Indian Journal of Microbiology (March 2007) 47:26–29

ORIGINAL ARTICLE

Paddy straw as substrate for ethanol production

L. Wati · S. Kumari · B. S. Kundu

Received: 20 June 2006/ Final revision: 15 January 2007 / Accepted: 16 January 2007

Abstract Pretreatment of paddy straw with 2% sodium hydroxide at 15 psi for 1 h resulted in 83% delignification. The hydrolysis of alkali treated paddy straw with a commercial preparation of cellulase for 2 h at 50°C resulted in release of 65% total reducing sugars. Maximum sugars were released at enzyme loading of 1.5% (v/v) .The fermentation of hydrolysate supplemented with nutrients by *S. cerevisiae* resulted in the production of 20–30 g L^{-1} ethanol after 48 h incubation which was further improved with addition of yeast nitrogen base and inoculated with 1% (w/v) yeast cells.

Key words Cellulose · ethanol · fermentation · paddy straw · S. *cerevisiae*

L. Wati (✉) · S. Kumari · B. S. Kundu
Department of Microbiology,
CCS Haryana Agricultural University,
Hisar - 125 004
India.
e-mail: lwkraj@hau.ernet.in
Tel: +91 / 1662 / 237720-26 Extn. 9292(O)

Introduction

Recently the demand of ethanol has increased considerably because of its use as gasohol in addition to other applications in industries which need production of alcohol on large scale[1]. Many efforts have been made in recent years to enhance ethanol production from different sources[2]. Fuel ethanol can become self-sustainable system only with the use of lignocelluloses as the other sources are limiting and costly[3,4]. Feasibility of lignocellulosic materials for ethanol production has been explored around the world depending upon availability. Lignification, silcification and crystallinity of cellulose are major barriers in the process of conversion of lignocellulosic biomass into ethanol. It is essential to alter or remove structural and compositional impediments to hydrolysis by pretreatment to improve rate of hydrolysis. Hydrolysis of cellulose by acids or cellulase results into monomers. Enzymatic methods have the advantage of being eco-friendly besides applicable under mild conditions of hydrolysis. These methods provide opportunities to develop technology for biomass ethanol production at competitive rate compared to other fuels.

Rice is a major crop grown worldwide with an annual productivity around 800 million metric tones that corresponds with large production of rice straw.

The straw is removed from the field by burning which is a common practice all over the world. The impact of open field burning of paddy straw on air quality has led to legislation, which will help in future to check this practice and will save plant nutrients. In the search for viable alternatives of biofuels, paddy straw has been pursued as suitable lignocellulosic waste for ethanol production in a process involving chemical pretreatment followed by enzymatic hydrolysis.

Materials and Methods

Paddy straw of Pusa–1 variety was collected from fields, dried at 50°±2°C, ground to different mesh size and analyzed for ash content, total nitrogen, cellulose, hemi cellulose and lignin using standard methods[5]. Commercial preparation of cellulase (Palkosoft super 720) was obtained from Maps Ltd. Ahmedabad (Gujrat) and enzyme activity was measured according to standard procedure[6].

Paddy straw was pretreated with 0.5 M sodium hydroxide at 1:10 (solid: liquid) for 1 h at 15 psi and the residue was freed of alkali by washing with water and dried at 50±2°C for subsequent use. Dry alkali treated paddy straw was suspended in citrate buffer (pH 5.0) at 1:10 (solid: liquid) in a conical flask of 250ml capacity. The enzyme was added at different concentrations (0.5–2.0%, v/v) and the reaction carried out at 50°±2°C in a shaking water bath for different time intervals (0.5–2.5h). The hydrolysate was centrifuged at 5000 rpm for 15 min and the total reducing sugars were estimated in the supernatant by di-nitrosalicylic acid method.[7] The saccharification value was calculated as:

$$\frac{\text{Reducing sugars produced}}{\text{Cellulose produced from substrate}} \times 0.9 \times \text{dilutions}$$

Strains of *Saccharomyces cerevisiae* were procured from the culture collection Department of Microbiology, CCS HAU, Hisar for fermentation of hydrolysate. The yeast cultures were maintained on medium containing 20.0 g glucose, 20.0 g peptone and 10.0 g yeast extract L^{-1} after subculturing at regular time intervals and stored in a refrigerator. The biomass of yeast after growth for 18 h at 30°±2°C in a medium containing 60.0g sucrose, 5.0g yeast extract and 5.0g peptone L^{-1} was centrifuged at 5000 rpm for 15min and inoculated into the hydrolysate at a conc. of 0.5–1.5% (w/v). The fermentation was carried out in 250 ml conical flasks containing 100ml hydrolysate supplemented with nitrogen (0.3**%** ammonium sulphate or urea), phosphorous (0.15% potassium di-hydrogen phosphate**)** and growth factors (0.5%yeast extract) or yeast nitrogen base (0.67%) or yeast extract (0.5%) and peptone (0.5%). The flasks were incubated at 30±2°C under stationary conditions and samples were analysed for ethanol content colorimetrically[8].

Results and Discussion

The paddy straw contained cellulose 35.0%, hemi cellulose 21.0, lignin 6.0, total nitrogen 1.24 and ash 16.0 % on dry weight basis. The enzymatic hydrolysis needs reduction in size of paddy straw to facilitate subsequent heat and mass transfer. The different mesh size paddy straw (0.5–4 mm) were delignified and used for hydrolysis. Increase in size of paddy straw decreased delignification and maximum of 83% delignification occurred at 0.5mm size, which was 50% at 2mm size. Paddy straw above 4mm was poorly delignified under test conditions (Table 1). Similar trend was also observed for hemicellulose degradation as a result relative concentration of cellulose in the residue increased. Similar observations were made by other workers for sodium hydroxide treatment of other substrates[11].

Alkali treated paddy straw of 0.5mm was finally selected for standardization of conditions for enzymatic hydrolysis. The enzyme preparation had an activity of 32 filter paper units (FPU)/ml. Loading of enzyme at a conc. of 0.5–2.0% (v/v) resulted in production of 37–65% total reducing sugars

Table 1 Composition of different particle size paddy straw (% dry weight basis) after alkali treatment.

Particle size (mm)	Paddy straw components		
	Cellulose	Lignin	Hemicellulose
0.5	72 (105.7)	1(83.3)	9 (57.1)
1.0	70 (100.0)	2 (66.7)	12 (42.9)
2 .0	64 (82.9)	3 (50.0)	18 (14.3)
3.0	57 (57.1)	5 (16.6)	18 (14.3)
4 .0	41(17.1)	6 (0)	20 (4.8)

* Figures in parenthesis indicate % change in the value due to alkali treatment as compared to the value for untreated sample

Table 2 Effect of cellulase concentration and incubation period on reducing sugars released from alkali treated paddy straw*

Incubation period (h)	Reducing sugars (% w/w)	Saccharification value	Enzyme conc. (%, v/v)	Reducing sugars (%, w/w)	Saccharification value
0.5	28.3	35.2	0.5	36.6	45.8
1.0	43.2	54.0	1	43.3	54.1
1.5	51.2	64.0	1.5	64.7	80.8
2.0	65.0	81.2	2.0	65.2	81.4
2.5	65.4	81.7	–	–	–

* Alkali treated paddy straw was used at 10% conc. and reaction was carried out at 50°±2°C in a shaking water bath.

Table 3 Effect of particle size on release of reducing sugars from alkali treated paddy straw*.

Particle size (mm)	Reducing sugars (% w/w)	Saccharification value
0.5	65.0	81.2
1.0	55.0	70.7
2 .0	46.4	65.2
3.0	38.0	59.9
4 .0	16.3	35.7

* Alkali treated paddy straw was used at 10% concentration and reaction was carried out at 50°C in a shaking water bath.

(Table 2). Maximum concentration of sugars was obtained at 1.5 % (v/v) suggesting that about 5FPU/g substrate of enzyme was optimum for hydrolysis of cellulose. Maximum sugars were generated at reaction time of 2h and further increase did not have any effect on sugar concentration. Sugar concentration decreased with increase in particle size of paddy straw and maximum was at 0.5mm size (Table 3).

The results from different workers with a cellulase fraction of *Trichoderma* sp.(IMB-Tr) and *Trichoderma reesei* obtained 30% reducing sugars from alkali pretreated paddy straw[12] while Vlasenko *et al*.(1997) showed the release of 37–46% sugars using 6.7% FPU/g of commercial *Trichoderma reesei* derived enzyme.

Table 4 Effect of nutrients, inoculum size and temperature on ethanol production from alkali treated paddy straw.

Parameters	Ethanol produced (gL^{-1})	
	Yeast strain 1	Yeast strain 2
Nutrients		
Yeast nitrogen base	30.7	27.8
Peptone + Yeast Extract(Y.E.)	23.6	20.7
Ammonium sulphate + Potassium di hydrogen phosphate + Yeast extract	30.0	25.9
Urea + Potassium di hydrogen phosphate + Yeast extract	26.0	22.6
Inoculum level (% w/v)		
0.5	23.6	22.2
1.0	30.7	27.5
1.5	30.7	28.0
Temperature (°C)		
30	31.6	27.8
37	21.3	20.2
40	15.7	16.8

The hydrolysate contained mainly sugars but for efficient fermentation by yeast nutrients are required. The recovery of ethanol was highest with yeast nitrogen base followed by ammonium sulphate, di-hydrogen phosphate and yeast extract (Table 4). The inorganic nitrogen source proved better among organic and inorganic nitrogen source supplemented to the hydrolysate.

Variable population of yeast in the fermentation was checked. One percent inoculum was optimum for fermentation (30.7 gL^{-1} ethanol). This shows that the cell number added was sufficient to produce highest amount of alcohol in 48h at 30±2°C.The variation in fermentation temperature (30–40°C) was checked for alcohol production under lab conditions. The optimum temperature for ethanol fermentation of hydrolysate by the yeast was 30±2°C. The increasing temperature had adverse effect as the alcohol production declined to 33% at 37°C.The results show that the feasibility is poor for fermentation of hydrolysate at a temperature suitable for cellulase activity. However, simultaneous fermentation could be possible with thermotolerent yeast[14] as cellulases are most active at 50°C.

Conclusion

Pretreatment of paddy straw with alkali followed by hydrolysis with commercial cellulase provide a good substrate for ethanol production by *S.cerevisiae*. This yeast can utilize only hexoses however some pentoses are released also and about 10% sugars in the hydrolysate remain unutilised. Further improvement in ethanol yield can be achieved using co-culture of hexose and pentose-fermenting yeast.

Acknowledgements Financial assistance from Indian Council of Agricultural Research is greatfully acknowledged.

References

1. Wheals AE, Barso LC, Alves DMG & Amorim HV (1999) Fuel ethanol after 29 years. Trends Biotechnol *17*: 482–487.
2. Ward OP (2002) Bioethanol technology development and Perspective. Adv Appl Microbiol 51:53–80.
3. Ballerini D, Desmarquest JP & Pourquic J (1994) Ethanol production from lignocellulosics: large scale experimentation and economics. Bioresource Technol 50:14–23.
4. Lee J (1997) Biological conversion of lignocellulosic biomass to ethanol. J Biotechnol 56:1–24.
5. Annonymous (1970) Official Methods of Analysis. Assoc. Official Agri Chemists 11th edn Washington, DC.
6. Ghosh, TK (1987) Measurement of cellulase activities. Pure Appl chem 59:257–268.

7. Miller GL (1959) Use of di nitro salicylic acid reagent for estimation of reducing sugars. Anal Chem **31**:426–428.
8. Caputi P, Vede JM & Brown T (1968) Spectrophotometric determination of chromic complex formed during oxidation of ethanol.Am J Enol Vitic 19:1601–1665.
9. Schell JS, Riley CJ, Dowe N, Farmer J, Ibsen KN, Ruth MI, Toon ST & Lumpkin RI (2004) A bioethanol process development unit: initial operating experiences and results with a corn fiber feedstock. Bioresource Technol 91:179–188.
10. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M & Ladish M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technol 9:637–686.
11. Sharma SK, Kalra KL & Kocher GS (2004) Fermentation of enzymatic hydrolysate of Sunflower hulls for ethanol production and its scale up. Biomass Bioenergy 27:399–402.
12. Peiris PS & Silva I (1987) Hydrolysis of rice straw to fermentable sugar by *Trichoderma* enzymes. MIRCEN J 3: 57–65.
13. Vlasenko E Yu, Ding H, Labavitch JM & Shoemaker SP (1997) Enzymatic hydrolysis of pretreated rice straw. Bioresource Technol 59: 109–119.
14. Ahring BK, Licht D, Schmidt AS & Sommer P (1999) Production of ethanol from wet oxidized wheat straw by *Thermoanaerobacter mathranii.* Bioresource Technol 68: 3–9.