Walisongo Journal of Chemistry Vol. 8 Issue 2 (2025), 259-272
ISSN: 2621-5985 (online); 2549-385X (print)
DOI: https://doi.org/10.21580/wjc.v8i2.28200

ENHANCED BIOGAS PRODUCTION FROM RICE STRAW THROUGH NAOH PRETREATMENT AND MOLASSES CO-SUBSTRATE ADDITION

Widiastuti Agustina Eko Setyowati[1*], Arin Ika Ningtyas[2], Fila Delfia Zai[3], Sri Mulyani[4] ,Elfi Susanti VH[5] ,Sri Retno Dwi Ariani[6] ,Suryadi Budi Utomo[7] ,Muhammad Hizbul Wathon[8]

Department of Chemistry Education, Faculty of Teacher Training and Education, Universitas Sebelas Maret, Surakarta, Indonesia

*Corresponding author: widiastuti_aes@staff.uns.ac.id

Abstract

This study investigates the effects of rice straw pretreatment using sodium hydroxide and the addition of molasses as a co-substrate on biogas production through anaerobic fermentation. The pretreatment process involved immersing rice straw in sodium hydroxide solutions at three different concentrations for twenty-four hours, while molasses was added at varying levels to evaluate its effect on the fermentation process. Compositional changes in the lignocellulosic biomass were analyzed using the Chesson–Datta method, which revealed significant alterations in cellulose, hemicellulose, and lignin contents. The lowest sodium hydroxide concentration produced moderate delignification while retaining a relatively high cellulose content, indicating its potential as the optimal pretreatment condition. Molasses addition consistently increased biogas production as dosage levels rose, with the highest yield observed at the maximum molasses concentration. The combined application of the lowest sodium hydroxide concentration and the highest molasses level resulted in the maximum biogas yield, demonstrating improved substrate biodegradability and fermentation efficiency. These findings highlight the practical potential of integrating NaOH pretreatment with molasses co-substrate addition to enhance the conversion efficiency of lignocellulosic agricultural waste into renewable energy through anaerobic fermentation.

Keywords: *rice straw, NaOH pretreatment, molasses, lignocellulose, biogas.*

Introduction

Indonesia has abundant natural resources and strong potential for developing renewable energy sources, especially new and renewable energy technologies (Dwivannie, Sasmita, and Pratiwi 2020). The increasing population has led to greater energy demand and the need for new energy solutions, including those based on biomass as a renewable energy source (Dwivannie et al. 2020). One of the most promising biofuels is biogas, a renewable energy source produced mainly from methane (CH_4, 50–75%), carbon dioxide (CO_2, 34–45%), and trace gases (Sabeeh et al. 2020) .

Traditionally, biogas has been produced primarily from animal waste, particularly cow dung, which has shown high biogas potential. For instance, 1 kg of cow manure can generate 90–310 liters of biogas (Wang et al. 2019). In recent years, there has been renewed interest in non-food lignocellulosic biomass because it is abundant and does not compete with food resources (Sabeeh et al. 2020). Rice straw is one such promising lignocellulosic material, widely available in Indonesia and considered an underutilized agricultural residue (Abdel daiem, Hatata, and Said 2022). Although rice straw has great potential, it is still minimally utilized and is often left in the field or openly burned after harvest.

Rice straw is a lignocellulosic resource with a high carbon-to-nitrogen (C/N) ratio, typically 50–70 (Ibrahim 2018), whereas the ideal C/N ratio for anaerobic digestion is around 20–30 (Deublein and Steinhauser 2011). Its complex structure, mainly composed of cellulose, hemicellulose, and lignin, makes it highly resistant to biodegradation (Aghbashlo et al. 2018). Lignin, a complex aromatic polymer, interacts strongly with cellulose and hemicellulose, hindering microbial access to the fibers and reducing hydrolysis and fermentation rates (Du et al. 2019). Its rigid structure decreases porosity and restricts enzyme action, leading to lower overall biogas yield (Du et al. 2019). To overcome these constraints, pretreatment is required to break down the recalcitrant structure of lignocellulosic biomass.

Pretreatment is essential to accelerate the decomposition of lignin-rich lignocellulosic materials, which cannot readily degrade during hydrolysis (Taherzadeh and Karimi 2008). Alkaline pretreatment, particularly with Sodium hydroxide (NaOH), is known to facilitate delignification, increase surface area and porosity, and enhance microbial accessibility (Sidiras and Koukios 1989; Ouahabi, Bensadok, and Ouahabi 2021). Through this process, lignin is disrupted and cellulose crystallinity is reduced, enabling more efficient enzymatic hydrolysis (Tan et al. 2021). Although NaOH pretreatment significantly improves methane production (Mirmohamadsadeghi et al. 2021; Peyrelasse et al. 2021), studies examining low NaOH concentrations, especially the unlocking mechanism of 5 g NaOH per gram of rice straw, remain very limited. Even moderate NaOH concentrations of 0.25 N, 0.5 N, and 0.75 N may support partial delignification without substantial cellulose loss. However, with increased use of alkali pretreatment, detailed characterization of post-treatment rice straw solids is often overlooked. Such analysis is crucial to clarify the behavior of lignin, cellulose, and hemicellulose fractions and their correlation with biogas production. The Chesson–Datta method serves as a valuable tool for identifying compositional changes and predicting the effectiveness of pretreatment strategies (Ouahabi et al. 2021).

An additional option to increase biogas production is the application of co-substrates. Molasses, a byproduct of the sugar industry, contains simple sugars such as sucrose, glucose, and fructose that provide an easily fermentable source for microbial metabolism (Kalemba and Barbusiński 2017). Molasses has also been identified as a good source of essential nutrients, including nitrogen, phosphorus, and potassium, which stimulate methanogenic microbial growth (Wen et al. 2024). However, its concentration must be optimized, as excessive amounts can lead to rapid acidification and inhibit methanogens due to a sharp decline in pH (Fuess et al. 2017).

Although previous studies have reported the individual benefits of NaOH pretreatment and molasses addition on biogas production, comprehensive investigations into their combined effects on rice straw digestion remain limited. Hence, the novelty of the present study lies in the systematic evaluation of the synergistic interaction between sodium hydroxide pretreatment and molasses co-substrate addition, an approach that has not previously been explored for rice straw substrates. Furthermore, this study provides a detailed compositional analysis using the Chesson–Datta method to link variations in lignin, cellulose, and hemicellulose with biogas yield. By integrating chemical pretreatment with co-substrate enrichment, this work proposes a low-cost and sustainable strategy to enhance the biodegradability and biogas potential of lignocellulosic agricultural residues, offering scientific and practical significance for renewable energy applications in developing countries.

Experimental Section

Materials

The materials used in this study consisted of chemical reagents and biological components essential for the anaerobic digestion process. Rice straw, weighing 18 grams per batch, served as the main lignocellulosic substrate. Cow dung was used as the inoculum, providing naturally occurring anaerobic microorganisms. Molasses was added as an additional carbon and micronutrient source at concentrations of 1%, 2%, and 3% v/v. NaOH solutions at concentrations of 0.25 N, 0.5 N, and 0.75 N were used to pretreat the substrate to enhance its biodegradability. Urea, at 0.3 grams per batch, was added to supply nitrogen. EM-4 (Effective Microorganisms-4) served as a microbial activator. Hydrochloric acid (HCl) and concentrated sulfuric acid (H_2SO_4, 98%) were used for pH adjustment and chemical analysis. Distilled water (aquadest) was used throughout the procedures for dilution and rinsing.

All reagents were of analytical grade, and all instruments were properly calibrated before use. Reactions involving volatile components and temperature-sensitive steps were conducted under controlled laboratory conditions to maintain safety and data integrity.

Instruments

The equipment used in this study included an anaerobic batch digester (custom-fabricated reactor), a high-speed blender for substrate homogenization, and a combustible gas detector (CGD02B Mestek) for biogas measurement. Additional laboratory instruments included a precision electronic balance (±0.01 g accuracy), 100 mL and 250 mL beakers, graduated cylinders, glass stirring rods, metal spatulas, filter paper, conical flasks, drying ovens (60°C and 85°C), watch glasses, glass funnels, 100 mL volumetric flasks, dropper pipettes, porcelain crucibles, and a muffle furnace capable of reaching 550°C.

This section was adapted from Sumardiono et al. (2022), with modifications involving the addition of molasses as a co-substrate and the use of a 7% EM-4 concentration.

Procedure

The investigation was organized into three consecutive experimental stages, each aimed at enhancing biogas yield from rice straw by applying alkaline pretreatment and supplementing with molasses as a co-substrate. Each stage addressed a distinct research question and was carried out under tightly controlled laboratory conditions to ensure repeatability and comparability of measurements. After determining the optimal NaOH concentration for pretreatment, all treatments were replicated three times (n = 3) to allow for appropriate statistical evaluation of the generated data.

Alkali Pretreatment of Rice Straw Using NaOH (0.25 N, 0.5 N, and 0.75 N)

In the initial phase of the study, rice straw was pretreated with NaOH at concentrations of 0.25 N, 0.5 N, and 0.75 N to evaluate the effects of varying alkalinity on lignocellulosic degradation. Separately prepared batches of 18 g of air-dried straw were immersed in 200 mL of the corresponding solution and allowed to react for 24 hours under ambient laboratory conditions. This alkaline exposure was intended to cleave ester and ether linkages within lignin and hemicellulose, thereby increasing cellulose accessibility for subsequent microbiological assays. Following treatment, the residues were neutralized by rinsing with distilled water until the eluate reached an approximately neutral pH, then air-dried under atmospheric conditions prior to compositional analysis. This controlled pretreatment procedure enabled systematic examination of the influence of NaOH concentration on the chemical structure and resulting biodegradability of rice straw.

Initial Chemical Characterization of Rice Straw

In the second phase, chemical characterization was performed to establish the baseline lignocellulosic profile of untreated rice straw. Cellulose, hemicellulose, and lignin were quantified using a modified Chesson–Datta approach. Approximately 1 gram of oven-dried straw was subjected to analysis. Sequential chemical treatments separated each structural component according to its solubility and reactivity, enabling accurate gravimetric measurement of the individual fractions. The resulting compositional data served as a reference against which the alkaline pretreatment introduced in Step I could be compared. By examining changes in composition before and after sodium hydroxide treatment, this phase clarified the structural modifications induced by the chemical pretreatment.

FTIR Characterization of Untreated and Optimally Pretreated Rice Straw

In the fourth phase, FTIR was employed to characterize the molecular structure of rice straw before and after alkaline treatment. Analysis included untreated straw and material exposed to the sodium hydroxide concentration identified as most effective in Step III. The purpose of the FTIR analysis was to identify shifts in functional groups and to document modifications in the chemical linkages associated with cellulose, hemicellulose, and lignin. By comparing the spectra of the two sample sets, this phase highlighted the structural rearrangements induced by alkali exposure, thereby supporting the rationale for selecting the optimal pretreatment regimen.

Effect of Molasses Co-substrate Addition on Biogas Yield

The fifth phase examined the effect of molasses addition on biogas production from rice straw pretreated with the optimal sodium hydroxide concentration established in Step III. Four experimental groups were prepared for this assessment: 1) Control: untreated straw with no molasses added; 2) Treatment A: alkaline pretreatment followed by the addition of 1% molasses (v/v); 3) Treatment B: alkaline pretreatment with 2% molasses (v/v); and 4) Treatment C: alkaline pretreatment with 3% molasses (v/v).

Determination of Optimal NaOH and Molasses Combination for Biogas Production

Multiple treatments combining various NaOH concentrations with different levels of molasses were evaluated to determine their effect on biogas yield. The combination that consistently produced the highest and most stable gas output was identified as the optimal formulation for digesting rice straw.

Throughout the experiment, researchers recorded daily and cumulative biogas volumes (g/L), digester pressure (cmH_2O), fermentation temperature (°C), and relative humidity (%RH). After digestion, the residual substrate was analyzed for cellulose, hemicellulose, and lignin content using the Chesson–Datta procedure.

Results and Discussion

The experimental findings indicate that pretreating rice straw with NaOH and adding small amounts of molasses noticeably enhanced its degradation during anaerobic digestion. Each result is discussed according to its respective test stage, covering changes in chemical composition, trends in gas production, and structural modifications identified through FTIR spectroscopy. Statistical analyses were conducted to confirm that the observed patterns were significant rather than due to random variation.

Effect of NaOH Pretreatment on the Chemical Composition of Rice Straw

The results show that the treatment of lignocellulosic feedstocks with sodium hydroxide significantly improves their biodegradability by cleaving ester bonds that connect lignin to structural carbohydrates, namely cellulose and hemicellulose. Bali et al. (2015) reported that alkaline pretreatment, particularly with sodium hydroxide, reduces the degree of polymerization and makes cellulose substantially more accessible. During pretreatment, the NaOH solution disrupts the lignin network and breaks ester linkages, thereby loosening the rigid fibrous structure of the material. With the matrix opened, cellulose surfaces become more exposed and more readily hydrolysed by enzymes in subsequent stages of bioconversion (Bali et al. 2015).

The delignification mechanism involves hydrolysis and partial oxidation, which increase lignin solubility and expose fermentable polysaccharides to microbial activity during anaerobic digestion. The following reaction illustrates this process.

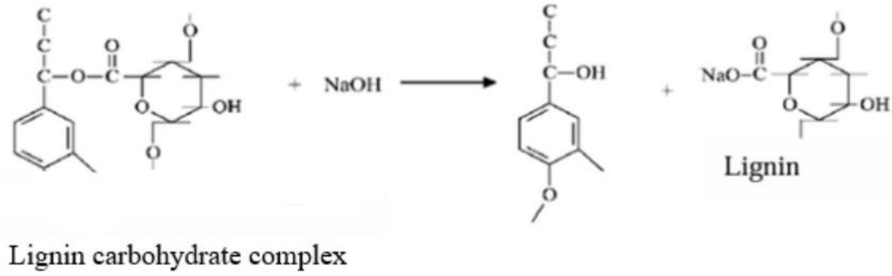

Figure 1. Reaction scheme of complex carbohydrate–lignin with NaOH

This reaction alters the ester linkages between lignin and carbohydrates (cellulose and hemicellulose), enabling the release of these polysaccharides through hydrolysis. Lignin, a key component of plant cell walls, is strongly bonded to carbohydrates via ester linkages, forming a recalcitrant matrix that hinders biomass utilization for bioenergy applications. When NaOH is introduced, it acts as a delignifying agent by breaking these linkages, resulting in lignin solubilization and increased carbohydrate availability for fermentation.

The analysis of cellulose, hemicellulose, and lignin content was conducted using the Chesson–Datta method. The delignification percentages for each treatment are presented in Table 1.

Table 1. Cellulose, Hemicellulose, and Lignin Content of Rice Straw Before and After Pretreatment

Treatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Delignification (%)
Without Pretreatment	32.23 ± 0.002	30.10 ± 0.002	8.70 ± 0.000	-
NaOH 0,25 N	22.56 ± 0.001	28.90 ± 0.001	7.46 ± 0.001	14.17 ± 0.013
NaOH 0,5 N	21.86 ± 0.001	28.26 ± 0.001	7.40 ± 0.002	14.94 ± 0.020
NaOH 0,75 N	16.00 ± 0.001	33.70 ± 0.000	7.03 ± 0.001	19.15 ± 0.013

As shown in Table 1, untreated rice straw contained 8.70% lignin, 32.23% cellulose, and 30.10% hemicellulose. Following NaOH pretreatment, lignin content progressively decreased, reaching a minimum of 7.03% at 0.75 N NaOH. The corresponding delignification efficiencies were 14.17% (0.25 N), 14.94% (0.5 N), and 19.15% (0.75 N). However, the highest delignification level at 0.75 N was accompanied by a significant reduction in cellulose (down to 16.00%), suggesting possible degradation or loss of fermentable components.

0.25 N NaOH provided a favourable balance between lignin removal and cellulose retention (22.56%), making it the most efficient treatment condition.

Hemicellulose levels fluctuated slightly but peaked at 33.70% under the 0.75 N treatment, indicating partial redistribution or solubilization. Statistical analysis using the Kruskal–Wallis test confirmed significant differences ($p < 0.05$) in cellulose, hemicellulose, and lignin content across treatments, validating the chemical effects of NaOH pretreatment.

Effect of NaOH Pretreatment on Biogas Production

NaOH pretreatment significantly affected the physical and chemical structure of rice straw, improving its digestibility. Physically, the treated biomass appeared softer and more fragmented, indicating the breakdown of rigid lignocellulosic matrices. The biomass was treated with sodium hydroxide to observe the resulting structural changes and their effect on biogas production efficiency. Alkaline pretreatment, particularly using NaOH, one of the most commonly applied bases, facilitates microbial degradation of lignocellulosic biomass by removing lignin and hemicellulose components. Additionally, this method reduces the degree of polymerization and crystallinity and disrupts the linkages between lignin and other polymers (Mankar et al. 2021).

Daily and cumulative biogas production profiles showed that all pretreatment levels enhanced biogas yield compared to untreated straw. The 0.25 N NaOH treatment resulted in the highest cumulative biogas production at 51.61 g/L, with peak production occurring on day 14. Treatments with 0.5 N and 0.75 N NaOH produced 50.48 g/L and 45.92 g/L, respectively. Although 0.5 N generated a biogas volume similar to 0.25 N, the more substantial cellulose loss observed in this treatment made 0.25 N the more efficient option overall.

Previous studies have also demonstrated that lower NaOH concentrations are more effective in increasing biogas yield without inhibiting fermentative microorganisms. Therefore, 0.25 N can be considered an optimal pretreatment concentration for rice straw, providing an appropriate balance between lignin degradation and maintaining microbial activity for anaerobic fermentation (Wang et al. 2019).

A one-way ANOVA yielded a p-value of 0.075, indicating that the differences among treatments were not statistically significant at the 95 percent confidence level. Although the statistical analysis suggests that the tested NaOH concentrations did not produce significant differences in biogas output, the observed trends remain consistent with previous studies showing that NaOH pretreatment can improve feedstock quality and enhance biogas production. Further research involving different NaOH concentrations, extended pretreatment durations, or varied operational conditions may provide deeper insights into the role of NaOH in optimizing biogas production.

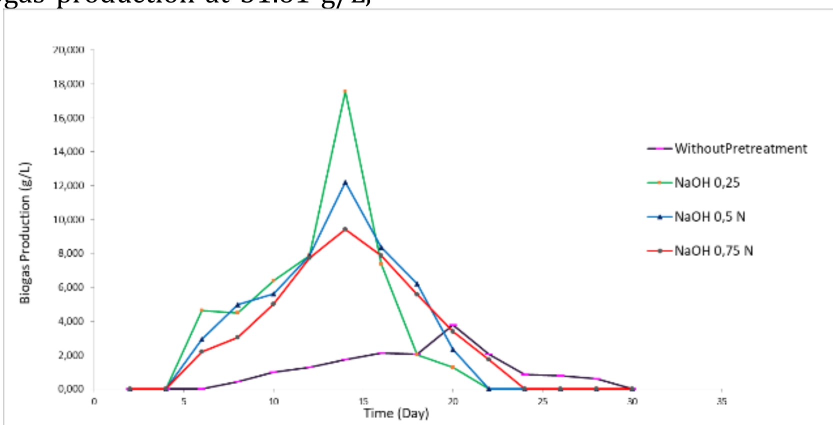

Figure 2. Daily Biogas Production Chart

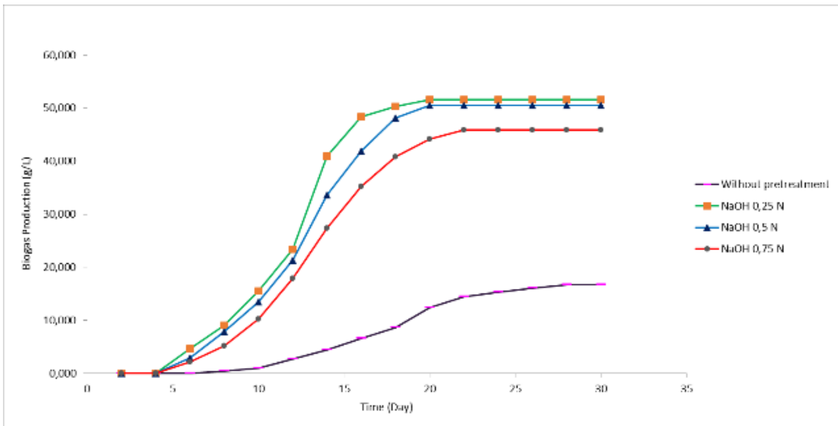

Figure 3. Cumulative Biogas Production Chart

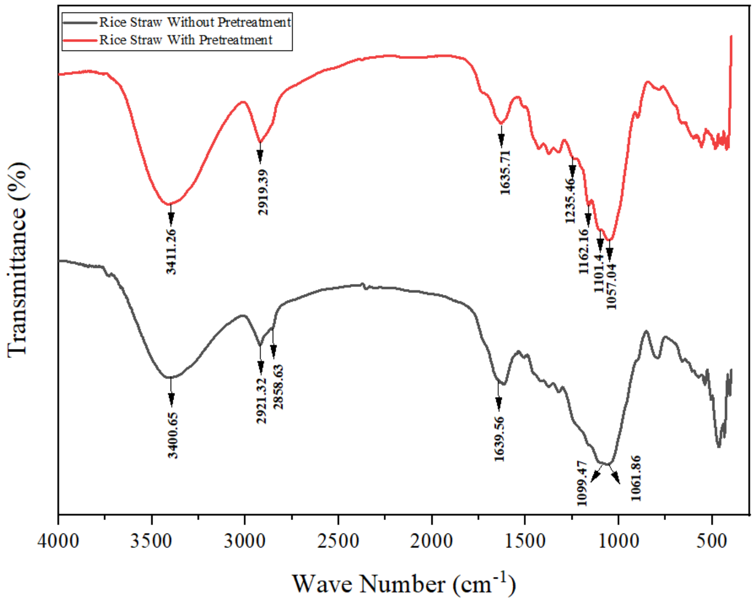

Figure 4. Characterization of Rice Straw using FTIR

FTIR Analysis of Structural Modifications

FTIR spectroscopy was used to analyze structural changes in rice straw before and after NaOH pretreatment. The spectra showed significant changes in several functional groups, confirming chemical modifications within the lignocellulosic components.

Based on the data presented in Table 2, the pretreatment process appeared to significantly affect the chemical structure of the straw, as indicated by shifts in absorption bands and the appearance or disappearance of several peaks in the IR spectra. The O–H stretching vibration corresponding to hydroxyl groups in cellulose, lignin, and phenolic compounds shifted from 3400.65 cm^{-1} in untreated samples to 3411.26 cm^{-1} in treated samples. This shift indicates the disruption of hydrogen bonding, suggesting molecular rearrangement due to alkali treatment (Tsegaye, Balomajumder, and Roy 2019).

In the aliphatic region, the double peaks observed at 2921.32 cm^{-1} and 2858.63 cm^{-1} in untreated straw, associated with C–H stretching of methyl and methylene groups in hemicellulose and lignin, were reduced to a single peak at 2919.39 cm^{-1} after pretreatment (Dumlu et al. 2021). This simplification suggests partial degradation of hemicellulose and lignin structures. Similarly, the C=O stretching vibration linked to aromatic carbonyl groups, characteristic of lignin, shifted from 1639.56 cm^{-1} to 1635.71 cm^{-1},

Volume 8, Nomor 2, 2025

indicating a reduction in lignin content (Rahnama et al. 2014).

A new absorption peak appeared at 1235.46 cm^{-1} following pretreatment, which can be attributed to the C–O stretching of acetyl groups in hemicellulose. This observation suggests that hemicellulosic side chains began to open during processing (Rahnama et al. 2014). The glycosidic region also showed significant spectral shifts: the initial peaks at 1099.47 cm^{-1} and 1061.86 cm^{-1} shifted to 1162.21 cm^{-1} and 1057.04 cm^{-1}, respectively (Tsegaye et al. 2019). These changes reflect modifications in the molecular framework of cellulose and hemicellulose.

In summary, the FTIR spectra demonstrate that the pretreatment step significantly altered the lignocellulosic architecture of rice straw by breaking hydrogen bonds, reducing hemicellulose and lignin content, and exposing additional functional groups. Such structural changes are crucial for enhancing biomass conversion into bioethanol or other biochemicals, as a more accessible structure accelerates enzymatic hydrolysis. These findings are consistent with earlier studies emphasizing the role of pretreatment in improving overall biomass conversion efficiency (Mosier et al. 2005; Sun and Cheng 2002).

Table 2. Interpretation of IR Spectra on Straw Without Pretreatment and Straw with Pretreatment

Absorption Zone (cm^{-1})	Peak (cm^{-1})		Interpretation of Functional Groups
	Straw Without Pretreatment	Straw With Pretreatment	
3650-3400	3400,65	3411,26	O-H stretching of hydroxyl groups (cellulose, lignin, phenolic compounds)
3000-2840	2921,32 2858,63	2919,39	C-H stretching of CH_3/CH_2 (hemicellulose, lignin)
1750-1600	1639,56	1635,71	C=O stretching of aromatic carbonyl groups (lignin, esters, aldehydes)
1240–1220	-	1235.46	C–O stretching of acetyl groups in hemicellulose
1200-1050	1099,47 1061,86	1162,21 1057,04	C-O/C-C glycosidic bonds of cellulose and hemicellulose

Effect of Molasses Addition on Biogas Production

This study examines how different concentrations of molasses, 1%, 2%, and 3%, affected the biogas yield from rice straw pretreated with 0.25 N NaOH, the concentration selected after earlier screening. By supplementing the substrate with molasses, the experiment aims to provide fresh, soluble carbon that methanogenic microorganisms can readily utilize, thereby promoting higher gas output during anaerobic digestion.

The measurement results showed an increasing trend in biogas production with higher molasses concentrations, with the 3% treatment producing the highest biogas volume compared to the 1% and 2% treatments. The daily biogas production curve showed a more significant increase under the 3% molasses treatment, particularly during the initial days of fermentation, indicating more optimal methanogenic activity. This can be seen in the following figures.

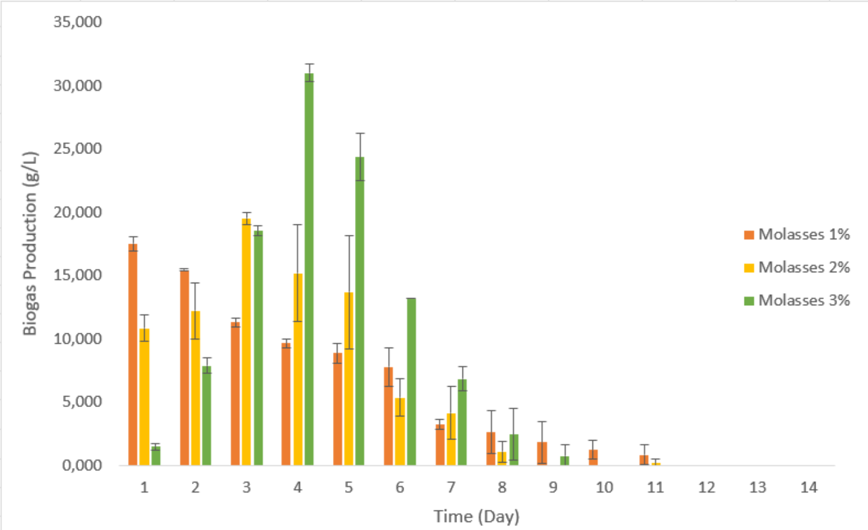

Figure 5. Daily Biogas Production on 0.25 N NaOH Pretreatment with Molasses Addition

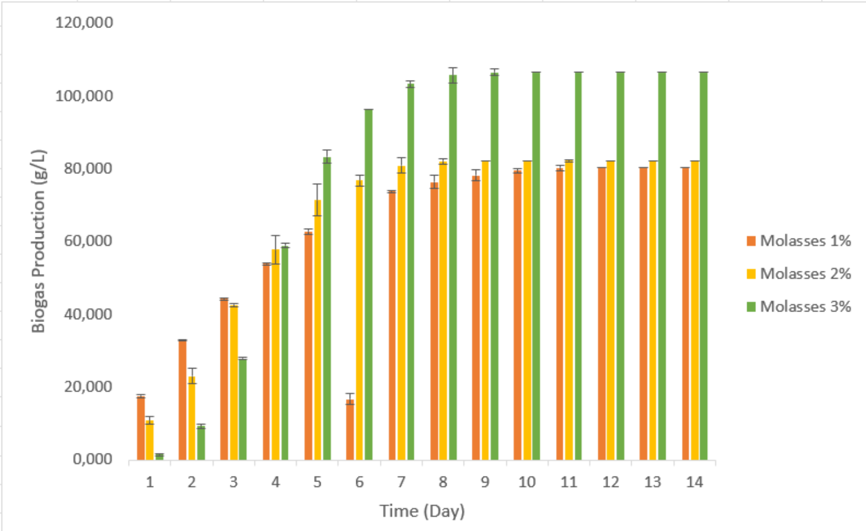

Figure 6. Cumulative Biogas Production on 0.25 N NaOH Pretreatment with Molasses Addition

This phenomenon indicates that molasses supplementation enhances the biodegradation rate of lignocellulosic waste and accelerates the peak of biogas production (Andersen, Jensen, and Mikkelsen 2015). Lower molasses concentrations may not supply enough substrate to maximize anaerobic microbial activity. An optimal concentration is crucial for maintaining fermentation stability; insufficient substrate limits microbial activity, whereas excessive concentrations may lead to osmotic inhibition or a rapid pH drop due to the accumulation of organic acids (Mischopoulou et al. 2017).

Molasses addition following 0.25 N NaOH pretreatment further increased biogas production. The 3% molasses treatment produced the highest cumulative yield of 106.53 g/L, peaking on day 4. In comparison, the 2% and 1% treatments produced 82.24 g/L and 80.32 g/L, respectively. As a source of readily fermentable sugars, molasses likely accelerated methanogenic activity during the early fermentation phase. However, statistical analysis using ANOVA revealed no significant differences among molasses treatments ($p = 0.610$), suggesting that although the trend is positive, higher concentrations or longer fermentation durations may be required to achieve statistical significance.

The results of this study indicate that although molasses shows potential as a co-substrate, its use at concentrations between 1% and 3% does not produce a statistically significant effect on biogas production. This highlights the importance of optimizing the combination of substrate composition, molasses concentration, and operational parameters in the anaerobic fermentation process to achieve improved outcomes. Therefore, future studies are recommended to test higher molasses concentrations, such as 5%, to determine whether a significant increase in biogas production can be observed. Additionally, evaluating a broader concentration range (e.g., 1%, 3%, 5%, 7%, and 10%) may offer clearer insights into the most effective molasses level for enhancing anaerobic digestion. Further research should also consider variables such as fermentation time, pH, temperature, and substrate characteristics to optimize the overall process.

Combined Effect of NaOH Pretreatment and Molasses Co-substrate Addition

This study aims to evaluate the extent to which the combination of pretreatment using a 0.25 N NaOH solution and the addition of molasses as a co-substrate could enhance biogas production from rice straw. NaOH pretreatment is known to break down the complex lignocellulosic structure of rice straw, increasing its accessibility for microbial degradation during anaerobic fermentation (Zhao, Zhang, and Liu 2012 ; Hendriks and Zeeman 2009). Meanwhile, molasses, as a readily fermentable carbon source, is expected to boost the activity of methanogenic microorganisms involved in biogas production (Avena et al. 2024 ; Guang et al. 2017).

Table 3. Biogas Production from Rice Straw under Various Combinations of NaOH and Molasses Pretreatment

Treatment	Total Biogas (g/L)	Peak Production (day)	Increase Vs. Control (%)
Control (without pretreatment)	16.658	20	-
NaOH 0,25 N	51.612	14	+209.833
NaOH 0,5 N	50.479	14	+203.032
NaOH 0,75 N	45.918	14	+175.651
NaOH 0,25 N + Molasses 1%	80.316 ± 0.633	1	+382.147
NaOH 0,25 N + Molasses 2%	82.24 ± 1.193	3	+393.697
NaOH 0,25 N + Molasses 3%	106.526 ±0.548	4	+539.489

As shown in Table 3, rice straw without pretreatment produced only 16.658 g/L of biogas, with peak production occurring on day 20. In contrast, NaOH pretreatment alone at concentrations of 0.25 N, 0.5 N, and 0.75 N significantly increased biogas yields to between 45.918 and 51.612 g/L, while advancing peak production to day 14. These results represent improvements of 175.65% to 209.83% relative to the untreated control, indicating that alkaline pretreatment enhances substrate biodegradability and accelerates microbial activity during fermentation.

Additional improvements were observed when molasses was introduced as a co-substrate. When combined with 0.25 N NaOH pretreatment, molasses at 1%, 2%, and 3% (v/v) increased cumulative biogas yields to 80.316, 82.240, and 106.526 g/L, respectively. These represent relative increases of 382.15%, 393.70%, and 539.49% compared to the control. Molasses supplementation also advanced peak production to day 1 (1%), day 3 (2%), and day 4 (3%), suggesting that molasses not only enhances total biogas yield but also stimulates early-stage microbial metabolism.

Among all treatments, the 3% molasses condition produced the highest biogas output, indicating that this concentration may provide an optimal balance of readily fermentable carbon and nutrient availability to support microbial growth. Although statistical analysis ($p > 0.05$) showed no significant differences among molasses concentrations, the consistent upward trend in biogas production suggests a positive correlation between molasses dosage and biogas yield (Sun et al. 2016).

In summary, the combination of 0.25 N NaOH pretreatment and 3% molasses supplementation shows considerable promise for enhancing the anaerobic digestion of rice straw. Future studies should explore a broader range of molasses concentrations, extended fermentation durations, and untreated co-substrate controls to further elucidate the synergistic effects between pretreatment strategies and nutrient co-feeding.

Conclusion

NaOH pretreatment significantly modified the chemical structure of rice straw, particularly its cellulose, hemicellulose, and lignin content, as shown through the Chesson–Datta analysis and confirmed by the Kruskal–Wallis test ($p < 0.05$). Among the concentrations tested, 0.25 N NaOH provided the most effective balance between lignin removal and cellulose preservation, yielding the highest biogas production (51.612 g/L), although the increase was not statistically significant ($p = 0.075$). The addition of 3% molasses further improved biogas yield to 106.526 g/L and advanced the peak production to day 4, indicating enhanced microbial activity during the early fermentation phase. Although statistical analysis ($p = 0.610$) showed no significant differences among the molasses concentrations, a clear upward trend was observed as the concentration increased. The combination of 0.25 N NaOH and 3% molasses resulted in a 539.49% increase over the untreated control, demonstrating strong potential for enhancing the anaerobic digestion efficiency of lignocellulosic biomass. Further studies using broader molasses concentration ranges and microbial community profiling are recommended to optimize this approach for large-scale applications.

Acknowledgments

The authors express their sincere gratitude to the Ministry of Education, Science, and Technology and the Institute for Research and Community Service (LPPM), Universitas Sebelas Maret, for the support and funding provided through the Non-State Budget (Non-APBN) Research Implementation Assignment Program under contract number 371/UN27.22/PT.01.03/2025. This support has greatly contributed to the successful completion of this research.

References

Abdel daiem, Mahmoud M., Ahmed Hatata, and Noha Said. 2022. "Modeling and Optimization of Semi-Continuous Anaerobic Co-Digestion of Activated Sludge and Wheat Straw Using Nonlinear Autoregressive Exogenous Neural Network and Seagull Algorithm." *Energy* 241:122939. doi: 10.1016/j.energy.2021.122939.

Aghbashlo, Mortaza, Mohsen Mandegari, Meisam Tabatabaei, Somayeh Farzad, Mohamad Mojarab Soufiyan, and

Johann F. Görgens. 2018. "Exergy Analysis of a Lignocellulosic-Based Biorefinery Annexed to a Sugarcane Mill for Simultaneous Lactic Acid and Electricity Production." *Energy* 149:623–38. doi: 10.1016/j.energy.2018.02.063.

Andersen, Rasmus Lund, Karen Møller Jensen, and Marie Just Mikkelsen. 2015. "Continuous Ethanol Fermentation of Pretreated Lignocellulosic Biomasses, Waste Biomasses, Molasses and Syrup Using the Anaerobic, Thermophilic Bacterium Thermoanaerobacter Italicus Pentocrobe 411." *PLoS ONE* 10(8):1–16. doi: 10.1371/journal.pone.0136060.

Avena, Louise Grace, Michelle Almendrala, Rumina Rodriguez, and Kristine Gayle Mojica. 2024. "Anaerobic Digestion of Distillery Wastewater and Napier Grass: Characterization and Process Performance Parameters." *E3s Web of Conferences* 530:3005. doi: 10.1051/e3sconf/202453003005.

Bali, Garima, Xianzhi Meng, Jacob I. Deneff, Qining Sun, and Arthur J. Ragauskas. 2015. "The Effect of Alkaline Pretreatment Methods on Cellulose Structure and Accessibility." *ChemSusChem* 8(2):275–79. doi: 10.1002/cssc.201402752.

Deublein, Dieter, and A. Steinhauser. 2011. "Biogas from Waste and Renewable Resources." John Wiley & Sons.

Du, Jing, Yuting Qian, Yonglan Xi, and Xiwu Lü. 2019. "Hydrothermal and Alkaline Thermal Pretreatment at Mild Temperature in Solid State for Physicochemical Properties and Biogas Production from Anaerobic Digestion of Rice Straw." *Renewable Energy* 139:261–67. doi: 10.1016/j.renene.2019.01.097.

Dumlu, Lütfiye, A. S. Ciggin, Stefan Ručman, and Nuriye Altınay Perendeci. 2021. "Pretreatment, Anaerobic Codigestion, or Both? Which Is More Suitable for the Enhancement of Methane Production From Agricultural Waste?" *Molecules* 26(14):4175. doi: 10.3390/molecules26144175.

Dwivannie, Violla, Aryo Sasmita, and Etty Pratiwi. 2020. "Karakteristik PH Dan Suhu Dalam Proses Pembuatan Biogas Dari Substrat." *JOM FTEKNIK* 6(May):1.

Fuess, Lucas Tadeu, Luma Sayuri Mazine Kiyuna, Antônio Djalma Nunes Ferraz Júnior, Gabriela Félix Persinoti, Fábio M. Squina, Marcelo Loureiro García, and Marcelo Zaiat. 2017. "Thermophilic Two-Phase Anaerobic Digestion Using an Innovative Fixed-Bed Reactor for Enhanced Organic Matter Removal and Bioenergy Recovery From Sugarcane Vinasse." *Applied Energy* 189:480–91. doi: 10.1016/j.apenergy.2016.12.071.

Guang, Zhao, Shuangli Ji, Ting Sun, Fang Ma, and Zhonglin Chen. 2017. "Production of Bioflocculants Prepared From Wastewater Supernatant of Anaerobic Co-Digestion of Corn Straw and Molasses Wastewater Treatment." *Bioresources* 12(1). doi: 10.15376/biores.12.1.1991-2003.

Hendriks, A. T. W. M., and G. Zeeman. 2009. "Pretreatments to Enhance the Digestibility of Lignocellulosic Biomass." *Bioresource Technology* 100(1):10–18. doi: 10.1016/j.biortech.2008.05.027.

Ibrahim, Hassan Al-Haj. 2018. "Bio-Energy Production from Rice Straw a Review." *Recent Advances in Petrochemical Science* 5(5):69–75. doi: 10.19080/rapsci.2018.05.555671.

Kalemba, Katarzyna, and K. Barbusiński. 2017. "Anaerobic Co-Digestion of Sewage Sludge and Molasses." *E3s Web of Conferences* 22:75. doi: 10.1051/e3sconf/20172200075.

Mankar, Akshay R., Ashish Pandey, Arindam Modak, and K. K. Pant. 2021. "Pretreatment of Lignocellulosic Biomass: A Review on Recent Advances." *Bioresource Technology* 334(March):125235. doi: 10.1016/j.biortech.2021.125235.

Mirmohamadsadeghi, Safoora, Keikhosro Karimi, Reza Azarbaijani, Laleh Parsa Yeganeh, İrini Angelidaki, Abdul-Sattar Nizami, Rajeev Bhat, Kavya Dashora, Virendra Kumar Vijay, Mortaza Aghbashlo, Vijai Kumar Gupta, and Meisam Tabatabaei. 2021. "Pretreatment of Lignocelluloses for Enhanced Biogas Production: A Review on Influencing Mechanisms and the Importance of Microbial Diversity." *Renewable and Sustainable Energy Reviews* 135:110173. doi: 10.1016/j.rser.2020.110173.

Mischopoulou, Magdalini, Sotirios D. Kalamaras, Panagiotis Naidis, Thomas A. Kotsopoulos, and Petros Samaras. 2017. "Start-up and Steady-State Results of a UASB Reactor Treating High PH Baker's Yeast Molasses Wastewater for Methane Production." *Journal of Chemical Technology and Biotechnology* 92(4):775–80. doi: 10.1002/jctb.5055.

Mosier, Nathan, Charles Wyman, Bruce Dale, Richard Elander, Y. Y. Lee, Mark Holtzapple, and Michael Ladisch. 2005. "Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass." *Bioresource Technology* 96(6):673–86. doi: 10.1016/j.biortech.2004.06.025.

Ouahabi, Yasmine Ryma, Kenza Bensadok, and Abdeldjalil Ouahabi. 2021. "Optimization of the Biomethane Production Process by Anaerobic Digestion of Wheat Straw Using Chemical Pretreatments Coupled with Ultrasonic Disintegration." *Sustainability (Switzerland)* 13(13). doi: 10.3390/su13137202.

Peyrelasse, Christine, Abdellatif Barakat, Camille Lagnet, Prasad Kaparaju, and Florian Monlau. 2021. "Anaerobic Digestion of Wastewater Sludge and Alkaline-Pretreated Wheat Straw at Semi-Continuous Pilot Scale: Performances and Energy Assessment." *Energies* 14(17):5391. doi: 10.3390/en14175391.

Rahnama, Nooshin, Hooi Ling Foo, Nor Aini Abdul Rahman, Arbakariya B. Ariff, and Umi Kalsom Md Shah. 2014. "Saccharification of Rice Straw by Cellulase From a Local Trichoderma Harzianum SNRS3 for Biobutanol Production." *BMC Biotechnology* 14(1). doi: 10.1186/s12896-014-0103-y.

Sabeeh, Mariam, Zeshan, Rabia Liaquat, and Ayesha Maryam. 2020. "Effect of Alkaline and Alkaline-Photocatalytic Pretreatment on Characteristics and Biogas Production of Rice Straw." *Bioresource Technology* 309(February):123449. doi: 10.1016/j.biortech.2020.123449.

Sidiras, D. K., and E. G. Koukios. 1989. "Acid Saccharification of Ball-Milled Straw." *Biomass* 19(4):289–306. doi: 10.1016/0144-4565(89)90073-5.

Sun, Li, Tong Liu, Bettina Müller, and Anna Schnürer. 2016. "The Microbial Community Structure in Industrial Biogas Plants Influences the Degradation Rate of Straw and Cellulose in Batch Tests." *Biotechnology for Biofuels* 9(1). doi: 10.1186/s13068-016-0543-9.

Sun, Ye, and Jiayang Cheng. 2002. "Hydrolysis of Lignocellulosic Materials for Ethanol Production: A Review." *Bioresource Technology* 83(1):1–11. doi: 10.1016/S0960-8524(01)00212-7.

Taherzadeh, Mohammad J., and Keikhosro Karimi. 2008. *Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review*. Vol. 9.

Tan, Jinyu, Yan Li, Xiang Tan, Hongguo Wu, Hu Li, and Song Yang. 2021. "Advances in Pretreatment of Straw Biomass for Sugar Production." *Frontiers in Chemistry* 9(June):1–28. doi: 10.3389/fchem.2021.696030.

Tsegaye, Bahiru, Chandrajit Balomajumder, and Partha Roy. 2019. "Alkali Delignification and Bacillus Sp. BMP01 Hydrolysis of Rice Straw for Enhancing Biofuel Yields." *Bulletin of the National Research Centre* 43(1). doi: 10.1186/s42269-019-0175-x.

Wang, Hongliang, Yunqiao Pu, Arthur Ragauskas, and Bin Yang. 2019. "From Lignin to Valuable Products–Strategies, Challenges, and Prospects."

Bioresource Technology 271(July 2018):449–61. doi: 10.1016/j.biortech.2018.09.072.

Wen, Peng, Liyuan Zhang, Manlin Wei, Baiyila Wu, Ming Xiao, Runze Zhang, Ji Ju, Chenyang Dong, Liu Du, Yongjie Zheng, Meili Bao, Hailin Bao, and Xiaoping Bao. 2024. "Effects of Lactobacillus Plantarum (L) and Molasses (M) on Nutrient Composition, Aerobic Stability, and Microflora of Alfalfa Silage in Sandy Grasslands." *Frontiers in Microbiology* 15. doi: 10.3389/fmicb.2024.1358085.

Zhao, Xuebing, Lihua Zhang, and Dehua Liu. 2012. "Biomass Recalcitrance. Part I: The Chemical Compositions and Physical Structures Affecting the Enzymatic Hydrolysis of Lignocellulose." *Biofuels Bioproducts and Biorefining* 6(4):465–82. doi: 10.1002/bbb.1331.