

Synthesis of Bioethanol from Artocarpus Heterophyllus Peel by Fermentation using Saccharomyces Cerevisiae at Low Cost

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Abstract

The most common way to produce bio-ethanol is fermenting raw materials using suitable microorganism. For this fermentation process to produce ethanol from Artocarpus Heterophyllus which is generally known as jack fruit, Saccharomyces Cerevisiae is used as a microorganism. Saccharomyces is used to produce high yield of ethanol due to its naturally adopting property and its high tolerance rate of ethanol and chemical inhibitor. Ethanol, unlike gasoline, is an oxygenated fuel that contains 35% of oxygen, which reduces particulate and Nitrogen Oxide emission from combustion. Ethanol can be made synthetically from petroleum or by microbial conversion of biomass through fermentation process. The main types of raw materials for ethanol production using biological method are cellulose, carbohydrate and sugar. Ethanol production procedures from biomass which are rich in carbohydrates, consists of feedstock preparation or pretreatment, hydrolysis, fermentation and product separation and purification. In this work, jackfruit peel is taken as a substrate due to its high carbohydrate content. The essential hydrolysis and fermentation step, which provides fermentable sugar and ethanol, can be carried out by using enzyme or microorganism. This is carried out using Saccharomyces Cerevisiae generally known as bakery yeast which has been used traditionally for fermented food and alcoholic drink production.

Keywords- Ethanol, Artocarpus Heterophyllus, Fermentation, Saccharomyces Cerevisiae, Micro Organism

I. INTRODUCTION

Ethanol is a colorless liquid. It is soluble in water as well as in ether, acetone, benzene, and other organic solvents. Anhydrous alcohol is hygroscopic; it achieves certain stability after absorption of water to the extent of 0.3- 0.4%. The main parameters of anhydrous ethanol Boiling point is 78.39°C Liquefaction temperature -114.15°C, Refractive index at 20°C-1.36048, Flash point (in closed cup) 13°C, Dynamic viscosity - 1.19 m Pa s⁻¹ Calorific value, Lower-29, 895 kJ Kg⁻¹, Upper-29,964 kJ Kg⁻¹, Azeotropic mixture consists of 95.57% ethanol and 4.43% water by volume. Therefore, normal distillation allows yield of 95.57% ethanol by volume. Chemically ethanol is dominant due to the presence of a functional group – OH in the compound, which enables industrially important chemical reactions, such as dehydration, halogenations, recovery of esters, and oxidation (Ullmann, 1990 b).

The biologically produced ethanol contains about 5% water. Gasoline and ethanol mixtures are called as gasohol. E10, sometimes called gasohol, is a fuel mixture of 10% ethanol and 90% gasoline that can be used in the ICEs of most modern automobiles. Hydrated (or azeotropic) ethanol is ethyl alcohol that contains approximately 5% water. Hydrated ethanol derived from sugar, or ethanol derived from wheat starch, may be used for production of diesohol. The alcohol can be concentrated by distillation to produce up to 96% ethanol. Removal of the remaining 4% water requires special treatment. The major effect of diesohol on engine performance is a significant reduction in visible smoke and particulate emissions. Engine thermal efficiency increases by up to eight percent when operating on diesohol. There is also a significant overall reduction in emission of carbon dioxide.

Ethanol or ethyl alcohol produced by hydrolysis and then fermentation processes from biomass is called as bioethanol. Carbohydrates (hemicelluloses and cellulose) in plant materials can be converted to sugars by hydrolysis process. Fermentation is an anaerobic biological process in which sugars are converted to alcohol by the action of microorganisms, usually yeast. The resulting alcohol from the processes is ethanol. Bioethanol is a fuel derived from renewable sources of feedstock; typically plants such as wheat, sugar beet, corn, straw, and wood. Bioethanol is a petrol additive/substitute.

II. MATERIALS AND METHODS

Artocarpus Heterophyllus Peel was collected from the markets and the microorganism Saccharomyces Cerevisiae was collected from nearby bakery. Saccharomyces Cerevisiae was kept in cold place until it is used for the process. Artocarpus Heterophyllus peel was pretreated to use further process. Pretreatment Hydrolysis and fermentation, Centrifugation and Distillation for separation, Analytical methods.

A. Pretreatment

The collected Artocarpus Heterophyllus peel was dried in an oven at 50⁰ C until its moisture content was completely removed. After that the dried peel was grounded to fines using ball mill and sieved by using sieve shaker. The above process is repeated to get the particle of 1mm size.

B. Hydrolysis and Fermentation

In this process Hydrolysis and Fermentation takes place simultaneously where hydrolysis is the process of converting carbohydrate molecules into sugar and fermentation is the process of converting sugar into ethanol. This simultaneous saccharification and fermentation process is carried out by adding 100gm of Artocarpus Heterophyllus Peel with 10gm Saccharomyces Cerevisiae and is made up to 250ml using distilled water. The mixture is taken in a 250ml standard measuring Flask. The SMF is tightly closed and kept at room temperature under anaerobic condition for 72hours. After fermentation, the product mixture was separated into liquid and solid phases using a centrifuge operated at 120 rpm for 5 minutes. There were two layers formed during the centrifuge. The upper layer consists of desired product and the lower layer consists of waste which is a residual mixture of Artocarpus Heterophyllus peel and Saccharomyces Cerevisiae. The upper layer is used for further distillation. In the distillation process, the product from the centrifuge is introduced in a distillation column and the temperature of the distillation column is maintained at 78.3⁰C i.e., boiling point of ethanol. Once the boiling point reaches the condensates starts to drop and is collected in a conical flask. The same process is carried out until the condensate drop stops. The collected product composition is analyzed using gas chromatography.



Fig. 1: Fermentation of Artocarpus Heterophyllus peel by Saccharomyces Cerevisiae

C. Analytical Method

Gas chromatography with a flame ionization detector using an HP-FFAP column (2.5 m length, 0.32 mm ID.) was used to analyze components of the ethanol product. While the injection ports were kept at 250⁰C, 30 μ L of the sample was injected by an apparatus which used nitrogen carrier gas with a flow rate of 20 ml/min. The column oven was operated isothermally at 15⁰C. The combustion gas was a mixture of hydrogen and air. 99% ethanol was used as the internal standard. Scanning was carried out to produce a chromatogram which showed peak areas of ethanol as volumetric percentages, the effects of following parameters were analyzed. They are Fermentation time composition of Artocarpus Heterophyllus Peel Temperature Shaking rate Composition of Nutrient.

III. RESULTS AND DISCUSSION

A. Confirmatory Test



Fig. 2: Gas chromatographic tests for reference

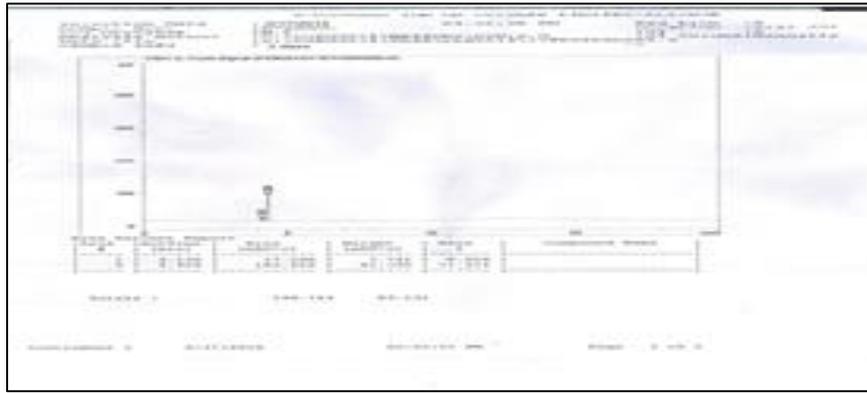


Fig. 3: Gas chromatographic tests for given ethanol sample

Ethanol of standard ethanol was determined as 4.189 to 4.401mts and the retention time of the test sample were matching and it is confirmed the presence of ethanol in the given sample was determined as 4.130 to 4.326. Retention times of both the reference standard given sample is confirmed by using retention time of ethanol in gas chromatography. Retention time is defined as the time interval between injection and rejection of sample in gas chromatography. From the above Fig. 2 & 3 of gas chromatography test, the retention time of standard ethanol was determined as 4.189 to 4.401 and the retention time of the given sample was determined as 4.130 to 4.326. Retention times of both the reference standard and test sample were matching and it is confirmed the presence of ethanol in the given sample.

B. Effect of Fermentation Time

Table 1: Effect of Fermentation time on Ethanol yield and Purity

S.NO	Fermentation time(hrs)	Ethanol Yield (ml)	Purity (%)
1.	24	59.3	88.425
2.	36	65.2	91.344
3.	48	74	99.205
4.	72	87.6	99.993

The *Artocarpus Heterophyllus* Peel of 80gm is fermented using 10gm of *Saccharomyces Cerevisiae* which is made up to 250ml and the fermentation time of the process is varied as 72h, 48h, 36h and 24h. For these fermentation time the ethanol yield and purity was measured and tabulated in Table 1 Effect of Fermentation time on Ethanol yield and on Purity. The graph is plotted between Effect of Fermentation time Vs Ethanol yield in Fig 4 and Effect of fermentation time Vs Purity of Ethanol in Fig.5.

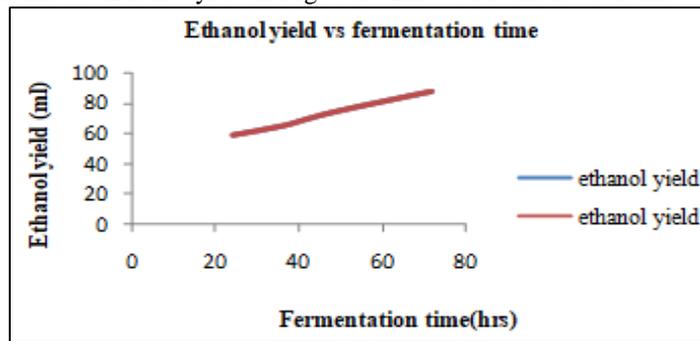


Fig. 4: Effect of Fermentation time on Ethanol yield

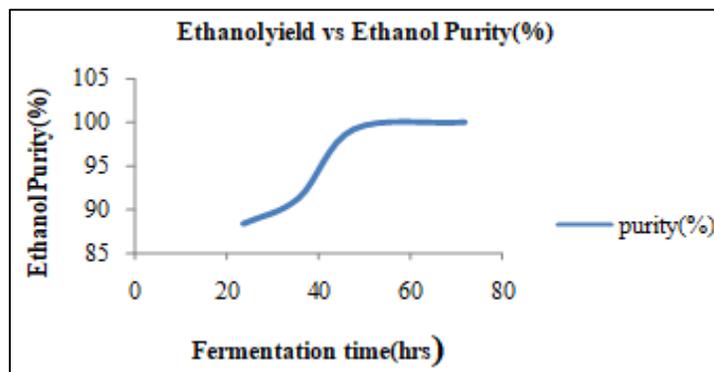


Fig. 5: Effect of Fermentation time on Purity of Ethanol

From the Fig 4.it is inferred that as the fermentation time increases, yield of ethanol also increases. From the Fig 5 it is inferred that as the fermentation time increases, the purity also increases and it is stabilized after 48h. The maximum ethanol yield of 87.6ml is achieved at 72h with highest purity of 99.993%.

C. Effect of Various Compositions

Table 2: Effect of Composition of Peel on Ethanol yield and Purity

S.NO	<i>Artocarpus Heterophyllus</i> peel composition(gm)	Water composition (ml)	Ethanol yield(ml)	Purity of Ethanol (%)
1.	20 gm	220	55	42.923
2.	40 gm	200	72	61.440
3.	60 gm	180	125	76.539
4.	80 gm	160	163	79.597
5.	100 gm	140	180	97.847

Artocarpus Heterophyllus Peel of various compositions such as 20gm, 40gm, 60gm, 80gm and 100gm were fermented using 10gm of *Saccharomyces Cerevisiae* which is made up to 250ml for 24h fermentation time. For these compositions of peel, the ethanol yield and purity was measured and shown in Table .2 Effect of Composition of *Artocarpus Heterophyllus* peel on Ethanol yield and effect of composition of *Artocarpus Heterophyllus* peel on Purity. The graph is plotted between effect composition of peel Vs ethanol yield in Fig 6 and effect of composition of peel Vs purity of ethanol in Fig.7.

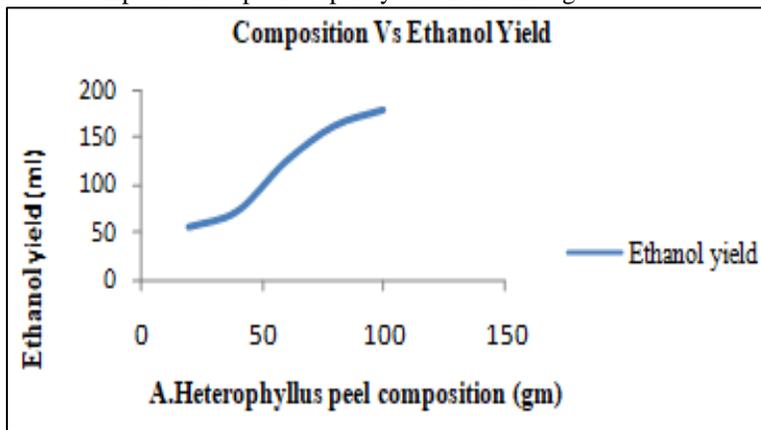


Fig. 6: Effect of Composition of A.Heterophyllus Peel on Ethanol yield

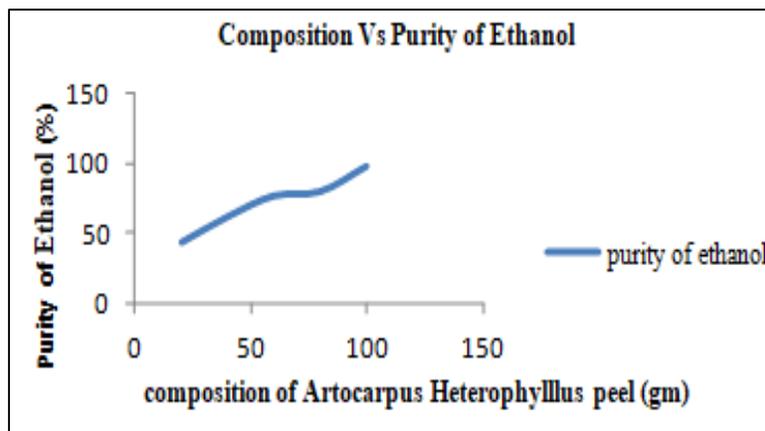


Fig. 7: Effect of Composition of A.Heterophyllus Peel on Purity of Ethanol

D. Effect of Temperature

Table 3: Effect of Temperature on Ethanol yield and Purity

S.NO	Temperature (° C)	Ethanol yield (ml)	Purity (%)
1.	10	42	86.183
2.	20	78	94.385
3.	30	67	99.592
4.	40	59	80.983
5.	50	35	17.155

The temperature of the process is varied as 10°C, 20°C, 30°C, 40°C and 50°C. The fermentation time for this process is 24h. For these temperatures the ethanol yield and purity was measured and shown in Table 3 effect of temperature on ethanol yield and on

purity. The graph is plotted between effect of temperature Vs ethanol yield in Fig 8 and effect of temperature Vs purity of ethanol in Fig 9.

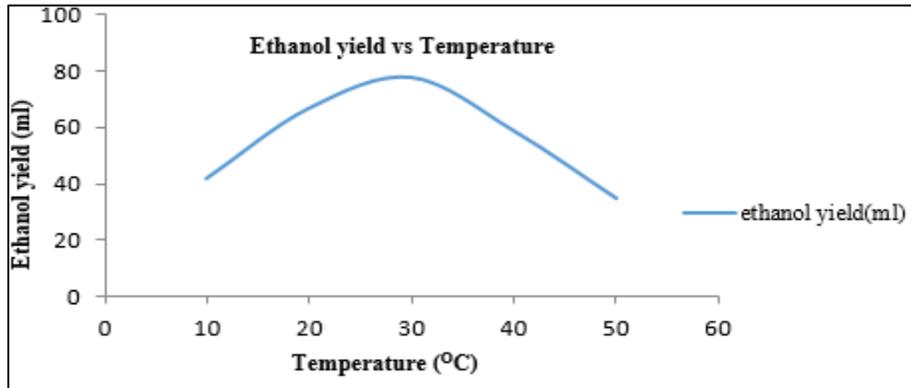


Fig. 8: Effect of Temperature on Ethanol yield

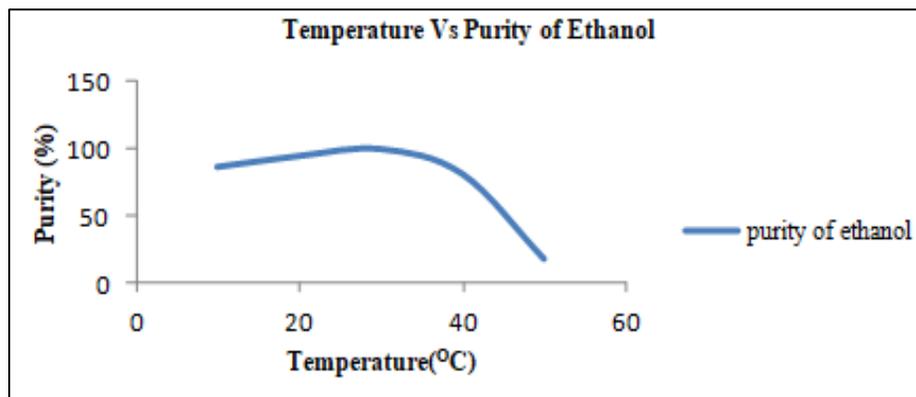


Fig. 9: Effect of Temperature on Purity of Ethanol

From the Fig 8 & 9, it is observed that maximum yield and purity was obtained at room temperature. The maximum ethanol yield of 67 ml is achieved at 30°C with highest purity of 99.592%.

E. Effect of Shaking Rate

The shaking rate is varied as 30 rpm, 60 rpm, 90 rpm and 120 rpm at room temperature for 1h. The fermentation was carried out for 24h. For these shaking rates the ethanol yield and purity was measured and shown in Table 4 effect of shaking rate on ethanol yield and on purity. The graph is plotted between effect of shaking rate Vs ethanol yield in Fig 10 and effect of shaking rate Vs purity of ethanol in Fig 11.

Table 4: Effect of shaking rate on Ethanol yield and Purity

S.NO	Shaking rate (rpm)	Ethanol yield (ml)	Purity (%)
1.	30	41.5	80.817
2.	60	53.9	98.456
3.	90	68	98.705
4.	120	75	99.047

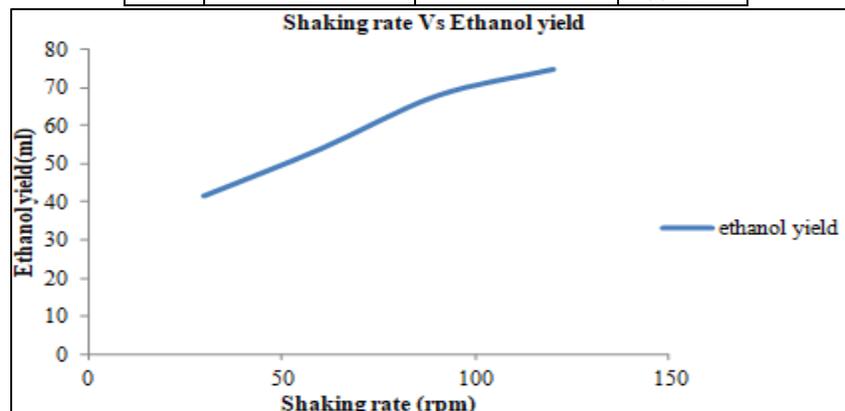


Fig. 10: Effect of shaking rate on Ethanol yield

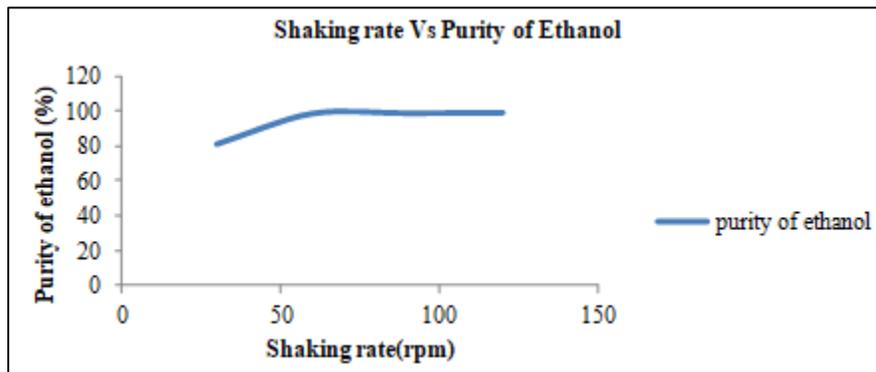


Fig. 11: Effect of shaking rate on Purity of Ethanol

From the Fig 10 & 11 it is observed that as the shaking rate increases, yield and purity of ethanol also increases. The maximum ethanol yield of 75 ml is achieved at 120rpm with highest purity of 99.047%.

F. Effect of Nutrient

Table 5: Effect of Nutrient on Ethanol yield and Purity

S.NO	Composition of zinc (gm)	Ethanol yield (ml)	Purity of ethanol (%)
1	0.5	30	57.5
2	1	47	60.7
3	1.5	53	71
4	2	68	82
5	2.5	75	91.5

Zinc is taken as micro nutrient at various compositions such as 0.5gm, 1gm, 1.5gm, 2gm and 2.5gm at room temperature. The mixture is shook at 120rpm for 1h and the fermentation time is 24h. For these various compositions of micro nutrients the ethanol yield and purity was measured and shown in Table 5 effect of amount of nutrient on ethanol yield and on purity. The graph is plotted between effect of amount Nutrient Vs ethanol yield in Fig12 and effect of amount of nutrient Vs purity of ethanol in Fig.13.

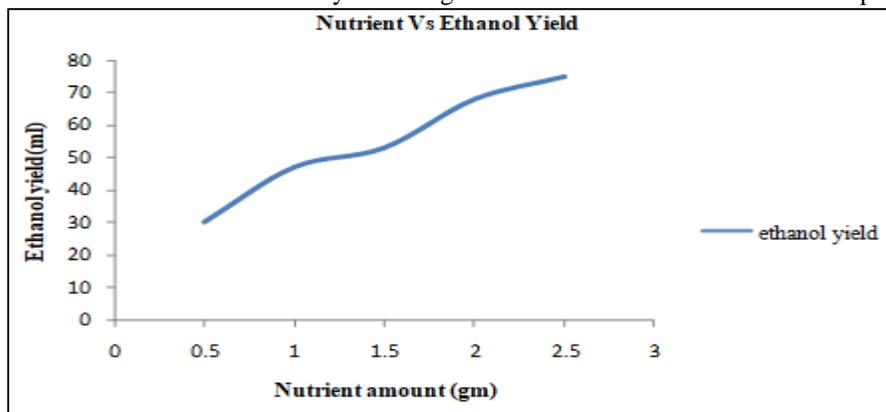


Fig. 12: Effect of Nutrient on Ethanol yield

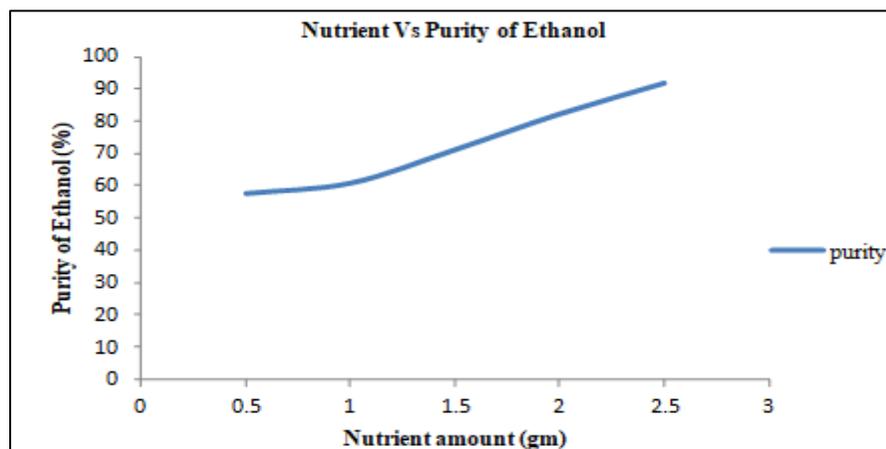


Fig. 13: Effect of Nutrient on Purity of Ethanol

From the Fig 12 & 13 it is inferred that as the composition of zinc increases, the yield and purity of ethanol also increases. The maximum ethanol yield of 75 ml is achieved for 2.5 gm of zinc with highest purity of 91.5%.

IV. CONCLUSION

Results obtained from this work have demonstrated that *Artocarpus Heterophyllus* Peel, the residual products from food wastes, are satisfactory material for ethanol production. The experiment was carried out to produce ethanol from *Artocarpus Heterophyllus* peel using *Saccharomyces Cerevisiae*. In the fermentation, all the processes were carried out in an anaerobic (no oxygen) environment. The effect of various parameters such as composition of *Artocarpus Heterophyllus* peel, temperature, shaking rate, fermentation time, shaking rate and nutrients were studied and the optimum conditions were obtained.

The result shows that the ethanol yield is increased by increasing the *Artocarpus Heterophyllus* Peel composition, Fermentation time, shaking rate. At the same time by increasing the temperature, it is found that the ethanol yield is minimized. According to the results obtained room temperature is optimum temperature to achieve higher yield of ethanol. And also the purity of ethanol is increased by increasing the *Artocarpus Heterophyllus* Peel composition, Fermentation time, shaking rates. Additionally the effects of nutrients were studied in this process by adding zinc of increasing composition the rate of fermentation, i.e., the fermentation time is reduced and the ethanol yield is increased. The experiment was done in an economical manner and the material and energy balances were calculated.

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