



Production of bioethanol from pretreated rice straw, an integrated and mediated upstream fermentation process

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Abstract

In addition to being a low-cost byproduct of rice cultivation, rice straw also has a high output volume (731 million tons annually) and is, thus, an ideal feedstock for bioethanol production. Somewhere between 34 and 47% cellulose, 19 and 27% hemicellulose, 5 and 24% lignin, and 18% ashes may be found in rice straw. The lignocellulose in rice straw was broken down into sugar using a variety of chemical (acid and alkali) treatments in this investigation. The processing of lignocellulose with alkali or acid was successful in producing a sugar. The sugar yield was greatly improved by combining chemical pretreatment with subsequent enzyme treatment. Finally, *Saccharomyces cerevisiae* yeast was used to ferment the pretreatment samples across the three groups (alkali, acid, and enzymes), and the resulting ethanol concentration was determined. Scanning electron microscopy (SEM) revealed greater degree of porosity in both pretreated samples that has further supported by Fourier transform infrared spectroscopy (FTIR) indicated some constituents had changed in cross-linking reaction between the C–O–H, C–O–C, and other functional groups. After 3 days of fermentation, practically all fermentable sugar (glucose) was transformed to bioethanol (40%) from a sample that had been prepared with 1% acid/enzyme. The synthesis of bioethanol slowed by 45% due to the lengthier fermentation time of up to 6 days. After 6 days of fermentation using *Saccharomyces cerevisiae* yeast, ethanol concentration was maximum when rice straw was subjected to acid pretreatment followed by enzyme treatment. In this experiment, the ethanol output was around 0.40 g/g.

Keywords Rice straw · Fermentation · Bioethanol · *Saccharomyces cerevisiae* · Lignocellulose · SEM · FTIR

1 Introduction

The global warming problem and continuous decrease of fossil fuel source have worried the world for decades now and the alternative energy such as gasohol or biodiesel has been increasingly consumed. Therefore, bioethanol serving as gasohol additive has been consecutively produced and improved as well. At present, bioethanol production is based on sugar (from sugar cane) or starch (from maize or cassava) as substrates. The price of ethanol made from biomass substrates is substantially higher than the price of ethanol made from fossil fuel since biomass feedstock accounts for around 40% of the cost of producing bioethanol [1]. The negative aspects of bioenergy and bio product systems driven by edible resources have become more numerously evident in the light of the most updated global developments, especially the war in Russia and Ukraine and its negative impact on the food and biofuel markets [2]. Therefore, to achieve the basic objectives of these systems, they must be based on various

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sources of biomass, varying from agricultural and forestry (woody) biomass to municipal solid waste, including food waste and energy crops grown on marginal land [3, 4].

Therefore, it has become a highly intriguing issue for academics to study how to reduce the cost of ethanol production by employing lignocellulosic materials such as bagasse, wood wastes, corncob, or rice straw as an alternative feedstock. To make the process economically viable, thorough process development and optimization are still necessary [5].

The best technology for converting lignocellulose to bioethanol should be selected based on overall economics (lowest cost), environment (lowest pollutants), and energy (higher efficiency). As the global population has continuously risen, so has the need for plentiful, cheap food. Given that rice is the most widely eaten staple meal on every continent, it naturally produces vast quantities of byproducts like straw and husks. It is well-known that the rice grains alone account for all of the money spent on rice farming [6]. Our changing climate is one of the most pressing problems we face today. The transportation sector is responsible for a significant amount of overall CO₂ emissions, and one strategy for lowering these emissions is to replace fossil fuels with the so-called biofuels like bioethanol [7].

Sugarcane, maize, and wheat are just few of the crops utilized to make ethanol today. However, it is vital to examine additional raw materials to reduce environmental effects, competition across crops for food and fuel production, and enhance cost efficiency. Using lignocellulosic material like agricultural or wood leftovers for the so-called second-generation bioethanol production is seen as a potential strategy. As logging trash and forest sector waste, softwood is the most common source of raw material for ethanol production [8–10]. Rising energy costs may be attributed to both diminishing oil sources and rising global energy demand. In addition, burning fossil fuels produces harmful byproducts including air pollution and carbon dioxide (CO₂), which is thought to be a major human contribution to the greenhouse effect. Alternative renewable energy sources, such as biomass, are becoming more economically viable as a result of these considerations [11].

The Kyoto Protocols require several developed countries to cut their CO₂ emissions by around 5% below their 1990 level by 2010, with additional cuts mandated after that. Substituting CO₂-neutral biofuels like ethanol for petrochemically generated transport fuels is one way to fulfill the world's rising energy demand and reduce CO₂ emissions [12]. There has been a lot of research on using ethanol produced from renewable resources as an alternative fuel or oxygenate additive to conventional fossil fuels in the last several decades. There are a lot of lignocellulosic

materials accessible, and they are inexpensive and renewable. The lignocellulosic waste material rice straw is widely available [13].

About 731 million tons are produced per year, with the majority going to Asia (667.6 million tons), Europe (3.9 million tons), the Americas (37.2 million tons), and Oceania (20.9%) (1.7 million tons). The biggest annual production of bioethanol from a single biomass feedstock is possible with this quantity of rice straw [14]. Due to the high energy consumption in the distillation process, the cost of producing ethanol is based not only on the yield but also on the concentration of ethanol in the fermentation broth. Here, two strip-per columns and a rectification column are used to heat-integrate by running at different pressures to boost the ethanol content in the broth after fermentation to 94%. Lower than a 4% ethanol concentration, energy needs begin to rise significantly [15]. The addition of starch-rich material to the lignocellulosic process increases the ethanol concentration in the broth, decreasing the energy demand in distillation and the associated costs.

Hydrolysis of the cellulose and hemicellulose to monomeric sugars, followed by fermentation of these sugars to ethanol, is the process by which bioethanol may be created from lignocellulosic material. The so-called simultaneous saccharification and fermentation (SSF) technique combines hydrolysis and fermentation into a single operation, offering a number of benefits over the separate saccharification and fermentation methods (SHF). By using SSF, the final product does not block glucosidase; therefore, fewer reactors are required. Even more so, SSF has been shown to provide a higher total ethanol output than SHF [16].

Cellulases may break down cellulose, the main component of lignocellulosic biomass, into glucose. The amount of lignin and hemicellulose present, as well as the crystallinity of the cellulose fibers, may all influence how well lignocellulosic biomass hydrolyze. Since lignin and hemicellulose are detrimental to the materials' performance, a pretreatment technique must be implemented to eliminate them, lessen cellulose crystallinity, and expand their porosity. To pretreat lignocellulosic materials, dilute-acid hydrolysis has been developed and shown to be effective. The cellulose hydrolysis is greatly enhanced by using this pretreatment approach, which results in high reaction rates. Dilute-acid hydrolysis of lignocellulosic feedstock may recover up to 95% of the hemicellulose sugars, depending on the substrate and circumstances utilized [17].

Rice straw is a common agricultural byproduct in all regions where rice has been farmed. There is around 39% cellulose, 27% hemicellulose, and 12% lignin in a typical sample. Microorganisms use glucose and other

fermentable sugars produced from the breakdown of cellulose and hemicellulose to produce ethanol. Microorganisms that may degrade lignocellulose, such as bacteria, fungus, actinomycetes, and protozoa, are likely present in nature but have not yet been identified by scientists [18]. The yeast *Saccharomyces cerevisiae* is widely used in batch fermentations to transform carbohydrates into ethanol for use in food, drink, and biofuels. Despite its evident significance, our understanding of the physiological restrictions that limit the pace of glycolysis and ethanol generation is lacking. Locating these limitations is a crucial first step in optimizing organisms and processes for quicker ethanol production. It is possible that the ethanol production capacity of current fermentation plants might be increased, and the cost of future facilities reduced because of such enhancements [19].

Therefore, significant reductions in costs might be attained by decreasing the amount of energy required for the distillation process. Reaching the maximum feasible ethanol concentration in the SSF stage is crucial since the cost of distillation drops as the ethanol concentration in the distillation feed rises [20]. To do this, one strategy is to raise the substrate concentration in the SSF. Research has shown that SSF can produce a high yield of ethanol when utilizing 5% water-insoluble solids (WIS), but that yield drops when employing a larger dry matter (DM) percentage. The goal of this research was to investigate the effects of a combination of physical and chemical pretreatments on the sugar yield from fermenting rice straw with *Saccharomyces cerevisiae* to produce ethanol (yeast).

2 Materials and methods

Because of its availability and the fact that it does not immediately compete with the food market, rice straw was employed as a biomass feedstock, making it an excellent candidate for bioethanol production. This straw has been air dried. Smaller chunks of straw (2–10 mm) were obtained after cutting and sieving. Then, they put it in a room-temperature storage facility.

2.1 Pretreatment of lignocellulosic materials

Since cellulose is located deep inside the matrix, surrounded by lignin, pretreatment is required. To disrupt the crystalline structure of cellulose, it was necessary to first destroy the lignin seal and hemicellulose sheathing over cellulose. This was necessary before the cellulose in the biomass could be broken down into sugar monomers. The pretreatment procedure, usually followed by enzymatic

hydrolysis, was responsible for this disturbance [21, 22]. Physical, chemical, and biological approaches are now used in practice. Acids, alkalis, and organic solvents are all used in the pretreatment process.

2.2 Alkali pretreatment

Roughly 50 g of dry rice straw was finely chopped (H2 c length) and suspended in various concentrations of sodium hydroxide (NaOH): 1, 2, 3, 4, and 5%. The samples then spent an hour in an 85 °C water bath. Cheese cloth was then used to press the hydrolysate. Luff school technique [23] was used to determine the reducing sugar content in the juice.

2.3 Pretreatment with acids

Using a ratio of 1:10 (w/v), 50 g of chopped dry rice straw was suspended in acid solution (1, 3, 5, 7, and 9% H₂SO₄). The mixture was autoclaved at 121 °C for 15 min. The samples were then incubated for 1 h at 85 °C in a water bath. Cheese cloth was then used to press the hydrolysate. Luff school, a technique established by Zhang et al. [24], was used to determine the reducing sugar content of the juice.

2.4 Sugar test

A total of 50 g of alkali pretreated rice straw juice in a flask was taken and 125 mL water was added in it along with 15 mL (1N HCL) and then heat it for 2 min. After heating, it was cooled down. A 500-mL flask was taken, and 15 mL (1N NaOH) was added in it. The flask was added which contains 125 mL water, 2 g sample, and 15 HCL in the 500-mL flask, to complete the volume of 500 in 500-mL flask. Take another small flask water added along with 5 mL Fehling solution A and 5 mL Fehling solution B in it. A total of 17 mL from the 500-mL flask in the Fehling solution flask was added. The small Fehling solution flask was heated, and methyl blue indicator was added after 1.5 min of the boiling. Then, it was titrated against the solution present in 500-mL flask. Upon the color change, the titration was stopped.

$$\frac{\text{Factors} \times \text{constant} \times \text{reading factor}}{\text{Volume of dil. molasses} \times \text{reading on pipette} + 17 \text{ ml}} \quad (1)$$

2.5 Hydrolysis

The hemicellulose and cellulose in lignocellulosic biomass were broken down into monomeric sugars for fermentation. It is possible to do this by either acid, alkali, or enzymatic hydrolysis.

2.6 Alkali/enzymatic hydrolysis

Enzymatic hydrolysis was then used to break down the cellulose and hemicellulose into sugars that the yeast could use. An assortment of cellulase enzymes hydrolyzed the material [25]. The most optimal alkali pretreatment condition (5%) was used for alkali/enzyme pretreatment. The material was treated with NaOH, then squeezed through cheesecloth. In order to extract the enzyme combination, the residual pulp was blended with distilled water (10:1 w/w on rice straw base) containing 0.8% v/w of enzyme mixture. Sample pH was lowered to 4. After 12 h of incubation at 55 °C, the sample was taken out of the water bath (1st enzyme). Cheese cloth was then used to crush the sample, and the resulting pulp was put to use in a second enzymatic process (2nd enzyme). First press juice was used to calculate the exact amount of sugar in the product, with terms like “1st enzyme” and “2nd enzyme” being used to describe the two stages of the process.

2.7 Acid/enzyme hydrolysis

For acid/enzyme pretreatment, the optimal acid hydrolysis concentration was chosen (1%). Several cellulase enzymes hydrolyzed the material. The sample that had been treated with H₂SO₄ was squeezed tightly through cheesecloth. The pulp was blended with distilled water including an enzyme cocktail (0.8% v/w on rice straw base) and the juice was saved. Sample pH was lowered to 4. After 12 h of incubation at 55 °C, the sample was taken out of the water bath (1st enzyme). The sample was then squeezed using cheese cloth, and the resulting pulps were suitable for the second enzymatic hydrolysis (2nd enzyme). First press juices such

as “1st enzyme” and “2nd enzyme” were used to calculate sugar concentrations.

2.8 Scanning electron microscopy and Fourier transform infrared spectroscopy

Scanning electron microscopy (SEM) of control untreated rice straw, pretreated with alkali, and acid treated was carried out by S-3700N (Hitachi) at 15 kV with 500 magnification mode by field emission scanning electron microscope (FESEM) at Pakistan Institute of Technology for Minerals and Advanced Engineering Materials (PITMAEAM) PCSIR, Lahore, Pakistan, to study the morphological changes. Fourier transform infrared spectroscopy (FTIR) of control untreated rice straw, pretreated with alkali, and acid treated was conducted by Agilent Technologies Cary 630 model at Food and Biotechnology Research Center PCSIR, Laboratories Complex, Lahore, Pakistan.

2.9 Fermentation

Following pretreatments, hydrolysis results in sugar, which is then fermented to produce ethanol. For this, the most often utilized microbe is *Saccharomyces cerevisiae*, also known as baker’s yeast, which ferments both glucose and mannose. Fermentation tests were performed on materials that had been processed with acid or enzymes, either with or without a subsequent detoxification process. A total of 2108 CFU/mL was the initial yeast count in the fermentation sample. Ethanol was distilled and measured after 3, and later 6, days of fermentation. Sugar concentrations were monitored during the fermentation process. The following equation was used to calculate ethanol yield, as given by Dave et al. [26].

$$\text{Ethanol yield} = \text{Measured ethanol in sample (g)} \div \text{Theoretical ethanol (g)} \quad (2)$$

$$\text{Theoretical ethanol (g)} = \text{amount of initial suar content (g) in fermentation solution} \times 0.5 \quad (3)$$

3 Results and discussion

3.1 Effect of alkali pretreatment method on sugar

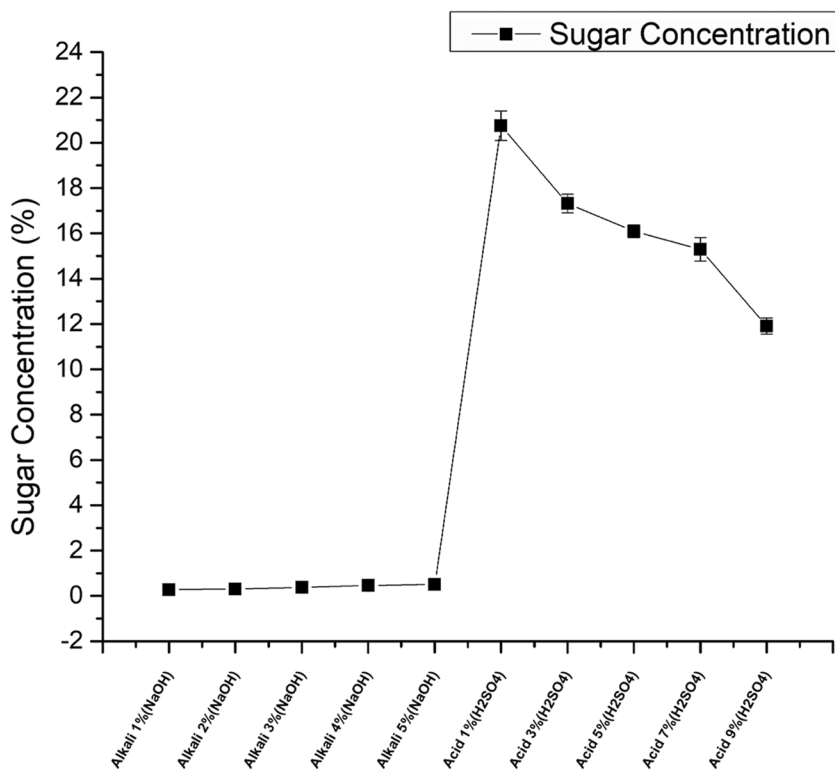
Taking off the lignin and some of the hemicellulose using an alkaline pretreatment makes the cellulose more accessible to the enzymes used in the subsequent hydrolysis steps. Alkaline pretreatment was more effective for lignin-rich biomass. Sodium hydroxide was employed in the alkaline pretreatment procedures. The sugar content of sample was found to be somewhat higher after being exposed to a higher alkalinity concentration (Table 1). When the sample was

Table 1 Sugar yield of alkali pretreated rice straw

Sr. no	Wt. of rice straw (g)	Pretreatment (NaOH)	Incubation time (h)	Sugar (%)
1	100	Alkali 1%	1 h	0.28 ± 0.01 ^a
2	100	Alkali 2%	1 h	0.31 ± 0.03 ^b
3	100	Alkali 3%	1 h	0.38 ± 0.07 ^c
4	100	Alkali 4%	1 h	0.46 ± 0.02 ^d
5	100	Alkali 5%	1 h	0.51 ± 0.04 ^e

Each value is the mean of three replicates and the ± specifies the standard deviation (SD) between the replicates. Values in each column that are followed by different letters vary significantly from each other

Fig. 1 Sugar content in alkali and acid pretreated rice straw



treated with 1% alkali beforehand, the sugar concentration dropped to an extremely low 0.28%.

When sample was pretreated with 2% alkali, 0.31% sugar content was observed, for 3% alkali 0.38%, and for 4% alkali 0.46% as described below (Fig. 1). But a slight increase in the sugar content was observed 0.51%, when sample was pretreated with 5% alkali.

3.2 Sugar response to acid pretreatment

Hydrolysis of the cellulose component was likewise catalyzed by the acid, and the resulting glucose was fermented to make ethanol. Pretreatment of lignocellulose with acid at 121 °C for 15 min resulted in the successful conversion of

the cellulose back into sugar. After acid pretreatment, sugar concentrations as high as 23.45% by weight were detected. It was found that when acid content was increased, sugar concentration decreased in the sample. The breakdown of furfural and hydroxymethyl furfural into their constituent monomeric sugars (xylose and glucose) may have been to blame. The use of 1% sulfuric acid resulted in sugar content as high as 20.75% (on a rice straw base). When the sample was pretreated with 3% H₂SO₄, 17.32% sugar yield was observed. When sample was pretreated with 5, 7, and 9%, sugar yield was observed as 16.1, 15.3, and 11.91% respectively as shown (Table 2). Higher concentration of acid led to decreasing the sugar yield in the sample (Fig. 1).

Our research focuses on two processes: the hydrolysis of cellulose in lignocellulosic materials to fermentable reducing sugar, and the fermentation of the sugars to ethanol. Cellulase enzymes were often used to speed up the hydrolysis, while yeast was responsible for the fermentation. Hydrolysis of cellulose is affected by lignin and hemicellulose concentration, fiber crystallinity, and porosity (accessible surface area) of the lignocellulosic resources.

Table 2 Sugar yield of acid pretreated rice straw

Sr#	Wheat straw (g)	Pretreatment (H ₂ SO ₄)	Incubation time (h)	Sugar (%)
1	100	Acid 1%	1	20.75 ± 0.65 ^e
2	100	Acid 3%	1	17.32 ± 0.42 ^d
3	100	Acid 5%	1	16.1 ± 0.28 ^e
4	100	Acid 7%	1	15.3 ± 0.52 ^b
5	100	Acid 9%	1	11.91 ± 0.36 ^a

Each value is the mean of three replicates and the ± specifies the standard deviation (SD) between the replicates. Values in each column that are followed by different letters vary significantly from each other

3.3 Alkali pretreatment

The alkali pretreatment improved enzyme availability to cellulose in the latter stages of hydrolysis by removing lignin and some of the hemicellulose. For biomass with a significant lignin concentration, alkali pretreatment worked best.

NaOH was used in alkali pretreatment procedures. The sugar content of the sample rose together with the alkalinity level. When the sample was treated with 1% alkali beforehand, the sugar concentration dropped to an extremely low 0.28%. The sugar concentration was observed to be 0.31% after the sample was processed with 2% alkali. However, after being pretreated with 5% alkali, sugar levels increased by 0.51%. The alkali pretreatment of cellulose increases the accessibility of cellulose to enzymes in the latter stages of hydrolysis [27] by removing lignin and a portion of the hemicellulose. An insoluble polymeric form of cellulose and hemicellulose is all that remain. In this method, alkalis including KOH, NaOH, and Ca (OH)₂ are heated to temperatures between 120 and 180 °C. It has been shown in other studies as well that alkaline treatment results in higher ethanol yields compared to acid pretreatment. It is because the formation of inhibitory byproducts is prevented, leading to more effective fermentation. The method's minor drawback is a slightly reduced sugar conversion rate. Therefore, biomass with a high percentage of lignin is ideal for alkaline pretreatment.

3.4 Acid pretreatment

Hydrolysis of the cellulose portion was likewise accelerated by the acid to provide glucose, which was then fermented to yield ethanol. Pretreatment of lignocellulose with acid at 121 °C for 15 min resulted in a successful conversion of the cellulose back into sugar. After acid pretreatment, the amount of sugar w/w measured reached as high as 20.75%. It was found that when acid content was increased, sugar concentration decreased in the sample. The breakdown of furfural and hydroxymethyl furfural into their constituent monomeric sugars (xylose and glucose) may have been to blame. The use of 1% sulfuric acid resulted in a sugar content as high as 20.75% (on a wheat straw base). Sugar production increased to 17.32% after being pretreated with 3% H₂SO₄. The sugar production was measured at 16.1, 15.3, and 11.91% after 5, 7, and 9% of the sample were subjected to pretreatment. As the acidity level became higher, the amount of sugar extracted from the samples went down a little.

According to Yang et al. [28], pretreatment with diluted acid has become the standard practice for preparing lignocellulosic materials for further processing. In addition to being cheap and easy to implement, this technique also benefits from gentle working conditions and environmentally friendly chemicals. The hemicelluloses in the biomass are hydrolyzed for 5–60 min at 130–200 °C in the dilute-acid pretreatment. Pretreatment times may be cut in half when temperatures are increased. Nitric acid has also shown promising results in cellulose-to-sugar conversion yields, but its greater price makes it less cost-efficient than sulfuric acid.

Researchers Monteiro et al. [29] found that in dilute-acid hydrolysis, the hemicellulose fraction depolymerizes at a lower temperature than the cellulosic portion. When higher temperatures (or longer residence time) are applied, the hemicellulosic generated monosaccharides break down and give birth to fermentation inhibitors such as furan compounds, weak organic acids, and phenolic compounds, which might impede the hydrolysis process. These fermentation inhibitors have been shown to reduce the efficiency with which fermenting bacteria produce ethanol. Acid hydrolysates may regain their fermentability by detoxification, but the process is expensive.

What this research demonstrates that pretreatment must be successful in terms of forming sugars or the capacity to create sugars by enzymatic hydrolysis, preserving carbohydrates without degrading them, and preventing the creation of inhibitory byproducts during the later hydrolysis and fermentation processes. Physical, physicochemical, chemical, and biological procedures have all been utilized for the pretreatment of lignocellulosic materials in the bioconversion of ethanol.

3.5 Hydrolysis

The hemicellulose and cellulose in lignocellulosic biomass were broken down into monomeric sugars for fermentation.

3.6 Effect of alkali pretreatment and subsequent enzyme treatment

Cellulose fibers and hemicellulose were typically converted to fermentable sugars by disintegration guided by enzymatic hydrolysis. Enzymes called cellulases were responsible for the hydrolysis. The most optimal alkali pretreatment condition (5%) was used for alkali/enzyme pretreatment. As can

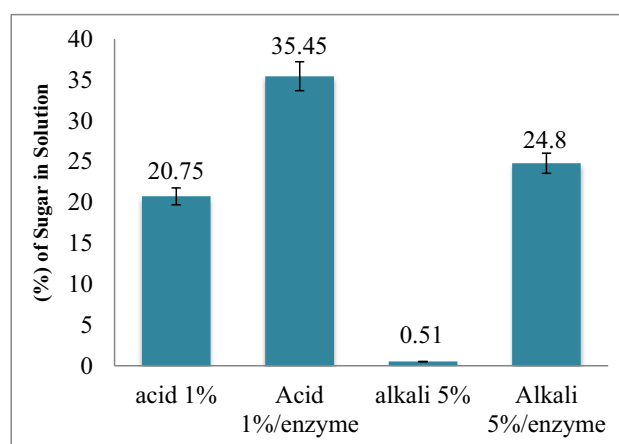


Fig. 2 Sugar concentration in alkali pretreated and subsequent enzyme treated

Table 3 The sugar yield and lignocellulose conversion of differently processed rice straw

Sr. no	Pretreatment	Sugar (%)
1	Alkali 5%	00.51 ± 0.07 ^a
2	Alkali 5%/enzyme (0.8% v/w)	24.8 ± 1.23 ^c
3	Acid 1%	20.75 ± 1.62 ^b
4	Acid 1%/enzyme (0.8%v/w)	35.45 ± 1.48 ^d
5	Acid 1%/enzyme (4% v/w)	38.30 ± 1.16 ^e

Each value is the mean of three replicates and the \pm specifies the standard deviation (SD) between the replicates. Values in each column that are followed by different letters vary significantly from each other

be seen in Fig. 2, the addition of alkali to a pretreatment sample before enzymatic hydrolysis results in a significant increase in sugar content (24.8% on a rice straw base). The optimal acid/enzyme hydrolysis condition was determined to be 1%. Technical enzyme applications improved lignocellulose material sugar conversion. As demonstrated in Fig. 2, acid pretreatment and subsequent enzyme treatment resulted in a significantly greater sugar yield (35.45%) compared to acid pretreatment alone (20.75%) (see Table 3 for details). More research on different biomass can be done by these methods to clarify effective scarification. Multiple approaches have been reported in literature to improve the enzymatic hydrolysis yield of pretreated biomass such as fed-batch approach, reducing the enzyme-lignin binding resulting in 15% increase in glucan conversion [30].

3.7 Scanning electron microscopy (SEM) of rice straw and FTIR

For observing the structural modifications in rice straw, SEM was used with alkali and acid pretreatment. SEM

micrographs demonstrated that the surface texture and morphology after both pretreatments were pointedly different from those of untreated (Fig. 3). The SEM micrograph of untreated sample shows a non-porous, smooth, and more compact surface while greater degree of porosity can be seen on both pretreated samples. Size and number of pores are greater in the acid pretreated substrate demonstrating more lignin breakdown which indicates that a large portion of lignin and hemicellulose can be removed by pretreatment. The untreated substrate shows notable characteristics/changes in the morphology of treated samples. The possible reason for this maybe the breakdown of lignin xylan bond caused by acid/base pretreatment.

FTIR measurements were conducted to study the effect of pretreatment on rice straw compared with untreated. Major peaks at 2918, 2916, 2914, 2851, 1634, 1034, and 1023 cm^{-1} were noted in control, acid treated, and alkali treated rice straw samples as shown in Fig. 4 (A, B, C). The band at 2800–3000 cm^{-1} is related to C–H stretching vibrations of CH₂ and CH₃ groups. The peak around 1600–1800 cm^{-1} corresponding to the C=O stretching vibration is mainly caused by free carbonyl groups; therefore, it is a typical hemicellulose marker. The spectra around 1200 cm^{-1} contain several bands in the C–O stretching region which were most probably cellulose-related absorptions, and these bands were sharpened by treatment. All the above variations might indicate some constituents had changed in cross-linking reaction between the C–O–H, C–O–C, and other functional groups. Except the NaOH-RS, the removal of some polysaccharide constituents could be verified from the decline of bands around 1730 cm^{-1} and the increase of the spectra around 1200 cm^{-1} after treatments.

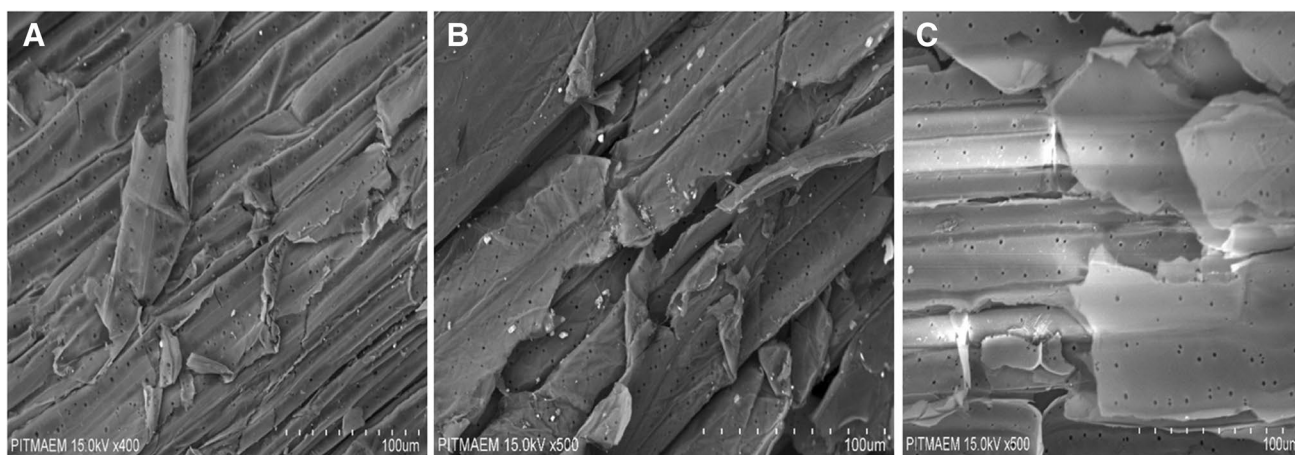


Fig. 3 Scanning electron microscopy of rice straw. **A** Untreated rice straw. **B** Rice straw treated with alkali. **C** Rice straw treated with acid

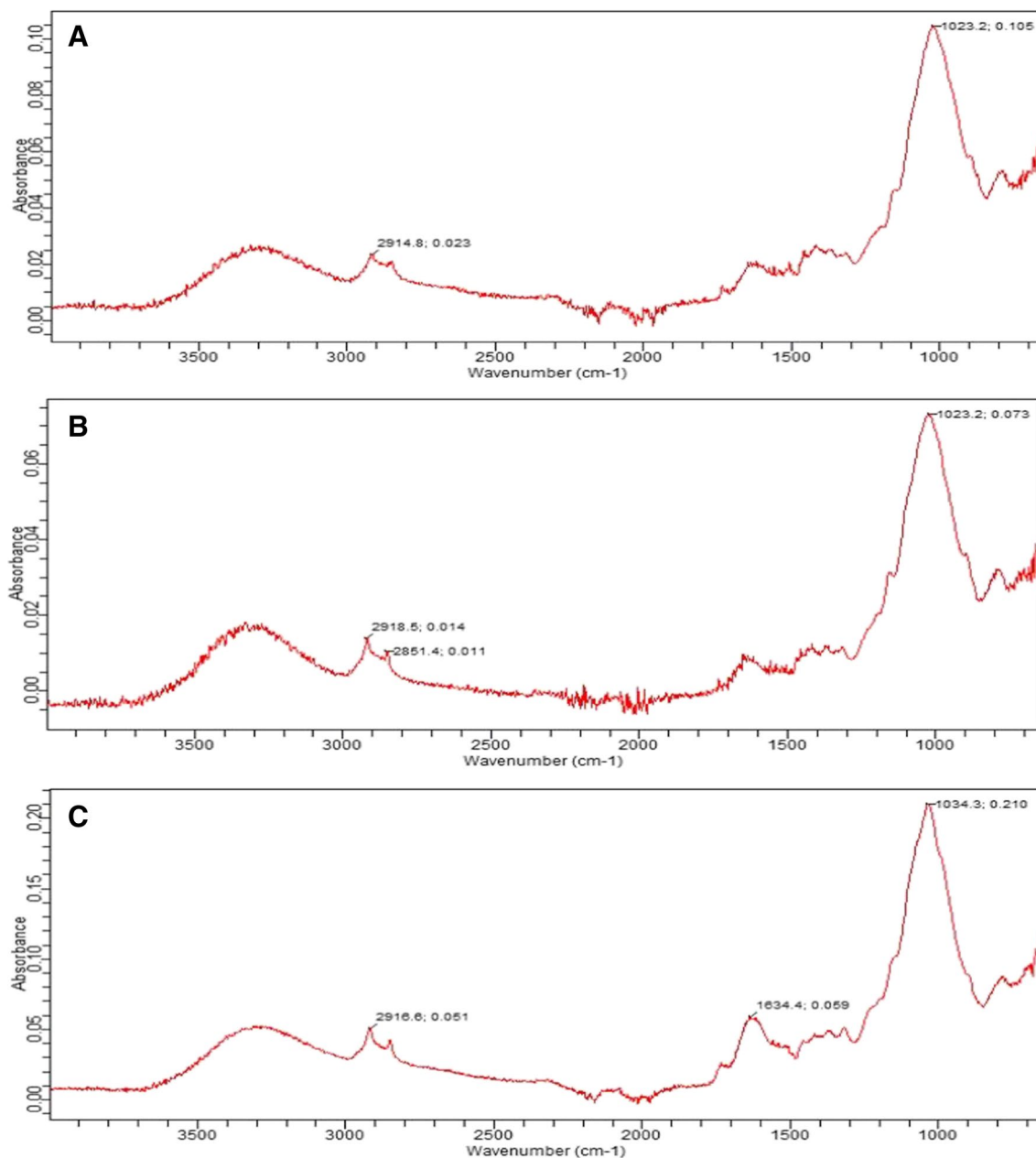


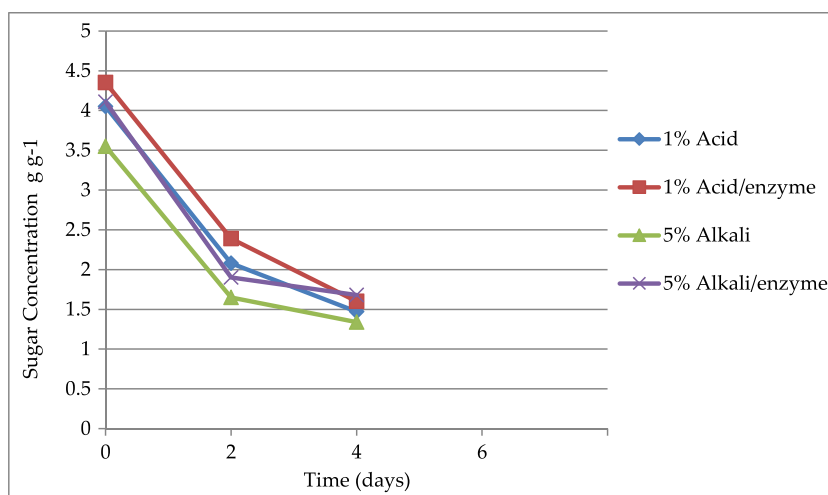
Fig. 4 FTIR of rice straw. **A** Untreated rice straw. **B** Rice straw treated with 1% acid. **C** Rice straw treated with 5% alkali

Table 4 Fermentation sugar concentration (1 g of sugar per 100 g of solution)

Sr. no	Fermentation time (days)	A g/100 g solution	B g/100 g solution	C g/100 g solution	D g/100 g solution
1	0	4.05 ± 0.12	4.35 ± 0.08	3.55 ± .1	4.11 ± 0.15
2	3	2.08 ± 0.05	2.39 ± 0.14	1.65 ± 0.07	1.90 ± 0.02
3	6	1.47 ± 0.09	1.60 ± 0.06	1.34 ± 0.16	1.68 ± 0.08

A = acid pretreatment sample; B = 1% acid/enzyme pretreatment sample; C = 5% alkali pretreatment sample; D = 5% alkali/enzyme pretreatment sample

Fig. 5 Sugar concentration during fermentation



3.8 Fermentation

Ethanol was made by fermenting sugar samples. Fermentation tests were conducted on materials that had previously been subjected to acid/enzyme pretreatment, either with or without subsequent detoxification. *Saccharomyces cerevisiae* yeast was employed in the fermentation procedure. Within 3 days of fermentation, almost all the glucose that could be fermented by *S. cerevisiae* was transformed into bioethanol. Up to 6 days of fermentation duration had a little impact on bioethanol yield (Table 4). Sample sugar concentration dropped from 5 to 6% w/v to approximately 1% w/v after 6 days of fermentation. This is the same as a sugar conversion rate of 65% to bioethanol. According to Fig. 5, the average production of ethanol in this experiment was 0.42 g/g.

3.8.1 Fermentation of alkali pretreated wheat straw

When the pretreated rice straw juices were subjected to fermentation initially for 3 days, it was observed that negligible amount of sugars were converted to bioethanol because of the less exposer of cellulosic and lignin content. However, bioethanol production is only marginally affected by fermentation times of up to 6 days as shown and described in Table 4.

3.8.2 Fermentation of alkali/enzymatic pretreated wheat straw

When alkali/enzymatic pretreated rice straw juice was subjected to fermentation initially for 3 days, that was a drastically increase of bioethanol concentration in alkali pretreated and subsequent enzyme treated sample was achieved as shown in Table 4.

3.8.3 Fermentation of acid pretreated wheat straw

When acid pretreated wheat straw juice was subjected to fermentation initially for 3 days, it was observed a measurable amount of sugars were converted to bioethanol from those samples which were pretreated with 1% acid as mentioned in Table 4.

3.8.4 Fermentation of acid/enzyme pretreated wheat straw

When acid/enzyme pretreated rice straw juice was subjected to fermentation initially for 3 days, positive effect was observed on conversion of sugar to bioethanol. As indicated in Table 4, the bioethanol production was noticeably greater i.e., 0.42 g/g in the acid pretreatment and subsequently enzyme treated specimen than in the acid pretreated sample alone.

The sugar produced during hydrolysis is fermented to produce ethanol, as shown in this research. Fermentation tests were performed on materials that had been processed with acid or enzymes, either with or without a subsequent detoxification process. *Saccharomyces cerevisiae* yeast was employed in the fermentation procedure. Within 3 days of fermentation, almost all the glucose that could be fermented by *S. cerevisiae* was transformed into bioethanol. The output of bioethanol was little impacted by increasing the fermentation duration to as much as 6 days. After 6 days of fermentation, the sugar concentration in the sample dropped from 4.5 to 1.5% w/v. This is the same as converting sugar into bioethanol at a rate of 50–60%. In this investigation, the ethanol output was almost 0.40 g/g.

Ibarra-Daz et al. [31] investigated whether enzymatic saccharification of acid or alkaline pretreated lignocellulosic yields fermentable sugars. Cellulases and hemicellulases that might be used for the hydrolysis of pretreated lignocellulosic are abundant in bacteria and fungi. Cellulases, xylanases,

hemicellulases, and mannanases are the four main hydrolytic enzymes found in enzymatic cocktails. The average ethanol production in this investigation was 0.42 g/g. Possible xylose makes up the remaining 35–45% w/v of sugar. *Saccharomyces cerevisiae* is unable to ferment the pentose sugar xylose. The sugar produced during hydrolysis was the subject of research by Annamalai et al. [32], who found that it may be fermented into ethanol. The yeast *Saccharomyces cerevisiae* is the most often employed microbe for this purpose. *Saccharomyces cerevisiae* is capable of fermenting both glucose and mannose. In this experiment, the filtrate from the pretreated samples was fermented. The purpose of the fermentation was to determine whether the potential pretreatment byproducts impacted the fermentation process. Fermentable sugar concentrations in the filtrates were determined using high-performance liquid chromatography. Each of the filtrates and the standard were supplemented with glucose to achieve a concentration of fermentable sugars equal to 50 g/L. pH was maintained at 5.5 throughout fermentation, and the temperature was maintained at 30 °C.

The cost of bioconversion of biomass to liquid fuel must be less than the cost of gasoline for the fuel to be competitive and gain economic acceptability. But researchers' attempts to improve the efficacy of biomass conversion technology have made this goal appear considerably closer to fruition than it did even a few years ago. However, there is still much room to reduce the price of converting biomass into ethanol. Two crucial factors in achieving low-priced ethanol production are the price of feedstock and cellulolytic enzymes. About 40% of the cost to make ethanol is attributable to the price of the biomass feedstock used in the process. Hamelinck and Faaij [33] have completed a short-term (5–10 years) and long-term (20–30 years) examination of the performance, critical technologies, and economic elements of bioethanol, such as cost per kilometer driven.

4 Conclusions and future directions

Rice straw has previously undergone a variety of chemical (acidic, alkaline) pretreatment procedures to convert lignocellulose material to sugar. Multiple enzyme mixtures and varying amounts of acid (H₂SO₄) and alkali (NaOH) have been used in these processes before. When comparing these processes, it became clear that acid treatment followed by enzymatic treatment is the most effective approach for transforming lignocellulose into glucose. There was a sugar content increase of up to 37% after using this technique. Low rice straw loading was also cited as a reason for the low ethanol concentration of 10 g/L that was produced in this research. Therefore, increased wheat straw loading is required to generate greater concentrations of ethanol appealing for industrial use.

Our research led us to the conclusion that an H₂SO₄ pretreatment procedure followed by an enzyme treatment is the most efficient way of producing ethanol under moderate pretreatment conditions. A rise in the usage of renewable energy sources is expected as the globe works to minimize its emissions of greenhouse gases. Materials from lignocellulosic biomass are among those being considered for use as a sustainable energy alternative. Ethanol, either pure or blended with gasoline, has excellent properties as a fuel. In the USA, cellulosic biomass resources are used to make ethanol, a liquid fuel. Bioethanol's high octane makes it a promising addition to the transportation fuel market. Bioethanol has the potential to be a clean-burning fuel that decreases pollution and carbon monoxide emissions. Also the world should focus on green energy and green projects which can also be a substitute option to avoid CO₂ emissions. The accuracy and reliability of these methods should be improved by evolving economic accounting and environmental impact assessment methods.

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Data availability All the data generated in this research work has been included in this manuscript.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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