BIOALCOHOL PRODUCTION FROM RICE SOUP BY FERMENTATION AND DISTILLATION

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ABSTRACT

Rice is a starch-rich raw material that can be used for production of bioalcohol. But the use of rice for bioalcohol production will cause a food shortage. So rice soup/ rice water which is thrown out as a byproduct of cooked rice can be used for bioalcohol production since it is a rich source of starch. Moreover rice soup is easily available in large quantities and cheap. Production of bioalcohol from rice soup is a two-stage process; the pretreatment of starch followed by fermentation. For this purpose, some efficient enzymatic procedures have been used for the pretreatment of rice soup. The different sources used are: 1) Direct amylase enzyme 2) Immobilized amylase 3) Aspergillus sp. 4) Yeast isolated from banana. The amylase enzymes, Aspergillus sp. and yeast isolated from banana were used for the pretreatment of starch, to breakdown starch to glucose. Yeast was inoculated to the pretreated starch and kept for anaerobic fermentation. The duration and the amount of alcohol produced varied with the pretreatment adopted. All the samples were distilled to produce alcohol. Various analysis were performed to confirm the production of bioalcohol and iodine test to detect the presence of ethanol was also performed.

Keywords: bioalcohol, rice soup, pretreatment, fermentation

I. INTRODUCTION

Bio-alcohol is probably the most commonly used non-fossil alternative transport fuel in the World. Fermenting and distilling sugar crops, starch crops that have been converted into simple sugars or processing cellulose bio-mass can produce bio-alcohols. These bio-alcohols are mainly bio-ethanol and bio-methanol. One of the benefits of this fuel is that it is biodegradable and has low toxicity. The sugars and starches in plants are the product of photosynthesis, the process by which plants use the energy from sunlight to make sugar, so the ultimate source of the energy is the sun (Marina et al 2002).

Rice soup or rice water is the water which is drained out after boiling rice. It contains almost all the nutrients, starch in major. Ratio of water to rice is one of the most important parameter considered while cooking rice. Most rice is perfectly cooked when the final moisture content is between 58% and 64% moisture. At lower moisture contents, the rice is firmer. The final moisture content is a matter of preference and preference can differ with the type of rice and final cooked application. The math is very simple; 100 grams of rice with initial moisture content of 12% needs 110 grams of water to be fully cooked at 58% moisture. 100 grams of rice with initial moisture of 12% needs 145 grams of water to be fully cooked at 64% moisture. In the cooking process, two cups of water to one cup of rice ratio is preferred. (Fitbit: Nutritional

Information, Calories in Roland Red Rice, 2011).The main objective of this study was to produce bioalcohol from rice soup by fermentation and distillation.

II. REVIEW OF LITERATURE

The white rot fungus *Trametes hirsuta* produced ethanol from a variety of hexoses: glucose, mannose, cellobiose and maltose, with yields of 0.49, 0.48, 0.47 and 0.47 g/g of ethanol per sugar utilized, respectively. In addition, this fungus showed relatively favorable xylose consumption and ethanol production with a yield of 0.44 g/g. *T. hirsuta* was capable of directly fermenting starch, wheat bran and rice straw to ethanol without acid or enzymatic hydrolysis. (Kenji Okamoto et al, 2011)

Bioethanol production from rice washing drainage and rice bran derived from a rinse-free process of rice manufacturing was established using the combinations of an enzymatic procedure and an ultra-sonic procedure of rice washing drainage for a sample pre-treatment. The maximum ethanol concentration was attained at 6.2% (v/v). (Masanori Watanabe et al, 2009).

Ripening changes were studied in plantains of three cultivars, two Horn types and one French type, and compared with those in bananas ripened under the same conditions. Bananas contained 1% starch when fully ripe and none when overripe, whereas plantains contained about 9% starch when fully ripe and 3% when overripe (composition expressed as percentage fresh weight). (John Marriott et al 2006)

III. MATERIALS AND METHODS

A) MATERIALS

1. RICE SOUP: Rice soup was prepared using Palakkadan matta rice or Red Rice of Kerala. This Red Rice of Kerala contains more starch compared to other rice variety, hence preferred. During cooking, the ratio of rice: water taken is 1:2. After cooking, the remaining water was drained and collected and used as rice soup or rice water.
2. BANANA: Overripe banana exposed to atmosphere for few days was used to isolate yeast. When the pulp of the banana was exposed to atmosphere, atmospheric yeasts attack the plantain pulp which appears as black portions in the pulp.
3. BREAD: Bread was kept in a wet atmosphere so that fungal growth occurred. Aspergillus sp. was isolated from this fungus infected bread.

B) METHODS

1. RICE SOUP PREPARATION

Rice soup was made using Palakkadan matta rice or Red Rice of Kerala. For one cup of rice two cups of water was used for cooking. After boiling the water was collected and used as rice soup or rice water (Fitbit: Nutritional Information, Calories in Roland Red Rice, 2011).

2. GENERAL METHODOLOGY

The flow chart for the preparation of Bioalcohol from rice soup has been shown in Fig 1. The complex starch is converted to simple sugar by various pretreatment methods. Rice soup provides the starch source and it was taken in different dilutions. Pretreatment methods include the use of commercially available amylase enzyme, immobilized amylase, *Aspergillus sp.*, and yeast isolated from banana. This was kept undisturbed for incubation at room temperature.

Pretreatment converts starch to simple sugar and DNS assay was conducted to detect the production of reducing sugar. The dilution which showed maximum production of reducing sugar was kept for anaerobic fermentation with 15ml of 5% yeast. Chromic acid test for alcohols, iodine test for ethanol and the odor was checked every day for the

production of bioalcohol. Simple distillation was performed when the sample showed maximum result in chromic acid test.

Starch (rice soup:water 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0)

↓

Pretreatment(amylase enzyme, immobilized amylase, *Aspergillus sp.*, and yeast isolated from banana)

↓

Incubation at room temperature

↓

Starch gets converted to sugar

↓

DNS Assay

↓

Anaerobic fermentation (15ml 5%yeast)

↓

Chromic acid test for alcohol content estimation

↓

Iodoform test for ethanol estimation

↓

Simple distillation

↓

Bioalcohol

Fig 1: Flow Chart for the preparation of Bioalcohol from rice soup

3. VARIOUS PRETREATMENT METHODS

a) Using commercially available amylase and commercially available yeast: 10ml of 1% amylase enzyme was added to each conical flask with rice soup.

b) Using immobilized amylase and commercially available yeast: 2g sodium alginate was weighed and dissolved in 50ml distilled water at 60˚C. This viscous solution was cooled for 1 hr. 25ml of 1% amylase was mixed with 50ml of sodium alginate mixture in the ratio 1:2. 2.5ml of gluteraldehyde was added to this mixture and mixed well. This mixture was taken in a micropipette and added dropwise to ice cold 0.2M calcium chloride solution to get calcium alginate beads, and left it for 30mins for proper hardening of beads. Later this was washed with 0.9% sodium chloride solution and obtained the immobilized amylase enzyme. 12g of immobilized enzyme was added to each conical flask with different dilutions of rice soup.

c) Using *Aspergillus sp*. and commercially available yeast: White, cotton like fungus was inoculated from fungus infected bread to sterilized potato dextrose agar plates and kept for incubation at room temperature. After 24hrs white cottony growth was seen which turned black in the next days. This was sub-cultured and slant was also prepared. Lactophenol cotton blue staining was also performed. 3 loops of this Aspergillus sp. were inoculated to the conical flasks with different concentrations of rice soup.

d) Using yeast isolated from banana: Pulp of over riped banana which was in contact with atmosphere was inoculated to sterilized YM agar plates and kept for incubation at room temperature. Colonies appeared after 24hrs of incubation. Sub cultured the selected colonies and prepared slants. From this slant culture, yeast cells were inoculated to rice soup of different concentrations.

e) Using yeast isolated from banana and commercially available yeast: Yeast isolated from banana was inoculated to rice soup of different concentrations.

4. INSTRUMENTAL ANALYSIS

a) Simple distillation: All the fermented samples were taken for distillation. Since the boiling point

of ethanol is 78.37˚C, the distillation temperature was set at 79˚C. Simple distillation was performed, were the more volatile will evaporate first and as it passes through the condenser it will be cooled by the water circulating around condenser. Thus the more volatile alcohol will be coming out first and is collected in a conical flask kept at the end of the condenser. The four samples which showed positive result in iodine test and chromic acid test was used for simple distillation.

5. CHEMICAL ANALYSIS

a) Estimation of starch content in rice soup: 1% starch solution was prepared that is 1g in 100ml distilled water (1000mg/100ml). 7 clean test tubes were taken and labeled it like B, S1, S2, S3, S4, S5, and T. To the test tubes labeled as S1, S2, S3, S4, and S5, added 2ml, 4ml, 6ml, 8ml, and 10ml starch solutions correspondingly. Then to the test (T) 5ml rice soup was added. Distilled water was added to each test tube to make up the solutions in the test tube 20ml. 0.1ml of iodine solution was added to each test tube. OD value was taken at 610nm. Plotted OD Vs concentration graph. (Nuffield Foundation).

b) Iodoform test for ethanol: 1ml of test solution was taken in a clean test tube and 25 drops of Iodine solution was added and vortexed. To this mixture, 10 drops of sodium hydroxide was added and gently swirled for few minutes. The dark colour of iodine starts to fade when observed after 2mins. The solution in the test tube became cloudy and then a yellow precipitate of triidomethane (iodoform) was seen. This gave a distinct antiseptic smell (Nuffield Foundation).

c) DNS assay: 7 clean test tubes were taken and marked as B,S1,S2,S3,S4,S5,and T. To S1, S2,S3, S4,S5 added 0.2,0.4,0.6,0.8,1.0ml glucose standard and to T added 0.5ml of test solution. Made up the solution in each test tube to 1ml using distilled water. Added 3ml of DNS to each test tube. Kept in boiling water bath for 10 mins. Added 1ml of 40% sodium potassium tartarate to each test tube. Added 10ml distilled water to each test tube. O.D value was at 540nm. Plotted OD Vs concentration graph. (Miller GL. 1959)

d) Estimation of alcohol content: Taken 7 clean test tubes and marked it as B, S1, S2, S3, S4, S5 and T. To the test tubes marked as S1, S2, S3, S4, S5 added 0.1, 0.2, 0.3, 0.4, 0.5ml of ethanol and to T added 0.5ml of test solution. Made up the solution in each test tube with distilled water to 2.5ml. Added 6.25ml of chromic acid to each test tube. Now added 0.5ml of 40% sodium potassium tartarate to each test tube. Incubated at 80˚C for 15mins. Added 2ml distilled water to each test tube. The OD value was taken at 600nm. Plotted OD Vs ethanol concentration graph and from it estimated the ethanol content.

6. MICROBIAL STUDIES

a) Lactophenol cotton blue staining (yeast morphology): Cleaned glass slide was taken. Placed a drop of lactophenol cotton blue stain on this slide. To the drop of stain added a loopful of the sample culture. Thin layer of this mixture was made by spreading. Kept for drying at room temperature. Observed under microscope for oval shaped and budding colonies. (Jorgensen Laboratories)

b) Lactophenol cotton blue staining for fungal morphology: Cleaned a glass slide. A drop of lactophenol cotton blue stain was added to this slide. A little of the fungal culture was added and mixed with this stain. Kept for drying. Observed under microscope. (Jorgensen Laboratories).

IV. RESULTS AND DISCUSSIONS

Various studies have been conducted to study the production of bioalcohol from rice soup by various methods of pretreatment techniques.. The results were tabulated, analyzed graphically and discussed in detail.

Initially the starch concentration in the rice soup was found by colorimetric method and the concentration of starch in 100ml of rice soup was found to be 3.5%.

1. CHROMIC ACID TEST FOR ALCOHOL CONTENT:

a) Using commercially available amylase and yeast: After 3 days of anaerobic fermentation, by chromic acid test it was found out 1.9% of alcohol was produced from rice soup and a maximum of 24% on 17th day.

b) Using immobilized amylase and yeast: This method started producing alcohol from 2nd day of anaerobic fermentation and by chromic acid test 1.8% alcohol was found. The maximum was 14.4% on the 19th day.

c) Using Aspergillus sp. and yeast: In this method alcohol production started from the 4th day onwards with 1.6% of alcohol and on the 17th day it produced 9% alcohol, the maximum.

d) Using yeast isolated from banana: After 36 days of anaerobic fermentation alcohol was not produced.

e) Yeast isolated from banana and yeast: After 2 days of anaerobic fermentation, by chromic acid test it was found 1.9% alcohol was produced and 21.6% on 17th day.

Table: 1 Percentage bioalcohol produced on first day and the highest value of production.

Methods	First day of alcohol production	% alcohol on first day	Highest value obtained	Number of days taken to attain the highest value
Amylase and yeast.	3rd day	1.9%	24%	17
Immobilized amylase and yeast.	2nd day	1.8%	14.4%	19
Aspergillus sp. and yeast.	4th day	1.6%	9%	17
Yeast isolated from banana.	-	–	–	–
Yeast isolated from banana and yeast.	2nd day	1.9%	21.6%	17

Biological pretreatment offers some conceptually important advantages such as low chemical and energy use, but a controllable and sufficiently rapid system has not yet been found. Chemical pretreatments have serious disadvantages in terms of the requirement for specialized corrosion resistant equipment, extensive washing, and proper disposal of chemical wastes. Biological pretreatment is a safe and environmentally-friendly method for pretreatment of starch (Taniguchi et al., 2005).

On pretreatment, initially the reducing sugar concentration increases and reaches a higher value. After that it will start decreasing since the reducing sugars are getting converted to ethanol. Any crop variety that contain sugar or starch that can be converted to sugar by pretreatment can be used for ethanol production. There are several bacteria and yeasts which can convert the sugar to ethanol. These organisms under anaerobic conditions, convert sugar to cellular energy and also produce ethanol and carbondioxide as metabolic waste product (Rasmussen et al 2010).

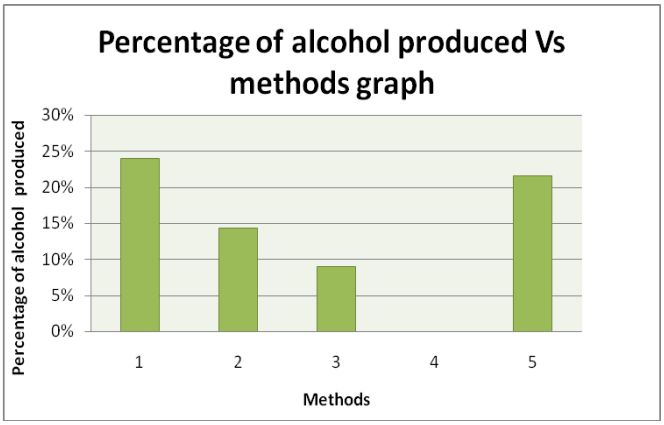

Fig 2: Graph showing the highest percentage of alcohol obtained in each pretreatment method.

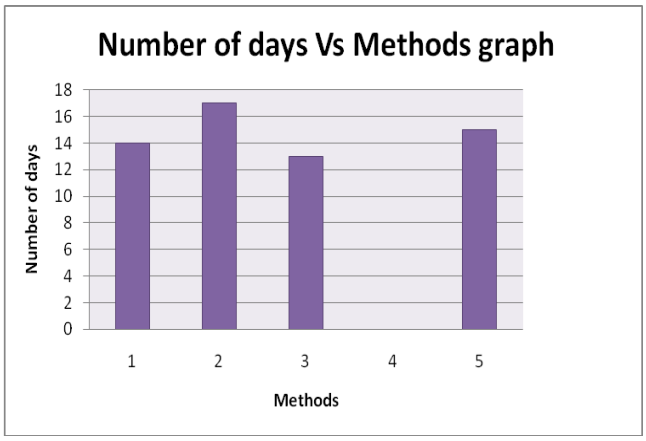

Fig 3: Graph showing the number of days taken for attaining the highest percentage of alcohol

2. IODOFORM TEST FOR ETHANOL

Among the five different pretreatment methods performed four gave positive result for the iodoform test for ethanol. The samples which were pretreated with commercially available amylase, immobilized amylase, Aspergillus sp and yeast isolated from banana, and fermented with yeast exhibited positive results for the test. Whereas the one which was pretreated with yeast isolated with banana alone, gave a negative result for ethanol test. This sample even gave a negative result for alcohol test also as it was not fermented with yeast.

CONCLUSION

Rice soup which is a waste product of cooked rice is cheap and low cost, and also contains lot of starch which can be converted to glucose.

In this work, rice soup was used for the production of bioalcohol. So it's waste product utilization also. Initially the starch content in rice soup was found out as 3.5% by colorimetric method. Since the rice soup contains starch, it should be converted to glucose i.e. pretreatment. Pretreatment is an important step in this ethanol production. For improving the efficiency of pretreatment four methods were adopted. These are using direct amylase enzyme, immobilized amylase enzyme, Aspergillus sp. and yeast isolated from banana. DNS assay was conducted to detect the presence of glucose on pretreatment. After the pretreatment the rice soup was fermented by yeast. Anaerobic condition was maintained during fermentation. Iodoform test and chromic acid test were conducted to detect the presence of ethanol and bioalcohol. After the fermentation the samples were distilled to separate out bioalcohol.

In the first method that is using direct amylase and yeast it started producing alcohol from the 3rd day onwards and attained a maximum of 24% on the 17th day. When immobilized amylase enzyme and yeast was used a maximum of 14.4% was obtained on the 19th day and the initial production started from the 2nd day onwards. Whereas in case of Aspergillus sp. and yeast the production started on the 4th day and attained a maximum of 9% on the 17th day. The sample in which yeast isolated from banana was inoculated didn't produce alcohol. Even though the DNS assay was positive, iodoform test and chromic acid test gave a negative result. That means the rice soup is getting converted to glucose but the fermentation is not occurring. So in the next method the yeast isolated from banana was used as pretreatment method and commercially available yeast was used for fermentation. Then it produced alcohol from the 2nd day to

the 17th day with a maximum of 21.6%.

Finally, it was concluded that among the various pretreatment methods the use of direct amylase enzyme is the most efficient method as it produced maximum percentage of alcohol in minimum number of days. Rice soup is a good source for bioalcohol production and also for waste product utilization.

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