

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 (CH₄) and carbon dioxide (CO₂). 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 by 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.