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A comparative overview of Bio-ethanol production from Organic Residues of Agro waste Materials

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Abstract
Bio-ethanol production from waste organic material is low coast technique. Carbohydrates are the constituent component of different agriculture materials. Rice straw, corn cob and peel of fruits and potatoes are rich in carbohydrates [1]. These carbohydrates of different collected materials being converted into alcohol. These conversion obtained by fermentation by the help of specific yeast variety. After fermentation and different treatment 41%-46% alcohol were obtained from different materials after alkali treatment. Bio-ethanol are the low coast biofuel. Treated and purify bioethanol generally used in petrol vehicles for transportation [2]. The production coast is very low and material can be obtained easily anywhere in India as well as other parts of the world. Obtained Percentage of bio-ethanol is differ from each other material because its depend on the constituent % of carbohydrate in material. Conversion of alcohol are chiefly depends on carbohydrate amount in sample.

Keywords: yeast, fermentation, % of alcohol, alkali treatment, purification, agro material

1. Introduction
Ethanol production from molasses and other starch containing product are existed since past. Indian is one of the largest agricultural producer country among world's producers. Different types of crops, fruits, vegetables, herbs and economical products are produced throughout *india*. There are different kinds of agricultural residue or waste materials lefts after processing. These waste materials are useless to animals as well as mankind. These waste materials generally thrown outside or in dust bin. Accumulation of this agro waste caused polluted to the environment. Sometime these agro waste rooted in environment and emit unpleasant smell in environment. These all waste agro materials can be utilized to produce the economical or commercial products and also eradication of pollution. We are demonstrating the use of this waste materials to bio ethanol production [3].
Alcohol is one of the most common usable chemicals of the modern world. Alcohol has been utilizing for centuries in social, medical or research purpose. Ethyl alcohol is most common type class of alcohol ($C2H5OH$) chemically comprising ethane and one hydroxyl group [4].
8.3yl alcohol is clear, colorless liquid with a characteristics, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Melting point of Ethyl alcohol is –114.1°C, boils boiling points is 78.5°C, and have density of 0.789 g/mL at 20°C. Very low freezing Point make it useful as low-temperature purposes, as antifreeze agent used in radiators of automobile.
Ethyl alcohol has been produced by human by traditional fermentation process, of starch containing materials as well as other materials at industrial level. Consumption of ethyl alcohol is very old, in different cultural of world population, however it has been consumed in INDIA as religious, cultural occasional event as holly ceremonies since thousand years. Ethyl alcohol soluble in water and highly inflammable liquid. Primary used of alcohol is consumption as drink, while other use of alcohol are existed. Currently bio-ethanol is being produced worldwide by only converting sugar molasses to ethanol at industrial level, which is not even sufficient to cope up with the demands of chemical industries and breweries. So as high demand of ethyl alcohol is increasing day by day. It is need to increase the production of alcohol using agricultural wastes, in addition to molasses and grains, as agricultural wastes or residue are abundantly available throughout the INDIA [5].
The Government of India has made it mandatory for all petroleum refineries to blend 10% ethanol with refined petroleum products out of which 5% with immediate effect. To fulfill this requirement it is an uphill task for the government to provide and Supply bulk ethanol to petroleum industries at affordable rates [6].

2. Materials
2.1 Waste agro materials
- Potatoes peel
- Paddy/rice straw
- Banana peel
- Corn cob

2.2 Chemicals: analytical grade (Fischer and Qualigens).
- enzymes
- Phenol
- Sulphuric Acid
- NaOH
- Different media
- Potassium dichromate

2.3 Yeast
S. cerevisiae variety ellipsoideus DYPBBI Y 0020

2.4 Equipment's

Serial no	Instrument	Model no	Company
1	Orbital shaker incubator	Cintex Ind. Ltd.	CTC-81
2	Water bath	Pooja Lab	WBS-200
3	Hot air oven	Metlab	-
4	Spectrophotometer	Thermo	4001/4
5	Laminar Air Flow	ACCO	AC-071-03
6	Refrigerator	Haier	HRB-331HP4
7	Microscope	-	Olympus

3. Method
3.1 Determination of total carbohydrates by Phenol Sulphuric Acid Method [7].
3.1.1 Standard Curve preparation for Phenol Sulphuric Acid method

Standard stock solution of concentration 0.1 gm/l was made for standard curve. 5% phenol was prepared by mixing 5 ml of phenol with 95 ml of distilled water.10 test tubes with each having 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml of stock solution respectively was Made. Final volume was made 1ml by adding distilled water. Blank was made using 1ml of distilled water. To each tube 1 ml of 5% phenol and 5 ml of 98% sulphuric acid was added. The tubes were covered with aluminum foil and kept in ice bath for 30 minutes. O.D. was Obtained at 490 nm.

3.1.2 Preparation of samples for carbohydrate determination

a) For making a homogeneous suspension of potato, banana peel of 0.05 mg/ml following steps were taken. Peels were grinded to make a fine powder.5.0 mg of powdered peels were added to 10ml of 1N NaOH and heated to 90˚C to make a fine suspension.1.0 ml of the above suspension was diluted with 9.0 ml of distilled water. .So as to make a final dilution of 0.050 mg/ml.

b) Control Preparation 0.50 ml from 0.10 gm/l of glucose standard stock solution was taken as control.

c) Determination of total carbohydrates in unknown samples. From the suspension of peels, 0.50 ml of sample was taken in a test tube. The final volume was made to 1.0 ml with distilled water. A blank was made using 1.0 ml of distilled water. To each tube 1.0 ml of the above diluted sample and 1.0 ml of 5% phenol solution and 5 ml of 98% sulphuric acid was added to each tube. The tubes were then placed in an ice bath for 30 minutes. The optical density was taken at 490 nm in a spectrophotometer. The percent total carbohydrate was calculated with the help of standard curve.

3.2 Fermentation of prepared material
1. Stock culture: the stock of strain *Saccharomyces cerevisiae* was maintained in YPD media and stored in refrigerator.
2. Preparation of inoculums: inoculum of the above mentioned strain of yeast was prepared by transferring of a loop full of inoculums to conical flask containing 50 ml of YPD broth. It was incubated at 25 ˚C for 48 hrs. To allow the culture to come in a log phase of growth. A 50% inoculums size was used for fermentation. The inoculum was checked microscopically for growth and sterility. The growth of final sample was measured by checking OD at 540nm.

3.3. Determination of alcohol contents
The fermented samples were centrifuged to remove the yeast cells and other solids present in the sample. The alcohol content of these samples was determined by potassium dichromate.

3.3.1 Principle of potassium dichromate method: Potassium dichromate is a strong oxidizing agent. It oxidizes the hydroxyl group of primary

Alcohol whereas chromium from dichromate reagent gets reduced. Reduced form of the reagent changes color from orange to dark brown. The intensity of the color is proportional to the concentration of alcohol.

3.3.2 Preparation of standard for potassium dichromate method
Take 6 clean and dry test tubes. To each tube add 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of 2 % absolute Alcohol. Adjust total volume of each tube to 3 ml using distilled water. Then add 4 ml of potassium dichromate reagent to each tube. Reaction gets completed after the addition of 1 ml of concentrated H_2SO_4 to each tube. Measure the O.D. at 620 nm using blank sample.

4. Distillation
After fermentation of different samples yeast cells were separated by filtration process. Left liquid part was distilled by claisen condenser apparatus. Fractions were collected up to 78.5°C. Obtained fractions were analyzed for ethanol percentage (v/v)

By optical density method by using spectrophotometer [8].

5. Result and discussion
Above overview revealed the interesting facts about the bioenergy production, as we collected different type waste agro residue, like peels of potatoes, bananas and other too. Satisfactory results were obtained. Table 1 predict the carbohydrate contents in different peels as we used as material. Table 2 showing Standard curve for potassium dichromate method for determination of alcohol percentage after fermentation process of sample. Table 3 revealed total sugar content (%) and conversion of sugar to alcohol (%) at various incubation. Table 4 showing total sugar content (%) and conversion of sugar to alcohol (%) at various incubation Period of fermentation from banana peels. Table 5 total sugar content (%) and conversion of sugar to alcohol (%) at various incubation Period of fermentation from rice straw. Table 6

total sugar content (%) and conversion of sugar to alcohol (%) at various incubation Period of fermentation from corn cob. Table 7 comparative contrast between all materials and final obtained alcohol %. Figure 1 demonstrate the % of sugar conversion to alcohol after chemical pretreatment, enzymatic hydrolysis and fermentation. As depicted in table 1 the maximum carbohydrate found in potato 92.5% and minimum in the rice straw 82%. The carbohydrate is the main source for the conversion of sugar to alcohol so more the carbohydrate more will be the alcohol. Bioethanol as an alternative fuel is a green and renewable energy source which helps in conservation fossil fuel uses, dependency, containment and controlling carbon foot print age for a healthy and eco-friendly future. India enforced 10% mandates for ethanol blending in petrol [9].

Table 1: carbohydrate contents in different peels.

Serial no	sample	Solid amount (gm)	Total carbohydrates (gm)	% of carbohydrates
1	Potato peels	1	0.925	92.5
2	Banana peels	1	0.882	88.2
3	Rice straw	1	0.820	82.0
4	Corn cob	1	0.856	85.6

Table 2: Standard curve for potassium dichromate method for determination of alcohol.

Serial no	Standard ethanol (ml)	Potassium dichromate reagent (ml)	Distilled water (ml)	Conc.H2SO4 (ml)	O.D.at 620 nm
1	blank	4	3.0	1	0.000
2	0.2	4	3.0	1	0.150
3	0.4	4	3.0	1	0.236
4	0.6	4	3.0	1	0.446
5	0.8	4	3.0	1	0.695
6	1.0	4	3.0	1	0.750

Table 3: total sugar content (%) and conversion of sugar to alcohol (%) at various incubation Period of fermentation from potato peel.

Serial no	Fermentation (hrs.)	Sugar %	% conversion of sugar to alcohol
1	0 hrs.	92.0	0.00
2	24 hrs.	42.8	30.2
3	48 hrs.	15.3	39.6
4	72 hrs.	6.2	46.2
5	96 hrs.	3.5	46.8

Table 4: total sugar content (%) and conversion of sugar to alcohol (%) at various incubation Period of fermentation from banana peel.

Serial no	Fermentation (hrs.)	Sugar %	% conversion of sugar to alcohol
1	0 hrs.	72.0	0.00
2	24 hrs.	32.2	28.8
3	48 hrs.	15.5	36.4
4	72 hrs.	8.2	42.4
5	96 hrs.	4.8	44.3

Table 5: total sugar content (%) and conversion of sugar to alcohol (%) at various incubation Period of fermentation from rice straw.

Serial no	Fermentation (hrs.)	Sugar %	% conversion of sugar to alcohol
1	0 hrs.	60.0	0.00
2	24 hrs.	34.2	26.5
3	48 hrs.	18.6	37.8
4	72 hrs.	6.7	40.2
5	96 hrs.	4.8	41.4

Table 6: total sugar content (%) and conversion of sugar to alcohol (%) at various incubation Period of fermentation from corn cob.

Serial no	Fermentation (hrs.)	Sugar %	% conversion of sugar to alcohol
1	0 hrs.	72.0	0.00
2	24 hrs.	28.7	32.3
3	48 hrs.	16.9	40.4
4	72 hrs.	8.3	45.3
5	96 hrs.	4.2	46.0

Table 7: Alcohol contents in different samples after 96 hrs. of fermentation.

Serial No.	Samples	Initial solid %	Sugar%	Alcohol %	Remaining sugar (unfermented) (gm)	% conversion of sugar to alcohol
1	Potato	10	9.2	4.3	0.350	46.8
2	Banana	10	7.2	3.18	0.480	44.3
3	Rice straw	10	6.0	2.48	0.480	41.4
4	Corn cob	10	7.2	3.3	0.420	46.0

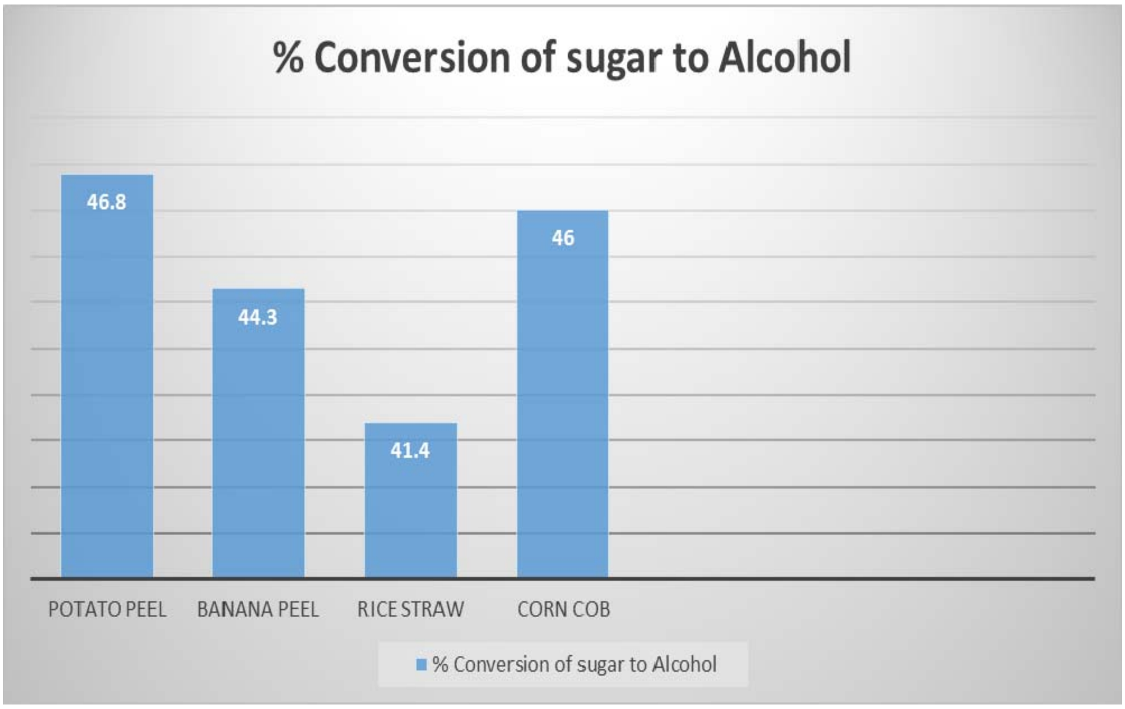

Fig 1: percentage of sugar conversion to alcohol after chemical pretreatment, enzymatic hydrolysis and fermentation

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