machine Learning
MnP	Manganese Peroxidase
MTCC	Microbial Type Culture Collection and Gene Bank
N	Nitrogen
NaOH	Sodium Hydroxide
NC	Negative Control
OD	Optical Density
OTU	Operational Taxonomic Unit
P	Phosphorus
PBS	Phosphate Buffer Saline

PC	Positive Control
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PERMANOVA	Permutational Multivariate Analysis of Variance
PS	*Pycnoporus sanguineus*
PSI	Per Square Inch
QC	Quality Control
QIIME	Quantitative Insights into Microbial Ecology
RDA	Redundancy Analysis
RF	Random Forest
RS	Rice Straw
S	Sulphur
sCOD	Soluble Chemical Oxygen Demand
SDG	Sustainable development Goals
SEM	Scanning Electron Microscopy
SVM	Support Vector Machine
TC	Total Carbon
TL	*Trichoderma longibrachiatum*
TS	Total Solids
VFAs	Volatile Fatty Acids
VS	Volatile Solids

Preface

Increasing demand for food, expensive labor, and shortage in agriculture, majorly in South and Southeast Asia, has resulted in opting for mechanized combine harvesters for cutting crops. The cutting of rice crops leaves behind significant straws in the field. Rice straw (RS) has low digestibility because of its high content of silica and lignin and is not a preferable feed for cattle. This leftover straw must be removed from the fields to sow the next crop. Farmers find open field burning as the easiest solution with the added benefit of pest control. However, this deteriorates the soil quality reducing nutrients like Nitrogen (N), Potassium (K), Phosphorus (P), and Sulphur (S), and results in increased air pollution that causes respiratory diseases in humans. Rice straw is one of the most commonly occurring lignocellulosic discards, as nearly 1.35 tonnes of straw is generated per tonne of rice produced. Only 20% of it is utilized, and the rest goes to waste. RS is mainly composed of cellulose (35.97% to 41.93%) and hemicellulose (24.99% to 28.73%), which can be hydrolysed to fermentable carbohydrates required for energy production. However, rice straw also contains significant percent of lignin that makes it recalcitrant to be used as a feedstock for energy production.

Anaerobic digestion (AD) is a waste-to-energy system that occurs naturally in the environment in the presence of microbes that possess the capacity to decompose organic matter in the absence of oxygen, producing biogas as a byproduct, constituting of methane (CH_4) and carbon dioxide (CO_2). AD has been employed for decades to convert lignocellulosic biomass into methane, biofertilizers, and several other beneficial products. The application of anaerobic digestion (AD) for the management of rice straw (RS) provides a way to generate bioenergy to tackle greenhouse gas emissions caused by conventional burning and tilling of straw back into the fields.

However, most studies do not discuss the selectivity of the inoculum and the role of the microbial community in AD operation. The first objective of this study was to compare the performance of raw versus digested cattle manure as inoculum sources for anaerobic digestion of rice straw and find convincing evidence for the outperformance of the microbial community of one over another to produce biogas. It was found that the microbial community of digested manure (ADS) at the start of the experiment resembled that of raw cow dung (CD) after 45 days of anaerobic digestion of rice straw showing 116 common bacterial genera in comparison to just 9 at the beginning of the experiment. Dominant bacterial genus included *Clostridiales*, whose abundance increased from 9.86% to 38.40% in ADS but decreased from 38.16% to 7.53% in CD from day 0 to day 45, followed by an increase in *Prevotella* and decrease in *Pseudomonas* as the days progressed. *Methanobrevibacter* was the dominant genus of hydrogenotrophic archaea whose abundance increased from 8.20% to 64.25% in ADS and decreased from 61.41% to 8.98% in CD from day 0 to day 45. The reactors inoculated with ADS produced 197.13 ± 41.33 mL/g VS of cumulative biogas and 59.43 ± 14.75 mL/g VS cumulative methane from rice straw, which were 7.07 times and 401.33 times higher than the reactors inoculated with raw CD, respectively. The highest accumulation of volatile fatty acids (VFAs) in samples containing raw CD as inoculum resulted in poor buffering and reduced biogas production. On the contrary, despite having high VFA values, ADS performed the best, indicating the establishment of a better acclimatized microbial community that was resilient to pH fluctuations. This provided evidence that the acclimatization of the inoculum plays a crucial role in determining the feedstock degradation rate and trend of AD operation to allow higher energy recovery from lignocellulosic residues.

Despite the fact that rice straw possesses a substantial amount of lignin and exhibits limited biodegradability, it can be subjected to pretreatment methodologies to alter its composition, features, and structure. This, in turn, leads to enhancement in anaerobic digestion and the subsequent production of methane. Thermal processes such as steam explosion, hydrothermal carbonization, and microwave irradiation enhance the accessibility of lignocellulose to microbial activity. However, these methods need substantial energy input and may generate inhibitory substances, hence raising concerns about their long-term sustainability. The susceptibility of lignocellulose to enzymatic hydrolysis is heightened subsequent to treatments including alkali, acid, and ozone. However, the implementation of chemical treatments can be associated with significant expenses, alterations in the composition of rice straw, as well as potential risks to both human health and the environment due to the utilization of hazardous substances.

The utilization of fungal treatment of rice straw for anaerobic digestion to harness bioenergy has low input costs and anticipated higher benefits. Fungi possess the ability to release a diverse array of lignocellulose-degrading enzymes, which exhibit considerable efficacy in breaking down the resistant constituents of rice straw. Consequently, this enzymatic activity leads to a substantial enhancement in the methane production yield of rice straw during anaerobic digestion.

The second objective of this study compared the role of thermal, alkali and fungal pretreatment in improving biogas production from rice straw. Rice straw treated with 1% NaOH and 2% NaOH generated the largest cumulative biogas output of 388.40 ± 59.87 mL/g VS and 360.37 ± 43.08 mL/g VS (23.11% and 14.22% increase compared to positive control) after 45 days. Methane production peaked at 134.35 ± 9.49 mL/g VS in reactors with 1% NaOH-treated rice straw, followed by 128.09 ± 12.23 mL/g VS in reactors with 2% NaOH. All samples except NC generated more than 91.66% biogas by day 36 and then saw a rapid decline in slope after day 36. Rice straw treated with *Pycnoporus sanguineus* for 10 days (PS-10D) produced the highest amount of biogas

(309.25 ± 12.36 mL/g) and methane (102.24 ± 89.62 mL/g) among fungal treatments, but it was less than control, thermal and NaOH-treated rice straw. Increasing the treatment time was negatively correlated with biogas and methane production. However, it was observed that PS-treated samples performed better than the control and NaOH-treated samples till day 18. This may be because of simpler carbon availability during the initial days and then its rapid depletion in the fermentation media. This observation made us dive deeper into it and explore the potential of fungal treatments for 10 days at a higher substrate loading in the next objective.

The third objective of this study was to evaluate the usefulness of fungal strains *Trichoderma longibrachiatum* (TL) and *Pycnoporus sanguineus* (PS) in improving hydrolysis and bioavailability of rice straw in anaerobic digestion (AD). The fungal treatment was carried out for a shorter duration of 10 days (10-day treatment performed the best according to results of objective 3). The fungal treatment of rice straw for 10 days sby PS and TL increased biogas production by 20.79% and 17.85% and reduced soluble chemical oxygen demand (sCOD) by 71.43% and 64.70%, respectively as compared to control. The AD samples containing fungal-treated rice straw showed higher lignocellulolytic enzyme activities contributing to better process performance. The taxonomic profile of microbial communities in treated samples showed increased diversity that could sustain consistent system performance and exhibit enhanced resilience against pH fluctuations. Metagenomic analysis revealed a 60.82% increase in Proteobacteria and an 11.58% increase in Bacteroidetes in PS and TL-treated rice straw samples, respectively, resulting in improved hydrolysis.

The last objective of this study utilized advanced machine learning (ML) algorithms to build prediction models that demonstrate the efficacy of anaerobic digestion (AD) and the benefits of pre-treatments involving physical, chemical, and biological methods as a sustainable approach for rice straw disposal. The efficiency of anaerobic digestion of rice straw relies on operational factors such as temperature, pH, soluble chemical oxygen demand (sCOD), volatile fatty acids (VFAs), and the activity of lignolytic enzymes. Modelling anaerobic processes is a valuable tool for anticipating important elements that affect process performance, such as the production of methane and biogas. Machine learning-based process modelling is more efficient than mechanistic models. Machine learning has the capacity to reveal concealed correlations among multiple input variables and output predictions. In this study, the independent variables include the day of observation, pH, cellulase and xylanase enzyme activity, sCOD (soluble chemical oxygen demand), and volatile fatty acids (VFAs). The dependent variables include biogas production and methane production. This study tested several supervised ML models like Linear Regression, Support Vector Machine (SVM), Decision Trees, Random Forest (RF), Gradient Boosting to model biogas and methane. The accuracy from the different ML models used for biogas prediction was in the order: XGBoost (92%) > GB (91%) = RF (91%) > SVM (85%) > DT (79%) > LR (76%). The accuracy followed the order XGBoost (91%) > GB (83%) > RF (87%) > SVM (63%) > DT (61%) > LR (56%) for methane prediction.