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Fermentation Kinetics and Ethanol Production from Different Corn Grains Varieties

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Abstract— **Study of fermentation kinetics in ethanol production from damaged corn grains is crucial aspect for economical yield enhancement. Two samples of corn flour, namely control and damaged, with different carbohydrate and fibrous content, were used as substrates. Samples were liquefied and saccharified using commercial α-amylase and glucoamylase, for production of fermentable sugars; Amount of fermentable sugar obtained after hydrolysis of damaged and control corn grains were 97 $gL^{-1}$and 127 gL^{-1} respectively. Enzyme hydrolysates were then fermented to produce ethanol in batch mode. Glucose was consumed promptly in both cases, control and damaged; ethanol production was considerably higher in control (56 g/L), compared to damaged (45 g/L). Submerged fermentation of damaged corn grains flour represented about 80 % of total ethanol production, when fine corn grains flour was used as substrate. In damaged corn flour ethanol productivity and yield was 1.12 g/L/h and 0.18 g/g flour respectively. In control corn flour ethanol productivity and yield was 1.16 g/L/h and 0.22 g/g flour. Maximum sugar consumption rate (Sm) for damaged and control corn were 3.22 g. $(L .h)^{-1}$ and 3.2 g. $(L .h)^{-1}$ respectively. Maximum product formation rate for damaged and control corn (Pm) were 1.38g. (L .h)-1 and 1.35g. (L .h) -1 respectively. Fermentation efficiency of damaged and control corn grains were 91.3% and 91.7%.**

Keywords— **Fermentation kinetics, Ethanol production, Damaged corn grains**

I. INTRODUCTION

Bio-fuels are being actively encouraged in the transportation sector. Research work is focused on the development of renewable resources, sustainable development, green energy, eco-friendly process, etc., in the transportation sector. Increasing the use of bio-fuels for energy generation purposes is of particular interest nowadays because they allow mitigation of greenhouse gases, provide means of energy independence and may even offer new employment possibilities. Bio-ethanol is by far the most widely used bio-fuel for transportation worldwide. Ethanol can be produced from various sugary substrates such as molasses, starchy materials like corn, wheat and potato (**Maiorella *et al.*. 1981**) and cellulolytic materials (**Deshpande *et al.*, 1983**), due to increasing demand for ethanol which is an alternative energy source (**Lynd *et al.*, 1991**). Sweet sorghum has the potential of becoming a useful energy crop (**Fikret-Kargi *et al.*, 1983**). However, fresh starchy materials are required for human consumption. A large quantity of different grains is spoiled every year in India because of unfavourable climatic conditions and inadequate transport and storage facilities. Damaged grains are those which are unfit for human consumption. The damage includes blackened, broken, cracked, attacked by fungi, insect damaged, partially softened by being damp, dirty and bad smell, etc. Ethanol production from damaged sorghum grains is feasible (**Gawande and Patil 2014).** Damaged sorghum grains are non edible could be utilized optimally for ethanol production (**Gawande and Patil 2016)**. Non edible damaged corn grains were utilized for ethanol production in co-culture at 25% substrate concentration using co-culture of Aspergillus niger NCIM 1248 and Saccharomyces Cerevisiae MTCC 170 **(Gawande and Patil 2017)**. In this research, two varieties of corn healthy/fine (control) and, damaged/blackened corn were used as fermentation substrates. Both of them were evaluated to study kinetics of the sugar consumption and ethanol production during fermentation, using a 5 L fermenter.

II. MATERIALS AND METHODS

A. SUBSTRATE

Control grains used were commercial yellow dent maize obtained from a local market, Nagpur and used as a control for experiment. These grains were purposely blackened by sprinkling water on them and keeping them in damp conditions spread on a clean jute bag, covered with damp cotton. Damaged grains samples were cleaned by removing debris and other contaminants by washing and drying

in oven at 60^{0}C for 36 hours. Finally damaged grains were milled and flour was kept in airtight container for further use. 3,5-Dinitrosalicylic acid (DNSA), soluble starch, maltose, dextrose, and other chemicals were purchased from E. Merck Ltd (India). Composition of damaged and fine corn grains is given in table 1.

Table I. Composition (W/W%) of Damaged and Fine Corn Grains

	Control corn grains	Damaged corn grains
Starch	70.5	40.3
Protein	12.8	10.8
Crude fibre	2.5	0.9
Lipid	2.62	2.6

B. LIQUEFACTION

Ground meals (25 g dry basis) with 0.02% of calcium hydroxide were mixed with distilled water to obtain mashes with 25% (wt/vol) solids. pH was initially adjusted to 5.6 with 0.1N HCl and temperature was increased to 85^{0}C in a shaking water bath (Bio Technics, India). When slurries reached 80 ^{0}C, 0.15% amylase (Hi-Media Laboratories) was added. Mashes were maintained at 90^{0}C during 120 min. In order to determine the progressive extent of starch hydrolysis, aliquots
were taken before enzyme addition, and after 20 min time interval.

C. SACCHARIFICATION

Commercial glucoamylase from Hi-media was used for saccharification of liquefied slurry. pH was initially adjusted to 4.5 with 0.1N HCl and temperature was brought to 70^{0}C in a shaking water bath (Bio Technics, India). When slurries reached 70^{0}C, 0.8% glucoamylase (Hi-Media Laboratories) was added. Mashes were maintained at 70^{0}C during 260 min. Saccharification was monitored by the yield of sugars as estimated by DNS method. Amount of glucose generated indicates the percentage of saccharification.

D. MICROORGANISM AND MAINTAINANCE OF CULTURE

The microbial cultures used for fermentation i.e. *Sacchromyces* cerevisiae MTCC 170 (Growth temperature 25^{0}C, pH 5.5) was procured from IMTECH, Chandigarh. The strains were maintained on slants of YEPD agar medium containing 0.5% yeast extract, 1% peptone, 2% glucose and 2% agar.

E. MEDIA

Growth medium used for preparing was S.cerevisiae contained in grams per 100 mL glucose, 2; peptone, 1; yeast extract, 0.5; and potassium dihydrogen phosphate, 0.1. The fermentation medium used for ethanol production from starch was identical to growth medium except that substrate used was damaged sorghum and corn flour (25%). 0.1N NaOH and 0.1N HCl was used to get desired pH. The damaged grains flour was pretreated with fungal amylase to extract the starch present in it. The pretreated solution was filtered, and the supernatant was analysed for the reducing sugar concentration. The amount of starch present in the sample was then analyzed by anthrone method.

F. ETHANOL PRODUCTION IN 5 L FERMENTER

About 2.5 L of enzymatic hydrolysate obtained from the enzymatic hydrolysis was collected in a 5 L batch fermenter (B-Lite, Sartorious Private Limited, Mumbai, India). Hydrolysate was neutralized and supplemented with a nutrient solution in the fermentation medium. The fermenter-containing hydrolysate was heated to a temperature of 80^{0}C for 30 min and agitated at 250 rpm, followed autoclaving, prior to inoculation. This was done for uniform mixing of the nutrient solution with the fermentation medium and elimination of any contamination. Fermentation was performed at temperature 30^{0}C and pH 5.6 for 72 h. The fermenter was inoculated with 8% of yeast inoculum. The agitation speed was maintained at 150 rpm, and the pH was maintained using sterilized 1N

HCl and 1N NaOH solutions throughout the experiments. Samples were drawn at 12 h intervals and analyzed for residual sugar and ethanol concentration. Yeast growth was monitored by measuring the optical density of the culture at 600 nm (OD600). Cell biomass was determined by making a pellet of cells by centrifugation, drying them at 70°C and expressing the dry weight as g/100 ml of growth medium. Reducing sugars was estimated by the 3,5 DNS method (Miller, 1959). Estimation of ethanol was done by spectrophotometer (**Caputi et al., 1968**).

G. FERMENTATION PARAMETERS

The ethanol volumetric productivity (g/L/h) was calculated as the ratio of ethanol concentration (g/L) at the end of the run to the fermentation time (t, h). The yield of ethanol to consumed sugar

(g/g) was defined as ratio of ethanol concentration to the sugar consumption ($So–S_f$), So initial sugar concentration and S_f final sugar concentration). Sugar conversion (%) calculated as a ratio of sugar consumption to the initial sugar concentration. Ethanol yield (Yp/s, g/g): Mass of product (ethanol) formed per mass of substrate consumed

III.RESULTS AND DISCUSSIONS

A. REDUCING SUGARS DURING LIQUEFICATION

Starch content in control corn is more compared to damaged corn (Table 1). Hence amount of reducing sugar released after 1 hour of liquefication from damaged corn grains was more compare to that from sound grains. Thus liquefaction progress of damaged corn is 7% slower as compared to healthy sorghum (Figure). After 2 hours of liquefication reducing sugar released became constant from both types of damaged grains. Thus 2 hours of liquefication time was suitable to complete starch breakdown.

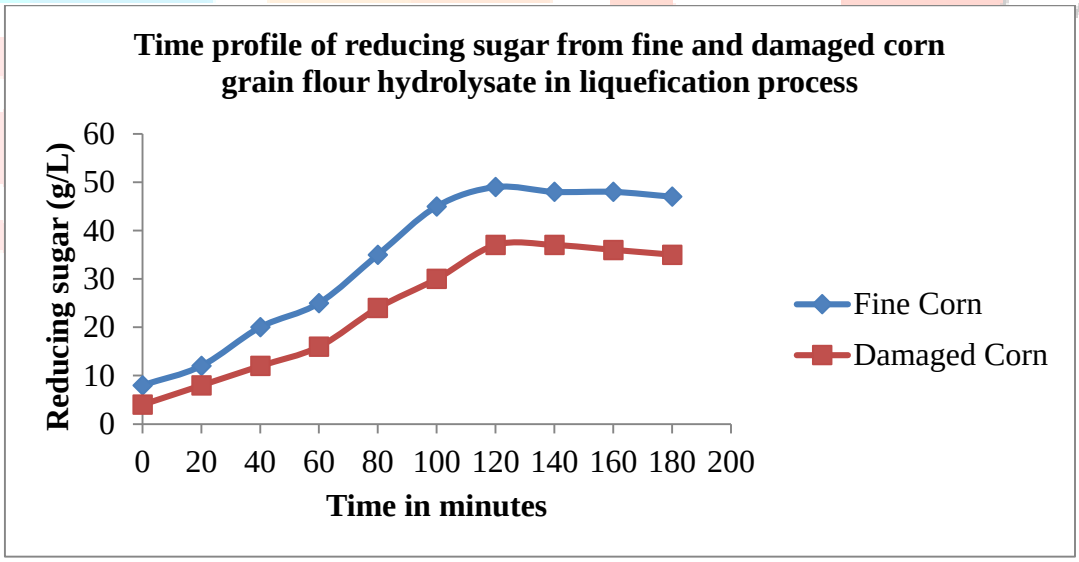

Fig 1: Reducing sugar production from fine and damaged corn grains flour during liquefication process

B. REDUCING SUGARS DURING SACCHARIFICATION

Saccharification progress of damaged grains were slower as compared to healthy grains. This could be due to enzyme inhibition due to mycotoxins present in the blackened pericarp. After 4 hours of saccharification reducing sugar released became constant from both types of damaged grains. This indicates that 4 hours of hydrolysis time was suitable to complete starch breakdown.

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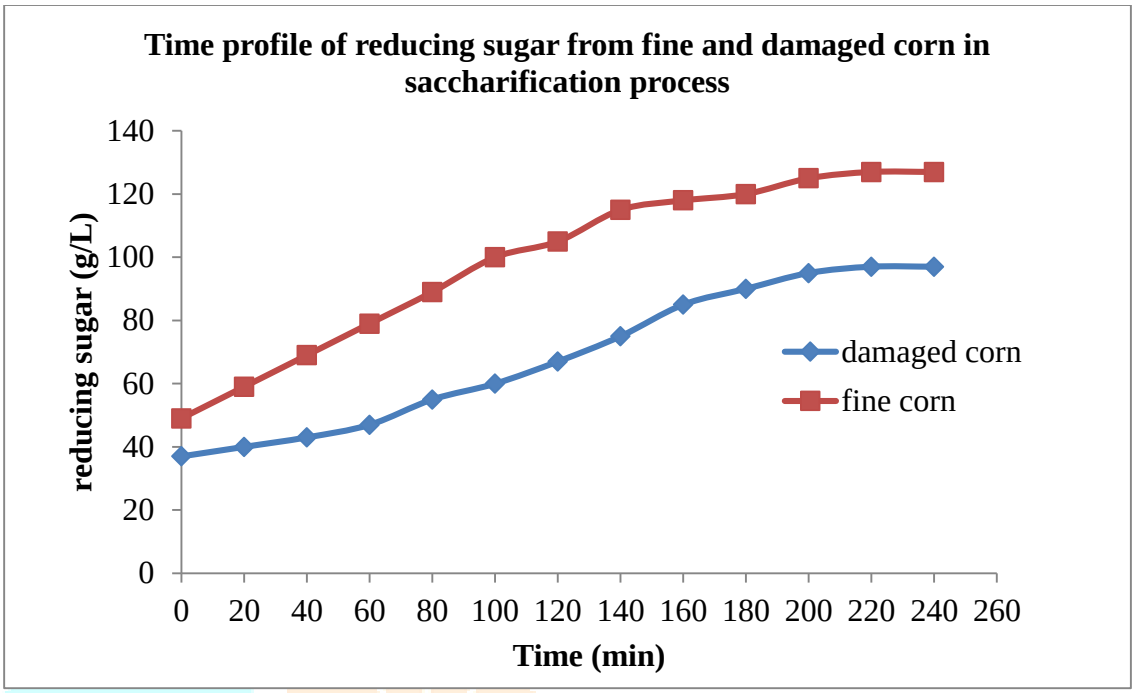

Fig 2: Reducing sugar production from fine and damaged corn grains flour during saccharification process

C. STUDY ON FERMENTATION KINETICS FOR ETHANOL PRODUCTION

Kinetic study divides fermentation into 3 stages (Figure 3 and Figure 4). Damaged corn grains has a faster initial total sugar reduction and ethanol production than fine corn grains. For damaged corn grains noticeable decrease takes place after 6th hour, whereas for fine corn grains noticeable decrease takes place after 10th hour. Therefore it is easier for inoculated yeast cells to go through adjustment to fermentation in enzyme hydrolysates of damaged variety of corn grains than healthy/fine variety of corn grains. This is explained by the difference in the initial quantity of sugars in the two varieties of the grains. Sugar consumption and ethanol production are low for first 10 hours of fermentation in enzyme hydrolysates of fine corn grains. Therefore studying influence of substrate composition on the kinetics of fermentation is important to increase yield of ethanol. For the initial stage of fermentation, it also shows that starting with a higher concentration of sugars in fine grains that has mixed sugars is less efficient in utilizing substrate by yeast compared to a lower concentration of mixed sugars in damaged grains. Most rapid glucose consumption and ethanol production occur at time between 6 hour and 36 hour for damaged corn flour hydrolysates and 10 hour and 48 hour for fine corn flour hydrolysates (Figure 3 and 4). Even though most glucose seems to be absorbed by the 36th hour for enzyme hydrolysates of damaged corn flour and 48th hour for enzyme hydrolysates of damaged corn flour, ethanol concentration continues to increase slightly in both cases. This is due to remaining fermentable sugars; Fructose and sucrose that were hydrolyzed to glucose and resulted in ethanol production after the initial glucose was consumed.

There was reduction of more than 90% reducing sugar concentration over initial sugar concentration from damaged corn grains hydrolysates with simultaneous production of 43 g/L and 56 g/L of ethanol after 36 and 48 hours of fermentation period using cells of S. Cerevisiae MTCC 170.

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At final stage, ethanol concentration increased very slowly by fermentation due to the release of glucose from residual sucrose. When this experiment was run for 72 h, there was little change after the 48thhour of ethanol production. There may be decline in cell biomass due to lack of nutrients and production of toxic metabolites, resulting in death of a few cells.

In this study, damaged corn had a smaller ethanol yield compared to fine corn grains. However, rates of sugar consumption and ethanol production were higher for damaged corn grains due to its initial lower concentration of sugar. This was verified by the fermentation kinetic parameters; Ethanol concentration of damaged and fine corn are 43 (g/L) and 56 (g/L) at the end of 36 and 48 hours period respectively.

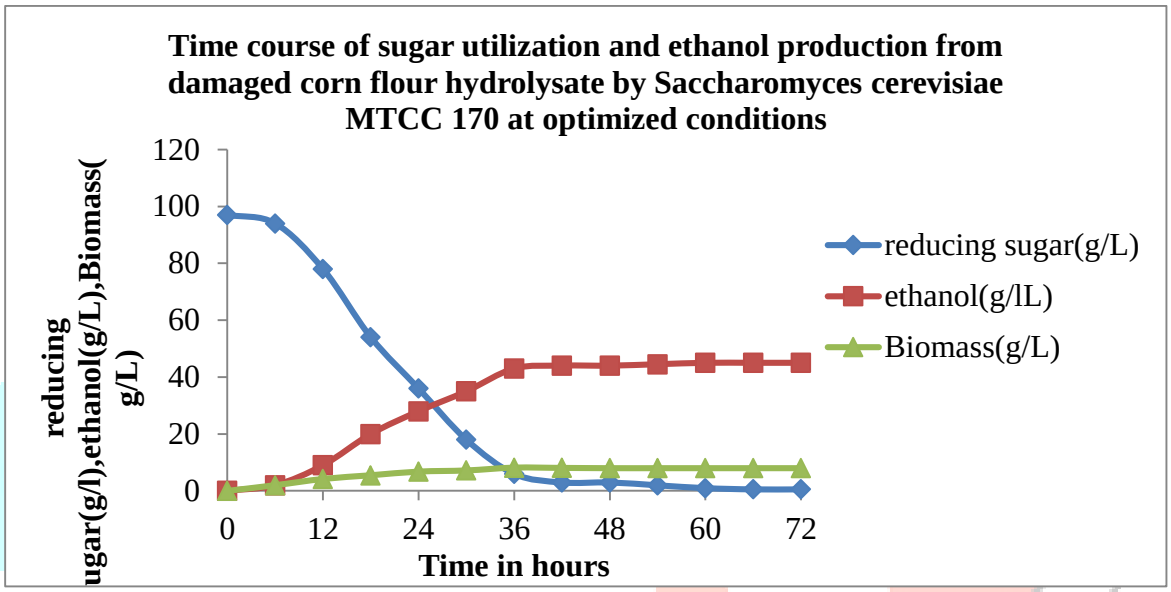

Figure 3 Time course of sugar utilization and ethanol production from damaged corn flour hydrolysate by Saccharomyces cerevisiae MTCC 170 at optimized conditions

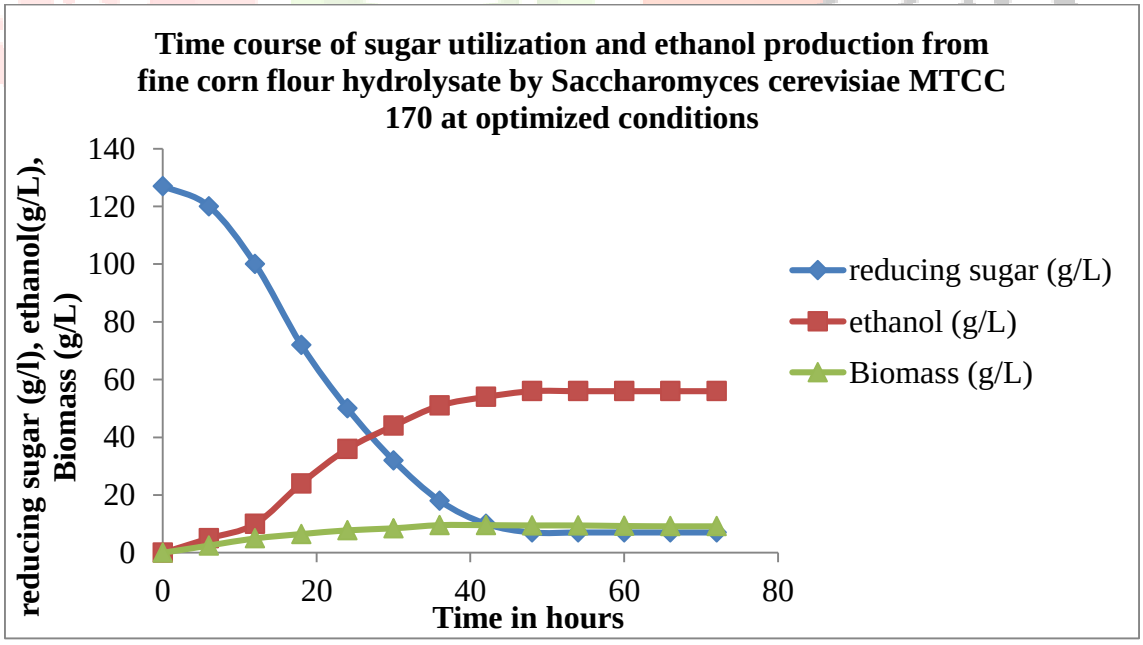

Figure 4 Time course of sugar utilization and ethanol production from fine corn flour hydrolysate by Saccharomyces cerevisiae MTCC 170 at optimized conditions

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Table II. Fermentation Kinetics Of Saccharomyces Cerevisiae (MTCC 170) In Batch Fermentation For Ethanol Production From Damaged Corn Grains Hydrolysate

Parameters		
Initial Sugar concentration (S_0, g/L)	97	127
Residual sugar (S, g/L)	0.57	7.51
Final biomass concentration (X, g/L)	8	9.2
Sugar consumed (%)	99.4	92.8
Ethanol (P, g/L)	45	56
Volumetric ethanol productivity (Qp,g/L/h)	1.12	0.78
Ethanol yield on flour (Yp/s, g/g)	0.18	0.22
Fermentation efficiency	91.3	91.7
Maximum sugar consumption rate (Sm) g. $(L .h)^{-1}$	3.22	3.2
Maximum product formation rate (Pm)	1.38	1.35

Fermentation kinetic parameters were determined; maximum sugar consumption rate (Sm), maximum ethanol production rate (Pm), maximum ethanol concentration, P, at end of fermentation period and ethanol yield, Yp/s, for both varieties of corn grains. There were higher sugar consumption and ethanol production rates for hydrolysates of damaged corn flour than for hydrolysates of fine corn flour (Table 2 and 3). Hydrolysed damaged corn flour had a sugar consumption rate of 3.22 g/(L·h), which means the rate of the consumption of total sugar was 3.22 g/(L·h) during the first 36 h of fermentation, as there was a linear decrease during this period. For fine corn grins this linear decrease lasted nearly 48 h with a maximum consumption rate of 3.2 g/(L·h). This explains sugar consumption and ethanol production for hydrolysates of damaged corn flour and hydrolysates of fine corn flour were almost same.

Finally under optimum conditions ethanol concentration from damaged corn at fermenter level reached up to 45 g/L, ethanol productivity 1.12 g/L/h, ethanol yield 0.18 g/g-flour. For control corn grains ethanol concentration were 56 g/L, ethanol productivity 1.12 g/L/h, ethanol yield 0.18 g/g-flour at optimum conditions. Submerged fermentation of damaged corn grains flour under optimized condition represented about 70 % of total ethanol production, when fine corn grains flour 56 (g/L) was used as substrate. Fermentation efficiency of damaged and control sorghum grains were 91.3 % and 91.7%. Maximum sugar consumption rate (Sm) for damaged and control corn were 3.22 g. $(L .h)^{-1}$ and 3.2 g. $(L .h)^{-1}$ respectively. Maximum product formation rate for damaged and control corn (Pm) were 1.38 g.$(L.h)^{-1}$ and 1.35 g.$(L.h)^{-1}$ respectively.

IV. CONCLUSION

Ethanol production varies depending on quality of grains and amount of starch in grains. In this paper, it is observed that damaged corn grains had reduced ethanol yield compared to control corn grains. This negative result was mainly due to dry matter losses during poor storage conditions for observed unfavorable effect. However, from fermentation kinetic study it was found that rates of sugar consumption and ethanol production of both varieties of grains were almost same. This was verified by studying fermentation kinetic parameters which indicates robustness of yeast facing biotic-damaged feedstocks. Conversions into bioethanol of damaged grains were lower but in terms of fermentation efficiency, damaged grains have similar efficiency to that of control grains. Damaged kernels can be acquired at a discount price by biorefineries and consequently converted into bioethanol with similar efficiencies. In this way improvement of yield thereby reduction in cost of ethanol production is possible and this method is useful for further ethanol production process performance enhancement.

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