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RESEARCH ARTICLE

EFFECT OF DIFFERENT PROCESSING PARAMETERS ON BIOETHANOL PRODUCTION FROM CORN (Zea *mays*) COB BY ENZYMATIC HYDROLYSIS

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ABSTRACT

Corn (*Zea mays*) Cob is rich source of cellulose, so this cellulosic material was utilized for ethanol production. The cellulosic material was pretreated with 1% H_2SO_4 at 108^0 C Temp. by taking 1:5 proportion (solid : liquid). The pretreated cellulosic material was hydrolyzed with cellulase enzyme (conc. in FPU) at 60^0 C Temp., fermented by *sacchromyces serevisiae 3090* at 30^0 C Temp. for 72 hrs. It was found that the hydrolysis time was increased at the interval of 12 hrs., the yield of reducing sugar and ethanol yield was increased i.e. 50.30% & 28.67 % resp. The particle size was inversely proportional to ethanol yield. Also substrate concentration should be minimize as such as possible for hydrolysis because the reducing sugar & ethanol yield (24.79% & 23.99%) had a similar variation trend with cellulase dosages varying from 10 to 30 FPU/ gm. substrate.

INTRODUCTION

Maize (*Zea mays*) known as corn utilized in more diversified ways than any other cereals. With its high percentage of carbohydrates, lipids and proteins, it is nutritious for human consumption. Apart from its nutritional qualities its waste are also utilized to produce bioethanol and bio gas etc. Basically the corn cob is preferred over other agricultural byproducts due to its composition which is easily convertible to bioethanol. The corn cob is lignocellulosic biomass comprises of cellulose, hemicellulose and lignin (Yu and Zang, 2004). Cellulose is a linear, crystalline homopolymer with a repeating unit of glucose strung together beta-glucosidic linkages. The structure is rigid and harsh treatment is required to break it down. Hemi-cellulose consists of short, linear and highly branched chains of sugars. In contrast to cellulose, which is a polymer of only glucose, a hemicellulose is a hetero-polymer of D-xylose, D-glucose, D-galactose, D-man-nose and L-arabinose . Ethanol production has been taken into estimation depending upon the ratio of hexosans (glucan, galactan and mannan) and pentosans (xylan, arabinan) in each biomass source (Sun and Cheng, 2002). In 2006, global production of bioethanol reached 13.5 billion gallons, up from 12.1 billion gallons in 2005. Bioethanol currently accounts for more than 94 per cent of global biofuel production, with the majority coming from sugar cane (Lin and Tanaka, 2006). With the increase in demand of fuel, it seems worthwhile to investigate the renewable sources of bioethanol. In present investigation, efforts were made to utilize the lignocellulosic waste of agricultural produces (corn cob) for production of bioethanol by enzymatic hydrolysis.

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MATERIALS AND METHODS

Materials: Corncob from different local varieties viz. PMH-19, Sweet corn and surya, were provided by Sorghum Research Station, Marathwada Agricultural University, Parbhani. Culture for ethanol production was *Saccharomyses cerevisiae (3090)* obtained from National Chemical Laboratories (NCL), Pune.

Chemical analysis of sample: The moisture, crude protein, crude fat, total carbohydrate, reducing sugar and ash content of samples were determined by using standard methods (AOAC, 1990). While hemicelluloses and cellulose estimation was carried out by method prescribed by Ranganna (1995).

Hydrolysis yield of reducing sugar: It was determined by measuring the reducing sugar and by using formula (Ming chen, 2006).

Preparation of samples: Corn cobs were sun dried 1 to 2 days to remove moisture. These cobs were ground to fine

particle size in hammer mill. The fine powder of corn cob with different particle size viz. 0.5, 1.0 and 1.5 mm were packed in polythene bags of 250 guage. These bags were stored at room temperature in dry condition until further use.

PROCEDURES

The ethanol was produced by treating the corn cob with acid and enzymatic hydrolysis followed by fermentation of hydrolysate of the pre-treated substrates by the microbial culture of *Saccharomyces cerevisiae (3090)* (Spindler *et al.*, 2004).

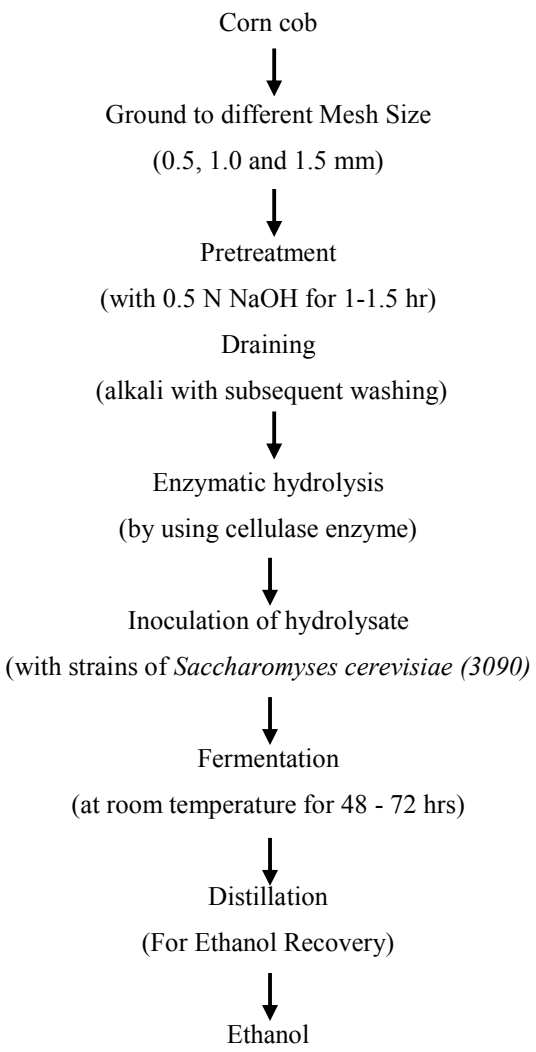
Pre-treatment: Sample of corn cob with different particle sizes were taken and treated with 0.5N NaOH at 1:6 (S:L). This treatment only used in enzymatic hydrolysis. This pretreatment was done to the lignin. This mixture was allowed to stand for 1-1.5 hrs. at room temperature and atmospheric pressure. After definite time residues was free from traces of alkali and dried at 50-52^{o}C in hot air oven and substrate was hydrolyzed by enzymes.

Enzymatic Hydrolysis: Cellulase enzyme was used to convert the polysaccharide to monosaccharide. Alkali treated dried corn cob powder was suspended in citrate buffer of pH 5.0 (Cellulase activity is optimum at pH 5.0). The enzyme cellulase was added at different concentration ranging from 10, 20 and 30 FPU (1FPU = 0.13g) respectively. The enzymatic hydrolysis was carried out at 52^{o}C in a shaking water bath for the period of 6 hrs. Total reducing sugars present in hydrolysate was estimated using Fehling's solution method. Finally it was subjected to fermentation (Ming *et al.,* 2006).

Fermentation: Both hydrolysates were subjected to fermentation using the strains of *Saccharomyses cerevisiae 3090*. This microbial culture was subjected into the subculture using the MGYP media. This broth was used for the fermentation hydrolysate material. The level of innoculum used for 2 per cent of hydrolysate (Daniel and Cynthia, 2004). Fermentation was carried out for 72 hrs under room temperature. During fermentation free sugars were converted into ethanol (Harikrishna *et al.,* 2001).

Distillation: Supernatant was collected from flask of fermenter and transferred to distillation unit. Then it was distilled at the temperature of 80-82^{o}C for 2 hrs. Further, ethanol was collected from distillation unit.

Production of ethanol using cellulase Hydrolysis



RESULTS AND DISCUSSION

Utilization of agricultural waste produce in production of bioethanol is an innovative concept where lignocellulosic material from corn cob could be hydrolyzed by enzymatic method, however it requires optimization of variation processing parameters. The results pertaining to different studies processing parameters are summarized under following suitable headings.

Table 1: Effect of hydrolysis time on the yield of ethanol

Sample No.	Particle size(mm)	Enzyme conc. (FPU)	Hydrolysis time (hr)	Reducing sugar (%)	Hydrolysis yield (%)	Fermentation time (hr)	Ethanol Yield (%)
A	0.5	20	12	18.40	23.82	78	10.48
B	0.5	20	24	29.90	38.71	78	17.04
C	0.5	20	36	40.50	52.44	78	23.08
D	0.5	20	48	47.50	61.51	78	27.07
E	0.5	20	60	50.30	65.13	78	28.67
SE	-	-	-	1.545	2.480	-	0.872
CD at 5 %	-	-	-	4.860	7.804	-	2.745

Table 2: Effect of enzyme concentration on the yield of ethanol

Sample No.	Particle size (mm)	Enzyme conc. (FPU)	Hydrolysis time	Reducing sugar (%)	Hydrolysis yield (%)	Fermtn. time (hr)	Ethanol Yield (%)
A	0.5	10	6	8.50	11.00	78	4.80
B	0.5	20	6	16.10	20.84	78	9.17
C	0.5	30	6	24.50	31.72	78	13.96
SE	-	-	-	0.395		-	0.292
CD at 5 %	-	-	-	1.367		-	1.010

Table 3: Effect of substrate concentration on the yield of ethanol

Sample No.	Particle size (mm)	Quantity (gm)	Enzyme conc. (FPU)	Reducing sugar (%)	Hydrolysis yield (%)	Fermtn time (hr)	Ethanol Yield (%)
A	0.5	50	20	24.70	31.98	78	14.07
B	0.5	100	20	43.50	56.33	78	24.79
C	0.5	150	20	42.10	54.50	78	23.99
SE	-	-	-	0.213	0.213	-	0.137
CD at 5 %	-	-	-	0.737	0.737	-	0.474

Effect of hydrolysis time on the yield of ethanol

During present investigation, the efforts were made to optimize various processing parameters. During initial trails enzymatic treatment was kept constant at the rate of 20 FPU for all samples with varying rates of hydrolysis time periods. The results pertaining to effect of different hydrolysis time on yield of ethanol is presented in Table-1. It is observed from table-1 that hydrolysis time was one of the factor which affect on the yield of ethanol. 100 gm of corn cob was hydrolyzed by cellulase enzyme at 20 FPU concentration at pH 5 and 50^{o}C temperature for 12-60 hrs time. The simple's sugar i. e. glucose, xylose, arabinose and cellobiose were produced during hydrolysis. The reducing sugar concentration was reached upto 50.30 per cent and prolonged hydrolysis time (60 hr) helped in the increasing hydrolysis yield which resulted in high ethanol yield. From the Table 1, we found that hydrolysis time was increased at the interval of 12 hrs. The yield of reducing sugar and ethanol was also increased. Sample E was hydrolyzed for 60 hrs to get 50.30 per cent reducing sugars which was fermented by *Saccharomyses cerevisiae (3090)* to get 28.67 per cent ethanol. This yield was higher than other samples i.e. sample A, B, C & D which having lower hydrolysis time. Thus hydrolysis time was important factor for significant yield of ethanol.

Effect of enzyme concentration on the yield of ethanol

It is observed from table-2 that as the cellulase contributes significantly to the biomass conversion. The cellulase dosage should be minimized as such as possible. Hydrolysis experiments were performed with 100 gm substrate and different cellulase concentration (presented as filter paper activity) per gram of substrate at pH 5 and 50^{0} C temperature. The reducing sugar concentration, hydrolysis yield and ethanol yield had a similarly variation trend i.e. increased sharply with cellulase dosage varying from 10 to 30 FPU per gram substrate. So finally these different concentrations the yield of ethanol get varied. Sample C treated at 30 FPU enzyme concentrations to get 13.96 per cent ethanol yield. This yield was more than sample A and B which hydrolyzed at 10 and 20 FPU concentration respectively to get 4.80 and 9.17 per cent ethanol respectively.

Effect of substrate concentration on the yield of ethanol

It is observed from table-3 that substrate concentration on enzymatic hydrolysis were investigated at fixed ratio (20 FPU) of cellulase to substrate as showed in table 4, reducing sugar concentration, hydrolysis yield and ethanol yield showed an opposite variation trend i.e. as substrate concentration increasing, the reducing sugar increased but the hydrolysis yield and ethanol yield decreased .This may be due end product feed back inhibition caused by high reducing sugar concentration. .Sample B having 100 gm substrate was hydrolyzed. and fermented to get 24.79 per cent ethanol as compared to sample C (23.99 per cent) and fermented to get 24.79 per cent ethanol as compared to sample C (23.99 per cent) which was less than sample B because of feedback inhibition.

CONCLUSION

On the basis of obtained results, it could be concluded that corn cob varieties contain maximum quantity of total carbohydrate i.e. 89.50 per cent which can be better utilised for conversion into fermentable sugar. In enzymatic hydrolysis of corn cob using cellulase concentration (20 FPU), particle size 0.5mm, hydrolysis time 60 hrs and fermentation time 78 hrs also yield the same value of ethanol yield (28.67 per cent). For the higher production of ethanol enzyme concentration (range 10-35 FPU) was superior.

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