Madras Agric. J., 101 (1-3): 102-104, March 2014

Sugarcane Bagasse as Substrate for Ethanol Production

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Pre treatment of sugarcane bagasse with 2% sodium hydroxide at 1.40 kg cm^{-2} for 1 h resulted in 83% delignification. The hydrolysis of alkali treated bagasse 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 of incubation, which was further improved with addition of yeast nitrogen base and inoculated

Key words : Cellulose, Ethanol, Fermentation, Sugarcane bagasse, S. *cerevisiae*

Recently, the demand of ethanol has increased considerably because of its use as gasohol, in addition to other applications in industries, which demands production of alcohol on large scale (Wheals, 1999). Many efforts have been made to enhance ethanol production from different sources (Ward, 2002). Fuel ethanol can become self-sustainable system, only with the use of lignocelluloses as other sources are limiting and costly (Ballerini, 1994)

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 as compared to other fuels.

For production of fuel ethanol, it is desirable to use cheaper and more abundant substrates. When producing ethanol from maize (made up of starch chains) or sugarcane (in the form of either cane juice or molasses), the raw material constitutes about 40–70% of the production cost (Quintero et al., 2008). By using waste products from forestry, agriculture and industry, the costs of the feedstocks may be reduced. Lignocellulose (complex polymer made up of cellulose hemicellulose and lignin) is considered as an attractive feedstock for the production of fuel ethanol, because of its availability in large quantities at low cost (Cheng *et al.*, 2008); and for reducing competition with food, but not necessarily with feed. Today, the production cost of ethanol from lignocellulose is still too high, which is the major reason why ethanol from this feedstock has not made its breakthrough yet.

Numerous studies for developing production of ethanol from lignocellulosics have been carried out in the world. However, the main limiting factor is the higher degree of complexity inherent to the processing of this feedstock. This is related to the nature and composition of lignocellulosic biomass (which contain up to 75% of cellulose and hemicelluloses). Cellulose and hemicelluloses should be broken down into fermentable sugars in order to be converted into ethanol or other valuable products (xylans, xylitol, hydrogen and enzymes). But this degradation process is complicated, energy-consuming and non-completely developed (Sánchez and Cardona, 2008). With the advent of modern genetics and other tools, the cost of producing sugars from these recalcitrant fractions and converting them into products like ethanol can be significantly reduced in the future.

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Materials and Methods

Sugar cane bagasse of CO-86032 variety was collected from Sugar cane Breeding Institute (SBI),Coimbatore and 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 (AOAC,1970). Commercial preparation of cellulase (Palkosoft super 720) was obtained from Maps Ltd. Ahmedabad (Gujrat) and enzyme activity was measured according to standard procedure (Ghosh, 1987).

Sugarcane bagasse was pretreated with 2 % sodium hydroxide at 1:10 (solid: liquid) for 1 h at 1.40 kg cm^{-2} steam under pressure and the residue was freed of alkali by washing with water and dried at 50+2°C for subsequent use. Dry alkali treated bagasse was suspended in citrate buffer (pH 5.0) at 1:10 (solid: liquid) in a conical flask of 250 ml 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 (0.5–2.5 h). 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 (Miller, 1959). 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 MTCC 181 were procured from Microbial Type Culture Collection (MTCC), Chandigarh for fermentation of hydrolysate. The yeast cultures were maintained on medium containing 20.0 g glucose, 20.0 g peptone and 10.0 g of yeast extract l^{-1} after sub culturing 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 60.0 g sucrose, 5.0 g yeast extract and 5.0 g of peptone l^{-1} was centrifuged at 5000 rpm for 15 min and inoculated into the hydrolysate at a concentration of 0.5-1.5% (w/v). The fermentation was carried out in 250 ml conical flasks containing 100 ml hydrolysate supplemented with nitrogen (0.3 % ammonium sulphate or urea), pho sph orous (0.1 5% 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 (Caputi, 1968).

Results and Discussion

The sugarcane bagasse contained cellulose (37.0 %), hemi cellulose (28.0 %), and lignin (18.0 %), respectively on dry weight basis. The enzymatic hydrolysis needs reduction in size of bagasse straw to facilitate subsequent heat and mass transfer.

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

Particle size (mm)	Sugarcane bagasse components		
	Cellulose	Hemicellulose	Lignin
0.5	72(105.7)	9(57.1)	1(83.3)
1.0	70(100.0)	12(42.9	2(66.7)
2.0	64(82.9)	18(14.3)	3(50.0)
3.0	57(57.1)	18(14.3)	5(16.6)
4.0	41(17.1)	20(4.8)	6(0)

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

The different mesh size bagasse (0.5-4 mm) were delignified and used for hydrolysis. Increase in size of bagasse decreased delignification and a maximum of 83% delignification occurred at 0.5 mm size, which was 50 % at 2 mm size. Bagasse above 4 mm was poorly delignified under test conditions (Table 1).

Table 2. Effect of cellulose concentration and incubation period on reducing sugars released from alkali treated sugarcane bagasse*

Incub-ation period (h)	Redu-cing sugars (% w/w)	Saccha rification value	Enzyme conc. (% v/v)	Redu-cing sugars (%, w/w)	Saccha rification value
0.5	28.3	35.2	0.5	36.6	45.8
1.0	43.2	54.0	1.0	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 sugarcane bagasse was used at 10% conc. and reaction was carried out at 50 °C+2 °C in a shaking water bath

Similar trend was also observed for hemicellulose degradation as a result, relative concentration of cellulose in the residue increased. This kind of observations were made by Sharma (2004) for sodium hydroxide treatment of other substrates

Alkali treated sugarcane bagasse of 0.5 mm 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 concentration of 0.5 – 2.0% (v/v)resulted in production of 37-65 % total reducing sugars (Table 2). Maximum concentration of sugars was obtained at 1.5 % (v/v), which revealed that about 5 FPU / g substrate of enzyme was optimum for hydrolysis of cellulose. Maximum sugars were generated at a reaction time of 2 h and further increase did not have any effect on sugar concentration. Sugar concentration decreased with increase in particle size of bagasse and the maximum was at 0.5 mm size (Table 3). The results from *Trichoderma reesei* obtained 30 % reducing sugars from alkali pretreated paddy straw (Peiris, 1987), while Vlasenko *et al.*(1997) showed the release of 37-46 % sugars using 6.7% FPU / g of commercial *Trichoderma reesei* derived enzyme.

Table 3. Effect of particle size on release of reducing sugars from alkali treated sugarcane bagasse*

Particle size (mm)	Reducing sugars (% w/w)	Saccharification value
0.5	65	81.2
1.0	55	70.7
2.0	46.4	65.2
3.0	38	59.9
4.0	16.3	35.7

* Alkali treated sugarcane bagasse was used at 10 % concentration and reaction was carried put at 50 °C in a shaking water bath

The hydrolysate contained mainly sugars, but for efficient fermentation by yeast additional nutrients are required. The recovery of ethanol was the 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 sources of nitrogen supplemented to the hydrolysate.

Variable population of yeast in the fermentation was checked. One per cent inoculum was optimum for fermentation (30.7 g l–1 ethanol). This shows that the cell number added is sufficient to produce the highest amount of alcohol in 48 h 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 showed that the feasibility was poor for fermentation

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

Parameters	Ethanol produced (g L^{-1})	
Nutrients	Yeast strain 1	Yeast strain
Yeast nitrogen base	30.7	27.8
Peptone+Yeast Extract (Y.E)	23.6	20.7
Ammonium sulphate+ Potassium di hydrogen	30	25.9
Urea + Potassium di hydrogen phosphate + Yeast extract	26	22.6
Inoculum level (% w/v)		
0.5	23.6	22.2
1	30.7	27.5
1.5	30.7	28
Temperature °C		
30	31.6	27.8
37	21.3	20.2
40	15.7	16.8

* Alkali treated sugarcane bagasse was used at 10 % concentration and reaction was carried put at 50 °C in a shaking water bath

of hydrolysate at a temperature suitable for cellulase activity. However, simultaneous fermentation could be possible with thermotolerent Yeast (Ahring, 1999), as cellulases are most active at 50°C.

Conclusion

Pre treatment of sugarcane bagasse 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 also released and about 10% sugars in the hydrolysate remained unutilized. Further improvement in ethanol yield can be achieved by using co-cultures of hexose and pentose fermenting yeasts.

Acknowledgement

The authors gratefully acknowledge the University Grand Commission (UGC), New Delhi for funding Rajiv Gandhi National Fellowship (RGNF) and Department of Bioenergy, Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore for technical and infrastructural support.

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Received: June 1, 2013; Accepted: July 30, 2013