Chapter 5

Comparison of thermal, alkali, and fungal pre-treatments on the anaerobic digestion of rice straw

5.1. Background

The disposal of rice straw through open dumping and burning is a widespread agricultural practice, particularly in South and South-East Asia [25]. Despite the damaging effects, the practice of open dumping and burning of rice straw continues due to its low cost and perceived convenience [26]. Biogas, which can be derived from organic wastes like rice straw, stands out as a crucial and prominent renewable energy source [27]. Rice straw is a widely used agricultural residue for anaerobic digestion (AD) due to its abundance and low cost. Despite its high lignin content and low biodegradability, rice straw can undergo pretreatment techniques to modify its composition, characteristics, and structure, improving anaerobic digestion and methane production.

Thermal treatments like steam explosion, hydrothermal carbonization, and microwave irradiation make lignocellulose accessible to microbes but need a lot of energy and may produce inhibitory chemicals, which may make them unsustainable [179]. Lignocellulose is more vulnerable to enzymatic hydrolysis after alkali, acid, and ozone treatments. However, chemical treatments can be costly, change rice straw's composition, and pose health and environmental dangers owing to the usage of harmful compounds [180].

The primary objective of this comparison analysis is to assess and compare the efficacy of thermal, alkali, and fungal pretreatment techniques in enhancing the anaerobic digestion process of rice straw.

5.2. Results and discussion

5.2.1. Cumulative biogas and methane production

The present study compared the performance of thermal, alkaline, and fungal treatment of rice straw for the enhancement of anaerobic digestion of rice straw. The study was conducted for a period of 45 days after which the reaction was stopped. The results of cumulative biogas and cumulative methane are presented in *Figure 5.1* and *Figure 5.2* respectively. Upon observation, it can be clearly seen that the highest cumulative biogas production of 388.40 ± 59.87 mL/g VS followed by 360.37 ± 43.08 mL/g VS (23.11% and 14.22% increase as compared to PC) was obtained from rice straw treated with 1% NaOH and 2% NaOH respectively, after 45 days. It was observed that the highest methane production of 134.35 ± 9.49 mL/g VS was observed in reactors containing 1% NaOH-treated rice straw, followed by 128.09 ± 12.23 mL/g VS in 2% NaOH-treated rice straw. The methane production from 1% and 2% NaOH-treated rice straw samples was 30.83% and 24.72% higher, respectively, as compared to PC. Thermally treated (autoclaved) rice straw produced 7.08% higher biogas (337.84 ± 33.48 mL/g VS) and 12.31% higher methane (115.33 ± 6.15 mL/g VS) as compared to PC. A study reported the production of 514 mL/g VS biogas, with around 59% of it being methane, by treating rice straw with 1% NaOH at room temperature for 180 minutes. The digester was run with an HRT of 15 days, and a temperature of 37 °C [181]. It can also be noted that autoclaved samples consistently outperformed PC in terms of biogas and methane production. It can further be observed that by day 36, more than 91.66% biogas had been generated in all the samples except for NC, attributing to a sharp decrease of slope beyond day 36. TL-treated samples produced the least methane. PS-treated samples performed better than the PC, and NaOH-treated samples only till day 18.

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 PC. A study by Albornoz et al., 2018 reported an inhibitory effect of fungal treatment by *Pleurotus eryngii*. On the contrary, the study conducted by Mustafa et al., 2016 showed that pre-treating rice straw with *Pleurotus ostreatus* and *Trichoderma reesei* resulted in a significant favorable effect. The methane yield from the treated rice straw was 263 L/kg VS, which represents a 120% increase compared to untreated rice straw. Additionally, the methane yield from the rice straw treated with *T. reesei* was 214 L/kg VS, showing a 78.3% increase compared to untreated rice straw. 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 sources 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.

Most commercial digesters are fed continually, in contrast to batch digesters [183]. PS treatment would hold significant potential to generate better short-term output. Hence, commercial digesters can be fed PS-treated rice straw that will turn-around digestible matter faster. TL-treated rice straw under-performed the PS-treated rice straw. However, the digestate resulting from the reactors using fungal-treated rice-straw might hold a better potential to be commercially used as fermented manure as the fungus breaks down the cellulose and hemicellulose structure more effectively [184]. Digestate from the alkali-treated feedstock might result in inhibitory/toxic intermediates in fermented organic manure, limiting its usage as a biofertilizer. In contrast, the fungal-treated feedstock will generate soil microbial community-friendly fermented organic manure and may result in better agricultural output. Among the fungal-treated samples, the lowest methane was recorded for PS-40D with a value of 80.05 mL/g/VS, which is 22% lower than PC. PS-10D performs better than PC till day 21. Beyond day 12, both NaOH samples perform better than PC, yielding the highest cumulative methane.

Fungal pre-treatments of lignocellulosic biomass are environmentally favorable and cost-effective, but they take longer time and lose carbohydrates. Ligninolytic enzyme activity and synthesis by white-rot fungus depend on fungal strain, substrate, moisture, incubation length, temperature, oxygen concentration, pH, and nutrition supplements. Optimizing pre-treatment increases fungal activity and ligninolytic enzyme production, improving lignin digestion [185].

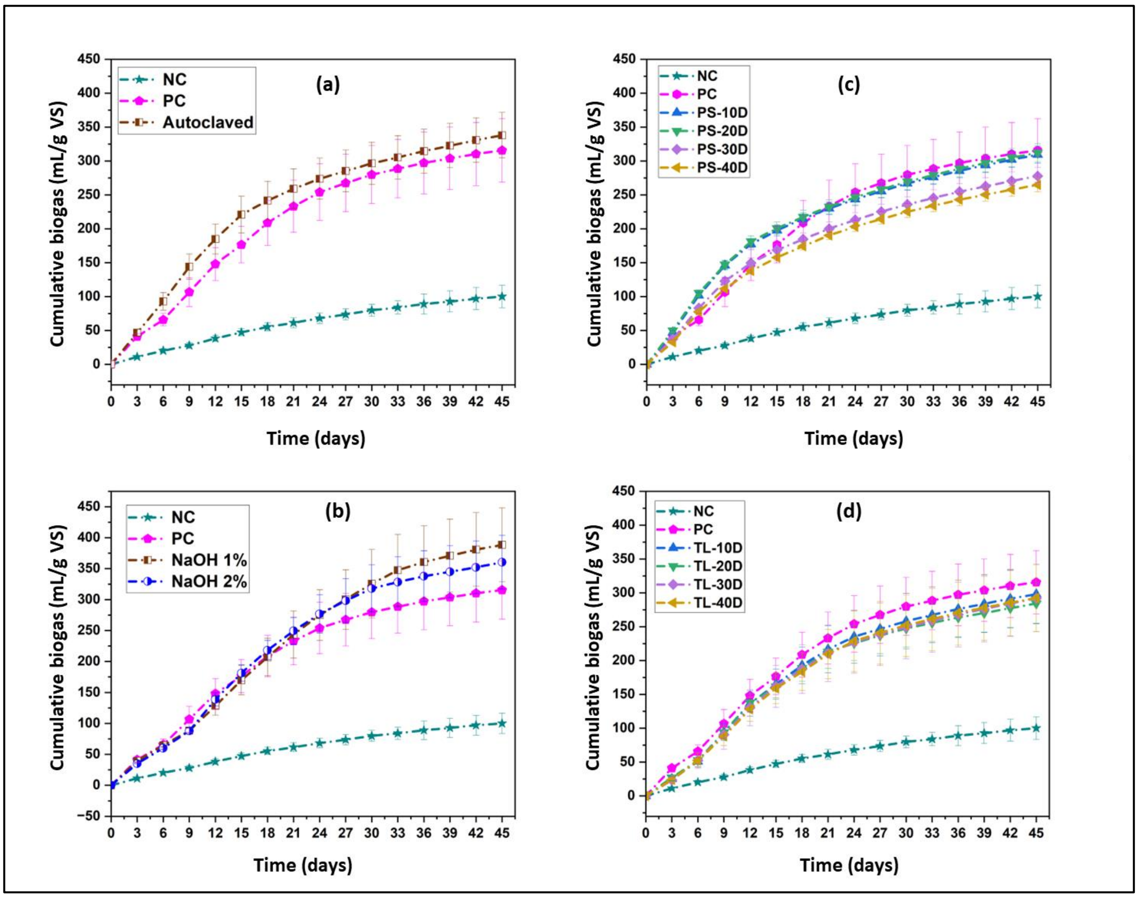

Figure 5.1. Cumulative biogas production from anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal treated rice straw

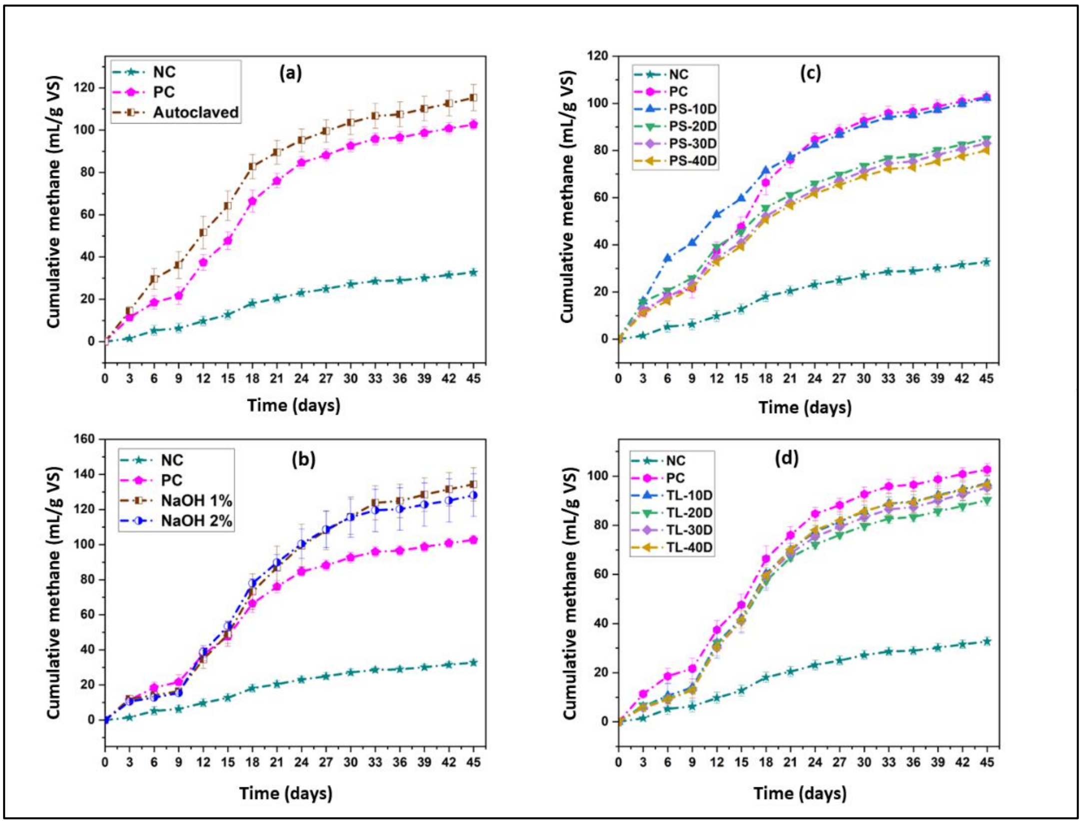

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Figure 5.2. Cumulative methane production from anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal treated rice straw

5.2.2. Biogas and methane production rate

The trend of biogas and methane production recorded every three days is presented in *Figure 5.3* and *Figure 5.4* respectively. The highest biogas recorded was for PS-10sD with a value of 723 mL on day 6. The reason for higher biogas production may be because the broken LCB present in the feedstock has simpler carbohydrates for anaerobic digestion. The lowest biogas is recorded for TL-20D and TL-10D. Here, as well, it becomes clear that the autoclaved sample is performing at par or better than the PC. The autoclaved rice straw consistently performed better than the PC. The production of biogas followed similar crests and troughs for all samples. The biogas production was higher during the initial days and then it started decreasing.

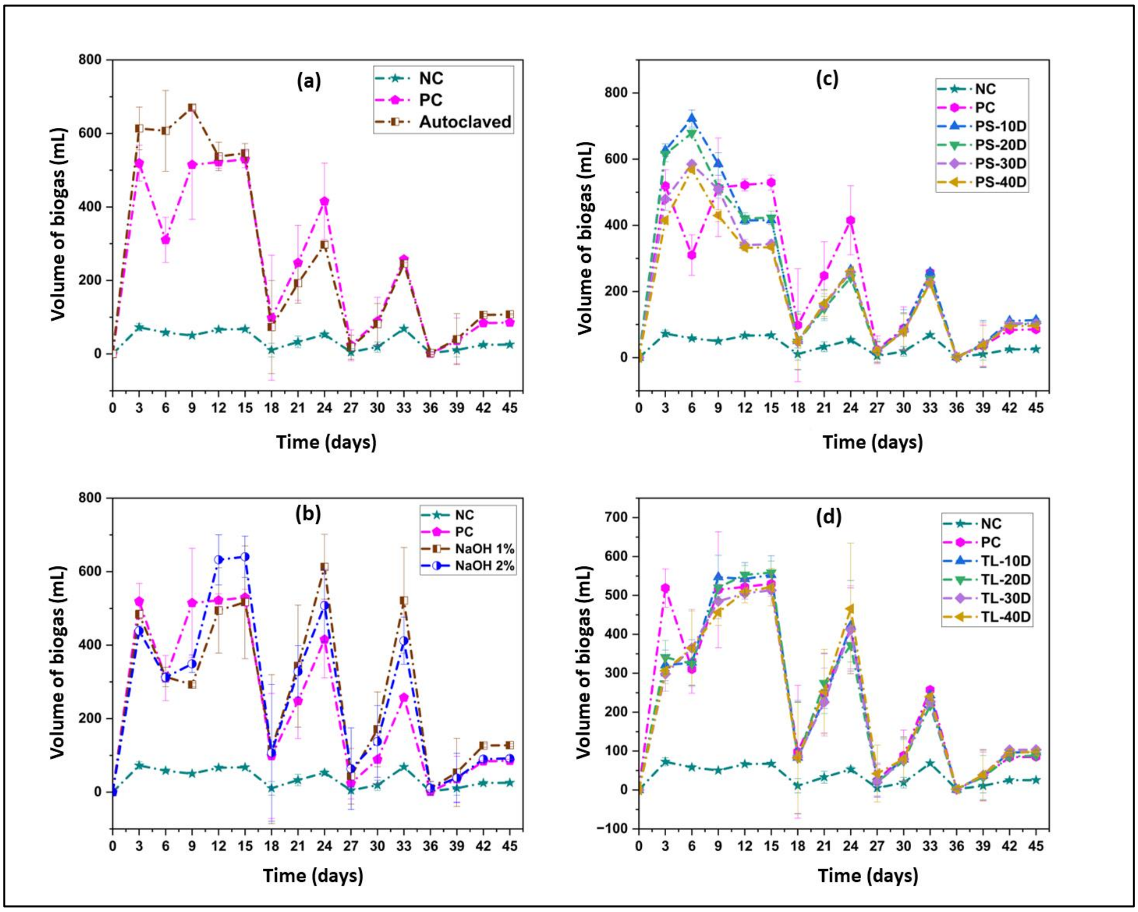

Figure 5.3. Volume of biogas produced (recorded every three days) from anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal treated rice straw

The highest level of methane was produced by 2% NaOH treated rice straw with a production value of 305.86 mL on day 18, and the lowest methane was produced by TL-20D treated sample with a value of 8.01 mL on day 36. Methane follows almost the same pattern as the biogas. In terms of methane concentration amongst treated samples, both the highest and lowest methane concentrations were observed for autoclaved samples, with values of 89.57% on day 18 and 8.15% on day 36. However, if the overall methane concentration is considered, then beyond day 18, NaOH-treated samples have the highest cumulative methane concentration on all the days. Hence, NaOH-treated samples not only generate the highest biogas but also generate the biogas richest in methane, thus making it more commercially suitable.

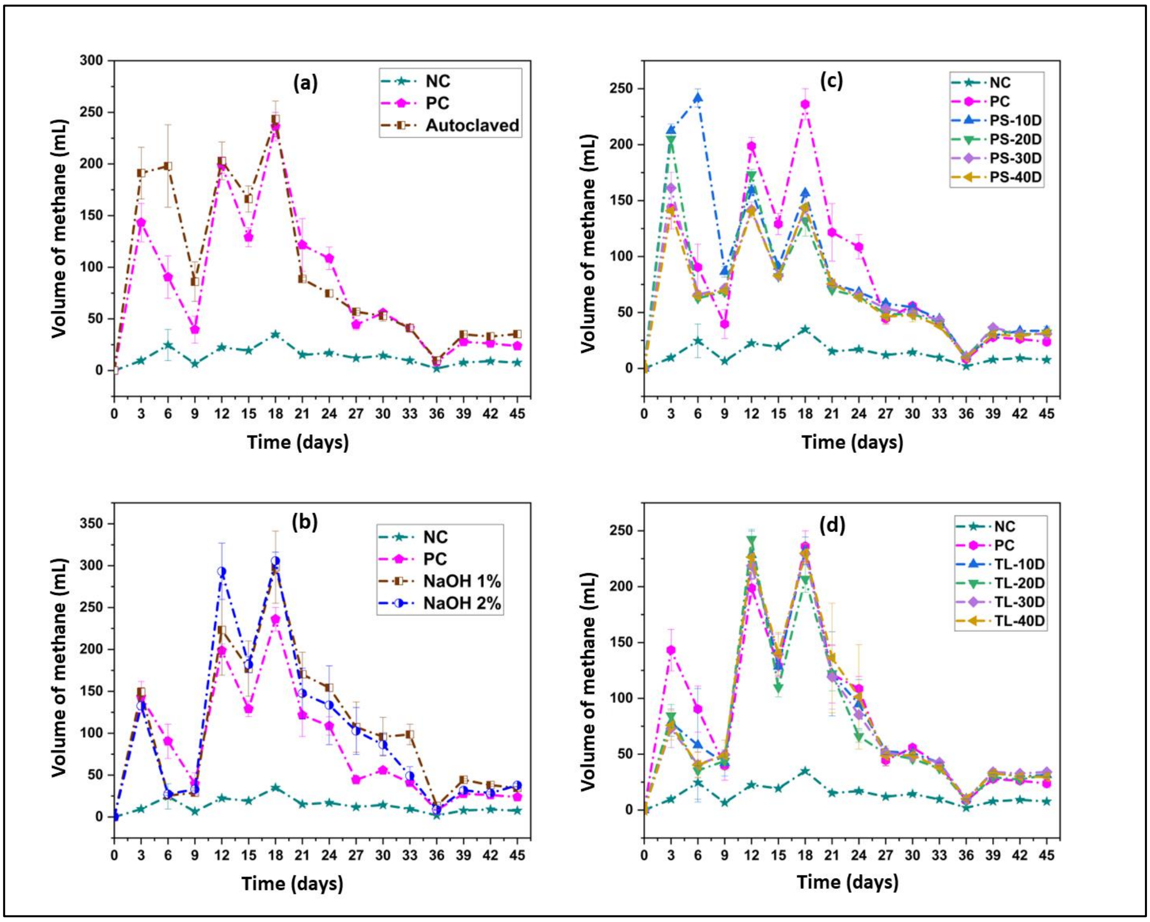

Figure 5.4. Volume of biogas produced (recorded every three days) from anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal treated rice straw

5.2.3. Interdependence of different operational parameters

The trend of VFA production and corresponding pH fluctuations has been presented in *Figure 5.5* and *Figure 5.5*, respectively. Volatile Fatty Acids are a subset of short-chain fatty acids that exhibit low molecular weight and a tendency to volatilize. They are essential in a variety of biological and environmental situations [186]. VFAs are important intermediates that are noteworthy for their contribution to microbial metabolism, fermentation, and anaerobic digestion [187]. Magnitude of their worth arises from their role as essential precursors for microbial synthesis and as critical markers for evaluating the deterioration of organic substrates in a variety of environments [188].

This introduction delves into the systematic investigation of the substantive significance that VFAs possess, outlining their importance in the breakdown of rice straw by anaerobic digestion. From the plots, it can be observed that VFAs reached local maxima around day 18 and beyond day 30. VFAs production declined to 0 between day 21 and day 30 for most samples with the lowest average VFAs produced on day 27. Upon analyzing the correlation between VFA values with biogas and methane generated, it was found that it is negatively correlated with a value of -33.34% and -25.57%, respectively. This further implies that the presence of higher VFAs corresponds to lesser conversion to biogas, indicating their accumulation impacts biogas generation and methane production detrimentally [189].

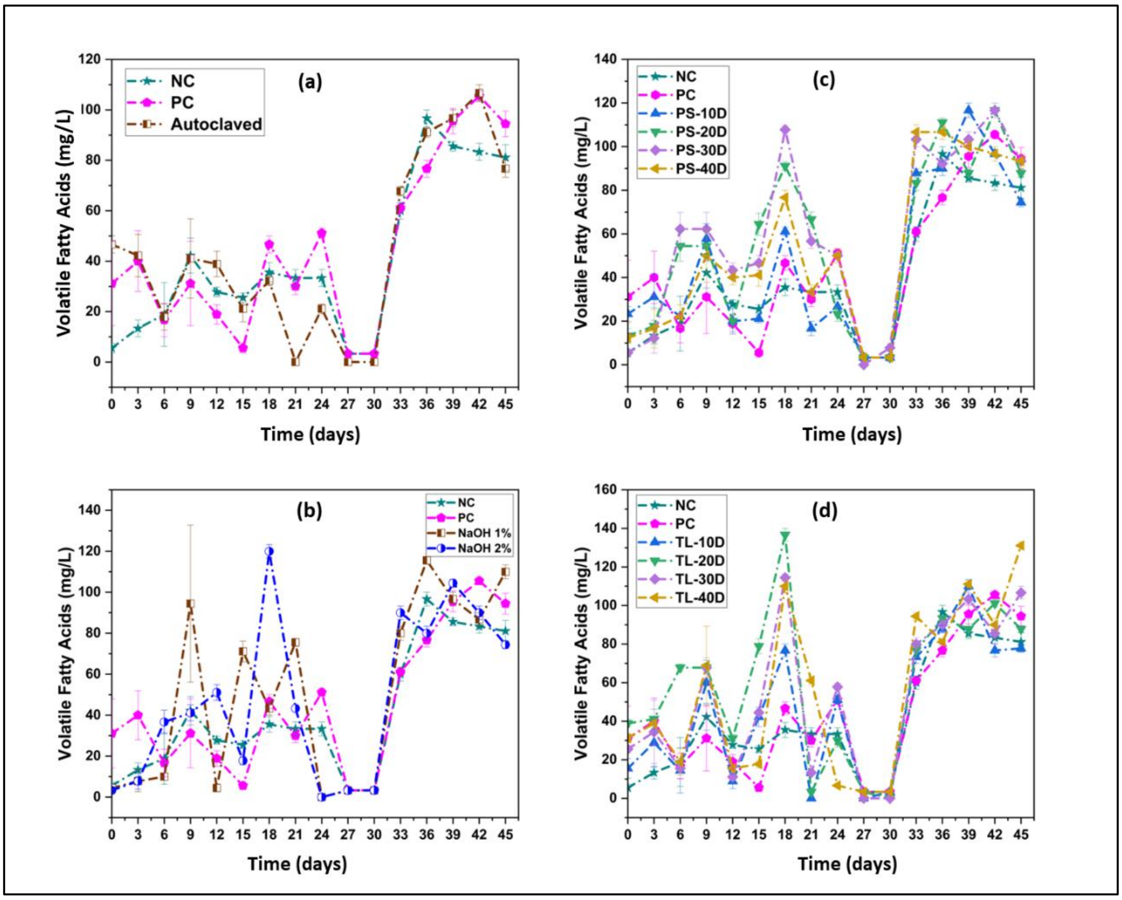

Figure 5.5. Trend of VFAs production from anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal-treated rice straw

It was observed that the pH values fluctuated the most for the initial days of anaerobic digestion. The lowest pH values were recorded on day 6. Post day 6, the pH values started increasing and the fermentation media turned mildly basic beyond day 15. The highest pH was observed for the autoclaved sample, with a value of 7.78 (mildly basic) on day 30. The lowest pH value of 5.76 was observed on day 6 for the 1% NaOH-treated sample. Except for TL-treated samples, all the samples started mildly basic and then went to mildly acidic after day 3. The TL-treated samples were mildly acidic from day 0 itself. All the samples were mildly basic after day 18. It is observed that for a higher value of pH (basic), the biogas production is lower, which is checked with a strong negative correlation of -61.09%. The lower pH values may be because of higher VFA production and utilisation.

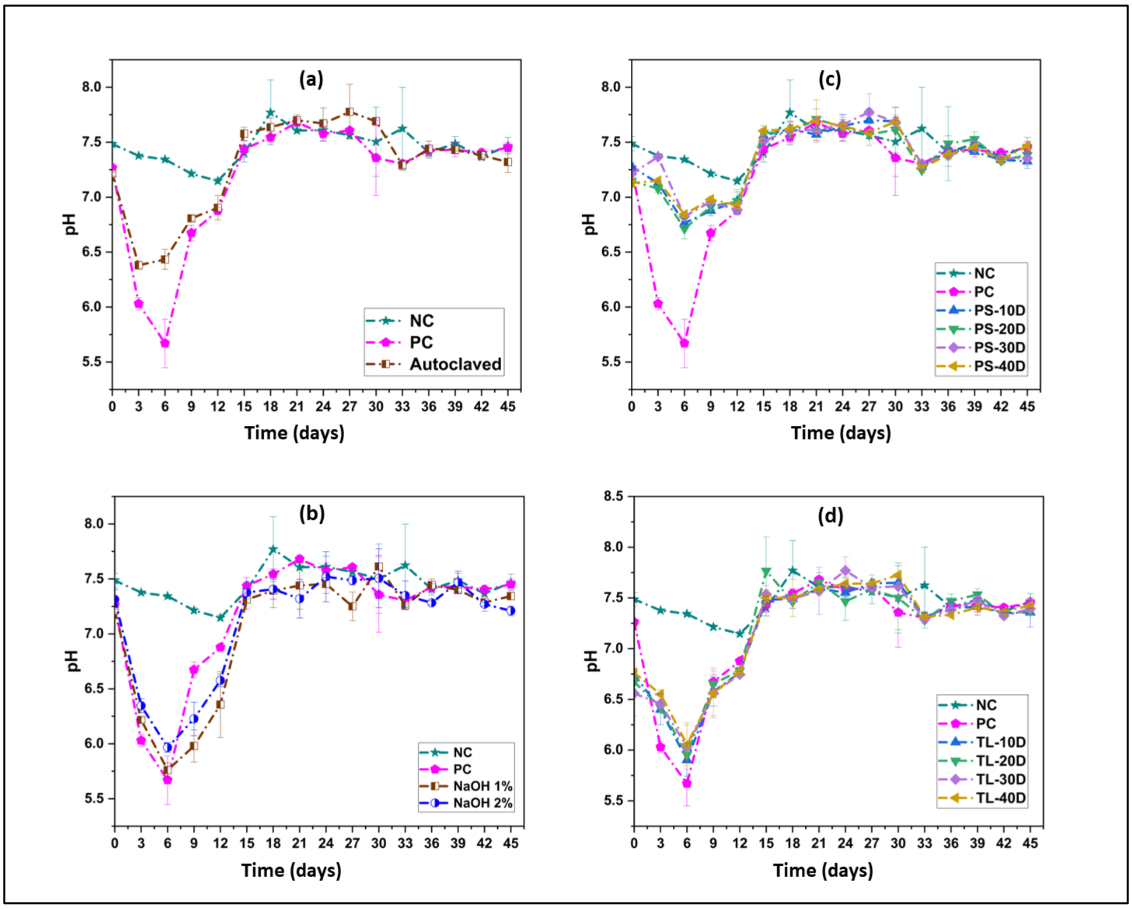

Figure 5.6. pH variations in anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal-treated rice straw

In the fields of environmental science and biological science, soluble chemical oxygen demand (sCOD) is an essential indicator for assessing the existence of organic matter in liquid systems [190]. As an analytical consequence, sCOD measures the amount of oxygen required for the chemical oxidation of organic and oxidizable inorganic components in a liquid sample [191]. sCOD is an essential component of liquid chemical analysis that provides insight into the oxygen consumption/requirement by the liquid sample. Investigating the complexities of soluble COD is, therefore, a voyage into the subtleties of scientific investigations in AD. The trend of variations in the sCOD is presented in *Figure 5.7*.

The highest sCOD value is recorded for TL-40D and TL-20D samples with a value of 53333.33 mg/L, and the minimum value is recorded for all the samples on day 15 or day 18 at a value of 16000 mg/L. It is fundamental to note that methane production is negatively correlated to sCOD, with a 39.68% negative correlation. High sCOD means high oxidisable carbon content in the sample on a particular day, corresponding to its reduced consumption to methane [192].

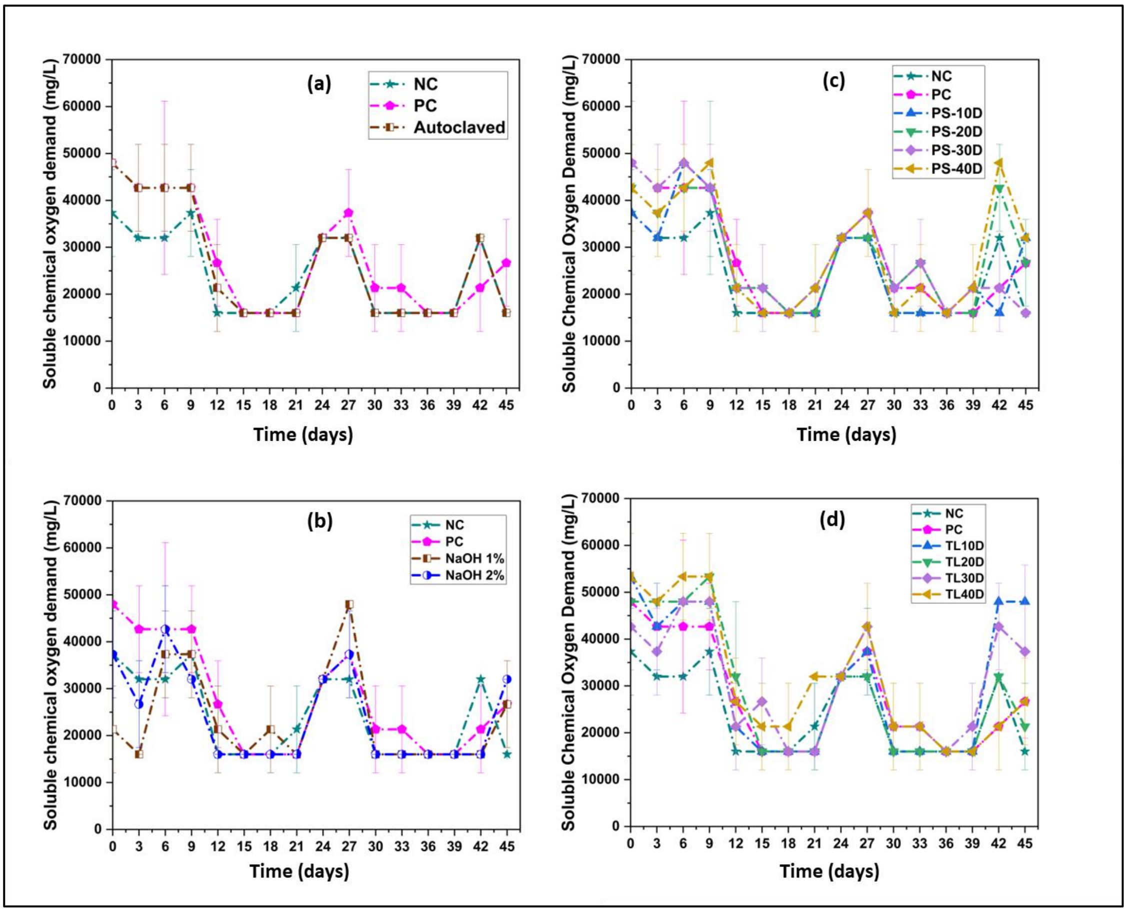

Figure 5.7. sCOD changes in anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal treated rice straw

5.2.4. Role of enzymes in the performance of AD of rice straw

The results of the changes in cellulase activity during the AD of autoclaved, alkali and fungal-treated rice straw are presented in *Figure 5.8*. Cellulase is the protagonist in the realm of anaerobic digestion. Meticulously studying cellulase activity enabled identifying the trend of microbial activity. As an essential cellulolytic enzyme, cellulase controls the hydrolysis of cellulose, a principal component of rice straw, into fermentable simple sugars [193]. Although the cellulase activity changes frequently over the days for all samples and follows different trends for different samples; correlation analysis yields that cellulase activity and biogas production are positively correlated with a value of 37.74%. It was observed that the cellulase activities were the highest for the fungal-treated samples during the initial days of AD. Thus, high cellulase activity is desirable to enhance biogas generation.

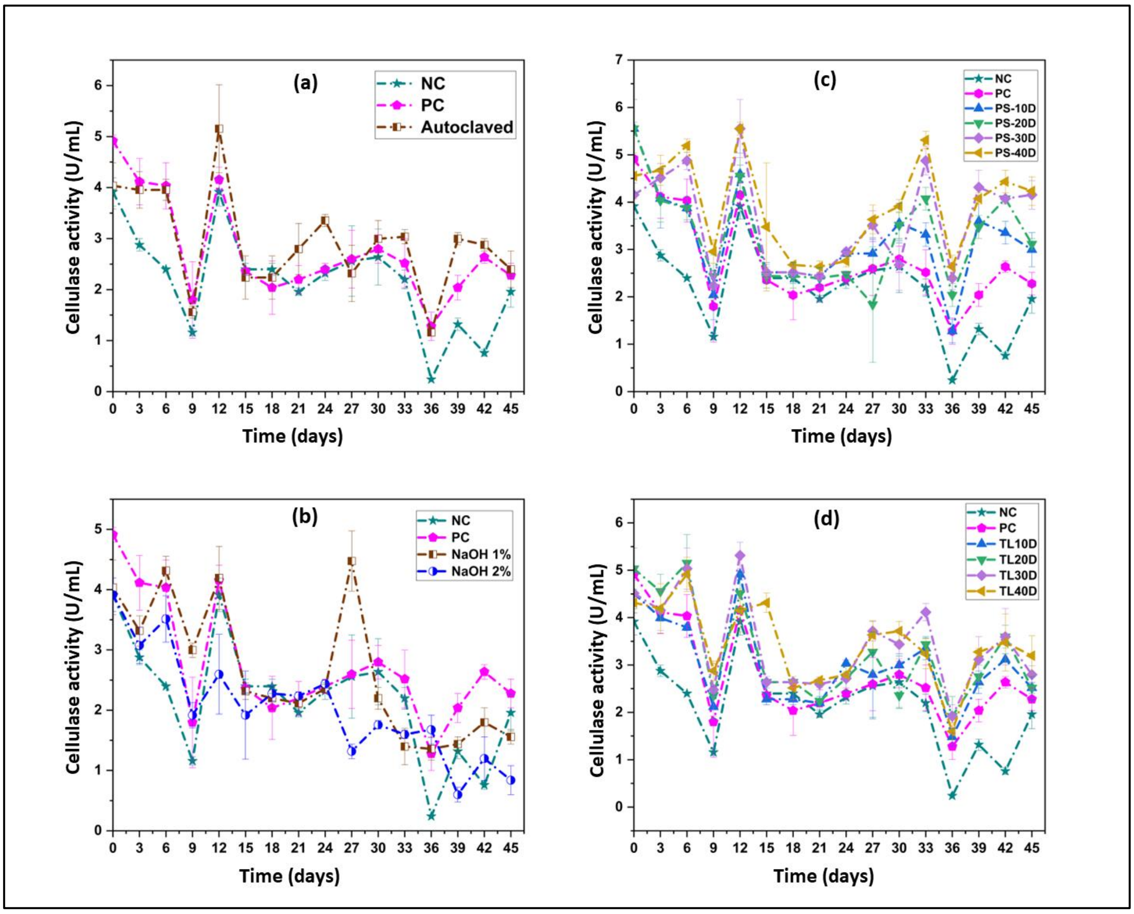

Figure 5.8. The trend of changes in cellulase enzyme in the anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal treated rice straw

Xylanase is a key factor in the complex and delicate process of anaerobic digestion (AD) for rice straw decomposition [194]. Xylanase, a cellulolytic enzyme, breaks down rice straw's main component, hemicellulose, into fermentable simple sugars [195]. Enzymatic alteration increases biodigester efficacy and controls biogas yield, methane content, and biodegradation kinetics [196]. By understanding xylanase engagement, AD techniques for sustainable rice straw management can be optimized.

As shown in *Figure 5.9*, the xylanase activity does not appear to be significantly correlated with biogas volume (2%) or methane volume (3.92%). Thus, high xylanase activity is desirable for better methane concentration and commercial viability. All samples had 3 local maxima during the duration of the experiment with second maxima to be highest in most cases.

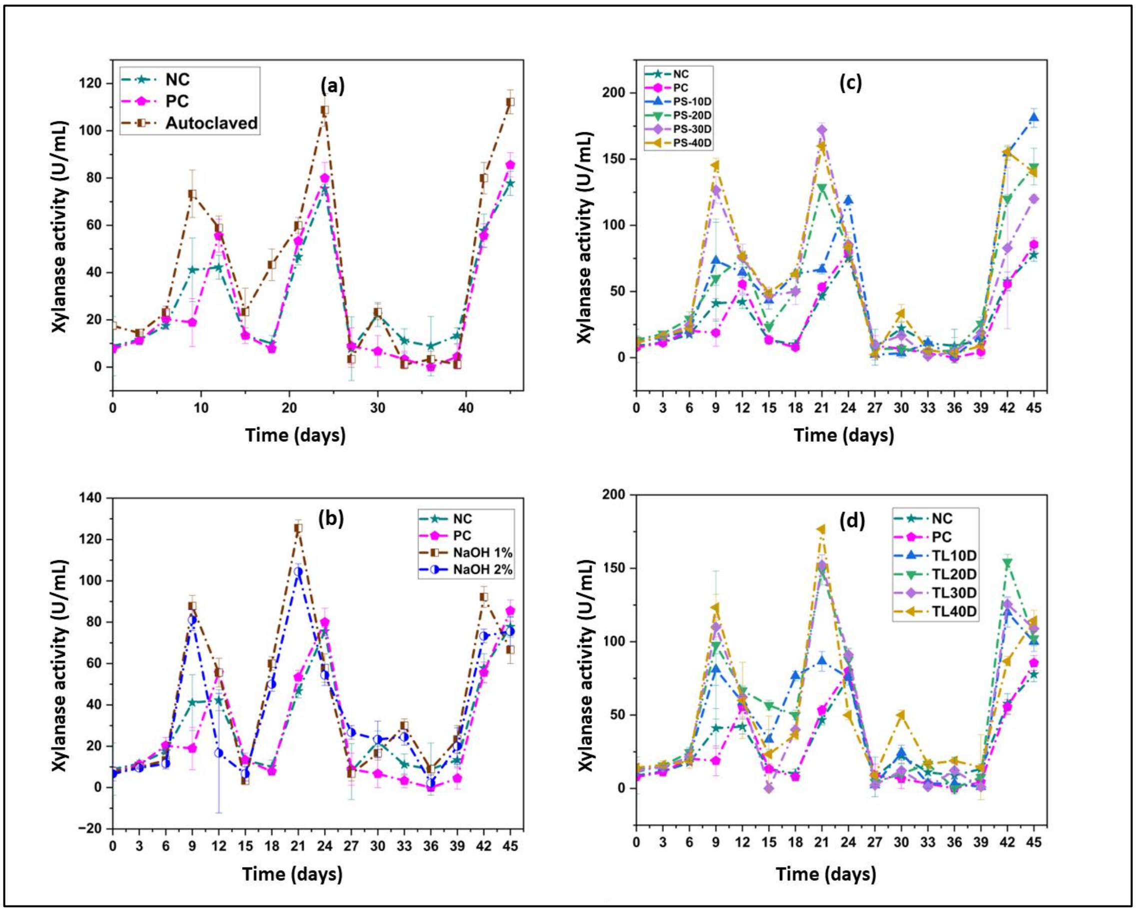

Figure 5.9. The trend of changes in xylanase enzyme in the anaerobic digestion of autoclaved, alkaline (NaOH 1% and NaOH 2%), and fungal-treated rice straw

5.3. Conclusion

This study analytically and critically compares the methods of pre-treatment of rice straw, including thermal, alkali, and fungal treatment, which can aid in resolving the dire problem of rice straw (parali) management and enable the commercial viability of AD systems. From the results presented in this study, pretreatment with 1% NaOH yielded the best results, followed by pretreatment with 2% NaOH in terms of biogas and methane production. The pretreatment of rice straw with 1% NaOH and 2% NaOH produced

23.11% and 14.22% higher biogas increase as compared to PC. Almost all samples produced more than 91% of the total biogas by day 36 except NC. The initial higher production of biogas and methane in PS-10D 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 thermal and alkali treatments have their drawbacks in terms of higher energy consumption and production of toxic intermediates, we further explored the potential of fungal treatment by *P. sanguineus* and *T. longibrachiatum* at an increased substrate loading to check the changes in biogas production and the results are presented in the next chapter.