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Sodium Hydroxide Hydrothermal Extraction of Lignin from Rice Straw Residue and Fermentation to Biomethane

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Abstract: The purpose of the NaOH pretreatment of rice straw with a recycling strategy was to enhance the economic efficiency of producing biomethane. Anaerobic digestion is used for converting rice straw into biogas. In this work, 5% NaOH and rice straw mixed samples were autoclaved at 121 °C for 20 min for lignin removal. The NaOH black liquor was separated using filtration for the subsequent treatment cycle. The NaOH liquor was utilized in one more subsequent recycling procedure to test its ability to remove lignin from the rice straw. The 5% NaOH treatment results in a reduction in rice straw (RC) lignin of 73.6%. The lignin content of the recycled NaOH-filtrated rice straw samples (RCF1) was reduced by 55.5%. The 5% NaOH-treated rice straw sample yields a total cumulative biogas of 1452.4 mL/gVS, whereas the recycled NaOH-filtered (RCF1) samples generate 1125.2 mL/gVS after 30 days of incubation. However, after 30 days of incubation, the untreated rice straw (RCC) bottle produced a total of 285.5 mL/gVS of biogas. The total increase in methane output after NaOH treatment is 6–8 times greater, and the biogas yield improves by 80–124%. We show here that the recycled NaOH black solution has still the effectiveness to be used for successive pretreatment cycles to remove lignin and generate methane. In the meantime, the NaOH black solution contains useful materials (lignin, sugars, potassium, and nitrogen) that could be purified for commercial purposes, and more importantly recycling the NaOH solution decrease the chances of environmental pollution. Thus, recycling NaOH decreased chemical consumption, which would provide net benefits instead of using fresh NaOH solution, had a lower water consumption, and provided the prospect of producing an optimum yield of methane in anaerobic digestion. This method will decrease the chemical treatment costs for biomass pretreatment prior to anaerobic digestion. Recycling of NaOH solution and the integration of pretreatment reactors could be a novel bioprocessing addition to the current technology.

Keywords: lignin; straw; heating; NaOH; pretreatment; biogas

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1. Introduction

One of the fundamental requirements for a sustainable way of life is energy. This need is primarily met by the energy generated from fossil fuel sources around the world. However, as these fossil fuel resources are depleted, there may be major energy shortages in the future [1]. The need for affordable, long-lasting energy is of the utmost importance. It must be both sustainable and cost-effective to meet the energy needs of human activity [2].

The primary problem is that the majority of the energy is derived from fossil fuels, which are expensive and generate greenhouse gases. Anaerobic digestion is a sustainable process for producing bioenergy from lignocellulosic waste biomass [3,4].

Rice straw production has increased as a result of rising energy consumption demands. The rice plant contains forty to sixty percent of its weight in rice straw, an agricultural waste. Rice straw contains 38–43% cellulose, 2–26% hemicellulose, and 15–20% lignin, depending on the variety and region [5,6]. Despite being one of the many crops that are commonly grown throughout the world, burning rice straw in open fields has a detrimental effect on the environment. The anaerobic digestion of rice straw to produce biogas is one possible method to use rice straw and minimize air pollution [7]. From anaerobic digestion, two useful products, biogas and digestate, are generated. Biogas can be used for lighting, electricity, and cooking. It can also be transformed into biomethane and used as a fuel for cars or injected into the pipeline to be transported as natural gas [8]. The composition of rice straw contains three major components: lignin, cellulose, and hemicellulose. Thus, rice straw's walls are tightly packed by layers of lignin, cellulose, and hemicellulose, and remain protected from enzymatic hydrolysis. In order to dissolve lignin and make cellulose and hemicellulose accessible to enzymatic action [9], a pretreatment step is therefore required before biogas production through anaerobic digestion [10]. Pretreatment aims to enhance surface area, promote lignocellulose structural opening, decrease lignin quantity, maintain hemicellulose, and decrease cellulose crystallinity. Additionally, biomass is processed to promote porosity in order to enhance enzyme access and assist the process of anaerobic digestion. When compared to acid or oxidative reagents, alkali pretreatment is the most efficient approach for rupturing the ester linkages between lignin, hemicellulose, and cellulose and limiting breakage of the hemicellulose polymers. According to previous studies, pretreatment with NaOH effectively increases the accessibility of enzymes to the cellulose while removing lignin and a small fraction of the hemicelluloses [11]. The majority of alkali pretreatment processes used in rice straw biogas production require heating and a substantial use of chemicals. Such pretreatment methods use a lot of water and produce harmful substances. Furthermore, treating alkali at high temperatures results in greater initial costs, increased treatment expenses, and most likely adverse environmental effects [12]. Biogas helps the economy by reducing poverty, offering affordable biofertilizer to increase soil fertility and reduce hunger, supporting new businesses, turning waste into usable products, and supporting global sustainability. Rice straw waste may be anaerobically digested to produce biogas, which small business owners can use to provide gas for cooking and farmers can use for electricity as well. Thus, the anaerobic digestion of waste biomass, such as rice straw, is a viable, sustainable, and profitable method for business owners to establish a biogas plant in an industrial area and supply energy to local populations [13]. It is estimated that crop waste production worldwide exceeds 9 billion tons. The huge quantity of crop waste can be efficiently managed with the advancement of anaerobic digestion technology. The production of biogas and its byproducts from crop waste can also help to solve challenges with food security, pollution in the environment, the cost of production, providing clean energy, and stability and sustainability in the economy [14].

To effectively pretreat crops and remove lignin, particularly rice straw, NaOH has shown a high rate of lignin removal efficiency compared to other alkalis, especially KOH and $Ca(OH)_2$ [15,16]. The treatment of rice straw with NaOH removes lignin and makes the cellulose component vulnerable to microbial hydrolysis. However, using a high concentration of alkalis not only increases the cost of the pretreatment process but also produces chemical inhibitors along with a high amount of volatile fatty acids (VFA), which stop the anaerobic digestion process [17]. Therefore, picking a suitable pretreatment method is a critical step for enhancing anaerobic digestion, but could also be economical for industrial applications [18].

NaOH black liquor can be reused for the pretreatment of biomass, rather than being wasted after initial treatment. Previously, NaOH with a high thermal heating process was applied and did not recycle this black liquor for further uses [15]. In such practices, the yield was improved, but the cost and environmental hazards were ignored. A few researchers filtered and recycled NaOH black liquor using extensive washing steps [19]. Because of such approaches, this important reagent is also unreasonably expensive for large-scale applications. The goal of this work was to reuse the black NaOH liquid repeatedly in order to identify a less expensive pretreatment procedure. In addition, the study focused on using NaOH in dilute concentrations with gentle heating conditions to remove lignin from rice straw as agribiomass. It was further noted that the effect of lignin removal on biogas yield and the anaerobic digestion process should be investigated.

2. Methodology

2.1. Materials and Methods

All essential chemicals, including NaOH, were bought from the Merck Group of Chemical Companies and Fisher Scientific. After being processed to a 40 mm mesh size, the rice straw was stored at room temperature in a polythene bag. The total solids (TS), volatile solids (VS), ash, moisture, lignin, hemicellulose, and cellulose were calculated using the normal NREL laboratory analytical process [20]. Using the oven-drying technique, the samples were dried in an air-drying oven for 24 h at 105 °C. This calculation Equation (1) was used to determine the moisture:

$$\%\text{moisture} = \frac{M_1 - M_2}{M} \times 100 \quad (1)$$

where M = initial sample weight/gm, M_1 = sample weight/gm. + container before drying, and M_2 = sample weight/gm + container after drying.

The moisture-free biomass was heated to 500 °C in a muffle furnace for 3 h, cooled to ambient temperature in a desiccator, and weighed to assess the samples' ash content. The ash was estimated as shown below in Equation (2).

$$\%\text{ash} = \frac{S_2 - S}{S_1} \times 100 \quad (2)$$

where S = burn dish weight, S_1 = sample without moisture, and S_2 = weight of sample plus dish after furnace ignition.

A clean crucible was heated for 24 h to 105–110 °C, and the substrate was weighted and dried in the oven set at 70 °C. The TS is calculated as shown in Equation (3):

$$\text{TS}\% = \frac{T - S}{S} \times 100 \quad (3)$$

where T = dried sample + dish and S = dish weight.

The volatile solids (VSs) were measured by heating the substrate to 450 °C for 1 h in a desiccator. The dry ash was collected from the muffle furnace, and VSs were calculated after cooling the sample as shown in Equation (4).

$$\text{VS}\% = \frac{V - D}{S} \times 100 \quad (4)$$

where V = substrate weight + dish and D = substrate weight + dish after ignition.

A 300 g dry sample was soaked in 3 mL of 72% sulfuric acid in a pressure tube for lignin measurement, and the sample was then incubated in a water bath at 30 °C for 60 min. A tiny glass rod was used to agitate the samples continuously. An amount of 84 mL of pure water was added to dilute the reaction to 4%. The reaction was stopped after full breakdown and cooled to ambient temperature. The soluble lignin was measured from the UV-visible spectrophotometer's absorbance value, and the insoluble lignin was measured from the weight of the ash after a rice straw sample that was oven-dried overnight was burned at 575 °C. The calculations were performed according to the NREL laboratory's analytical process [20]. The National Renewable Energy Laboratory's (NREL) established analytical method was used for the analysis of rice straw compositions of cellulose and hemicellulose sugars (hexose and pentose sugars) by using a high-pressure liquid chromatography (HPLC) system for rice straw samples.

2.2. Rice Straw Pretreatment

The experiment (Figure 1) was started using 5% NaOH for lignin degradation from rice straw (RC sample). All the rice straw samples (100 g/L) were immersed in 5% NaOH in a 100 mL solution in a 250 mL flask. The pH of NaOH black liquor was measured and it was 12.5 at the start of the reaction process. All the samples along with the control (rice straw dissolved in a 100 mL solution without NaOH) were then autoclaved at 121 °C for 20 min [21]. Under the same conditions, the control samples (RCC) were treated with distilled water. After heating, the black NaOH liquid was filtered and collected in a sterile flask. The solid rice straw residue was separated by filtration and rinsed with 100 mL of water. Before using it in an anaerobic digestion experiment, the washed solid residue was allowed to dry at room temperature. The 100 mL of water used in residue washing was added to the black liquor of NaOH. It was assumed that adding 100 mL of water to filtered black liquor of NaOH would reduce the starting concentration of 5% NaOH/100 mL by up to 2.5%. Again, 100 mL of filtered NaOH black liquid was added to a 250 mL flask containing 100 g/L of rice straw (RCF1) and autoclaved at 121 °C for 20 min. Under the same conditions, the control samples were treated with distilled water. The NaOH was collected in a sterile flask after being filtered, and the solid residue of the rice straw was separated and rinsed with 100 mL of water. With a spatula, the solid residue was separated and dried at room temperature.

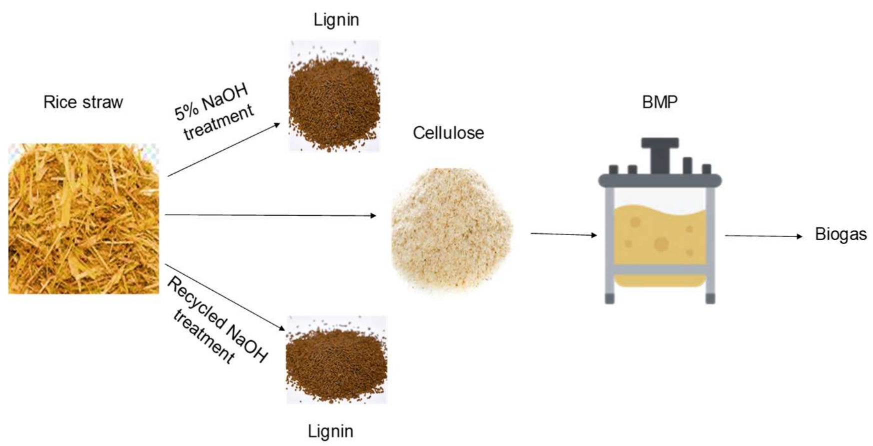

Figure 1. Experimental plan for 5% NaOH and recycled NaOH for lignin removal.

2.3. Scanning Electron Microscopy (SEM)

Scanning election microscope (SEM) machine was used to examine the pretreatment distortion and destructions on the structural surface of rice straw samples after and before treatment. The surface morphology of the untreated and pre-treated samples from both experimental groups were studied using a vacuum-desiccated SEM (S-3700 microscope Hitachi, manufacturer, Albany, CA, USA) with magnification ranges of 1000× to visualize the cracked and degraded tough structure of biomass samples as described previously [22].

2.4. Anaerobic Digestion Experiment

Anaerobic digestion experiments (ADE) were conducted on rice straw samples RC, RCF1, and RCC as a control sample. In each 250 mL serum bottle, the reactor volume was 100 mL. K_2HPO_4 (2.5 g/L), 3.5 g/L sodium bicarbonate solution, and 5 mL stock solutions of vitamin solutions thymine (0.01 g/L), folic acid (0.02 g/L), vitamin B12 (0.001 g/L), panthonic acid (0.05 g/L), riboflavin (0.05 g/L), and lipoic acid (0.05 g/L) were used as fermentation media. The fermentation media were autoclaved at 121 °C for 20 min. An inoculum of the full-scale anaerobic digester was enriched successively on fermentation media supplemented with 5 g/L alkali lignin, 5 g/L glucose, 5 g/L cellulose, and 5 g/L methanol under N_2-flushed conditions. This culture was used as an inoculum for biogas experiment. Based on the volatile solid (VS) content as food to microorganism ratio (F/M ratio), a 0.28 (S/I ratio) was determined for the ADE experiment. After flushing the serum bottles with N_2 gas for 6 min, the bottles were incubated at 40 °C and pH 8. There were control samples of inoculum without rice straw and rice straw without inoculum. Both untreated and pretreated rice straw sample fermentation tests were started in parallel to compare biogas and methane yield. The daily biogas production was assessed using the water displacement method, and serum vials were physically shaken on a daily basis. The GFM406 multichannel portable gas analyzer was used to determine the CH_4 and CO_2 composition of the biogas. The biogas data were examined using OBA, (https://biotransformers.shinyapps.io/oba1/, accessed on 20 March 2019). This biogas software package (R package) estimated the total biogas, methane, daily methane, daily biogas, rate of biogas, and methane. The R software derives all of these characteristics from daily biogas data for each bottle based on biogas volume and percent CH_4 [23]. The software makes use of the R function to estimate the theoretical and expected yield given a sample of raw data. Three groups make up the web-based interface known as the OBA Online Biogas App: (1) fundamental vectorized functions for typical conversions and calculations, (2) two data processing functions that compute BMP or comparable results from laboratory measurements, and (3) a function for predicting CH_4 production: basic biogas package operations. The oxygen demand, composition, molar mass, biogas volume, gas volume to moles, cumulative biogas, and methane production were all calculated using this software.

For the daily methane and cumulative methane yields for each of the examined samples, RCC, RC, and RCF1, a one-way ANOVA in Excel data analysis toolpak was carried out. Using Graphpad Prism 9.0.0, the standard deviation (SD), mean values, and significance between the data were determined.

3. Results

3.1. Pretreatment and Composition of Rice Straw

For each experimental analysis, the composition of the evaluated biomass must be found, because the chemical and biological processing generated changes in the physical properties, chemical structures, and chemical compositions of rice straw (RC). The selected rice straw for this study has 41.3, 22.2, and 20.3% cellulose, hemicellulose, and lignin, respectively. Total solids (TS), volatile solids (VS), and ash were 89.5, 80.2, and 9.6%, respectively, Table 1. The composition of the rice straw used in this study is comparable with that employed in an earlier article on rice straw that evaluated the effects of NaOH on lignin [24].

Table 1. The composition of rice straw.

Factor	Value
Moister (%)	9.6. ± 0.2
Volatile solid (VS %)	80.2 ± 2
Total solid (TS %)	89.5 ± 3
Ash (%)	7.2 ± 1
Hemicellulose	22.2 ± 2
Cellulose	41.3 ± 3
Acid-insoluble lignin	6.8 ± 2
Acid-soluble lignin	20.3 ± 1

The compositional value of rice straw, as indicated in Table 1, was estimated using a conventional NREL laboratory analytical process before NaOH treatment and was used to determine the level of delignification [20]. The samples treated with 5% NaOH showed 73.6% delignification for the RC samples. RCF1 samples, on the other hand, showed 55.5%

delignification. This was due to the addition of water to the NaOH black liquor for the following cycle after filtering. It was observed that the lignin value reduced with NaOH treatment in both RC and RCF1 samples. In contrast, no significant reduction in lignin removal was observed in untreated RCC samples (Figure 2).

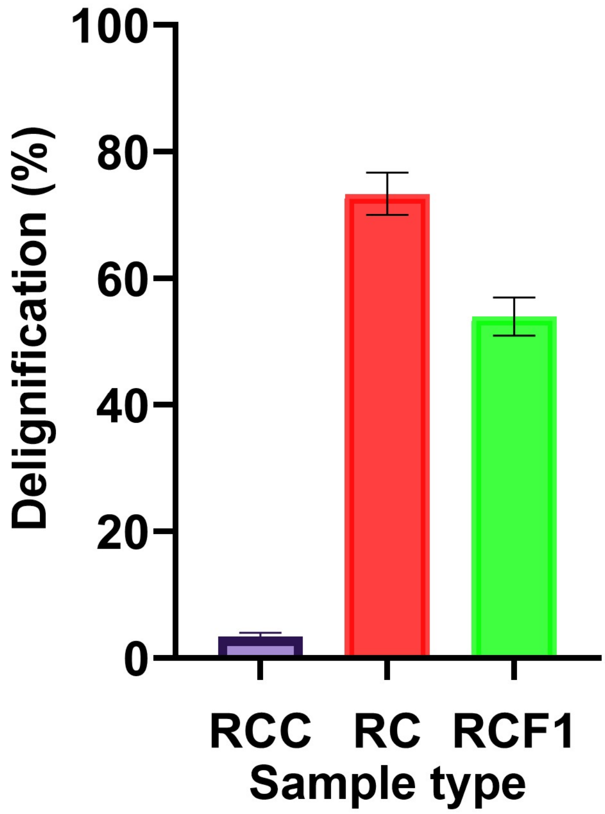

Figure 2. The percent value of delignification of rice straw with 5% NaOH treatment: RCC is the control sample, RC is the 5% NaOH-treated sample, and RCF1 represents the filtered sample.

3.2. SEM Analysis

SEM micrographs were used to compare the surface features of the untreated (RCC), 5% NaOH-treated (RC), and recycled filtered (RCF1) samples. The RCC images had a clean and smooth structure and were exceedingly compact. The dense structure of the rice straw was broken after NaOH pretreatment, and degradation was more visible on the surface of rice straw. The SEM micrographs of the autoclaved RCC samples without NaOH revealed that the hydrothermal pre-treatment without NaOH had a less damaging effect on their surface compared to the treated samples (Figure 3). The surface degradation effect was more intense and clearly visible in the case of the sample of the 5% NaOH-treated (RC) and recycled filtered samples (RCF1). In Figure 3, the deterioration effect was harsh for the 5% NaOH-treated (RC) sample, and gradually diminished in evident distortion on the surface of the recycled filtered sample (RCF1). The less severe effect in the SEM pictures of the recycled filtered sample (RCF1) supports the delignification rate and the relation of NaOH with lignin removal from the rice straw residue. Overall, the SEM micrographs clearly demonstrated ruptures in the silicon waxy structure, surface degradation, and morphological alterations in the rice straw as a result of heating and NaOH treatment, indicating that the fermentation process was accelerated.

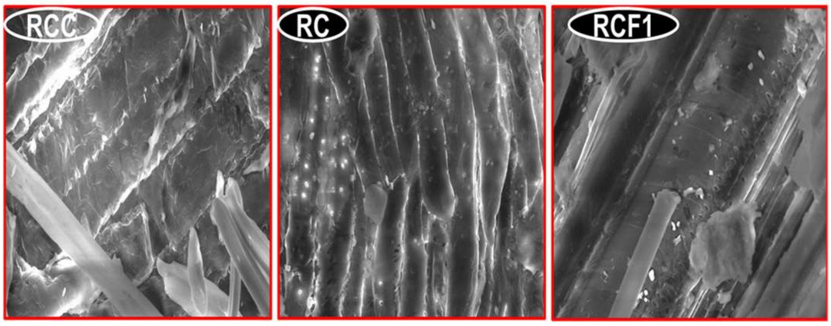

Figure 3. SEM images of the rice straw: RCC is control untreated sample, RC is 5% NaOH-treated sample, and RCF1 represents filtered sample of recycled NaOH treated with magnification of 1000× times.

3.3. Anaerobic Digestion for Biogas

Anaerobic digestion of the 5% NaOH-treated rice straw samples was compared to RCC samples that had not been treated. The improvement in biogas output in anaerobic digestion was investigated. The maximum daily biogas yields were 55.6 and 39.6 mL/gVS, respectively, from 5% NaOH-treated (RC) and recycled filtered sample (RCF1) bottles (Figure 4). The untreated RCC bottle yielded a lower biogas output at 14.7 mL/gVS. During the fermentation experiment using rice straw samples, it was observed that biogas production begins after 7 days of lag time in the case of untreated (RCC) bottles, but only 24 h in the case of treated RC and RCF1. Similarly, biogas generation continues until 27 days of anaerobic digestion for samples treated with 5% NaOH, whereas it stops after 24 days for untreated RCC bottles.

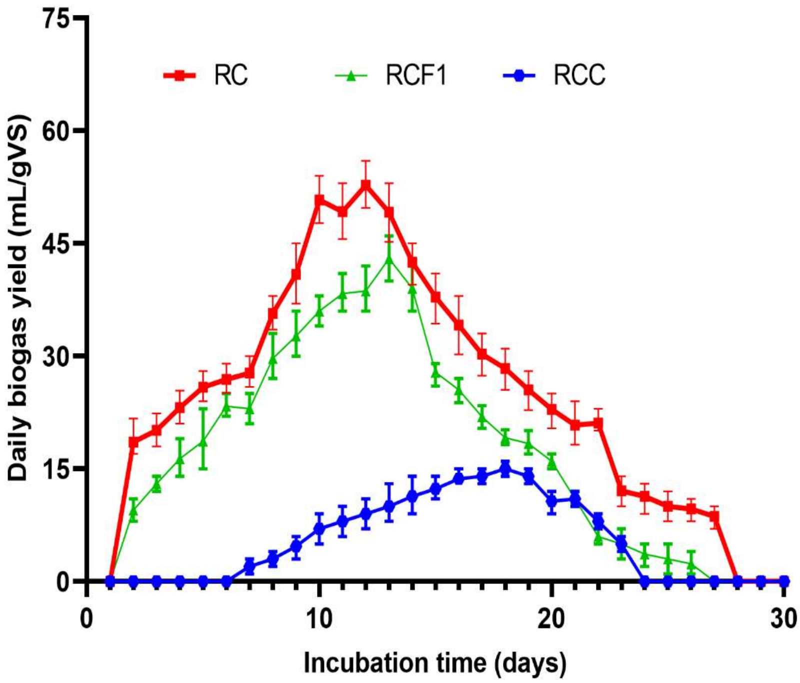

Figure 4. Daily biogas (mL/gVS): RCC is control untreated sample, RC is a 5% NaOH-treated sample, and RCF1 represents a filtered sample.

The daily methane output of the pretreated and untreated samples is shown in Figure 5. The greatest daily methane yield from RC and RCF1 bottles was 26.5 and 15.9 mL/gVS/d, respectively. The maximal daily methane output from the untreated RCC bottle was 4.5 mL/gVS/d.

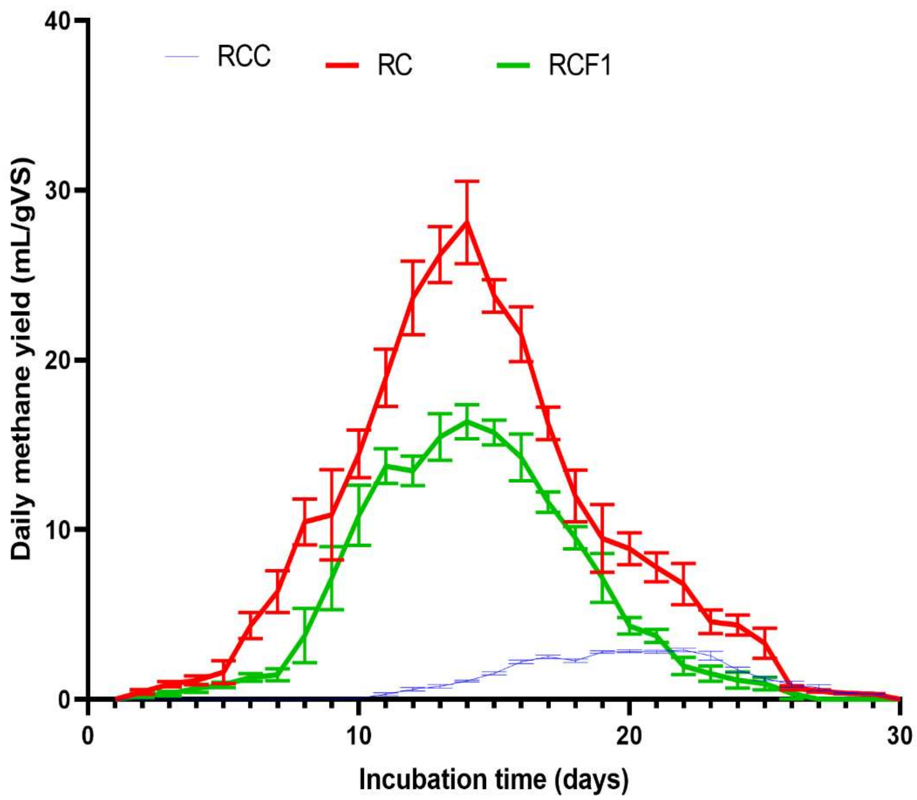

Figure 5. Daily methane (mL/gVS): RCC is control untreated sample, RC is a 5% NaOH-treated sample, and RCF1 represents a filtered sample.

After adding the inoculum to the reactor bottles to start the fermentation process, the daily production of biogas and methane began and after reaching its peak value, it continuously remained at a 30 mL per day yield for the next two weeks. The highest and optimum biogas production was observed from the 7th day until the 20th day of the fermentation process. The first two weeks of production showed the highest and maximum levels of biogas and methane. NaOH pretreatment enhanced the biodigestibility of RC and RCF-1 samples, facilitating anaerobic microbial utilization and reducing the time needed for digestion. This explains how the lignocellulose matrix was efficiently broken down by the NaOH pretreatment, supplying methanogens with enough organic materials to grow faster and produce more methane. However, in the untreated rice straw sample, the low biogas yield and low methane output provide indications that the lignin in the lignocellulose matrix of rice straw is not broken down, possibly leading to an inadequate supply of organic matter in the anaerobic reactor. As a result, during the lag phase of the anaerobic digestion time, the sample required additional time to generate biogas. As a result, the methanogens end up producing small amounts of methane and biogas.

The cumulative biogas from the fermented bottles of the treated and untreated samples is shown in (Figure 6). The maximum cumulative biogas yielded by the RC and RCF1 bottles was 1452.4 and 1125.2 mL/gVS, respectively. After 30 days of incubation, the maximum cumulative biogas from the untreated RCC bottle was only 285.5 mL/gVS.

Similarly, the cumulative methane yield in (Figure 7) for the treated and untreated samples shows a similar pattern. The RC and RCF1 bottles yielded the greatest cumulative methane levels of 396.7 and 274.4 mL/gVS, respectively. After 30 days of incubation, the total methane output from the untreated RCC bottle was only 30.2 mL/gVS.

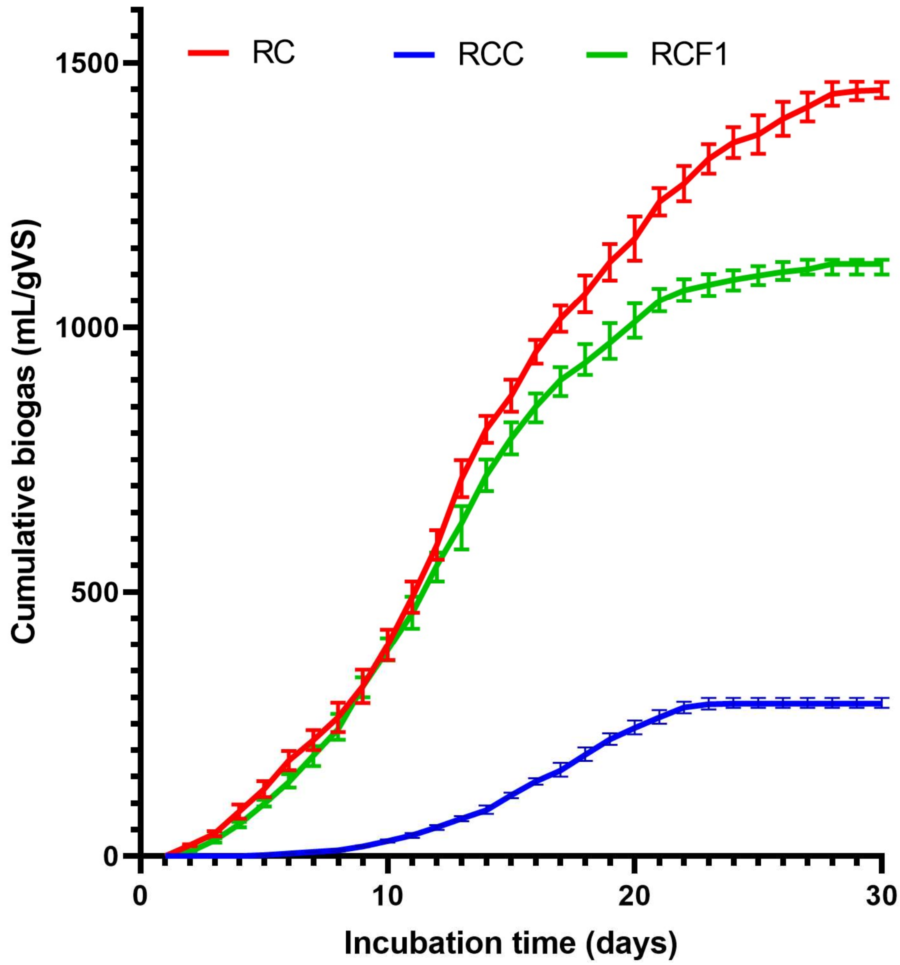

Figure 6. Cumulative biogas (mL/gVS): RCC is control untreated sample, RC is a 5% NaOH-treated sample, and RCF1 represents a filtered sample.

To observe a significant variance in the total methane production across all tests, a single-factor one-way ANOVA in the excel data analysis toolpak was used for the statistical difference, and the means of methane production among the three conditions—untreated, 5% NaOH-treated, and recycled-NaOH-solution-treated rice straw samples—were found. Table 2 displays the mean variance analysis (ANOVA) of single-factor results and multiple comparisons of the control and treatment samples. A total of 90 reaction samples and data from 30 of each sample were used for the substrate samples of untreated rice straw (RCC), treated rice straw (RC), and filtered NaOH-recovered liquor-treated rice straw (RCF1). According to Table 2, the mean methane yield for the RC and RCF1 samples at 95% probability is very different from that of the RCC sample. The average result consistently had a p-value of 1.33×10^{-8}, indicating that most of the variables for all the substrates under investigation—rice straw that had not been treated, rice straw that had been treated with recycled NaOH, and rice straw that had been treated with NaOH—were significant (RCF1). When RC was the substrate, followed by RCF1, the difference in methane output was significant, and it was possible to see a precise, significant difference in the value. The least amount of methane was produced by the RCC composed of rice straw that had not been processed. The cumulative methane yields from RC and RCF1 differ significantly from each other, as shown in Table 2 ($p > 0.05$). The yields of the treated samples and the RCC (control) samples are the same when compared to one another. The biogas yield was significantly affected by the NaOH treatment of the two RC and RCF1 substrates as a result. Increased lignin breakdown and cellulose digestibility, both of which encourage anaerobic digestion, are ascribed to this effect.

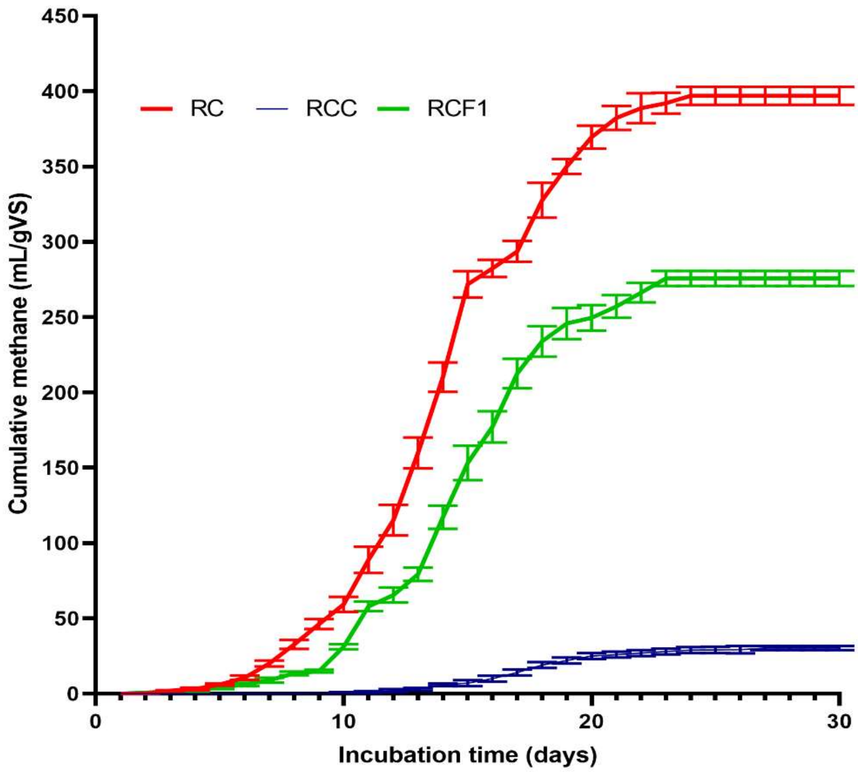

Figure 7. Cumulative methane (mL/gVS): RCC is control untreated sample, RC is a 5% NaOH-treated sample, and RCF1 represents a filtered sample.

Table 2. Comparison of cumulative methane yield of the RCC, RC, and RCF1 samples using one-way ANOVA.

ANOVA Summary						
Groups	Count	Sum	Average	Variance		
RCC	30	420.758	14.02527	183.934		
RC	30	6434.37	214.479	27,157.8		
RCF1	30	4468.662	148.9554	14,449.81		
ANOVA Results						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	626,811.8225	2	313,405.9	22.4978	1.33×10^{-8}	3.101296
Within Groups	1,211,954.77	87	13,930.51			
Total	1,838,766.592	89				

Table 3 displays the effects of several chemical pretreatments on certain straw biomasses. The table demonstrates that, although the same chemical pretreatment procedures were used, the effects of these pretreatments are not the same, e.g., 5% NaOH for rice straw and wheat straw. In a similar sense, it can be inferred that different procedures used for the same feedstock produced different outputs. It was found that after pretreatment with acids, NaOH, and biological and hydrothermal treatments, all reactions enhanced the biogas yield. In order to choose the appropriate chemical for the feedstock being used, this difference in yield increase can be further investigated using the same chemical concentration. In addition, it is necessary to identify the chemical concentration that can produce a yield increase compared to that of each substrate, due to differences in structural rigidity and responses to chemical effects. Our findings demonstrate that NaOH has a greater capacity to destroy lignin than other chemicals tested against all the substrates, wheat straw, corn straw, corn cob, rice straw, and sorghum stalk. It is also reported that the lignin-degrading bacteria also break down lignin and ultimately increase methane production, as shown in Table 3 for the batch fermentation method with *B. altitudinis* AN-2. The NaOH increased methane production in the treated samples compared to the untreated samples. This demonstrates that the choice of chemical pretreatment to be used will depend on the chemical, the treatment conditions, and the final products. The NaOH recycling pretreatment effect proved that it can be reused again and proved itself as a cost-effective chemical reagent compared to other chemical reagents used for lignin removal from complex biomass.

Table 3. Comparison of different chemical treatments and methane yields from biomass with the current study.

Substrate	Pretreatment Conditions	Methane Yield	Ref
Sugarcane bagasse	2% NaOH, Batch at 35 °C for 30 days,	222 mL CH_4/g VS	[25]
Wheat straw	3% NaOH, Batch at 37 °C for 30 days,	350 mL CH_4/g VS	[25]
Corn Straw	8% NaOH, 1L Erlenmeyer flask at 37 ± 1 °C	100.6 mL/g VS	[26]
Corn Straw	10% Ammonia, 1L Erlenmeyer flask at 37 °C	100.6 mL/g VS	[27]
Pinewood	Organosolv (150 °C, 1 h), at 39 ± 1 °C	387.4 mL/g VS	[28]
Rice straw	Hydrothermal 100 °C; 10 min	92.5 mL/g VS	[29]
Rice straw	Fungal treatment, Batch at 37 °C for 20 days	127 mL CH_4/g VS	[30]
Rice straw	NaOH+ hydrothermal	598.7 mLCH_4/g VS	
Sorghum stalk	H_2SO_4, 250 mL batch reactors at 37 °C	55% CH_4	[30]
Wheat straw	Acid steam, batch reactors at 37 °C	280 mL CH_4/g VS	[31]
Wheat straw	2% NaOH autoclaved, batch reactors at 37 °C	165.9 mL CH_4/g VS	[32]
Wheat straw	*Bacillus altitudinis* AN-2, batch fermentation	225.3 mL CH_4/g VS	[33]
Untreated straw	No treatment	78.5 mL CH_4/g VS	[33]
Corn cob	$Ca(OH)_2$ soaking (30 days), batch reactors	360.6 mL CH_4/g VS	[16]
Rice straw	5% NaOH autoclaved, batch reactors	430.8 mL CH_4/g VS	[15]
Rice straw	5% KaOH autoclaved, batch reactors	308.5 mL CH_4/g VS	[15]
Rice straw (RC)	5% NaOH autoclaved, batch reactors	396.7 mL CH_4/g VS	This study
Rice straw (RCF1)	5% NaOH autoclaved, batch reactors	274.4 mL CH_4/g VS	This study
Rice straw (RCC)	No treatment	30.2 mL CH_4/g VS	This study

4. Discussion

Anaerobic digestion is a wonderful and commonly used method for producing biogas from waste biomass and developing an effective waste management system. As a feeding substrate, animal waste such as manure and agricultural leftovers such as straw are commonly used. Rice straw is a crop leftover from rice, one of Pakistan's most important crops. Several factors, including lignin content, cellulose crystallinity, and particle size, limit the digestion of the hemicellulose and cellulose found in rice straws. Farmers burn the residual waste in the field, and there are no management mechanisms in the country either. If the correct management procedures are followed, this useful carbon source can be used for energy production. This research presented a recycled NaOH treatment of rice straw to remove lignin and expose hemicellulose and cellulose for effective digestion in the anaerobic fermentation process. The reprocessing of black NaOH liquid was utilized to test its effect in subsequent pretreatment and to ensure its reuse for biomass digestibility prior to anaerobic digestion. Initially, the rice straw's composition was measured, and the results of the contents were comparable to the composition of straw reported in earlier studies [34,35]. The 5% NaOH reduced the barrier lignin by 73.6%, which is comparable to the lignin removal potential of NaOH from rice straw residue found in prior work [15]. In prior research, we discovered that decreasing the alkali concentration under the same heating circumstances decreased the potential for lignin breakdown [15]. Similarly, in this study, a similar response for the lignin removal capacity of the recycled NaOH samples (RCF1) was observed. Its potential was reduced due to a decrease in NaOH concentration in the second reprocessing pretreatment. The effect of NaOH degradation on the surface of the rice straw samples also shows that the 5% NaOH samples caused higher degradation than the recycled samples. The RCC exhibits no significant structural degradation in the rice straw sample. Similarly, after pretreatment, there is damage and degradation on the surface of biomass straw [15,36]. During the rice straw fermentation experiment, it was discovered that biogas generation begins after 7 days of lag time in the case of untreated bottles, but just 24 h in the case of NaOH pretreatment bottles. This suggests that pretreatment reduces the time required for hydrolysis in the anaerobic fermentation process and aids in the start-up of biogas generation. Similarly, biogas generation continues for the samples treated with 5% NaOH until 27 days of anaerobic digestion, whereas biogas production stops after 23 days in the case of the untreated bottle. On the 10th day of the fermentation experiment, the concentrations of CH_4 and CO_2 were measured, and the methane concentration was 3%, rising to a maximum of 15.6% on the 20th day of the experiment in the untreated bottle. However, the concentration of CH_4 in the NaOH-treated bottles was 16% on the 6th day and rapidly grew to 57–67% on the 17–20th day of the fermentation experiment. Biogas and methane production ceased on the 27th and 28th days of the ADE experiment in the NaOH-treated bottles and on the 23rd day in the untreated bottle. The recycled RCF1 had a reduced biogas yield, which might be related to a decrease in the NaOH level during the filtration process for the second cycle, as well as the addition of an additional 100 mL of distilled water to adjust the volume for the following batch of the experiment. The explanation for the decrease in biogas output is obvious, because lignin removal and increasing biogas potential are directly related to the amount of lignin in lignocellulosic biomass [15]. Preferably, the biogas production and CH_4 percentage should be around 50–70% under standard temperature and pressure (STP) to be used for cooking, burning, and lighting. Only the NaOH-treated samples produced more than 65% raw biogas in this study, while the untreated samples produced just 15–17% CH_4 content. Similar observations of an increase in biogas have been reported previously in pretreatment with the alkalis $Ca(OH)_2$, KOH, and NaOH, depending on the concentration of the tested chemical and thermal heating from rice straw and wheat straw. The findings of this study and the related literature show that alkalis are a useful agent for delignifying biomasses, and that they should be utilized again rather than discarded. Lignin removal of waste biomass with NaOH pretreatment could be taken into consideration to proceed from lab research to an industrial-scale technique for less expensive biogas generation in order to enhance the anaerobic digestion process [37,38]. Such observations further highlighted the significance of pretreatment prior to the fermentation process in producing high-quality biogas. Even so, it is vital to remember that the pretreatment method must be affordable and may be adequate for pilot-scale waste biomass fermentation processing. The findings clearly show that processed rice straw enhances biogas and methane yields. When compared to the untreated sample, the NaOH samples produced roughly 6–8 times more biogas and methane. Overall, the NaOH-treated samples produced 85–124% more biogas than the untreated samples. The findings of this study are comparable to those of earlier studies [37,38]. The manipulated variables set for one-way ANOVA were RCC, RC, and RCF1 to compare the mean differences for daily methane yield and cumulative methane yield, and from the 90 data sets it is found that there is a significant improvement in the methane yield in both 5% NaOH-treated rice straw and 2.5% recycled-NaOH-treated rice straw samples. The P value of 0.0001 indicated that the results were significant. The results of this study demonstrated that pretreatment under mild circumstances improved the digestibility of the biomass straw and, as a result, increased output yield. It was also stated that NaOH black liquor should not be discarded. It should be recycled for the pretreatment purpose of disintegrating the lignin of lignocellulosic biomass before the fermentation process produces various bioproducts. To summarize this study, it was essential to reduce the cost of pretreatment for the efficient fermentation of rice stalks to methane. The rice straw pretreatment with 5% NaOH increased methane yield during initial testing. In the majority of instances, however, researchers discarded the NaOH solution after initial pretreatment, and recycling this black liquor solution as a treatment was not considered for lignin removal. We presumed that the reuse of alkaline pretreatment and the recovery of lignin are crucial issues for biorefinery in order to reduce pretreatment costs. For lignin removal from rice straw, an alternative strategy to the pretreatment procedure involving recycling the NaOH solution is presented in this study. We are able to demonstrate that the recycled NaOH solution removed lignin and increased methane yield. Relative to other pretreatment strategies, NaOH pretreatment is more effective at removing lignin and releases fewer inhibitors such as furfural and HMF. Through this method's design, not only was lignin removed, but the resulting NaOH solution was also recycled for the pretreatment of rice straw. Transformation of the recovered lignin revealed its potential in a variety of industrial fields. For instance, lignin has been identified as a highly valuable renewable polymer that can be used to manufacture phenolic resins and epoxies [39]. In addition, lignin can function as a binder and dispersant, such as in cement additives. During the pretreatment procedure, lignin and carbohydrates may be recovered for the ongoing utilization of biomass. In addition, no pollutant was discharged post alkali pretreatment. Improving alkaline pretreatment is both interesting and valuable because it concurrently reduces treatment costs and generates byproducts. In previous research, the recycling of a NaOH solution demonstrated an economic benefit, increased methane yield, and decreased pretreatment costs. According to the studies, black liquor recycling enhanced pretreatment efficacy and decreased chemical and water consumption by 40 to 60 percent [40]. Therefore, continuous-reactor studies are required to assess the economic and environmental benefits of recycling the black residue of NaOH, as well as the technical and financial viability and aggregate profitability of a biorefinery system. The results of this study demonstrated that pretreatment under mild circumstances improved the digestibility of the biomass straw and, as a result, increased output yield. NaOH black liquor should not be discarded, and should be recycled for the pretreatment purpose of disintegrating the lignin of lignocellulosic biomass before the fermentation process produces various bioproducts.

5. Conclusions

Two treatment options for lignin removal from rice straw were evaluated: NaOH and recycled NaOH. To accelerate the effect of NaOH on the rice straw substrate, mild thermal heating was utilized. The maximum dose examined in this study indicated that NaOH treatment considerably reduced lignin by up to 73.6%. The recycled filtered sample has a lignin removal percentage of 55.5%. This is due to NaOH concentration loss during the filtration process, which was neutralized by the addition of water in the second cycle. Additionally, no extra NaOH dose was utilized in the NaOH filtration process; therefore, the NaOH concentration fell in the subsequent heating cycles and the impact of treatment on lignin was reduced. The SEM findings clearly show that the NaOH treatment destroyed the surface morphology of the rice straw samples. The fermentation experiment findings showed a considerable improvement in biogas yield following the NaOH treatment when compared to the untreated sample. We concluded from this work that NaOH black liquor can be retested for lignin removal in agricultural waste biomass digestion to reduce the cost of chemical treatment for large-scale development.

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References

1. Arthur, P.M.A.; Konaté, Y.; Sawadogo, B.; Sagoe, G.; Dwumfour-Asare, B.; Ahmed, I.; Bayitse, R.; Ampomah-Benefo, K. Evaluating the Potential of Renewable Energy Sources in a Full-Scale Upflow Anaerobic Sludge Blanket Reactor Treating Municipal Wastewater in Ghana. *Sustainability* **2023**, *15*, 3743. [CrossRef]
2. Łysiak, G.; Kulig, R.; Al Aridhee, J.K. Toward New Value-Added Products Made from Anaerobic Digestate: Part 1—Study on the Effect of Moisture Content on the Densification of Solid Digestate. *Sustainability* **2023**, *15*, 4548. [CrossRef]
3. Kildegaard, G.; Balbi, M.D.P.; Salierno, G.; Cassanello, M.; De Blasio, C.; Galvagno, M. A cleaner delignification of urban leaf waste biomass for bioethanol production, optimised by experimental design. *Processes* **2022**, *10*, 943. [CrossRef]
4. Tsegaye, D.; Khan, M.M.; Leta, S. Optimization of Operating Parameters for Two-Phase Anaerobic Digestion Treating Slaughterhouse Wastewater for Biogas Production: Focus on Hydrolytic–Acidogenic Phase. *Sustainability* **2023**, *15*, 5544. [CrossRef]
5. Harun, S.N.; Hanafiah, M.M.; Noor, N.M. Rice straw utilisation for bioenergy production: A brief overview. *Energies* **2022**, *15*, 5542. [CrossRef]
6. Chen, C.; Deng, X.; Kong, W.; Qaseem, M.F.; Zhao, S.; Li, Y.; Wu, A.-M. Rice straws with different cell wall components differ on abilities of saccharification. *Front. Bioeng. Biotechnol.* **2021**, *8*, 624314. [CrossRef]
7. Shetty, D.J.; Kshirsagar, P.; Tapadia-Maheshwari, S.; Lanjekar, V.; Singh, S.K.; Dhakephalkar, P.K. Alkali pretreatment at ambient temperature: A promising method to enhance biomethanation of rice straw. *Bioresour. Technol.* **2017**, *226*, 80–88. [CrossRef]
8. Wang, Y.; Zhi, B.; Xiang, S.; Ren, G.; Feng, Y.; Yang, G.; Wang, X. China's Biogas Industry's Sustainable Transition to a Low-Carbon Plan—A Socio-Technical Perspective. *Sustainability* **2023**, *15*, 5299. [CrossRef]
9. Cui, C.; Yan, C.; Wang, A.; Chen, C.; Chen, D.; Liu, S.; Li, L.; Wu, Q.; Liu, Y.; Liu, Y.; et al. Understanding the Inhibition Mechanism of Lignin Adsorption to Cellulase in Terms of Changes in Composition and Conformation of Free Enzymes. *Sustainability* **2023**, *15*, 6057. [CrossRef]
10. Budiyono; Wicaksono, A.; Rahmawan, A.; Matin, H.H.A.; Wardani, L.G.K.; Kusworo, T.D.; Sumardiono, S. The effect of pretreatment using sodium hydroxide and acetic acid to biogas production from rice straw waste. *MATEC Web Conf.* **2017**, *101*, 02011. [CrossRef]
11. Anuradha, A.; Sampath, M.K. Optimization of alkali, acid and organic solvent pretreatment on rice husk and its techno economic analysis for efficient sugar production. *Prep. Biochem. Biotechnol.* **2022**, *53*, 279–287.
12. Syahri, S.N.K.M.; Abu Hasan, H.; Abdullah, S.R.S.; Othman, A.R.; Abdul, P.M.; Azmy, R.F.H.R.; Muhamad, M.H. Recent challenges of biogas production and its conversion to electrical energy. *J. Ecol. Eng.* **2022**, *23*, 251–269. [CrossRef]
13. Arshad, M.; Ansari, A.R.; Qadir, R.; Tahir, M.H.; Nadeem, A.; Mehmood, T.; Alhumade, H.; Khan, N. Green electricity generation from biogas of cattle manure: An assessment of potential and feasibility in Pakistan. *Front. Energy Res.* **2022**, *10*, 11485. [CrossRef]
14. Romero-Perdomo, F.; González-Curbelo, M.Á. Integrating Multi-Criteria Techniques in Life-Cycle Tools for the Circular Bioeconomy Transition of Agri-Food Waste Biomass: A Systematic Review. *Sustainability* **2023**, *15*, 5026. [CrossRef]
15. Shah, T.A.; Raheem, U.; Asifa, A.; Romana, T. Effect of Alkalis pretreatment on Lignocellulosic Waste Biomass for Biogas Production. *Int. J. Renew. Energy Res.* **2018**, *8*, 1318–1326.
16. Shah, T.A.; Tabassum, R. Enhancing Biogas Production from Lime Soaked Corn Cob Residue. *Int. J. Renew. Energy Res.* **2018**, *8*, 761–766.
17. Sumardiono, S.; Adisukmo, G.; Hanif, M.; Budiyono, B.; Cahyono, H. Effects of pretreatment and ratio of solid sago waste to rumen on biogas production through solid-state anaerobic digestion. *Sustainability* **2021**, *13*, 7491. [CrossRef]
18. Hidalgo-Sánchez, V.; Behmel, U.; Hofmann, J.; Borges, M.E. Enhancing Biogas Production of Co-Digested Cattle Manure with Grass Silage from a Local Farm in Landshut, Bavaria, through Chemical and Mechanical Pre-Treatment and Its Impact on Biogas Reactor Hydraulic Retention Time. *Sustainability* **2023**, *15*, 2582. [CrossRef]
19. Wang, W.; Tan, X.; Wang, Q.; Yu, Q.; Zhou, G.; Qi, W.; Zhuang, X.; Yuan, Z. Commentary on the Reuse of Black Liquor for the Enzymatic Hydrolysis and Ethanol Fermentation of Alkali-treated Sugarcane Bagasse. *J. Microbiol. Biotechnol.* **2017**, *6*, 228.
20. Sluiter, A.; Sluiter, J.; Wolfrum, E.J. *Methods for Biomass Compositional Analysis*; National Renewable Energy Laboratory (NREL): Golden, CO, USA, 2013.
21. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96*, 673–686. [CrossRef]
22. Qu, P.; Huang, H.; Zhao, Y.; Wu, G. Physicochemical changes in rice straw after composting and its effect on rice-straw-based composites. *J. Appl. Polym. Sci.* **2017**, *134*, 44878. [CrossRef]
23. Lou, X.F.; Nair, J.; Ho, G. Influence of food waste composition and volumetric water dilution on methane generation kinetics. *Int. J. Environ. Prot.* **2012**, *2*, 22–29.

24. Do, N.H.; Pham, H.H.; Le, T.M.; Lauwaert, J.; Diels, L.; Verberckmoes, A.; Tran, V.T.; Le, P.K. The novel method to reduce the silica content in lignin recovered from black liquor originating from rice straw. *Sci. Rep.* **2020**, *10*, 21263. [CrossRef] [PubMed]
25. Olatunji, K.O.; Ahmed, N.A.; Ogunkunle, O. Optimization of biogas yield from lignocellulosic materials with different pretreatment methods: A review. *Biotechnol. Biofuels* **2021**, *14*, 1–34. [CrossRef] [PubMed]
26. Song, Z.; Yang, G.; Guo, Y.; Zhang, T. Comparison of two chemical pretreatments of rice straw for biogas production by anaerobic digestion. *BioResources* **2012**, *7*, 3223–3236.
27. Song, Z.; Yang, G.; Liu, X.; Yan, Z.; Yuan, Y.; Liao, Y. Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion. *PLoS ONE* **2014**, *9*, e93801. [CrossRef]
28. Arreola-Vargas, J.; Ojeda-Castillo, V.; Snell-Castro, R.; Corona-González, R.I.; Alatriste-Mondragón, F.; Méndez-Acosta, H.O. Methane production from acid hydrolysates of Agave tequilana bagasse: Evaluation of hydrolysis conditions and methane yield. *Bioresour. Technol.* **2015**, *181*, 191–199. [CrossRef]
29. Luo, T.; Huang, H.; Mei, Z.; Shen, F.; Ge, Y.; Hu, G.; Meng, X. Hydrothermal pretreatment of rice straw at relatively lower temperature to improve biogas production via anaerobic digestion. *Chin. Chem. Lett.* **2019**, *30*, 1219–1223. [CrossRef]
30. Dahunsi, S.; Adesulu-Dahunsi, A.; Osueke, C.; Lawal, A.; Olayanju, A.; Ojediran, J.; Izebere, J. Biogas generation from Sorghum bicolor stalk: Effect of pretreatment methods and economic feasibility. *Energy Rep.* **2019**, *5*, 584–593. [CrossRef]
31. Nkemka, V.N.; Murto, M. Biogas production from wheat straw in batch and UASB reactors: The roles of pretreatment and seaweed hydrolysate as a co-substrate. *Bioresour. Technol.* **2013**, *128*, 164–172. [CrossRef]
32. Chandra, R.; Takeuchi, H.; Hasegawa, T.; Kumar, R. Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. *Energy* **2012**, *43*, 273–282. [CrossRef]
33. Shah, T.A.; Ali, S.; Afzal, A.; Tabassum, R. Simultaneous pretreatment and biohydrogen production from wheat straw by newly isolated ligninolytic Bacillus s strains with two-stage batch fermentation system. *BioEnergy Res.* **2018**, *11*, 835–849. [CrossRef]
34. Gustafson, V. Combined Production of Bioethanol and Biogas from Wheat Straw. Master's Thesis, Department of Chemical Engineering, Lund University, Lund, Sweden, 2015.
35. Cui, Z.; Shi, J.; Li, Y. Solid-state anaerobic digestion of spent wheat straw from horse stall. *Bioresour. Technol.* **2011**, *102*, 9432–9437. [CrossRef] [PubMed]
36. Zeng, J.; Singh, D.; Chen, S. Biological pretreatment of wheat straw by Phanerochaete chrysosporium supplemented with inorganic salts. *Bioresour. Technol.* **2011**, *102*, 3206–3214. [CrossRef]
37. Shah, T.A.; Ullah, R. Pretreatment of wheat straw with ligninolytic fungi for increased biogas productivity. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 7497–7508. [CrossRef]
38. Shah, T.A.; Charles, C.; Orts, W.J.; Tabassum, R. Biological pretreatment of rice straw by ligninolytic Bacillus s strains for enhancing biogas production. *Environ. Prog. Sustain. Energy* **2018**, *38*, e13036. [CrossRef]
39. Chen, Y.; Peng, N.; Gao, Y.; Li, Q.; Wang, Z.; Yao, B.; Li, Y. Two-Stage Pretreatment of Jerusalem Artichoke Stalks with Wastewater Recycling and Lignin Recovery for the Biorefinery of Lignocellulosic Biomass. *Processes* **2023**, *11*, 127. [CrossRef]
40. D'adamo, I.; Gastaldi, M.; Morone, P.; Rosa, P.; Sassanelli, C.; Settembre-Blundo, D.; Shen, Y. Bioeconomy of sustainability: Drivers, opportunities and policy implications. *Sustainability* **2021**, *14*, 200. [CrossRef]