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Production of Ethanol from Corn using Yeast (Saccharomyces Cerevisiae)

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ABSTRACT: Corn is one of the richest source for production of ethanol.Yeast fermentation was carried out simultaneously with saccharification process to produce ethanol.The fermentation process was optimized with respect to temperature and pH. Results revealed a temperature of 30°C and pH 5.0-6.0 as optimum for fermentation. Gas Chromatography method for estimating percentage of ethanol was employed. After optimizing these parameters, the experiment was performed. Under optimized conditions, Saccharomyces cerevisiae (yeast) was utilized for alcoholic fermentation using corn which produced 76.49% of ethanol.

KEYWORDS: Corn, ethanol, saccharification and fermentation

I. INTRODUCTION

The increasing demand for liquid fuels for transportation, increased world-demand for oil (gasoline), and the negative consequences of global warming have all contributed to the increased use of corn-based sugar to produce ethanol. Ethanol can be used as a substitute for gasoline to be burned in many of today's passenger cars and trucks. Most gas stations currently use 10% ethanol in their gasoline. However, it has also been used as 85% ethanol to 15% gasoline at some gas pumps called E85 or Flex fuel. Running this fuel in the gasoline motor typically does not require any mechanical modification. Not all gasoline motors are manufactured to run on E85 so it is best to check your car's owner's manual before fueling up with E85.

Ethanol has been used by humans since prehistory as the intoxicating ingredient in alcoholic beverages. Dried residues on 9000-year-old pottery found in northern China imply the use of alcoholic beverages even among Neolithic peoples. Its isolation as a relatively pure compound was first achieved by Islamic alchemists. Antoine Lavoisier described ethanol as a compound of carbon, hydrogen, and oxygen, and in 1808, Nicolas-Theodore de Saussure determined ethanol's chemical formula. Ethanol was first prepared synthetically in 1826, through the independent efforts of Henry Hennel in Britain and S.G. Serullas in France. Michael Faraday prepared ethanol by the acid-catalyzed hydration of ethylene in 1828, in a process similar to that used for industrial ethanol synthesis today. With the advent of distillation, which appears to have been discovered first in ancient Arabia, people were able to obtain beverages with higher ethanol content. In its strictest sense, **fermentation** (formerly called **zymosis**) is the anaerobic metabolic breakdown of a nutrient molecule, such as glucose, without net oxidation.Depending on which organism it is taking place in, fermentation may yield lactate, acetic acid, ethanol or other reduced metabolites . Normal fermentation processes typically cease when a beverage has achieved an alcohol content of 10 to 15 percent. Distillation is the process by which ethanol is boiled from the fermented mixture and captured, producing a liquid with a much higher concentration of alcohol.

Yeast alcohol is the most valuable product for the biotechnology industry with respect to both value and revenue. Approximately 80% of ethanol is produced by anaerobic fermentation of various sugar sources by Saccharomyces cerevisiae. Yeast alcohol technology has resulted in vast improvements during the last decade but profit margins were narrowed. Contamination, limited availability of raw materials and fermentation process design are the major

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limitations causing reduced alcohol yields and quality. In view of the importance of alcohol as an alternative for liquid fuel, several investigations in ethanol fermentations are currently reported. The price of the sugar source is an important parameter when considering the overall economy of production and it is of great interests to optimize alcohol yields to ensure an efficient utilization of carbon sources (Bai et al., 2008; Carlos et al., 2011).
Another crucial factor in fermentation is selecting potent microorganisms with the most commonly used microorganisms being yeasts, which can produce ethanol concentrations as high as 18% of the fermentation broth (Balat et al., 2008). Among the yeasts, S. cerevisiae still remains the prime species for ethanol production. Previous published reports showed that the ethanol tolerance and sugar utilization efficiency of yeast may be improved by altering the nitrogen sources in fermentation medium (Thomas et al., 1996; Yalçin and Özbas, 2004). Therefore strong economic incentives can be revealed by improving production processes resulting in a substantial growth for the ethanol industry in the near future (Carlos et al., 2011).
Recent studies have focused mainly on the genetic modification of S. cerevisiae to improve ethanol yields and efficient bioconversion of various substrates to alcohol (Cao et al., 1996).
The overall chemical reaction conducted by the yeast may be represented by the chemical equation

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$$

II. RELATED WORK

L. Matsakas et al, 2014,used household food wastes were utilized as raw material for the production of ethanol at high dry material consistencies.e included a distinct liquefaction/saccharification step to the process, which rapidly reduced the viscosity of the high solid content substrate, resulting in better mixing of the fermenting microorganism. This step had a positive effect in both ethanol production and productivity, leading to a significant increase in both values, which was up to 40.81% and 4.46 fold, respectively. He subjected remaining solids (residue) after fermentation at 45% w/v dry material (which contained also the unhydrolyzed fraction of cellulose), to a hydrothermal pretreatment in order to be utilized as raw material for a subsequent ethanol fermentation. This led to an increase of 13.16% in the ethanol production levels achieving a final ethanol yield of 107.58 g/kg dry material. By this he demonstrated the ability of utilizing household food waste for the production of ethanol at elevated dry material content. Finally, subsequent fermentation of the remaining solids could lead to an increase of the overall ethanol production yield.

M.Jeschke et al, 2012, found that Corn Stover is the most plentiful source of lignocellulosic biomass in the U.S. Sustainable utilization of corn stover as a feedstock for ethanol and other biofuels could help meet energy needs while delivering agronomic benefits. And also in fields where excess residue interferes with planting, impedes stand establishment, and ties up nitrogen, partial stover harvest can increase corn yields and potentially reduce production costs. Sustainable corn stover harvest requires that only a portion is removed from the field, leaving a sufficient amount behind to meet other critical needs, including mitigation of soil erosion, maintenance of soil organic matter, and sustained soil fertility.

U. G. AKPAN et al, 2008, achieved the conversion of organic waste (Old newspapers) and food waste (maize) were respectively carried out via acid and microbial hydrolysis, which yielded 42% and 63% fermentable sugar wort. This was then converted into ethanol by fermentation process using Saccharomyces Cerevisiae. 95% ethanol was obtained by fractional distillation of the fermentable wort and the total volume of ethanol produced from 2,500 grams of the organic and food wastes was 0.86 liters.Fermentation Kinetic parameters were evaluated. Considering the percentage fermentable sugar yield from the biomasses in study, it is more economical to produce ethanol from food waste (maize) than old organic waste (old newspaper).

Latif et al.(2001) obtained a maximum concentration of ethanol 27, 23, 21g L -1 (w/v) from 200 g L -1 (w/v) dry corn cobs by Saccharomyces cerevisiae, Candida tropicalis and the co-culture, respectively, after 96 h of fermentation. However, theoretical yields of 82%, 71% and 63 % were observed from 50 g L -1 dry corncobs for the above cultures, respectively.

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Production of ethanol from raw starch of spoiled quality sorghum and wheat grains was consummate by utilizing crude amylase preparation from B. subtilis VB2 and an amylolytic yeast strain Saccharomyces cerevisiae VSJ4. Different concentrations of spoiled wheat and sorghum starch from 10% to 30% (v/v) were used and 25% was established to be optimum for damaged wheat and sorghum starch yielding 4.40% (v/v) and 3.50% (w/v) ethanol respectively. While 25% raw starch of fine value wheat and sorghum grains gave yield of 5.60% (v/v) and 5.00% (w/v) respectively. The process was carried out at 35 °C, pH 5.8 and 200 rpm stirring speed for 4 days (Suresh et al. 1999).

III. METHODOLOGY

Material: Corn was obtained from the local market in Indore(Madhya Pradesh)

Enzymes: Alpha amylase and Gluco amylase.

Yeast: Baker's yeast Saccharomyces cerevisiae.

Methods:

Milling & Mashing: It is the process where the large particle of the feed is converting into smaller particles. It is the process of combining a mix of mild feed (like corn, etc.) and water and heating the mixture is needed. Mashing allows the enzyme to breakdown the starch in the grain into sugars, typically the feed into a form of slurry.500gm corn is mixed with 1.5 litre of distilled water.

Saccharification:

1. For selection of enzymes, alpha amylase was used for the saccharification process. In which the complex sugar molecules are converted to simple sugar molecules. So that the feed can be easily fermented by microorganism.

2. Addition of Yeast:**-** For converting the feed into the ethanol , fermentation process is used. In which Saccharomyces cerevisiae is used to ferment the feed. By the fermentation the ethanol was produced .

Separation:

1. After all the process was done two layers were formed one of the liquid biofuel and another of solid residue (slurry). The liquid was separated after the slurry is selled down from the upper surface of it.

2. The collected liquid was then passed through the filter paper for further separation of solid particles from liquid.

3. For more purification of ethanol distillation is done.

4. The pH level was maintained 5-6.

Distillation:

It is a process of separating the component substances from a liquid mixture by selective evaporation and condensation. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of mixture's components. In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction. The temperature was maintained at 78° C.

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Operation	Temperature	pH	Time
Mashing	~30°C	~6.0	5-10 min
Liquefaction	30-35°C	~6.0	120 min
Saccharification	40-45°C	~5.0	24 hrs
Fermentation	30-32°C	~5.0	~55-60 hrs

Fig.1 Table showing Summary of conversion of corn to ethanol

IV. RESULT & DISCUSSION

Saccharomyces cerevisiae (yeast) was utilized for alcoholic fermentation using corn which produced **76.49%** of ethanol

Effect of pH

pH between 4.0 and 8.0 were tested for fermentation using corn and temperature of 30 ± 1ºC.

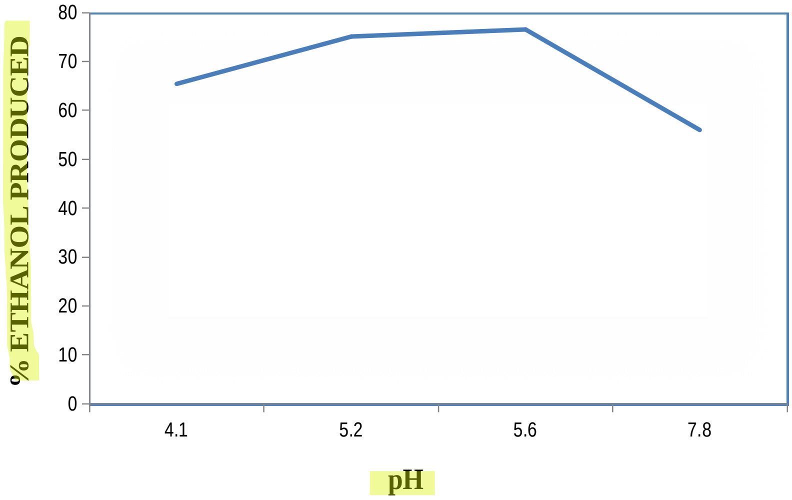

Fig.2. Graph between percentage ethanol produced vs pH

Organism

In fermentation, of the various ethanol producing micro-organisms (Bhatt *et al* 1987, Laplace *et al* 1992 and 1993)

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yeast belonging to *Saccharomyces cerevisiae* have been used most commonly. Ok and Hashinanga (1997) isolatedyeast from spoiled high sugar foods.

Skotnicki*et al* (1981) compared the rates of growth and ethanol production by 11 different strains of *Zymomonas* , with some strains being more tolerant of high sugar or ethanol concentration and high incubation temperature than others. One of the most promising ethanol producing organism is the bacterium

*Zymomonasmobilis*which is used to make palm wines. This bacterium canproduceupto 1.9 mol of ethanol from each mole of glucose fermented.

Renu Bansal and R.S. Singh (2003) did a comparative study on ethanol production from molasses using *Saccharomyces cerevisiae*&*Zymomonasmobilis*. Yeast was found to be more
ethanol tolerant and produced more ethanolat sugar concentration above 15% (v/v).

Uma and Polasa (1990) isolated S. *cerevisiae* from palm wine, which produced increased amounts of ethanol in yeast extract peptone dextrose medium. Bertolini*et al* (1991) isolated new strains of *S.cerevisiae* on basal medium containing 48% sucrose from fermenting sample collected from Brazilian alcohol factories. Isolated strains fermented concentrated sugarcane syrups as well as high sucrose solution in synthetic medium with conversion efficiency of 89-92%.

Most of the distilleries in India operate at a low efficiency because the yeast strains used are not of good quality. Fermentation efficiencies less than 90% are quite common while it should be 95% on an average. Secondly, exact conditions of temperature, pH and nutrients, which are essential for yeast fermentation, are not vigorously maintained. The **Chart** below lists some of the yeast strains used in distilleries and the amount of alcohol they produce.

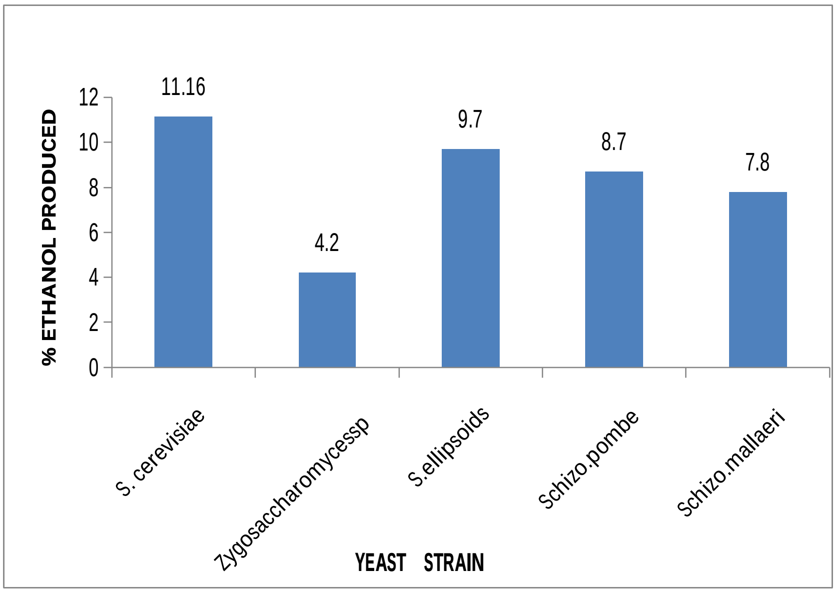

Fig.3. Chart showing percentage ethanol produced with respect to different yeast strain
(ref. Recycling, residues of agriculture and industry, pp202, M.S.Kalra)

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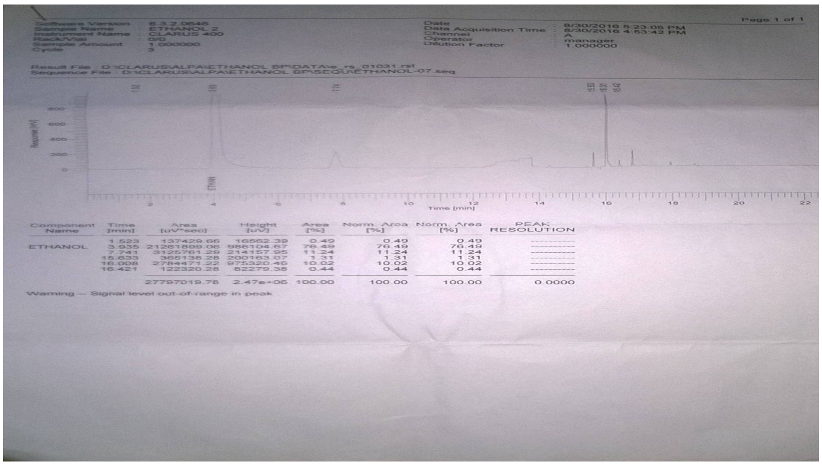
Gas chromatography

Gas chromatography (GC) is an analytical technique for volatile and semi-volatile compounds. Many ethanol analyses have done with GC since impurities in ethanol are basically volatile as well as ethanol itself (Hide et al., 2001, Campo et al., 2007, Rodrigues et al., 2008). A sample is vaporized at an injection port by heat. The sample vapor is sent to column packed with adsorbent or absorbent. Inside column, each component in sample is separated depending on its physical and chemical property. The end of column the concentration of each compounds are measured by a detector. There are many kinds of coatings for column. A coating should be chosen depending on the target compounds. Also, there are many kinds of detectors. Each detector has advantages and disadvantages. Thus, a detector should also be chosen carefully to detect target compounds. Gas chromatography-mass spectrometry (GC-MS) is an integrated system of two analytical equipments. Gas chromatography separates analytes and mass spectrometry identifies them. GC-MS accelerates ethanol analysis with its simultaneous separation and identification capavities. A typical GC chromatogram is shown in figure



Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]	Norm. Area [%]	Norm. Area [%]	PEAK RESOLUTION
	1.523	137429.66	16562.39	0.49	0.49	0.49	----------
ETHANOL	3.935	21261899.06	986104.67	76.49	76.49	76.49	----------
	7.741	3125761.29	214157.95	11.24	11.24	11.24	----------
	15.633	365138.28	200163.07	1.31	1.31	1.31	----------
	16.008	2784471.22	975320.46	10.02	10.02	10.02	----------
	16.421	122320.28	82279.38	0.44	0.44	0.44	----------
		27797019.78	2.47e+06	100.00	100.00	100.00	0.0000

Warning -- Signal level out-of-range in peak

Fig.4. Image of the result obtained by Gas Chromatography

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