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Research Paper :

Studies on effect of different hydrolysis method and particle sizes of corncob on bioethanol production

R.V. SALVE, **SYED IMRAN HASHMI** AND P.M. CHILKAWAR

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See end of the article for authors' affiliations

Correspondence to:
SYED IMRAN HASHMI
Department of Food Trade and Business Management, College of Food Technology, Marathwada Agricultural University, PARBHANI (M.S.) INDIA

ABSTRACT

Among the available agricultural products, corn cob is an abundant lignocellulosic raw material which could be a potential source of bioethanol production. This possible substrate contains cellulose which cannot be fermented by conventional yeast. For these reasons, corncob should be hydrolyzed first to simple sugars, followed by yeast fermentation of glucose to ethanol. In present investigation, efforts were made to utilize the agricultural waste *viz.,* corncob in production of bioethanol. Corn cob from different varieties were analyzed to judge there suitability for bioethanol production. The corncob variety with high lignocellulosic content was used for further studies, which was hydrolyzed by two different methods (*i.e.* acid and enzyme) to prepare corncob hydrolysate. The different particle sizes of corn cob flour were also considered during study so as to analyze the effect of particle size on the ethanol yield. The results revealed that amongst various local varieties, sweet corn variety was found to have maximum carbohydrate content (87 per cent), out of which 43.20 per cent was cellulose content. On hydrolysis, higher sugar concentration liberated from acid hydrolysate compared to enzyme hydrolysate, while particle size inversely correlates with the production of reducing sugar during hydrolysis. It is found that, Acid hydrolysis of corn cob with 0.5 mm particle size, 2 per cent inoculums level and fermentation period of 78 hr given maximum yield of 24.50 per cent of bioethanol.

Key words : Corncob, Bioethanol, Particle size, Fermentation, *Saccharomyces cereviseae*

Bioethanol is an alcohol made through the fermentation of plant sugars from agricultural crops and biomass resources. In the search for viable alternative of biofuel, corn cobs has been pursued as suitable lignocellulosic waste for bioethanol production in process involving chemical pretreatment (Lin and Tanaka, 2006). The lignocellulosic biomass comprises of cellulose, hemicellulose and lignin (Hayn *et al.*, 1993). During recent years considerable interest has been shown in the utilization of lignocellulosic materials as renewable resource for the production of industrially potential products such as ethanol, organic acids, single cell proteins, plastics, resins, chemical solvents etc. Lignification and crystallinity of cellulose are major barrier in the process of conversion of lignocellulosic biomass into bioethanol. It is essential to alter or remove structural and compositional impediments by pretreatment to improve rate of hydrolysis. Enzymatic methods have advantage of being eco-friendly besides applicable under mild condition of hydrolysis (Ghose, 2005). Bioethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels. Lignocellulosic biomass is a cheap, renewable, abundantly available resource and its conversion to glucose and other fermentable sugars has been considered, in the last few decades, to be an attractive route for ethanol production (Curreli *et al.*, 1997).

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 can also be utilized to produce bioethanol and bio gas etc. Basically the corn cob could be preferred over other agricultural byproducts due to its composition which is easily convertible to bioethanol (Cao *et al.,* 1996). Corn cobs are available as 20 per cent of the total weight of unshelled corns and are available as agricultural waste after shelling. The total production of corn cobs was 1.4 million tons in India while in the world 30.1 million tonnes of corn cobs used for furfural production due to its high hexose and pentose content (Kuhad and Singh, 1993). Considering the importance of lignocellulosic material in biothanol production, corn cob could be a good agriculture waste material for bioethanol production. Hence, present investigation was carried out with an objective to utilize lignocellulosic waste from corn cob in bioethanol production. In present study, different particle sizes of corn cob flour and different hydrolysate preparation methods (*viz.,* acid and enzyme hydrolysis), were also considered to judge their suitability in terms of final bioethanol yield.

METHODOLOGY

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 *et al.,* 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)*.

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 were free from traces of alkali and dried at 50-52^{0}C in hot air oven and substrate was hydrolyzed by enzymes.

Acid hydrolysis:

Acid hydrolysis was used for the conversion of polysaccharides into monosaccharides *i.e.* reducing sugar. The concentrated 2.5N sulphuric acid was added in 100g corn cob powder. This hydrolysate was boiled at 90-95^{0}C temperature for 4 hrs and centrifuged the solution at 5000 rpm. The supernatant was collected for alkali hydrolysis at pH 6 with 2N NaOH for neutralization of acid. Finally reducing sugars dissolved in the hydrolysate which was subjected to fermentation (Sanchez and modig, 2004).

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^{0}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^{0}C for 2 hrs. Further, ethanol was collected from distillation unit .

RESULTS AND DISCUSSION

The results obtained during present investigations are summarized as follows.

Proximate composition of corncob:

The representative corn cob samples from different varieties were studied to find proximate chemical composition and the result are presented in Table 1.

The data depicted in Table 1 showed that highest carbohydrate content was observed in sweet corn cob

Table 1: Proximate composition of different corn cob

Sr. No.	Variety	Carbohydrate (%)	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Cellulose (%)	Hemicellulose (%)
1.	PMH-19	86.00	1.95	0.86	2.39	8.8	39.00	33.80
2.	Sweet Corn	87.00	2.02	0.89	2.00	8.1	43.20	37.00
3.	Surya	85.90	2.10	0.80	2.10	9.1	39.50	32.50
	S.E.$\pm$	0.816	0.106	0.023	0.057	0.341	0.489	0.412
	C.D. (P=0.05)	0.282	0.368	0.082	0.199	1.180	1.692	1.424

(87.00per cent) where as the lowest was observed in Surya (85.90 per cent). The highest protein content was observed in surya variety (2.10 per cent) where as the lowest was observed in PMH-19 (1.95 per cent). The maximum crude fat content, ash, moisture was observed in sweet corn cob where as the lowest was observed in Surya variety. The carbohydrate make up of corn cob were also studied. It was revealed from table 1 that the highest cellulose and hemicellulose content was observed in sweet corn cob (43.20 per cent) and (37.00 per cent), respectively where as lowest was observed in PMH-19 *i.e.* 39.00 per cent cellulose and 33.80 per cent hemicellulose. These results are in agreement with those reported by Ryan and Spencer (2001) with slight variations. On the basis of results obtained with respect to chemical composition of different varieties of corncob, sweet corn variety was found to be superior. Hence, sweet corn cob was used for further research work during this study.

Acid hydrolysis of corn cob for ethanol production:

The data related to effect of particle size of corn cob on the production of ethanol using acid hydrolysate are summarized in Table 2.

It is revealed from Table 2 that as particle size of corn cob was increased from 0.5 mm to 1.5 mm, the yield of ethanol get decreased from 24.50 per cent to 19.60 per cent, so particle size of corn cob was inversely proportional to yield of ethanol. Sample A having 0.5 mm particle size was more effective for the ethanol production (24.50 per cent) which was significant than other sample B and C.

The hydrolysis of corn cob powder was done by 2.5 N concentrated H_2SO_4. This hydrolysis helps to convert the polymers into simple sugars and the hydrolyzed sample was fermented to get ethanol. The fermentation was carried out by *Saccharomyses cerevisiae (3090)* at 2 per cent innoculum and pH 5.0 for 78 hrs. It was found that as the particle size increases (0.5 mm to 1.5 mm), the reducing sugar was also found to decrease significantly (42.96 per cent to 34.38 per cent) and again per cent ethanol yield and specific gravity significantly decreased.

The finding of present investigation indicates that there were significant changes in reducing sugar and ethanol yield and non-significant change in specific gravity. Similar results were found by Yu and Zang (2004).

Enzymatic hydrolysis of corn cob for ethanol production:

The dosages of 20 FPU of cellulose enzymes were used for the hydrolysis of corn cob powder in order to produce reducing sugar which further get utilized by microbes to produce ethanol. Effect of different particle sizes on the properties and ethanol yield is summarized in Table 3.

Table 3 shows effect of particle size of corn cob on the production of ethanol by using enzyme and *Saccharomyses cerevisiae (3090)* stain. It is revealed from Table 3 that as particle size of corn cob was increased from 0.5 mm to 1.5 mm, the yield of ethanol get decreased from 9.60 per cent to 7.40 per cent, so particle size of corn cob was inversely proportional to yield of ethanol in case of enzymatic hydrolysis too. Sample A having 0.5 mm particle size was more effective for the ethanol production (9.60 per cent) which was significant than other sample B and A.

Table 2 : Effect of particle size of corn cob on the production of ethanol by using acid and *Saccharomyses cerevisiae (3090)* stain

Sample No.	Particle size (mm)	Inoculum level (%)	2.5N H_2SO_4 (ml)	Fermentation time (hr)	Reducing sugar (%)	Specific gravity	Ethanol yield (%)
A	0.5	2	500	78	42.96	0.90	24.50
B	1.0	2	500	78	36.84	0.88	21.00
C	1.5	2	500	78	34.38	0.82	19.60
				S.E.	0.623	0.032	0.395
				C.D. (P=0.05)	2.154	0.111	1.367

Table 3 : Effect of particle size of corn cob on the production of ethanol by using enzyme and *Saccharomyses cerevisiae (3090)* stain

Sr. No.	Particle size (mm)	Enz. conc. (FPU)	Hydrol. time (hr)	Reducing sugar (%)	Hydro. yield (%)	Innoculum level (%)	Fermentation time (hr)	Sp. gravity	Ethanol yield (%)
A	0.5	20	6	16.90	21.88	2	78	0.89	9.60
B	1.0	20	6	14.50	18.77	2	78	0.85	8.20
C	1.5	20	6	13.00	16.83	2	78	0.82	7.40
			SE	0.343	0.213	-	-	0.034	0.169
			CD at 5 %	1.185	0.737	-	-	0.120	0.587

The hydrolysis of corn cob sample was carried out by using cellulase enzyme at the rate of 20 FPU concentrations for 6 hrs. The fermentation was carried out by using *Saccharomyses cerevisiae (3090)* at 2 per cent innoculum and pH 5.0 for 78 hrs. It was found that as the particle size increased (0.5mm to 1.5 mm), the reducing sugar was found to be significantly decreased (16.90 per cent to 13.00 per cent) and hydrolysis yield was also significantly decreased (21.88 per cent to 16.83 per cent) . The per cent ethanol yield and specific gravity was also significantly decreased, as the particle size increased. These results are in well agreement with those reported by Ming *et al.* (2006) and Spindler *et al.* (2004).

The finding of present investigation indicate that there were significant changes in reducing sugar, hydrolysis yield and ethanol yield and non-significant change in specific gravity.

Conclusion:

From the obtained results following conclusion could be drawn.

– The results regarding proximate analysis of three different variety of corn cob indicated near about 85.50 to 87.00 per cent carbohydrate content along with cellulose concentration up to 45 per cent are the important consideration for the ethanol production.

– The *sweet corn variety* corncob contains maximum quantity of total carbohydrate *i.e.* 89.50 per cent which can be better utilised for conversion into fermentable sugar.

– The particle size also affected the amount reducing sugar and ethanol yield. 0.5 mm was found to yield maximum ethanol (24.50 per cent) with fermentation time 78 hrs with *saccharomyses cerevisiae (3090)*

– Significantly the highest quantity of ethanol (24.50 per cent) was produced by acid hydrolysis process with 0.5mm particle size of corn cob, 2 per cent innoculum of *saccharomyses cerevisiae (3090)* and 78 hrs fermentation time.

Authors' affiliations:
R.V. SALVE, D.M.V.M. College of Agri-Biotechnology, Hatta, BASMAT (M.S.) INDIA
P.M. CHILKAWAR, Department of Food Science and Technology, MGM's, College of Food Technology, AURANGABAD (M.S.) INDIA

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