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Enzymatic Saccharification of Bagasse Pith for Bioethanol Production by using Strain of Saccharomyces Cerevisiae

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Abstract

Lignocellulosic biomass is a potential renewable resource for the production of bioethanol. It is the most abundant plant material resource; its susceptibility has been curtailed by its rigid structure. Biofuels produced from various lignocellulosic biomasses such as agroforest residues have been recognized to have potention, to be available substitute to gasoline. Sugarcane processing generates a large content of bagasse pith. Disposal of bagasse pith is critical for both agricultural profitability and environmental protection. Sugarcane bagasse pith is a renewable source that can be used to produce ethanol and many other value added products. In present study, ethanol production from sugarcane bagasse pith hydrolysate by strain of yeast *Saccharomyces cerevisiae* was analyzed using response surface methodology. Pretreatment of bagasse pith by different acid (H_2SO_4) concentration shown that total Reducing sugar (TRS) 34.66 g/l i.e., maximum sugar content, was observed in case of dilute acid pretreatment, 10%w/w in Bagasse pith. The yield of sugar was highest in case of Bagasse pith enzymatic hydrolyzate with 6% v/w NAT Cellulase followed by 2% Glagosidase is 32.99 g/l. In batch fermentation, optimization of key process variables resulted in ethanol concentration was 7.73% (v/v).

Keywords: *Sugarcane; Bagasse pith; Pretreatment Lignocellulose; Saccharomyces* ***cerevisiae and Sachharification***

1. Introduction

Bioethanol is an attractive, sustainable energy source to fuel transportation. Our economy and lifestyle mostly rely on the use of fossil fuels because major energy source (about 80%) comes from fossil fuels (Demirbas, 2007). Depending on the production and consumption rates, the presently known reserves of fossil fuels will not appreciably run out for at least 100 years or more (Goldemberg, 2007). In addition, the unfettered use of fossil fuels shows negative impacts on the environment because of emission of greenhouse gases (CO_2, CH_4 and CO) resulting in global warming and pollution (Saratale et al., 2008). Based on the premise bioethanol can contribute to a cleaner environment and with the implementation of environmental protection laws in many countries, demand for this fuel is increasing. For large-scale biological production of fuel ethanol, it is desirable to use cheaper and more abundant substrates. India is the world's largest producer of sugar cane producing nearly 357 million tonnes per annum and nearly 60% of the cane is utilized for the production of sugar of which about $1/3^{rd}$ of the total sugarcane is obtained as residue so called bagasse containing appreciable quantity (up to 35% w/w) of the pith. Presently the pith which is considered to be the undesirable entity from view point of papermaking is separated and pith used as a source of fuel of biomass. Sugarcane bagasse has a complex structure, and is primarily composed of cellulose (20-50%), hemicelluloses, and lignin (15-30%) (Jain RK *et al*. 2011). The use of bagasse in the production of paper products is becoming increasingly more important (Taylor, 2000; World Centric, 2008). Conversion of lignocellulosic sugar hydrolysate into ethanol requires many other micro and macro elements apart from fermentable nitrogen which in right balance can always give optimum product yield. Statistical screening in this context provides a rapid whereby a perfect strategy can be materialized to improve targeted product yield (Dasgupta *et al.* 2013) Response surface methodology (RSM) explores the relationships between several explanatory operating variables and one or more response variables and has been widely applied for optimization of ethanol production from various substrates (Uncu & Cekmecelioglu 2011; Jargalsaikhan & Saraçoğlu 2009). Ethanol can be regarded as a more environment friendly fuel than gasoline because it releases only some amount of carbondioxide to atmosphere in compare to gasoline. Efforts were made to achieved maximam prehydrolysis and sachharification efficiency to obtain higher yields of fermentatble sugar and ethanol. The aim of the present study is to optimization the conditions of process for the pretreatment of biomass (prehydrolysis) and sachharification of the prehydrolysed basses pith through chemical and enzymatic route to produce maximum quantity of fermentable sugars to convert it into the bio ethanol by the help of *Saccharomyces cerevisiae*.

2. Material and Methods

2.1 Materials

2.1.1 Raw Material

The raw material used was bagasse pith which was procured from the Bagasse based paper mill. Procured Bagasse was subjected to depithing by CPPRI patented method.

2.1.2 Microorganism used
Saccharomyces cerevisiae i.e. obtained from biotechnology Lab, CPPRI
2.1.3 Enzyme used
Commercial Enzymes Cellulase I & Cellulase II

2.2 Methods
Two steps used for hydrolysis of Bagasse pith: 1. Pretreatment by dilute acid for maximum yield of sugar. 2. Enzymatic hydrolysis of pretreated Bagasse pith for maximum yield of fermentative sugar (i.e. glucose). Pretreatment of Bagasse pith was done by dilute acid pretreatment of H_2SO_4. After prehydrolysis residual biomass was subjected to yield analysis & the hydrolyzate was analyzed for TRS, TS, Xylose, and Lignin & Phenolics. The effect of temperature and pH on enzyme activity was checked between 40-70°C temperature ranges similarly the limit of pH was also established from 4.0 to 8.0. Enzyme activity is estimated by T.K.Gosh (1987) method. Bioethanol production then done by Fermentation of Enzymatic saccharified Hydrolyzate, was carried out by *Saccharomyces cerevisiae* under conditions given in Table 1.

Table 1: Condition for Fermentation of enzymatic hydrolyzate by *S.cerevisiae*

Parameters	**Conditions**
Inoculum size	10% (v/v)
Temperature	34˚C
pH	5.5
Retention time	Observation at 32 hrs

As fermentation was done, next step was to estimation of ethanol calorimetrically as described by Caputi et.al. 1968 .Total ethanol conc. in the medium was estimated by chromic acid method i.e. measuring absorbance at 584 nm using a spectrophotometer.

3. Results & Discussion

3.1 Conversion of Bagasse Pith into Bioethanol
A combined action of Microorganism i.e. Saccharomyces cerevisiae and commercial enzyme was needed for the production of ethanol. These agents carry out the process of fermentation that converts sugars to ethanol.
3.2 Pre Hydrolysis of Bagasse Pith
Dilute acid prehydrolysis of bagasse pith was done according to the procedure mentioned in experimental work section table 2. The pre-hydrolyzate was analyzed for various parameters like Total Reducing sugar (TRS), Total Sugar (TS), Lignin and Phenolics. These lignin-associated materials are more easily oxidized during the bleaching process (Lee, 2005)

Table 2: Result of Pretreatment by dilute Acid & Steam.

Parameter	BP/4/90	BP/6/90	BP/8/90	BP/10/90
Yield, %	81.85	78.28	75.40	74.14
T.R.S .g/l %	26.88	27.90	29.76	34.66
	16.12	15.94	19.83	23.10
T.S g/l%	17.96	17.37	23.24	23.83
	10.77	9.92	15.49	15.88
Xylose g/l %	5.399	12.85	17.44	20.46
	3.24	7.34	11.63	13.64
Lignin g/l %	1.86	1.95	1.97	2.31
	1.12	1.11	1.31	1.54

Above results showed that maximum sugar i.e. 34.66 g/l was obtained with 10% H_2SO_4 pretreatment but the difference between TRS at 10% and 8% is minimum i.e. they are approximately same. So, pretreatment with 10% H_2SO_4 was further preceded for the enzymatic Saccharification studies. Same work is done by Jain R.K *et al.* 2011.

The maximum enzyme activity was shown at temperature 50 °C and pH 5.0 for both FPase and CMCase activity.

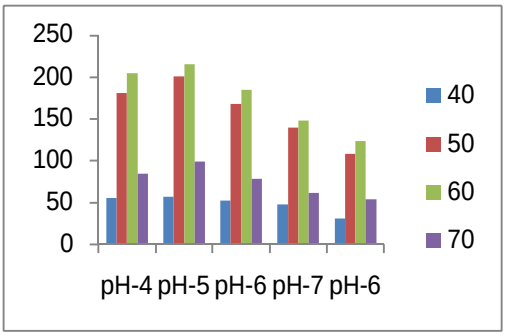
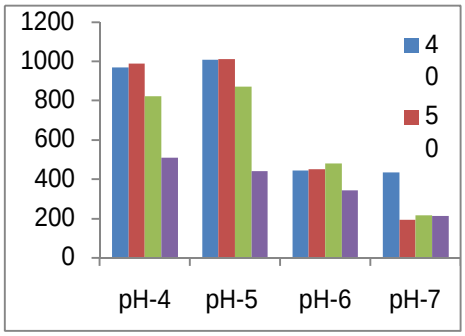


Fig:1 ***FPase activity***



CMCase activity

The Analysis of enzymatic saccharified hydrolyzate was for various parameters like Total Reducing sugar (TRS), Total Sugar (TS), Lignin and Phenolics was given in table 3.

Table 3. Result of Optimization of Enzyme concentration of Bagasse Pith.

Enzymatic sachharification Parameters	EBP/2/50/24	EBP/4/50/24	EBP/6/50/24
Yield, %	91.75	88.98	89.34
TRS (g/l) %	24.83	27.60	32.99
	16.88	18.49	20.45
TS (g/l) %	22.91	23.87	28.6
	30.92	31.03	38.61
Xylose(g/l)	6.75	4.8	10
%	4.59	3.21	6.2
Lignin (g/l)	0.78	0.84	0.95
	0.5	0.56	1.0

3.3 Enzymatic Sachharification

Fermentation of ethanol with initial enzymatic hydrolyzate TRS conc. 32.99 g/l was performed by *Saccharomyces cerevisiae.* TRS, Cell growth and ethanol concentration analyzed with different time interval shown in table 4. The process was carried out for 48 hrs. Interestingly, the combination of enzymes used for full hydrolysis did not include a pure cellobiase preparation like Novozyme 188, which was previously used for sugarcane hydrolysis (Martin et al., 2002). It is possible that cellobiase is present in some of the commercial enzyme preparations tested. Another explanation comes from the difference between steam-enzymatic treatments reported in other studies (Martin et al., 2002) and the alkaline–enzymatic approach presented here. Alkaline treatment delignifies more efficiently than regular steam explosion (Rodriguez-Vazquez and Diaz-Cervantes, 1994).

Table 4. Ethanol production from Bagasse pith Enzymatic Hydrolyzate by Saccharomyces cerevisiae

Time(hrs)	**Turbidity at 600nm**	**TRS (g/l)**	**Ethanol %(v/v)**
0	0.138	32.99	0.0
3	0.377	25.98	4.67
5	0.523	24.91	5.41
8	1.073	24.10	5.76
24	2.575	22.39	6.58
48	3.750	13.63	7.73

Result of the fermentation studies of enzymatic hydrolyzate Bagasse pith by *Saccharomyces cerevisiae* showed that ethanol production efficiency is 7.73 % (v/v).

4. Conclusions

The studies indicated that the bagasse pith available as a waste biomass from paper mill prove to be a potential substrate alternate to the conventionally used raw materials for the production of bio ethanol. It is important before sachharification to convert the cellulose ion to fermentable sugars under given optimized process conditions. Enzymatic sachharification route results it to higher yield of fermentable sugars thereby providing opportunity for higher bioethanol yield.

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