Chapter 8

Conclusion and future prospects

The present study has explored the potential of rice straw as a feedstock for anaerobic digestion while trying to establish the importance and role of inoculum age and its microbiome dynamics in the production of methane. The study has also compared thermal, alkali and fungal pretreatment methods and used ML algorithms to predict the production of methane and biogas. Following conclusions have been drawn from different chapters of this thesis:

- The inoculum comparison study evaluated the digestive efficiency of raw and digested manure and provided compelling evidence that emphasized the superior performance and greater process stability of acclimatized inoculum in determining the speed at which feedstock breaks down. This is crucial for achieving increased energy recovery from lignocellulosic residues.
- The production of biogas from rice straw was 7.07 times higher with digested manure compared to raw cow dung. Rice straw inoculated with digested manure produced the maximum cumulative biogas of 197.13 ± 41.33 mL/g VS, whereas the rice straw inoculated with raw cow dung had a significantly lower biogas production of 24.41 ± 3.94 mL/g VS (P-value < 0.05).
- The samples that utilized raw cow dung as the inoculum for anaerobic digestion of rice straw exhibited the greatest accumulation of volatile fatty acids (VFAs), which impaired the AD system's ability to maintain a stable pH level.
- The relative abundance of dominant microbial genera after 45 days of digestion of rice straw inoculated with raw CD was similar to the ADS inoculum used at the start of the experiment indicating inoculum acclimatization and aging plays a crucial role

in AD. Metagenomic studies have shown that a greater presence of hydrolyzers and hydrogenotrophic methanogens in digested manure led to improved efficiency in the anaerobic digestion of rice straw.

- Based on the findings of the pretreatment study, it was observed that pretreatment with 1% NaOH resulted in the highest biogas and methane generation, while pretreatment with 2% NaOH provided slightly lower results. The application of 1% NaOH and 2% NaOH as pretreatment for rice straw resulted in a biogas production increase of 23.11% and 14.22%, respectively, compared to the control.
- Among the fungal treated samples, rice straw treated with *Pycnoporus sanguineus* for 10 days (PS-10D) performed the best and produced 309.25 ± 12.36 mL/g VS biogas containing 102.24 ± 89.62 mL/g VS methane, but it was less as compared to control.
- Increasing the treatment time was negatively correlated with the biogas and methane production. The increase in the duration of fungal treatment inhibited the anaerobic digestion process. This inhibition may be a result of fast consumption of carbon source consisting of cellulose and hemicellulose thereby reducing the overall digestible carbon content.
- This gives an insight into the possibility of increasing the substrate loading in the digesters which was the next objective of the study. The initial higher production of biogas and methane in PS-treated samples can be leveraged in continuous commercial digesters. As commercial digesters are fed continuously, PS treatment could improve short-term output compared to batch feed employed in the trial.
- The 10- day fungal pretreated rice straw with *P. sanguineus* and *T. longibrachiatum* was used for anaerobic digestion at an increased substrate loading and biogas yield increased by 20.79% and 17.85% respectively. Higher activities of cellulase and

xylanase enzymes in AD systems with fungal treated rice straw are indicative of improved hydrolysis.

- The metagenomic analysis 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 60.82% increase in *Proteobacteria* in PS and 11.58% increase in *Bacteroidetes* in TL-treated rice straw samples resulting in improved hydrolysis.
- The ML models employed for biogas prediction achieved the following accuracy rates: XGBoost (92%), GB (91%) and RF (91%) performed equally well, SVM achieved 85%, DT 79%, and LR 76% accuracy. The methane prediction accuracy ranked as follows: XGBoost (91%) > Gradient Boosting (83%) > Random Forest (87%) > Support Vector Machine (63%) > Decision Tree (61.5%) > Logistic Regression (56%).

Future prospects

- Examine the effects of different important factors, such as temperature, pH, retention time, organic loading rate etc., on the efficiency of anaerobic digestion. Optimize these parameters to improve biogas production efficiency and promote substrate degradation.
- Explore microbial dynamics under different conditions to understand how community composition affects process stability and efficiency.
- Examine the possible advantages of co-digestion by the amalgamation of rice straw with other organic substrates, such as food waste and animal manure. Evaluate the combined impacts on the generation of biogas, nutrient composition, and the stability of the process.

- Carry out pilot-scale or demonstration studies to verify the viability of anaerobic digestion for rice straw on a bigger scale. Tackle obstacles pertaining to the scalability of technology, logistics, and the incorporation of existing agricultural techniques.
- Investigate techniques for extracting useful nutrients from the digestate generated by anaerobic digestion. Explore potential avenues for utilizing digestate as a fertilizer or soil amendment, making use of its high nutritional content.
- Perform a thorough techno-economic analysis (TEA) to assess the viability and efficiency of implementing large-scale anaerobic digestion plants for rice straw. Take into account variables such as initial investment, ongoing expenses, and prospective sources of income.
- Evaluate the ecological advantages and possible disadvantages of utilizing anaerobic digestion for the decomposition of rice straw. Take into account variables such as the mitigation of greenhouse gas emissions, the generation of energy, and the overall ecological viability of the procedure.