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ORIGINAL PAPER

Comparative study of ethanol production from sodium hydroxide pretreated rice straw residue using *Saccharomyces cerevisiae* and *Zymomonas mobilis*

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Abstract

Rice straw is a suitable alternative to a cheaper carbohydrate source for the production of ethanol. For pretreatment efficiency, different sodium hydroxide concentrations (0.5–2.5% w/v) were tested. When compared to other concentrations, rice straw processed with 2% NaOH (w/v) yielded more sugar (8.17 ± 0.01 mg/ml). An alkali treatment induces effective delignification and swelling of biomass. The pretreatment of rice straw with 2% sodium hydroxide (w/v) is able to achieve 55.34% delignification with 53.30% cellulose enrichment. The current study shows the effectiveness of crude cellulolytic preparation from *Aspergillus niger* resulting in 80.51 ± 0.4% cellulose hydrolysis. Rice straw hydrolysate was fermented using ethanologenic *Saccharomyces cerevisiae* (yeast) and *Zymomonas mobilis* (bacteria). Overall, superior efficiency of sugar conversion to ethanol 70.34 ± 0.3% was obtained with the yeast compared to bacterial strain 39.18 ± 0.5%. The current study showed that pretreatment with sodium hydroxide is an effective method for producing ethanol from rice straw and yeast strain *S. cerevisiae* having greater fermentative potential for bioethanol production than bacterial strain *Z. mobilis*.

Keywords Rice straw · Pretreatment · Enzymatic hydrolysis · Fermentation · Ethanol

Introduction

A large portion of the world's energy is generated by fossil fuels. However, there is a high demand for renewable bioenergy because fossil fuels are depleting and some of these fuels have negative environmental consequences (Allen et al. 2016). Biofuels being renewable and green energy resources are the most promising alternatives to fossil fuels (Khan et al. 2021). Lignocellulosic biomass, which includes forest residues, wheat and rice straw, and woody materials, is currently a hot topic in discussions about industrial-scale biofuel production (Kumari et al. 2021). In terms of feedstock, rice straw (RS) is a promising candidate due to its high content of cellulose and hemicellulose and its large annual global production of 370–520 million tonnes per year (Valles et al. 2021). Asia provides around 84% (826 metric tonnes) rice residue of the total world's output (980 metric tonnes) (Goswami et al. 2019). Rice straw accounts for 60% of all burned crop leftovers, with an estimated 50 million tonnes of rice residue burned in fields. However, the most recent estimates for rice-straw generation in India showed that the volume of residue generation has increased with yearly fluctuations based on rice grain production in the years 2012–13

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(157.85 M tonnes), 2013–14 (159.97 M tonnes), 2014–15 (158.22 M tonnes), 2015–16 (156.61 M tonnes), 2016–17 (164.55 M tonnes), and 2017–18 (169.14 M tonnes). In India, rice straw has traditionally been used for a wide range of applications, including cattle feed, rural arts and craft industries, and thatching of houses (the cost of thatching varies by region due to the quality of straw from different varieties being different for thatching purposes, but in general it is about INR 60–70 (about US$ 1) per square metre in remote areas where such houses are prevalent) (Bhattacharya et al. 2021).

Agricultural straw or crop leftovers left in the field after grain harvest is purposefully burned; this method of residue management is known as stubble burning or crop residue burning. Residue burning emits various pollutants into the air, harming the ecosystem. To understand the effect of residue burning on adjacent areas and cities, the influence on human health must be addressed. To solve the problem of residue burning, a number of technical solutions for ex-situ and in-situ residue management are being promoted in India (Kaur et al. 2022). However, no single approach is powerful enough to eliminate it totally. The use of rice straw biomass for large-scale ethanol production not only provides a rice residue control solution but also creates nonconventional biomass for ethanol production.

Rice straw has the potential to be a feedstock for the production of cellulase (Prajapati et al. 2021), xylanase (Singh et al. 2022), methane (Kumari et al. 2021), bioethanol (Wang et al. 2021), biohydrogen (Kim et al. 2022), chitosan (Tejas et al. 2022), xylitol (Singh et al. 2021), and butanol (Valles et al. 2021). Carbon dioxide mitigation, agricultural system integration, and technological and economic feasibility are the three most important features of a cellulosic bioenergy industry that is long-term. In this way, the manufacture of rice straw-based bioethanol is a viable option for reducing greenhouse gas emissions, as it is readily available as an agri-residue and can be blended with gasoline to make transportation fuel (Samar et al. 2021). More than 85% of India's petroleum needs are met by foreign suppliers. To reduce the country's reliance on imported fossil fuels, the Indian government has set targets for blending ethanol at 10 and 20% by 2022 and 2030, respectively, under the 2018 National Biofuel Policy (Sharma et al. 2021). Rice straw is converted to bioethanol by a process that includes pretreatment, enzymatic saccharification, and fermentation (Ashoor and Sukumaran 2020; Gundupalli et al. 2021). The most important step in this bioconversion is pretreatment as it disrupts the cellulose lignin complex and changes the structure and crystallinity of cellulose which will expose the carbohydrate content for better sugar yield during enzymatic hydrolysis and in turn will increase ethanol yield in subsequent steps during fermentation (Madadi et al. 2017). Lignocellulose hydrolysis and ethanol production from lignocellulose can be improved using a variety of pretreatment techniques (Bay et al. 2021). Among different pretreatment methods, alkaline pretreatment is one of the most effective techniques for the pretreatment of rice straw as it affects the cell wall directly and results in effective delignification (Wati et al. 2007).

Rice straw is swollen by alkalis like NaOH, which reduces the material's polymerization and crystallinity. Alkaline pretreatment is less energy-intensive than other pretreatment options because it is done at ambient temperatures and pressures. Alkali breaks the ester bonds that link lignin polymer and xylan, leading to an expansion of pores (Kumar et al. 2022). Furthermore, tiny changes in alkali concentration have no effect on the biomass's cellulose content, and the maximum saccharide part can be recovered because only a small fraction is solubilized. Enzymatic hydrolysis of pretreated biomass is an important step for the release of maximum fermentable sugars. Saccharification of any lignocellulosic biomass involves the action of a cocktail of three cellulolytic enzymes viz. endoglucanase, exoglucanases, and β-glucosidases (BGL) which act in a synergistic manner to hydrolyze cellulosic biomass (Kumar et al. 2023). *Aspergillus niger* is a well-studied fungus for the production of cellulases (Díaz et al. 2021). Brewer's yeast, *S. cerevisiae*, is the most widely used and conventional cell factory for commercial bioethanol production. Due to its high fermentation rate and ethanol tolerance, *S. cerevisiae* ferments hexoses efficiently and produces a high ethanol yield (Sindhu et al. 2016). But in the last decade, the bacterial strain *Z. mobilis* has drawn attention due to its fast growth rate and its ability to ferment sugars into ethanol. *Z. mobilis* is a rod-shaped, gram-negative, motile bacteria that do not produce spores. *Z. mobilis* has a number of benefits over yeast as an alternative organism for the manufacture of ethanol. These include increased rates of glucose uptake and ethanol production, and increased tolerance to ethanol (Joon Lee et al. 1979; Rogers et al. 1980).

Rice straw biomass has received relatively little attention in terms of its potential as a substrate for bioethanol production. In investigations of ethanol production, *S. cerevisiae* is the most widely studied microbe. The *Z. mobilis* has attracted the greatest attention among the ethanol-producing bacteria listed in the literature. These bacteria are thought to be the most technologically interesting, serving as an alternative to distillery yeasts. From an industrial standpoint, fermentation with *Z. mobilis* has numerous advantages: it metabolises sugars via the Entner-Doudoroff route, which results in a lower energy gain due to reduced biomass output, but a higher ethanol yield.

So, the present study was carried out with the use of NaOH for the pretreatment of a locally grown variety of rice straw, followed by hydrolysis of pretreated biomass

using crude enzymatic preparation from *A. niger* and finally comparative evaluation of *S. cerevisiae* and ethanologenic *Z. mobilis* for bioethanol production.

Materials and methods

Microorganisms

Aspergillus niger (The culture was obtained from the fermentation laboratory, Kurukshetra University, Kurukshetra, NCBI accession no. HM008328.1) was grown in Czapeck–Dox inorganic medium (g/L) with the following ingredients: $NaNO_3$ 2, KCl 0.5, $FeSO_4.7H_2O$ 0.01, KH_2PO_4 0.5, and $MgSO_4.7H_2O$ 0.5, pH 5.5. The culture *S. cerevisiae* NCIM 3280 (procured from National Collection of Industrial Microorganisms (NCIM), Pune, India) was routinely grown in YEPD broth (g/L) [Yeast extract 10, peptone 20, dextrose 20, pH 6.5]. The *Z. mobilis*(MTCC 2427) was procured from Microbial Type Culture Collection and Gene Bank(IMTECH, Chandigarh, India) was maintained on nutrient agar (g/L) (Beef extract 3, Peptone 5, Sodium chloride 8, Agar 15, pH 6.8).

Biomass preparation

Rice straw was procured from local fields of the Kurukshetra district of Haryana and used as substrate. To reduce the moisture content, the straw was air dried after washing. The dried biomass was pulverized in a blender and then sieved through a mesh size of 10 to get a particle size of 2 mm. Then, it was stored in an airtight container for further experiments.

Pretreatment of rice straw

The rice straw was pretreated in the autoclave at 121 °C, 15psi pressure for 30 min by taking ground rice straw in 250 ml Erlenmeyer flask with different concentrations of NaOH (0.5–2.5% w/v) at a biomass loading of 10%. The amount of sugar that came out of enzymatic hydrolysis with crude cellulase from *A. niger* was used to measure the efficiency of pretreatment. After alkaline pretreatment 10 ml distilled water was added to the flask and stirred for 0.5 h on a magnetic stirrer (REMI Magnetic Stirrers 2MLH) and the filtrate was collected after filtration through a muslin cloth. To remove any remaining alkali, neutralized the pH, the treated biomass was rinsed with distilled water. The presence of lignin in the alkaline extract was determined by measuring absorbance at 205 nm. The reducing sugar content of the extract was evaluated by the DNS method (Miller 1959). After air drying at room temperature, the pretreated biomass was either used directly for hydrolysis or stored in an airtight container for later use.

Compositional analysis of treated and untreated rice straw

Determination of cellulose content

To evaluate the cellulose content, 1 g of dry biomass was fluxed for 20 min with 10 mL of 80% acetic acid and 1.5 mL of nitric acid. The mixture was dried in a hot air oven at 105 °C until it reached a constant weight, and the difference between the initial and final weights was used to calculate the content of cellulose (%) (Ahmed et al. 2010).

$$\text{Cellulose Content (\%)} = \frac{w_2 - w_1}{w_0} \times 100$$

Determination of hemicellulose content

The method developed by Di Blasi et al. (1999) was used to determine the hemicellulose content. 10 ml of a 0.5 M solution of sodium hydroxide was added to 1 g of the dried biomass (w_0). The contents were heated at a temperature of 80 °C for 3.5 h and stirred constantly. After that, the contents were filtered through glass crucibles (dried and weighed, w_1) and washed with distilled water until the pH of the wash solution reached a neutral value. The solid residue (in the crucible) was dried in an oven at 105 °C to a constant weight (w_2). The difference in the weight of the sample before and after this treatment gave the hemicellulose content (%w/w), as follows:

$$\text{Hemicellulose Content (\%)} = \frac{w_0 - (w_2 - w_1)}{w_0} \times 100$$

Determination of lignin content

The amount of lignin in the biomass was calculated using a method developed by Yao et al. (2010). After being hydrolyzed for two hours at 20 °C with 72% sulfuric acid, the biomass was filtered. The lignin concentration was calculated by comparing the solid residue's pre- and post-acid hydrolysis weights after it had been dried at 105 °C in a hot air oven to a constant weight.

$$\text{Lignin Content (\%)} = \frac{w_2 - w_1}{w_0} \times 100$$

Determination of ash content

The Ash content was estimated by using methodology developed byErdei et al. (2010)0.1–5 g of dried powdered

biomass taken in an appropriately dried and weighed (W_1) crucible was kept in a muffle furnace at 650 °C for 4 h or until weight became constant, and weighed again (W_2) after cooling in a desiccator.

$$\text{Ash}(\%) = \frac{w_2 - w_1}{\text{Initial weight of sample}} \times 100$$

Fourier-transform infrared spectroscopy (FTIR)

At 10 scans per sample, FTIR spectra of rice straw (raw and alkali treated) samples were recorded with a resolution of 4 cm^{-1} in the wave number range of 3600–600 cm^{-1}.

Production of cellulases by A. niger under solid-state fermentation

To induce cellulolytic enzyme production the fungal isolate was grown on rice straw via SSF and for enzyme manufacture, the pretreated substrate (2 g) was moistened with Czapeck dox medium to obtain a 70% moisture level. The flasks were inoculated with 2×10^8 *A. niger* spores per/ml and incubated for 120 h at 28 °C. Following the incubation period, the enzyme was extracted using citrate buffer (0.05 M pH 4.8). The flasks were properly shaked for 1 h at 125 rpm at 30 ± 2 °C. Filtration through muslin cloth was used to separate the solids, which was followed by centrifugation (7200 × g for 15 min) at 4 °C. After biomass separation, the supernatant was employed as a crude enzyme. Four volumes of ice-cold acetone were used to precipitate the crude extract from SSF, and the precipitate was then resuspended in the appropriate volume of 0.05 M citrate buffer (pH 4.8). These solutions were employed to hydrolyze biomass. According to Ghose (1987), the filter paper assay was used to quantify the total amount of cellulase activity in crude enzyme preparation. p-nitrophenyl -D-glucopyranoside was used as a substrate to measure the activity of -glucosidase (Ghose 1987). The amount of an enzyme that releases 1 µmol of reducing sugars per minute, expressed as an international unit/gram (IU/gds) of dry solids, considered one unit of enzyme activity. The DNS method was used to calculate the total reducing sugars in the enzymatic hydrolysate of biomass (Miller 1959). Dehkhoda and Brandberg (2009) methods were used to concentrate the enzymatic hydrolysate.

Saccharification of pretreated rice straw

Enzymatic saccharification of alkali-treated biomass was carried out in a 100 ml screw cap flask with a solid loading of 5 and 10% (w/v) with different loading of crude unprocessed cellulases preparation, 200 µl 100 × antibiotic solutions of penicillin and streptomycin cocktail, and 100 µl Tween-80 surfactant. 0.05 M citrate buffer was used to make a total reaction volume of 20 ml (pH 4.8). The flasks were agitated at 200 rpm and kept at 50 °C for 48 h. As previously stated, the crude enzyme samples were prepared. After 24 h of incubation, the samples (0.5 ml) were extracted and the total reducing sugars were calculated using the DNS method. The saccharification efficiency was calculated as described by Saritha et al. (2012). Saccharification efficiency was calculated using the following formulae.

Saccharification efficiency (%) = (Amount of reducing sugars released × 100 × 0.9)/(amount of cellulose).

Ethanol production using rice straw hydrolysate

Bioethanol was produced from the sugar-rich hydrolysate produced by hydrolyzing alkali-pretreated rice straw with a crude enzymatic preparation. Hydrolysis of the pretreated biomass was carried out in a way that maximized the amount of sugar produced. Evaporation in a rotatory evaporator was used to concentrate enzymatic hydrolysate to improve sugar concentration, and the liquid's boiling temperature was kept at 80 °C. The hydrolysate was concentrated to a reducing sugars concentration of 5–20%. The synthetic sugars (YEPD with 20% glucose) were used as a control. The *S. cerevisiae* was used for fermentation for bioethanol production. The fermentation efficiency was also compared using bacterial strain *Z. mobilis* for fermentative production of bioethanol. Fermentation profile was studied for different time intervals (24-96 h), temperature (25–35 °C), pH (4–7), agitation speed (100–200 rpm), and hydrolysate from 5 and 10% solid loading. Ethanol concentration was measured by the method of Caputi et al. (1968) using purified ethanol as a standard. The fermentation parameters were calculated as:

Ethanol volumetric productivity (g/l/h) was calculated as the ratio of ethanol concentration (g/l) to the fermentation time t.

The yield of ethanol to consumed sugars (YP/S) was defined as the ratio of ethanol concentration to sugar consumption.

Sugar conversion (%) is calculated as a ratio of sugar consumption to the initial sugar concentration.

The efficiency of sugar conversion to ethanol (g, in percent) has been estimated by the ratio of ethanol yield to the theoretical value of ethanol yield (0.51%).

Results and discussion

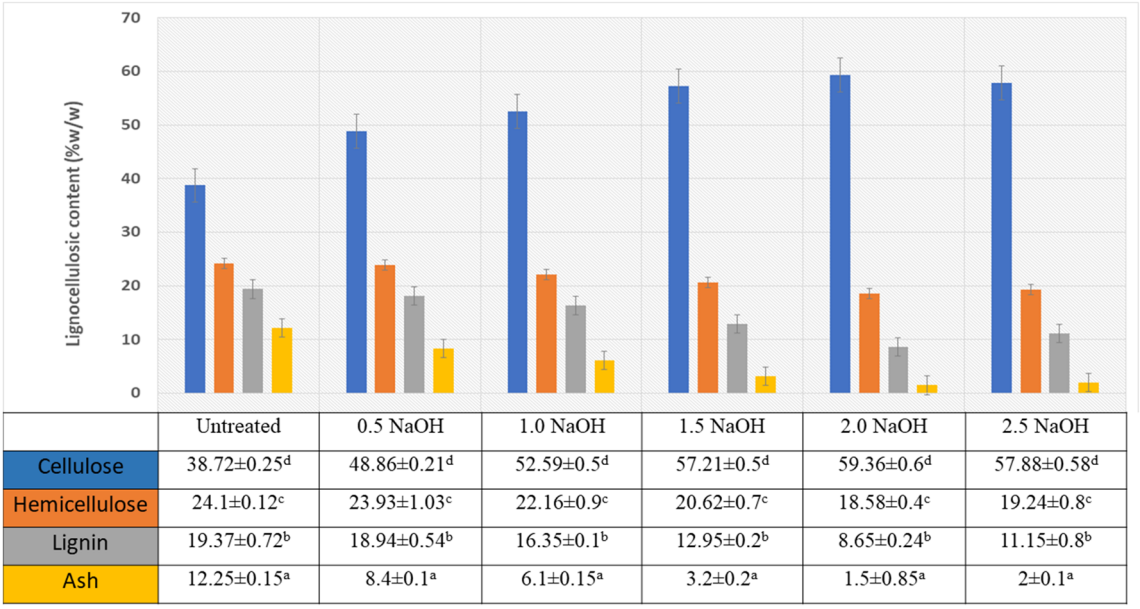
Efficiency of pretreatment at different alkali concentrations and its effect on compositional analysis of rice straw

The economics of large-scale processing of rice straw biomass for bioethanol production are affected by alkaline pretreatment, so the lowest concentration of this reagent is desired to weaken the barriers that cause lignocellulosic recalcitrance and convert the biomass into susceptible forms for saccharification by cellulolytic enzymes. So, rice straw powder was pretreated with different concentrations of NaOH (0.5–2.5% w/v), and reducing sugars obtained by enzymatic hydrolysis using crude cellulase were presented in Table 1. Pretreated rice straw (PRS) produced more fermentable sugars with 2% NaOH compared to 1.5 or 2.5% NaOH. Sugar yields were similar at 6.1 mg/ml for pretreated rice straw with 1.5% and 2.5% NaOH. According to the results obtained in the present study PRS with 2%, NaOH resulted in the maximum liberation of reducing sugars at 8.17 ± 0.01 mg/ml after 60 h of hydrolysis. So, PRS with 2% NaOH was used for further experiments. In the present study, the native or raw rice straw had 38.72 ± 0.25% cellulose, 24.10 ± 0.12% hemicelluloses, 19.37 ± 0.07% lignin, and 12.25 ± 0.15% ash (w/w) before alkali treatment (Fig. 1). The sugar yield decreased at the highest alkali concentration due to a higher pH, which changed the environment of enzymatic hydrolysis. While performing NaOH pretreatment of coastal bermuda grass at 121 °C it was observed that the increase in the

Table 1 Production of reducing sugars (mg/ml) by crude cellulase from *A. niger* from rice straw pretreated with different concentrations of NaOH

Time (h)	Reducing sugar (mg/ml)				
	NaOH concentration (%w/v)				
	0.5	1.0	1.5	2.0	2.5
0	0	0	0	0	0
12	0.94 ± 0.02^{a}	1.13 ± 0.05^{a}	1.28 ± 0.06^{a}	1.53 ± 0.03a	1.54 ± 0.04^{a}
24	1.25 ± 0.03^{b}	1.81 ± 0.02^{b}	2.43 ± 0.03^{b}	4.22 ± 0.04^{b}	3.82 ± 0.04^{b}
36	2.93 ± 0.02^{c}	3.47 ± 0.05^{c}	4.67 ± 0.03^{c}	6.12 ± 0.07^{b}	5.77 ± 0.03^{c}
48	3.45 ± 0.03^{d}	4.92 ± 0.06^{d}	6.00 ± 0.02^{d}	8.06 ± 0.12^{c}	6.13 ± 0.04^{d}
60	3.57 ± 0.03^{d}	4.94 ± 0.03^{d}	6.1 ± 0.08^{d}	8.17 ± 0.01^{c}	6.17 ± 0.06^{d}
72	3.59 ± 0.09^{d}	4.92 ± 0.08^{d}	6.15 ± 0.05^{d}	8.1 ± 0.07^{c}	6.18 ± 0.03^{d}

Values are mean ± SD and values with different superscript differ significantly ($p < 0.05$; Tukey test)



	Untreated	0.5 NaOH	1.0 NaOH	1.5 NaOH	2.0 NaOH	2.5 NaOH
Cellulose	38.72 ± 0.25^{d}	48.86 ± 0.21^{d}	52.59 ± 0.5^{d}	57.21 ± 0.5^{d}	59.36 ± 0.6^{d}	57.88 ± 0.58^{d}
Hemicellulose	24.1 ± 0.12^{c}	23.93 ± 1.03^{c}	22.16 ± 0.9^{c}	20.62 ± 0.7^{c}	18.58 ± 0.4^{c}	19.24 ± 0.8^{c}
Lignin	19.37 ± 0.72^{b}	18.94 ± 0.54^{b}	16.35 ± 0.1^{b}	12.95 ± 0.2^{b}	8.65 ± 0.24^{b}	11.15 ± 0.8^{b}
Ash	12.25 ± 0.15^{a}	8.4 ± 0.1^{a}	6.1 ± 0.15^{a}	3.2 ± 0.2^{a}	1.5 ± 0.85^{a}	2 ± 0.1^{a}

Fig. 1 Effect of different NaOH concentrations on the compositional analysis of rice straw biomass

concentration and retention time caused increased delignification, however, with a simultaneous decrease in the total solids as well as the carbohydrate polymers, ultimately affected the yield of total reducing sugars in the hydrolysis (Wang et al. 2010). Kataria and Ghosh, (2014) also achieved maximum hydrolysis yield from *Saccharumspontaneum*after its pretreatment at 121 °C using 0.5% NaOH and 120 min retention time. They also reported that the higher NaOH levels are favorable for larger delignification but are undesirable because they cause solubilization of the carbohydrates causing a reduction in the yield of holocellulose. This in turn may affect the yield of total reducing sugars in the subsequent hydrolysis.

The pretreatment of rice straw with 2% NaOH resulted in 53.30% cellulose enrichment with 55.34% lignin loss (Table 2).

The decrease in lignin and ash content following alkaline pretreatment was mostly responsible for the increase in cellulose content. The chemical composition differences of the untreated, and alkali-pretreated rice straw was studied by using FTIR. The solid obtained (alkali pretreated) was washed with water and dried in the oven. The FTIR spectra of the raw and pretreated were recorded in solid-state. As a consequence of the pretreatment, the profile of the FTIR spectra in Fig. 2 displays structural changes in the untreated and pretreated samples. As shown in Fig. 2, the observed bands near 3342 cm^{-1}, 1649 cm^{-1}, 1383 cm^{-1}, and 1043 cm^{-1} in the spectrum of untreated biomass shifted with the observation of new bands near 3309 cm^{-1}, 1756 cm^{-1}, 1366 cm^{-1}, and 1027 cm^{-1} in the spectra of the pretreated biomass. According to the FTIR spectra, pretreatment led to a significant drop in the intensities of polysaccharide bands, resulting in the formation of functional groups. Pretreatment with NaOH is an effective delignification process as it releases ester linkages and disrupts the alkyl and aryl linkages of lignin. Alkaline hydrolysis removes lignin and reduces cellulose polymerization and crystallinity, making it more accessible to enzyme hydrolysis. It has been proven

Table 2 Effect of NaOH pretreatment on biomass composition of rice straw

Pretreatment	%Change in biomass composition			
	Cellulose (%w/w)	Hemicellulose (%w/w)	Lignin (%w/w)	Ash (%w/w)
0.5% NaOH	26.18 ↑	0.70 ↓	6.35 ↓	31.42 ↓
1.0% NaOH	35.82 ↑	8.04 ↓	15.59 ↓	50.20 ↓
1.5% NaOH	47.75↑	14.43 ↓	33.14↓	73.87↓
2.0% NaOH	53.30 ↑	22.90↓	55.34 ↓	87.75 ↓
2.5% NaOH	49.48 ↑	20.16↓	42.43↓	83.67↓

Arrows ↑ and ↓ indicate % increase and % decrease, respectively, in the lignocellulosic content due to the pretreatment, compared to the untreated biomass

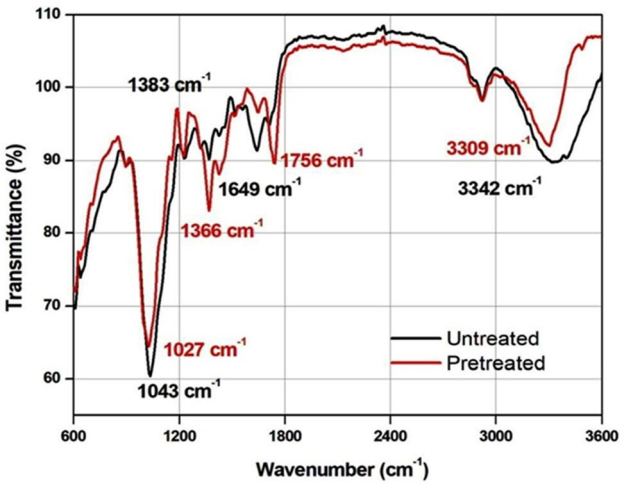


Fig. 2 FTIR spectra of untreated and pretreated rice straw

to be the most effective and beneficial technique. Due to the increased removal of lignin, alkali-treated biomass hydrolyzes better than acid-treated biomass. Lignin removal can also aid in removing non-productive cellulolytic enzymes bound to lignin during subsequent hydrolytic processes (Ying et al. 2018). Another study found that using 1% NaOH reduced lignin by 17.4% (Sharma et al. 2019). In this study, pretreatment with NaOH yielded a solid recovery of 57% on a dry weight basis, with the remainder being eliminated after washing. The alkali extract had an absorbance of 0.514 at 205 nm, indicating that degraded soluble lignin was present. After alkaline pretreatment and washing, the absorbance values of wash water were reduced (Table 3), indicating that lignin had been eliminated from the solids. Sugars were also found in the liquid portions of wash water.

The pretreated washed substrate was significant because it improved subsequent enzymatic hydrolysis by lowering pH and eliminating phenolic chemicals formed by lignin that inhibit enzymes. The enzymatic hydrolysate containing lignin was not fermented and requires prior detoxification and pH adjustment before fermentation. After alkali treatment and washing the lignin can be recovered easily at low pH from the liquid extract and wash waters by acidification of extract when lignin is precipitated (Zhang and Cai 2008) (Table 3).

Saccharification of pretreated rice straw

In addition to pretreatment, the success of enzymatic saccharification depends on the effectiveness and suitability of the enzymes for certain biomass. An enzyme produced by *A. niger* using pretreated rice straw as substrate under SSF had a cellulase activity of 1.6 ± 0.2 FPU/ml and the same was concentrated to have activity of 15.3 ± 0.2 FPU/ml as described in materials and methods (Table 4). Singh and

Bishnoi (2012) reported similar enzyme production. During saccharification, enzyme loading had a significant impact on sugar output and, as a result ethanol was recovered. The influence of enzyme concentration on PRS saccharification (with 2% w/v NaOH) was studied. Saccharification was carried out using PRS as the saccharification substrate at 5 and 10% solid loading. The reducing sugars production profile was studied throughout 120 h. As for the effect of incubation time, there was a significant difference ($p < 0.05$) in the production of reducing sugars from 0 to 120 h for all cellulase concentrations used during saccharification. The concentration of crude cellulase at the activity of FPase 15 U/g and β-glucosidase concentration of 235 U/g resulted in the production of 23.36 ± 0.2 and 29.33 ± 0.2 mg/ml reducing sugars for 5 and 10% hydrolysate, respectively (Table 5).

In the case of 10% loading, higher sugar release was observed (29.33 ± 0.2 mg/ml) compared to 5% loading in which 23.36 ± 0.2 mg/ml sugar was released. The saccharification efficiency was 50.24 ± 0.4, and 80.51 ± 0.4% for 5 and 10% loading, respectively. Thus, at higher solid loading higher concentration of reducing sugars was obtained in sugar hydrolysate but saccharification efficiency was lower. For the cost-effective production of ethanol, higher loading of the substrate and higher concentration of fermentable sugars are desired. To make the process economical minimum dose of the enzyme was desired. An increase in enzyme concentration up to 15 FPU/g resulted in an increased rate of hydrolysis of rice straw. However, a further increase in enzyme dose did not result in a significant increase in the hydrolysis of substrate. This might be due to improper mixing and suspension of the slurry. The increase in enzyme concentration should increase in hydrolysis rate but it will make the process uneconomical. But saccharification with high loading of the substrate causes an increase in viscosity of the medium which affects heat and mass transfer and results in higher energy consumption for mixing. Moreover, high substrate concentration produces more inhibitors which cause enzyme inhibition (Wang et al. 2016). Saccharification efficiency of 76% was obtained with 2.1% glucan loading of alkali-pretreated rice straw which was decreased to 50%

Table 3 Amount of sugars in the washing of pretreated solid and lignin level in alkaline extract

Washing No	Vol. used for extraction ml	Sugar mg/ml	Extracted volume	Total sugar extracted mg	Level of lignin extracted (absorbance at 205 nm)
1	10	1.06 ± 0.15	8.0	8.8 ± 0.1	0.514 ± 0.04
2	10	0.43 ± 0.15	8.5	3.46 ± 0.3	0.161 ± 0.02
3	10	0.23 ± 0.05	8.7	1.75 ± 0.05	0.113 ± 0.03
4	10	ND	9.0	–	0.077 ± 0.04
5	10	ND	9.5	–	0.042 ± 0.01

ND Not detected

Table 4 Enzyme activity of *Aspergillus* produced under SSF

Type of Enzyme preparation	FPU/gds	FPU/ml	β-Glucosidase activity/gds	β-Glucosidase activity/ml
Crude cellulase from *Aspergillus* produced through SSF	28.16 ± 0.2	1.6 ± 0.2	202.45 ± 0.5	10.4 ± 0.2
Concentrated cellulase	–	15.3 ± 0.2	–	235.3 ± 0.1

Table 5 Effect of different concentrations of different crude cellulolytic enzymes on fermentable sugars and saccharification efficiency

Time (h)	5% Loading (w/v)				10% Loading (w/v)			
	FPU/gds	β-Glucosidase (U/gds)	Fermentable sugar(g/L)	Saccharification efficiency (%)	FPU/gds	β-Glucosidase (U/gds)	Fermentable sugar(g/L)	Saccharification efficiency (%)
0	0.0	0.0	0	0	0	0	0	0
24	3.0	45	12.33 ± 0.2	42.06 ± 0.5	3	45	14.36 ± 0.4	24.37 ± 0.4
48	6.0	90	17.46 ± 0.3	60.18 ± 0.3	6	90	17.0 ± 0.1	29.67 ± 0.4
72	9.0	135	19.43 ± 0.1	66.18 ± 0.8	9	135	21.06 ± 0.4	36.48 ± 0.2
96	12.0	180	23.13 ± 0.1	79.18 ± 0.7	12	180	29.16 ± 0.2	50.21 ± 0.4
120	15.0	235	23.36 ± 0.2	80.51 ± 0.4	15	235	29.33 ± 0.2	50.24 ± 0.4

with 4.2% glucan loading (Sharma et al. 2019). A similar trend was observed in other studies also where the yield of fermentable sugar gets increased with an increase in enzyme concentration (Ouyang et al. 2009). In fact, with an increase in enzyme concentration more active sites will be available for binding of the substrate and conversion to fermentable sugars.

Fermentation of enzymatic hydrolysate by yeast and bacterial strain for bioethanol production

The fermentation process of concentrated sugar syrup obtained after hydrolysis of pretreated rice straw was utilized for ethanol production using yeast *S. cerevisiae* and bacterium *Z. mobilis* and synthetic sugar (glucose) was taken as control. The maximum fermentation efficiency of 90% was obtained in control containing synthetic sugar glucose (Table 6). The fermentation efficiency of 70.34 ± 0.3 and 39.18 ± 0.5% was observed in 10% sugar hydrolysate containing 29.33 ± 0.2 mg/ml sugar using *S. cerevisiae* and *Z. mobilis,* respectively. The ethanol yield of 0.36gg-1 was obtained with *S. cerevisiae* which produced 6.4 ± 0.3 g/L ethanol with 58.65% sugar consumption after 24 h (Table 7). While 10% sugar hydrolysate produced 3.07 ± 01 g/L ethanol with 55.23% sugar consumption using bacterial strain. The optimized cultural conditions of pH 6.0 times of 24 h and temperature of 30 °C using 10% hydrolysate for ethanol production were reported for both yeast and bacterial strain in the present study. The agitation rate has not affected bioethanol production significantly as the same bioethanol yield was obtained at high and low agitation speed. Microbial growth and metabolism for bioethanol production are effectively controlled by cultural parameters like pH, temperature, and time. The *S. cerevisiae* has been reported to produce maximum bioethanol at 30 °C and pH 6.0 (Łukajtis et al. 2018). Bioethanol production increased with increasing concentration of enzymatic hydrolysate this may be due to the higher concentration of enzymatic hydrolysate the uptake capacity of yeast cells for sugar increases and results in higher ethanol production. A low amount of bioethanol may be due to the presence of oligomeric sugars along with glucose in hydrolysate (Kumar et al. 2020). Łukajtis (2018) reported 8.8 g/L ethanol from alkaline pretreated wheat straw by *S. cerevisiae*. Enzymatic hydrolysate (20%) produced 18.07 g/L ethanol after 72 h using *S. cerevisiae* (Kumar et al. 2020). Kumar et al (2019) reported 78% fermentation efficiency from rice straw hydrolysate using an immobilized enzyme cocktail. In the majority of the studies, predetoxification of hydrolysate is involved before fermentation but in the present study no special detoxification is applied and biomass was simply washed with water to remove any kind of phenolic inhibitors generated after alkaline pretreatment. As evident from

Table 6 Fermentation parameters of enzymatic hydrolysate with *S. cerevisiae* and *Z. mobilis*

Parameter	Fermentation with *S. cerevisiae*			Fermentation with *Z. mobilis*		
	Control	5% Hydrolysate	10% Hydrolysate	Control	5% Hydrolysate	10% Hydrolysate
Initial sugar (g/L)	20	23.17 ± 0.2^{d}	29.15 ± 0.5^{d}	20	23.09 ± 0.4^{d}	29.19 ± 0.7^{d}
Residual sugar	05	13.27 ± 0.7^{c}	12.57 ± 0.3^{c}	12	14.28 ± 04^{c}	13.26 ± 0.5^{c}
Sugar consumption (%)	75	56.11 ± 0.8^{e}	58.65 ± 0.3^{e}	40	39.88 ± 0.6^{f}	55.23 ± 0.6^{g}
Ethanol (g/L)	6.8	3.30 ± 0.2^{b}	6.4 ± 0.3^{b}	3.5	1.6 ± 0.3^{b}	3.07 ± 0.1^{b}
Volumetric ethanol productivity Qp(g/L/h)	0.28	0.14 ± 0.2^{a}	0.26 ± 0.4^{a}	0.14	0.07 ± 0.2^{a}	0.13 ± 0.4^{a}
Ethanol yield Yp/s	0.45	23.3 ± 0.4^{d}	0.36 ± 0.4^{a}	0.43	0.18 ± 0.4^{a}	51.23 ± 0.4^{f}
Efficiency of sugar conversion to Ethanol (%)	90	58.43 ± 0.4^{f}	70.34 ± 0.3^{f}	45	37.76 ± 0.5^{e}	39.18 ± 0.5^{e}

Values are mean ± SD and values with different superscript differ significantly ($p < 0.05$; Tukey test)

Table 7 Ethanol production from rice straw using *S. cerevisiae*

Pretreatment method	Fermentation time (h)	Ethanol production ($g\ L^{-1}$)	Ethanol productivity ($g\ L^{-1}\ h^{-1}$)	Fermentation efficiency (%)	References
5% Maleic acid at 190 °C for 20 min	144	11.2	0.07	62.80	Jung et al. 2015
Combined ultrasonication surfactant and enzyme hydrolysis	72	14.8	0.20	61.25	Sindhu et al. 2016
Alkali + acid (HCl)	72	6.13	0.08	–	Hashem et al. 2013
Steam pretreatment by autoclaving at 121 °C for 30 min	48	1.13	0.02	28.0	Arora et al. 2016
1% NaOH at 121 °C for 30 min	24	4.03	0.16	66.38	Kumar et al. 2019
2% NaOH	24	6.4	0.26	70.34	Present study

the present study, the ethanol production from enzymatic hydrolysate was significantly higher using *S. cerevisiae* than *Z. mobilis*. The *S. cerevisiae* produced 79% more ethanol from 10% hydrolysate compared to *Z. mobilis*. Earlier comparative studies on ethanol production also supported *S. cerevisiae* as a better producer than *Z. mobilis*. Kaur et al (2018) produced 0.170 g/g and 0.137 g/g ethanol from rice straw hydrolysate using *S. cerevisiae* and *Z. mobilis*. A summary of different studies of ethanol production and fermentation efficiency from NaOH-pretreated rice straw using *S. cerevisiae* is represented in Table 7.

Conclusion

The pretreated rice straw with 2% NaOH has achieved 11% delignification with 20.9% cellulose enrichment. The current study shows the effectiveness of crude cellulolytic preparation from *A. niger* resulting in 80.51 ± 0.4% cellulose hydrolysis. Using *S. cerevisiae* 70.34% fermentation efficiency was achieved after 24 h without any detoxification of enzymatic hydrolysate. The results of this study concluded that *S. cerevisiae* and *Z. mobilis* differ significantly with respect to ethanol production *i.e.* better ethanol production was recorded using yeast strain compared to bacterial strain. The *S. cerevisiae* produced 79% more ethanol from 10% hydrolysate compared to *Z. mobilis*. The current study focuses on recent advances in improved bioethanol production: (1) highlighting current results from using novel biomass sources such as rice straw, (2) describing developments in pretreatment technologies for the conversion of lignocellulosic biomass, and (3) listing the use of enzyme cellulase and microbial strains during saccharification and fermentation processes. Keeping in view the need, feasibility, and progress in lignocellulosic bioethanol, rice straw is one of the most important, promising, and potential feedstock not only for bioethanol production but also for future biorefineries.

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Data availability Not available.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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