Ethanol production from Rice (Oryza sativa) straw by simultaneous saccharification and cofermentation

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Ethanol production from alkali treated rice straw was investigated by simultaneous saccharification and cofermentation (SSCF) using commercial cellulase and 3 different yeast strains viz., Saccharomyces cerevisiae HAU-1, Pachysolen tannophilus and Candida sp. individually as well as in combination at varied fermentation temperature and incubation time. Dilute alkali (2%) pretreatment of straw resulted in efficient delignification as observed by low residual lignin (12.52%) with 90.6% cellulose and 28.15% hemicellulose recovery. All the 3 yeast strains were able to produce ethanol from alkali treated rice straw and overall ethanol concentration varied from 5.30 to 24.94 g/L based on different fermentation time and temperature. Comparative analysis of ethanol production from different yeast strains combinations revealed maximum ethanol concentration of 23.48 g/L after 96 h incubation at 35°C with P. tannophilus individually and 24.94 g/L when used as co-culture with Saccharomyces cerevisiae.

Keywords: Bioethanol, Candida sp., Cellulose, Fermentation, Hemicellulose, Lignin, Pachysolen tannophilus, Saccharomyces cerevisiae

Energy security and climate change imperatives require large scale substitution of petroleum based fuels. Bioethanol, a substitute to fossil fuels not only reduces the reliance on oil imports and alleviates uncertainties caused by the fluctuations of oil price, but also secures reduction in environmental pollution problems due to its high oxygen content1. The fermentative production of ethanol using current starch-based technology suffers from raw materials shortage and high cost. A potential method for low-cost fermentative production of ethanol is to utilize lignocellulosic materials2.

The agricultural crop residue such as rice straw is one of the most abundant lignocellulosic wastes on earth. In terms of total production, rice is the third most important grain crop in the world after wheat and corn. About 1-1.5 kg of straw is produced from every kg of the grain harvested3. FAO’s global rice production for 2016-17 is estimated at 495.2 million tonnes (on milled basis) thus indicating abundant production of rice straw annually4. The options for the disposal of rice straw are limited due to its low bulk density, slow degradation in the soil, harbouring rice stem diseases and high mineral content5. Open air burning, the major practice for removing rice straw, pollutes air and leads to asthma and bronchitis in addition to pulmonary morbidity and mortality6. Consequently, use of rice straw in ethanol production would be beneficial both in terms of environmental concern as well as to the farmers.

Most process concepts for bioethanol production from rice straw start with the dissolution of lignin part (pretreatment), followed by the hydrolysis of polysaccharide part i.e., cellulose and hemicellulose (saccharification) and yeast-based fermentation of the resulting sugars. To compete with the price of petroleum, the cost of bioethanol production must be lowered down for the process to prove viable. One way to achieve the purpose is to perform the enzymatic hydrolysis together with the fermentation i.e., simultaneous saccharification and fermentation (SSF) instead of separate hydrolysis and fermentation (SHF). SSF has further been improved to include the cofermentation of multiple sugar substrates i.e., simultaneous saccharification and cofermentation (SSCF)7. The principal benefits of performing enzymatic hydrolysis together with fermentation, instead of a separate step after the hydrolysis, are the reduced end-product inhibition of the enzymatic hydrolysis and the reduced investment costs. The principal drawbacks, on the other hand, are the need to find favourable temperature for both the enzymatic hydrolysis and fermentation and a suitable fermenting organism for both hexose and pentose sugars.

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In the present investigation, we explored the suitable temperature and yeast strain for increased ethanol production and simplified technology operation by SSCF of rice straw.

Materials and Methods

Rice straw of variety “Pusa-1” was procured from farmers’ fields, Village Dabra, Hisar, Haryana, India. Before any pretreatment, it was dried at 50°C and comminuted to small pieces using Wiley grinder. A commercial preparation of cellulase enzyme (Palkosoft super 720) was donated by Maps India Ltd. Ahmedabad, Gujarat. The hexose fermenting yeast strain of *Saccharomyces cerevisiae* (HAU-1) was obtained from culture collection, Department of Microbiology, CCS HAU, Hisar. The pentose fermenting yeast strains *Pachysolen tannophilus* and *Candida* sp. were obtained from the CSIR-Institute of Microbial Technology (IMTECH), Chandigarh and Fermentation Technology laboratory, Department of Microbiology, CCS HAU, Hisar, respectively. All the strains were maintained on medium containing glucose 20.0, peptone 20.0 and yeast extract 10.0 (g L⁻¹) at pH 5.0 by regular sub-culturing and stored at 4°C until use. For inoculum preparation different yeast strains were grown at 30°C in yeast extract peptone sucrose (YEPS) medium containing yeast extract 5.0, peptone 5.0 and sucrose 60.0 (g L⁻¹).

Pretreatment of rice straw

Dried and comminuted rice straw after screening through 0.5 mm sieve was immersed in sodium hydroxide solution (2%) at 1:10 (solid: liquid) and autoclaved at 15 psi for 1 h. Residue was collected and washed extensively with tap water to make it alkali free and dried at 50°C for further use.

Simultaneous saccharification and cofermentation (SSCF)

Dry alkali treated rice straw was suspended in distilled water at 1:10 (solid: liquid). The enzyme was added at a concentration of 7.5 FPU/g substrate as optimized in our earlier study⁸ along with yeast biomass pre-grown in YEPS @ 1% (w/v) and yeast nutrient (urea @ 0.3%). All the 3 yeast strains were tested individually as well as in combination for their ethanol production potential. The flasks were incubated at different temperatures (30, 35 and 40°C) and the samples were analyzed for total reducing sugars and ethanol content at regular intervals after centrifugation at 5000 rpm for 15 min.

Analytical methods

The cellulose, hemicellulose and lignin content of paddy straw was estimated by determining acid detergent fibre (ADF) and neutral detergent fibre (NDF) in the samples as described by the standard procedure, Association of Official Agricultural Chemists⁸. Total nitrogen and organic carbon content was estimated by standard Kjeldahl’s method and volatile solids valuation⁹. The exoglucanase activity of commercial enzyme was estimated following the standard procedure recommended by the Commission on Biotechnology, IUPAC¹⁰. The endoglucanase activity was measured as the rate of reducing sugars formation during hydrolysis of 1% carboxymethylcellulose at pH-4.8 at 50°C. The total reducing sugars were estimated using the 3,5-dinitrosalicylic acid (DNS) method¹¹. Ethanol content was estimated by the method described by Caputi et al.¹². Calculation of carbohydrate (cellulose, hemicellulose and lignin) content in terms of mass balance after alkali treatment was as follows:-

\[
\text{Recovery} = \frac{C_a \times 100}{C_t}
\]

\[C_t = \frac{C_b \times 100}{R_s}\]

where, \(C_a\) = actual carbohydrate content after alkali treatment; \(C_t\) = theoretically expected carbohydrate content after alkali treatment; \(C_b\) = actual cellulose content before alkali treatment; and \(R_s\) = recovery of total solids

Statistical analysis

Data were analysed for statistical significance by the application of complete randomized design (CRD) with 3 replicates. A 5% probability level (\(p = 0.05\)) was used to accept or reject the null hypothesis.

Results

Rice straw contained 35.07% cellulose, 24.85% hemicellulose, 6.29% lignin, 49.82% total organic carbon and 0.85% nitrogen on dry wt. basis (Fig. 1). Treatment of dried and ground rice straw with sodium hydroxide (2%) resulted in 43.27% recovery of total solids. Cellulose, hemicellulose and lignin content in dry matter recovered after alkali treatment was 73.43, 16.16 and 1.82%, respectively (Fig. 1) while the recovery of cellulose and hemicellulose calculated in terms of mass balance (as described in M&M) was 90.6 and 28.15%, respectively. The residual lignin was
The exoglucanase and endoglucanase activities of commercial cellulase were 30 and 33 IU/mL (µ moles of glucose released/mL/min), respectively.

Simultaneous saccharification and cofermentation of alkali treated rice straw was carried out using commercial cellulase and different yeast strains. The ethanol production profile was studied with respect to temperature, incubation time as well as yeast strain combination. Fermentation study at 30°C revealed increased ethanol concentration with increased incubation time for all the 3 yeast strains individually as well as in co-culture. The effect of yeast strains revealed maximum 20.30 g/L ethanol production after 96 h incubation with *P. tannophilus* individually and 15% higher ethanol production i.e., 23.48 g/L when used in combination with *S. cerevisiae* (Table 1). Ethanol production was found to be statistically significant with respect to yeast strains (CD= 0.515; p=0.05). The ANOVA revealed a significant interaction between fermentation time and yeast strain combination (CD= 1.029; p=0.05).

Fermentation studies at 35°C showed only a slight difference in ethanol production after 72 and 96 h incubation. A comparative analysis of yeast strain combination divulged that individually *P. tannophilus* produced maximum ethanol 23.08 g/L after 72 h incubation while a slight acclivity (23.48 g/L) after 96 h incubation (Table 1). Combination of *P. tannophilus* with *S. cerevisiae* further hiked ethanol production to 24.68 g/L after 72 h incubation (Table 1). Ethanol production drastically reduced at 40°C with all the yeast strains individually as well as in combination. Combined effect of fermentation time and yeast strain revealed the production of maximum 11.67 g/L ethanol by co-culture of *P. tannophilus* and *S. cerevisiae* after 96 h incubation. Analysis of rice straw hydrolysate after SSCF at 35°C with *S. cerevisiae* and *P. tannophilus* for 72 h revealed 6.13% w/v residual sugars and 14.41% residual cellulose.
Discussion

Dilute alkali treatment of straw biomass dissolved the complex lignin network to a great extent with release of polysaccharides (cellulose and hemicellulose) for hydrolysis by cellulolytic enzymes. Delignification showed the apparent increase in cellulose fraction from 35.07% to 73.43% (about 110% rise) while decrease in hemicellulose fraction from 24.85% to 16.16% (about 35% fall) (Fig. 1). The increase in cellulose content is believed to be too significant to be only due to the lignin and hemicellulose removal. The apparent rise and fall in cellulose and hemicellulose fraction has been reported by other group of researchers as well. Kim et al.\(^1\) reported increase in cellulose content from 39.5 to 52.5 g/100 g dry wt. while decrease in hemicellulose content from 24.4 to 1.3 g/100 g dry wt. in two-stage pretreatment of rice straw. Karthik et al.\(^14\) reported 43.37% increase in cellulose content of sorghum biomass on dilute acid pretreatment. In terms of mass balance, cellulose recovery was more compared to hemicellulose. This could be attributed to the low degree of polymerization and amorphous nature of hemicellulose and its higher solubility in alkali.

Dry alkali treated rice straw was subjected to ethanolic conversion by simultaneous saccharification and cofermentation (SSCF). The SSCF of lignocellulosic residues generally proceeds with a lag phase due to inhibition by pretreatment byproducts and a compromise on saccharification temperature. The present investigation on SSCF of rice straw demonstrated significant ethanol production after 24 h of fermentation indicating the absence of lag phase. This may be due to the dilute alkali pretreatment which forms lesser inhibitory byproducts and these have further been removed by water washing procedure.

Increase in fermentation temperature from 30 to 35°C showed a marked increase in ethanol production at all fermentation time and yeast strains while further increase from 35 to 40°C exhibited explicit decrease (Table 1). Though ethanol production was statistically significant with respect to fermentation time (CD=0.345; p=0.05) at 35°C but seems unsubstantial in terms of energy cost of rice straw ethanol conversion as indicated by only a slight difference in ethanol concentration values after 72 and 96 h fermentation (Table 1). Comparing the effect of yeast strains, \(S.\ cerevisiae\) (hexose fermenting) in combination with \(P.\ tannophilus\) (pentose fermenting) brought about best fermentation as evidenced by maximum ethanol production (24.68 g/L) with this yeast strain combination (Table 1). Rice straw ethanolic conversion, thus found to take place optimally at 35°C with \(S.\ cerevisiae\) and \(P.\ tannophilus\) combination after 72 h incubation by simultaneous saccharification and cofermentation. Oberoi et al.\(^15\) studied ethanol production from sulfuric acid (2% w/v) pretreated rice straw fermented with 10% (w/v) hydrolysate-adapted \(Candida\ tropicalis\) ATCC 13803 by SSCF and reported that the adapted cells produced about 1.6 times more ethanol than non-adapted cells. Li et al.\(^16\) reported 21.1 g/L ethanol production within 80 h by simultaneous saccharification and fermentation of paddy straw from 10% (w/w) of lime-pretreated and CO\(_2\)-neutralized rice straw by sequential use of \(S.\ cerevisiae\) and \(Pichia\ stipitis\) with heat inactivation of \(S.\ cerevisiae\) cells prior to xylose fermentation.

Though most of the cellulose had been hydrolyzed to sugars, the fermented residue still contained about 14.41% cellulose. This may be either crystalline cellulose or cellulose bound to the residual lignin. The fermented residue being rich in protein due to yeast biomass could be used as feed supplement.

Conclusion

The current work shows successful ethanol production from rice straw by simultaneous saccharification and cofermentation. The simpler SSCF process, where the substrate, cellulase enzyme and the yeast are all present in the reactor initially, significantly reduces the initial investment cost and process economics. Use of thermotolerant yeast strains in SSCF to allow fermentation at temperatures closer to the optimal hydrolysis temperature may further result in better saccharification, and thereby increase ethanol yield. Genetic engineering of yeast strains for better utilization of pentose sugars may further improve the rice straw ethanol production.

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References


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