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Bioethanol production from fruit and vegetable waste using yeast

Abstract

Fossil fuels has a negative impact on environment as greenhouse gases are emitted which is harmful to both health as well as environment. Ethanol is a renewable fuel made from various waste materials such as plants collectively known as "biomass." Production of biofuel from various waste such as agricultural waste, municipal waste, etc., has been carried out with the objective of converting the waste to useful material. Any starch or sugar based material, referred as cellulosic material, which has high amount of cellulose present in its composition and which can be converted to a fuel and it improves the energy balance of ethanol, because cellulosic feed stocks require less fossil fuel energy to produce ethanol. Biomass used to bring out the process of converting non-food-based materials into cellulosic ethanol and also reduces the amount of fossil fuel energy used in production. Production of ethanol as an alternative fuel from food and agricultural waste was done by bio-processing. Wastes from fruits, such as peels from banana and orange, vegetable waste such as peels from potato and pea were subjected to simultaneous scharification and fermentation for 10 days by active culture of yeast Saccharomyces cerevisiae. The ethanol yield was determined at every interval of 48 hours. In the experiment it was shown that potato peels has the highest biomass yield, followed by orange peels, pea peels, banana peels and mix peels. The optimal ethanol yields was 3.759 mol/l, 2.972 mol/l, 5.5452 mol/l, 3.057 mol/l, 1.833 mol/l from carbohydrate content of 205.908 mol/l, 209.908 mol/l, 216.479 mol/l, 208.780 mol/l, 204.300 mol/l of Orange, Banana, Potato, Pea, and mix peel respectively. These indicate that ethanol yield from Potato and orange peel was significantly higher than pea, banana and mix peels.

Key words: *Biofuel, Ethanol production, Fruit waste, Fermentation, **S**accharomyces cerevisiae, Vegetable waste.*

Introduction:

Biofuels are an important factor as they substitute for fossil fuels and hence the production is increasing everyday. It can be produced from wood, straw, micro-algae or biological remains and energy management ensure the economic and sustainable production of second generation biofuels [4]. Biofuel production techniques depend upon the type of raw material, efficiency level, production volume, surrounding situation and end-users requirement etc. Algae are aquatic oxygenic autotrophic and can be served as a good source for the production. Some microalgae grow fast and their lipid and starch contents are high over 30% w/w which makes them easy to cultivate and hence increases the rate of production [4]. Cellulose is a polymer of glucose, which include paper, cotton, wood and starch food and is a simple sugar that is easily consumed by yeast to produce ethanol. Biofuel is a renewable source of energy and can be used as an alternative to conventional fossil fuels as it burns up to 75% cleaner than fossil fuels. Use of agricultural waste which has no economic value can be used for biofuel production and gives a better way of efficiently utilizing agricultural land. Sugarcane molasses, groundnut shells, rice husks, straw, corncobs, etc. are being studied as substrates for biofuel production. They are a wide range of fuels which can be derived using biomass or plant matter and animal waste as well as organic waste materials. It can also derived using industrial and municipal waste, and forestry agricultural residues [5]. Substrate which contains high amount of sugar can be used to produce bioethanol which means fodder crop which contains simple sugar in large amount [5]. The polymers like starch and cellulose are first broken down into simple sugars through chemical hydrolysis or enzymatic hydrolysis called saccharification, and then converted by fermentation process to ethanol and carbon dioxide[5]. In scarification, process starch is converted into simple sugars which are monosaccharide using microorganism or enzymes such as glucoamylase and α-

amylase [3]. Yeast, fungi, certain microalgae and genetically modified microorganisms are used as feedstock for biofuel production [1]. The yeast, *Saccharomyces cerevisiae*, produces ethanol by fermentation of glucose, but it is unable to ferment pentose sugars [7]. Sufficient biomass of yeast can be produced using fermenters and other advantages include smaller area for production as compared to plants, easy extraction method and ability to grow on a wide variety of media [7]. the present work various fruit and vegetable peels were used to convert biomass into ethanol for the production.

Material and Method

Routine Culture Maintenance.

Culture of *Saccharomyces cerevisiae* was maintained on YEPDA (1% yeast extract, 2% peptone, 2% agar) slant stored at 4°C.

Preparation of peels for Ethanol Production

Sterile banana, orange, potato, pea peels were collected and chopped in to small pieces and grinded with blender using sterile distilled water and pH was set to 5.5 then stored in refrigerator for further use.

Chemicals and Composition

Acidic Dichromate solution (0.01mol/l in 5mol/l of sulphuric acid): Add 125 ml of water and carefully add 70 ml of conc. Sulphuric acid to it with constant swilling. Cool down the flask and add 0.75 g of potassium dichromate. Dilute with distilled water and make the volume upto 250ml. Starch indicator solution: 1%starch solution prepare by adding 1 g of starch powder in freshly boiled water. Stir until dissolved. Sodium thiosulfate solution (0.03mol/l):-Add 7.44 g of sodium thiosulfate in 1000 ml of distilled water. Potassium iodide solution (1.2mol/l): Dissolve 5 g of KI in 25 ml of distilled water. Anthrone reagent: weigh 200 g of anthrone and mix it in 100 ml of H_2SO_4 The growth medium (Inoculum Medium) prepared for ethanol production consists of glucose (20 g/l), Ammonium sulphate (0.8 g/l), KH_2PO_4 (0.8 g/l), Magnesium sulphate (4 g/l), Yeast extract (3.2 g/l), in the production medium Glucose as carbon resource is replaced with agricultural waste (orange, banana, Pea, Potato, and mix peels) in 250 ml of conical flask containing 100 ml of distilled water(pH-5.5). The flasks were autoclaved at 121°C for 20 minutes. The cells of *Saccharomyces cerevisiae* were aseptically cultured in inoculum medium. Incubate culture at 30°C for overnight.

Ethanol Production

Medium (270 ml) was prepared using agricultural waste separately using Banana peels, Orange Peels, potato peels, pea peels, and mix peels and transferred to a Conical Flask. The media was autoclaved at 121^0C for 20 minutes and cooled. 10ml of previously activated culture of *saccharomyces cerevisiae* was added to the medium. The Flasks were cultured in aerobic conditions. The samples were withdrawn at regular time intervals every 48 h till ten days of incubation and qualitative estimation of ethanol was done using potassium dichromate method and at tenth day of incubation.

Estimation of total carbohydrate content:

Total carbohydrate content was measured using anthrone assay method. Stock standard solution was prepared using 0.1 g of glucose and was transferred into 100 ml of distilled water. 10 ml of peel sample was taken and transferred into 100 ml of distilled water. For Standard solution Take out 0.2 to 1ml of working standard solution of five different test tube and add water to bring the volume to 1ml in each test tube add 4ml of anthrone reagent and mix the contents as well and cover the test tube with bath for 10 min then cool the test tube to the room temperature and measure the optical density in a photoelectric colorimeter at 620nm. Simultaneously prepare a blank with 1ml of distilled water and 4ml of anthrone reagent. Construct a calibration curve on a graph paper, by plotting the glucose concentration on x-axis and absorbance at 620nm on the y-axis. Compute the concentration of the sugar in the sample from the calibration curve. While calculating the sugar concentration in the unknown sample, the dilution factor has to be taken into account.

Table I: Total carbohydrate content in various peels in mol/l.

Peels	Orange	Banana	Potato	Pea	Mix
Concentration of sugar (mol/l)	205.908	209.810	216.479	208.780	204.300

Quantitative estimation of ethanol using potassium dichromate method

Ethanol yield was measured by ethanol assay using potassium dichromate method. This method uses a redox titration to find the concentration of ethanol in an aqueous solution. The ethanol is oxidised to ethanoic acid by reacting it with an excess of potassium dichromate in acid. Sample was prepared and Diluted to 1:20 10 ml in 200 ml with distilled water. After dilution 10 ml of acidic dichromate was added in 250 ml beaker. Pipette out 1 ml of diluted sample into a sample holder and place it in a beaker containing potassium dichromate solution. Store the beaker in incubator overnight at 25-30^{o}c.Next morning discard the sample holder and rinse the walls of beaker with 100 ml distilled water and add 1 ml of KI solution to it and swirling to mix. Fill the burette with sodium thiosulfate solution and titrate each flask with it until brown iodine colour becomes pale yellow. Add 2 ml of starch solution and keep titrating until blue colour disappears and become colourless. Titrate the blank flask first, and repeat until concordant results are obtained. Then titrate each flask to obtain results.

The blank titration indicates how much amount acidic dichromate was present at the start. As no alcohol is present the full amount of the dichromate is still present. The blank titrations are carried out so the result can be compared with those of the sample titrations. Determine the average volume of sodium thiosulfate used for sample and for blank titration from concordant sample results. Subtract the volume of the sodium thiosulfate solution used for the sample titration from the volume used for the blank titration. This volume of the sodium thiosulfate solution is now used to determine the alcohol concentration. Calculate the number of moles of sodium thiosulfate in this volume. Using the equations, determine the relationship between the moles of sodium thiosulfate and the moles of ethanol.

- as 6 mol of $S_2O_3^{2-}$ is equivalent to 1 mol of $Cr_2O_7^{-2}$
- and 2 mol of $Cr_2O_7^{-2}$ is equivalent to 3 mol of C_2H_5OH
-then 1 mol of $S_2O_3^{-2}$is equivalent to 0.25 mol of C_2H_5OH

This ratio helps to calculate the moles of alcohol in the sample solution. As the sample was diluted to 1:20 so, the result need to be multiplied with the dilution factor 20. Convert the answer in moles per litre to percentage (grams per 100 ml) to compare with the figure given on the bottle of the alcoholic beverage tested.

Result

Fruit waste and vegetable waste like Potato peel, pea peel, banana peel, orange peel, were selected on the basis of the carbohydrate amount of the fruit and vegetables. The selection of organism *Saccharomyces cerevisiae* is done as it is ability to convert sugar into ethanol by fermentation process. The total carbohydrate content was measured using an throne assay for various peels. Highest amount of ethanol production was obtained in potato peels as it contains high amount of sugar which in the form of starch. Quantification of ethanol produced by the fermentation of different peel was carried out using potassium dichromate method. Obtained results are as follows.

Table II: shows total amount of sugar present in peels.

Peels	Sugar content in mole/l
Orange	205.908
Banana	209.810
Potato	216.479
Pea	208.780
Mix	204.300

Table III: The quantitative estimation of ethanol using potassium dichromate method in (g/l).

Peels	Day 1	Day 3	Day 5	Day 7	Day 9	Day 10
Orange	0.3455	5.394	20.012	69.102	55.280	23.034
Banana	0.3915	5.394	15.340	30.405	39.485	46.068
Potato	0.4847	5.698	34.551	46.068	138.205	19.740
Pea	0.6910	6.638	16.578	23.034	34.551	69.102
Mix	0.6449	5.394	23.578	17.275	19.740	12.562

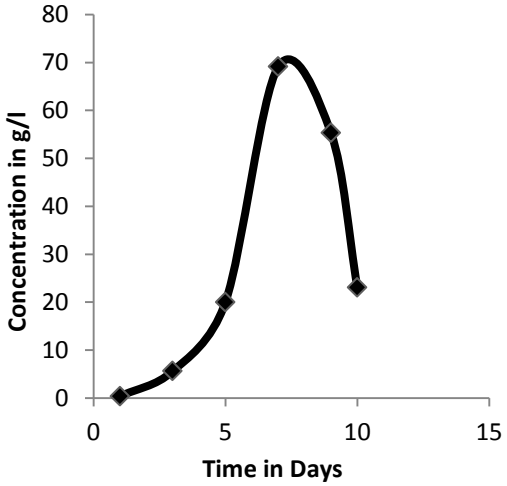


Fig I

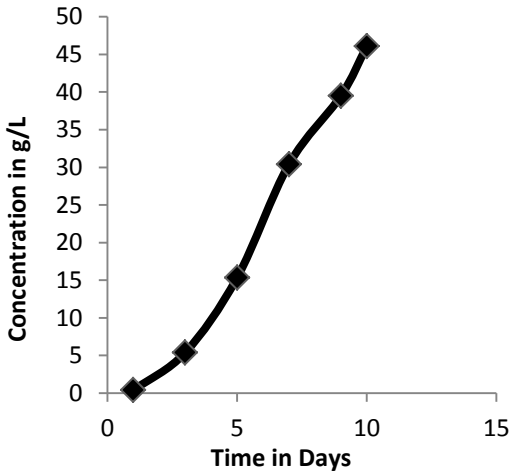
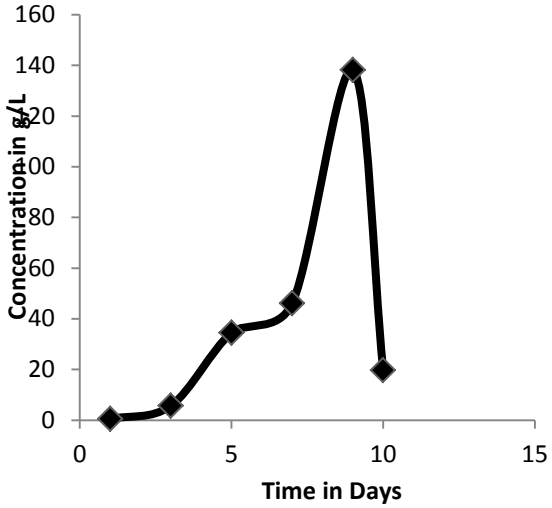


Fig II



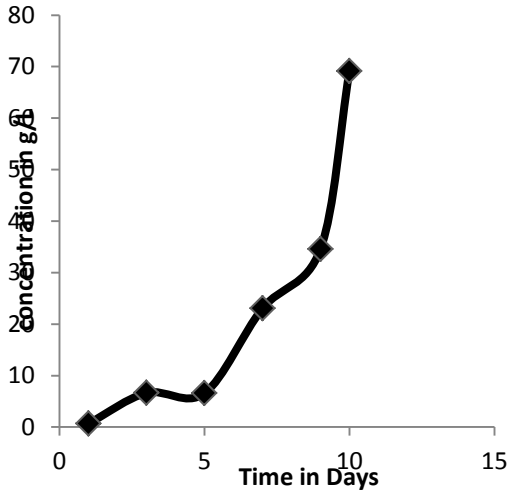


Fig III **Fig IV**

Above figures shoes ethanol production in the fermentation medium in using Orange, Banana, Potato, Pea, and Mix peels as a source of carbon.

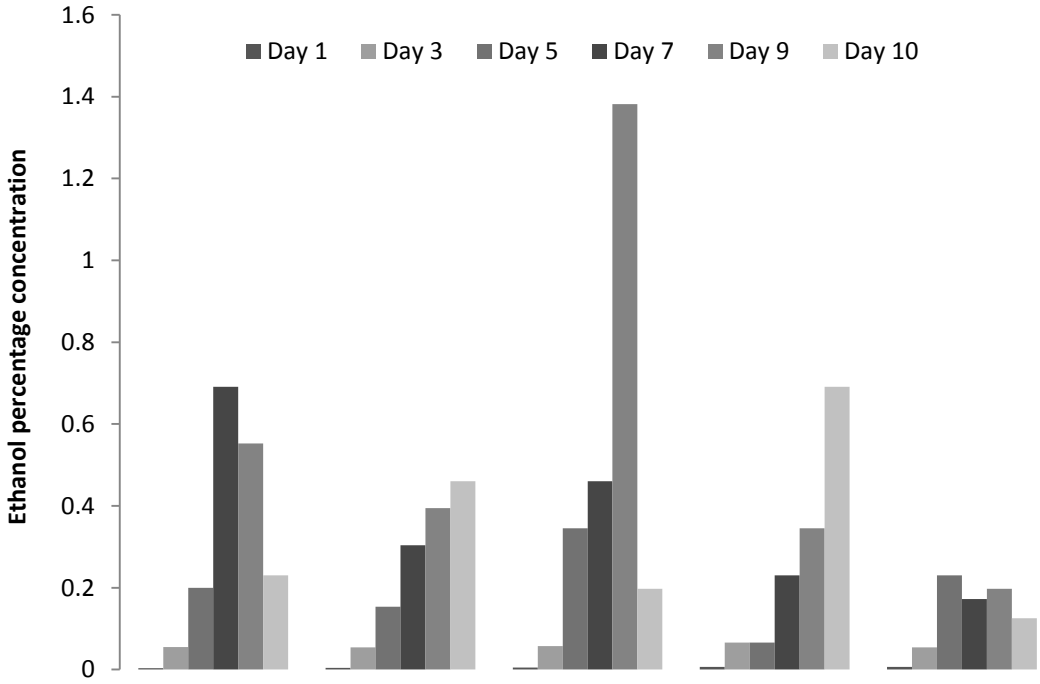


Fig V: Shows ethanol production in v/v % using peels as a source of carbon.

TABLE IV; Shows total ethnaolic content in g/l.

Peel	**Total ethanol content (g/l)**
Orange	28.91
Banana	22.84
Potato	40.79
Pea	23.43
Mix	13.10

It was seen that after fermentation using *Saccharomyces cerevisiae*, Potato waste produced 0.408% (40.79g/l) ethanol, Orange waste produced 0.288% (28.91g/l) ethanol, pea waste produced 0.234% (23.43 g/l) ethanol, banana waste produced 0.228% (22.84g/l) ethanol.

Table V: Shows conversion of sugar into ethanol present on day one in each peels.

Peels	Orange	Banana	Potato	Pea	Mix
Concentration of sugar (mol/l)	205.908	209.810	216.479	208.780	204.300
Ethanol produced (mol/l)	3.759	2.972	5.452	3.0572	1.833

Conclusion

From the above practice it can be concluded that, as the demand of alternative fuel increases every day, production of ethanol from renewable source such as agriculture waste, fruit waste, municipal waste, etc is imperative for meeting increased demand. These materials could prove as a cheap and abundant feedstock, and have potential to produce fuel bioethanol at reasonable costs. Production of ethanol from various carbon sources is carried out by engineering or by exploiting native fermentation pathways of various microbial hosts. From this experiment it is proved that the ethanol yield could be produced from fruit and vegetable waste as the substrates and high income or profit can be obtained using material which has high amount of sugar. Maximum activity is obtained by using potato peel as a substrate at 37°C as potato contains high amount of starch.

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Ashna Trivedi
Student
K K Shastri Government Science College
Ahmedabad

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