Bio-Ethanol and Citric Acid Production from Banana Peel and Pineapple Peel by Fermentation Process

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***Abstract*- Now a days due to generation of solid wastes, problems aroused regarding in its disposal .By utilizing the organic solid waste, disposal can be done in effective manner and alternative energy can be produced from usage of it. In the present study banana fruit peel and pine apple peel were used as raw source for the production at inoculum medium, important product such as citric acid and ethanol were produced using Trichoderma viride and saccharomyces cerevisiae by converting sugar to ethanol by fermentation under lab condition. The peel around 10g of pine apple and 2g of banana peel were mixed with potassium dihydrogen phosphate and peptone was added, sterilized at121^0c for 15 min. After sterlization the Trichoderma viride and saacharomyces cervisiae were inoculated.After inoculation the flasks were incubated at room temperature in an orbital mixure at 30^0c for 3 to 4 days.After 24 hrs an aliquot of sample was withdrawn, citric acid and ethanol was estimated using standard method.**

Keywords: Banana peel, Pineapple peel, Trichoderma viride, Saccharomyces cerevisiae ,Citric acid, Ethanol.

I. INTRODUCTION:

The rapid growth of cities in development world in recent decades has resulted in increased consumption of resources to meet growing demand of urban population and industry, situation leads to generation of large amount of waste in cities. Especially organic waste, when disposed by dumping in open spaces, water bodies etc... It creates harm to the human health as well as to the environment [1].

The another important need is fuel. Due to increased pollution and global warming, the research has been moved towards the development of alternative fuel that is eco friendly. Continued utilization of these fuel and poor regeneration practices of traditional sources of fuels are major challenges for fast growing human population. Nowadays due to industrial growth, fossil fuel were rapidly decreased and cost of petrol and diesel were increased and natural gas demand due to the scarcity of resources were increased [2].Biofuel is one of the examples for natural gas. Bioethanol and biodiesel are example for biofuel. Ethanol is viewed to be a alternative one. No other sustainable option for production of transportation fuel can match ethanol made from lignocelluloses biomass with respect to dramatic environmental, economic and infrastructure advantages .Currently there is a scope for ecological biofuels all over the world. The simultaneous saccharification and fermentation of banana peel and pineapple peel by Trichoderma viride and Saccharomyces cerevisiae ethanol can be produced. Saccharomyces cerevisiae generally found on fruits, sugary exudates of tree nectar of flowers etc. The usage of yeast is to produce ethanol by fermenting sugar solution with poor supply of oxygen. Ethanol is used as a solvent and it is safer alternative to Methyl Tertiary Butyl Ether, which is additive to gasoline to provide cleaner combustion.Hence demand for ethanol be increased by eliminating (MTBE) [4].

The aim of our study is to produce ethanol and citric acid from organic waste such as banana peel and pineapple peel by co-culture of Trichoderma viride and Saccharomyces cerevisiae

II. MATERIALS AND METHODS

A. SOURCE OF INOCCULUM

Trichoderma viride was obtained from Centre for Bioscience and Nanoscience Research (CBNR) isolated and maintained on 2% malt agar medium and used for further studies. Yeast strain Saccharomyces cerevisiae also obtained from CBNR for further studies.

B. SOURCE OF PEELS

The ripe banana peels and pineapple peels were obtained from markets in Coimbatore. These peels were used within 24 hours after collection.

C. FERMENTATION STUDIES

Dry banana peels of about 2g and pineapple peels of about 10g are taken. Then potassium dihydrogen phosphate of 0.5% and peptone of 0.5% added to the flask which contains these peels as separate. Then 100 ml distilled water is added to each flasks. Then Trichoderma viride is added in set of flask containing banana peel and pineapple peel as separate for the production of citric acid. Then both of Trichoderma viride and Saccharomyces cerevisiae is added in another set of flask for the production of ethanol. Then these flasks are placed in incubating shaker. Then fermentation process is studied for every 24 hours for about 3 days.

D. ESTIMATION OF CITRIC ACID

Citric acid produced in the peel can be estimated by titrating it with NAOH solution of 0.1N with phenolphthalein as an indicator. The end result will be pale pink colour. Burette solution was prepared by dissolving o.4g NAOH in 100ml istilled water. The pipette solution was prepared by taking 10ml of sample with 4-5 drops of phenolphthalein indicator.

$$\text{Normality of citric acid} = \frac{[N(NaOH) \times V(NaOH)]}{V(Citric\ acid)}$$

$$\%\ \text{of citric acid} = \frac{N \times Eq.wt \times 100}{\frac{volume}{of}\ filterate}$$

Where,

N = normality of citric acid.

V = volume of citric acid.

Eq.Wt = Equivalent weight of citric acid.

E. ESTIMATION OF ETHANOL

By taking distilled water of 0.9 ml and 0.1 ml of ethanol serial dilution is done in 5 eppendrop tubes and it is then centrifuged in centrifuge machine at 5000rpm for 5 min and it taken as standard solution.

then sample ethanol of 0.5 ml is taken in eppendrop tube and centrifuged as same as standard. Then by placing standard solution and samples in UV Spectrophotometer the optical density for each solution can be obtained .By plotting the graph between optical density and sample (µl/ml). The ethanol amount can be calculated.

F. ESTIMATION OF REDUCING SUGAR USING DNS METHOD

Sugar reduction can be estimated by using Dinitro Salicylic acid method .In this method sample of pineapple and banana fermented ethanol is taken in test tube as a separate of about 0.5 ml. Then DNS reagent of 0.5ml is added to it. In another test tube 0.5 ml distilled water with DNS reagent is added. Then it is heated and the sample is taken and placed in UV Spectrophotometer and optical density is noted. By plotting optical density and sample (µL).sugar reduction can be calculated.

III. RESULT AND DISCUSSION

The yield of both the fruits was determined by obtaining absorbance of sample at 197nm which represents the optical density. The result shows that from first day to third day of fermentation for banana the yield was increased from 0.31(OD) to 0.78 (OD) and citric acid production was increased from 72% to 75%. Similarly for pineapple ethanol yield was increased from 0.75(OD) to 1.5(OD) and citric acid production was increased from 71.04% to 81.12%. Reduction of sugar for banana was 0.081 (OD) and pineapple was 0.060 (OD). The PH was 6.

TABLE 1 STANDARD SOLUTION OPTICAL DENSITY VALUE FOR DAY 2

Sample	Optical density(OD)
Sample1(0.9ml distilled water+ 0.1 ml ethanol)	0.152
Sample2 (0.9 ml distilled water+ 0.1 ml sample 1)	0.549
Sample3(0.9 ml distilled water+ 0.1 ml sample 2)	0.559
Sample4(0.9 ml distilled water+ 0.1 ml sample 3)	0.829
Sample 5 (0.9 ml distilled water+ 0.1 ml sample 4)	1.5

GRAPH(1)

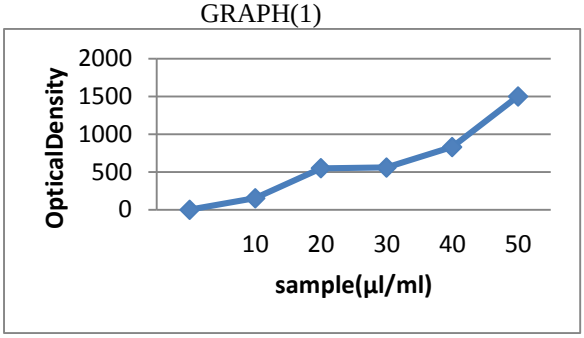

Fig1: Day 2 - Standard solution graph- optical density vs sample (µl/ml)

TABLE 2 STANDARD SOLUTION OPTICAL DENSITY VALUE FOR DAY 3

Sample	Optical density
Sample1(0.9ml distilled water+ 0.1 ml ethanol)	0.981
Sample2(0.9 ml distilled water+ 0.1 ml sample 1)	0.981
Sample 3(0.9 ml distilled water+ 0.1 ml sample 2)	0.843
Sample 4(0.9 ml distilled water+ 0.1 ml sample 3)	0.769
Sample 5(0.9 ml distilled water+ 0.1 ml sample 4)	3.00

GRAPH (2)

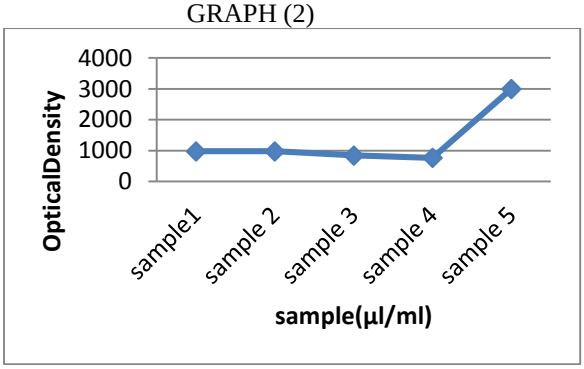

Fig 2: Day 3- Standard solution graph- optical density vs sample ((µl/ml)

TABLE 3 ETHANOL PRODUCTION BY TRICHODERMA VIRIDE AND SACCHAROMYCES CEREVISIAE

Waste material	Optical density at 197 nm for			(µl/ml)		
-	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
banana	0	0.310	0.785	0	35	47
pineapple	0	0.75	1.5	0	14	28

GRAPH(3)

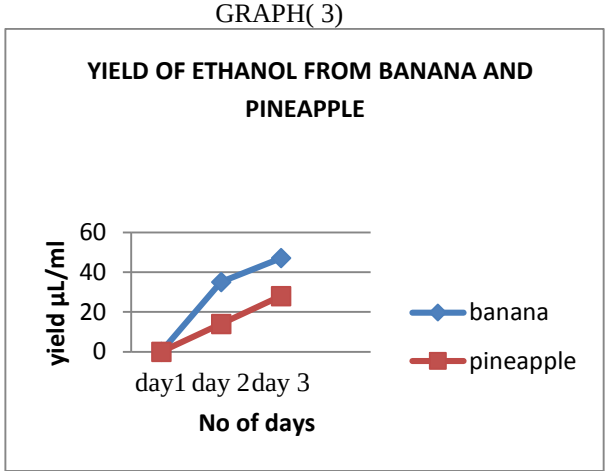

Fig 3 **:** Yield of ethanol from samples – yield(µl/ml) vs no of days

A. CALCULATION OF CITRIC ACID

DAY 1

BANANA

%Of Citric acid = 0

PINEAPPLE

%Of Citric acid = 37%

DAY 2

BANANA

Volume of NAOH = 7.5 ML

Normality of citric acid = (0.1*7.5)/ 10

=0.075 N

%Of Citric acid = (0.075*96*100)/10

=72%

PINEAPPLE

Volume of NAOH = 7.4 ML

Normality of citric acid = (0.1*7.4) / 10

=0.074N

% Of Citric acid = (0.074*96*100) /10

= 71.04%

DAY3

BANANA

Volume of NAOH = 7.9 ML

Normality of citric acid = (0.1*7.9)/ 10

=0.079 N

%Of Citric acid = (0.079*96*100)/10

=75.84%

PINEAPPLE

Volume of NAOH = 8.45 ML

Normality of citric acid = (0.1*8.45) /10

=0.074N

% OF Citric acid = (8.45*96*100) /10

= 81.12%

GRAPH(4)

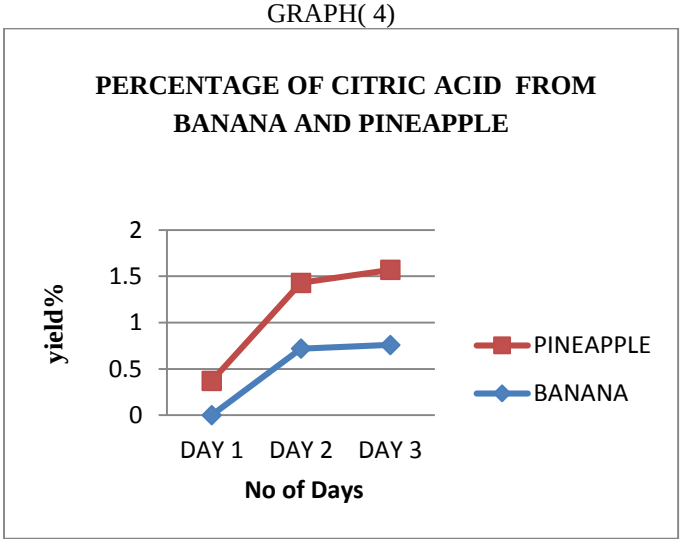

Fig 4: Yield of citric acid by usingTrichoderma viride from samples - Yield(µg/ml) vs no of days

B. DISCUSSION

The maximum yield of ethanol is obtained in banana and the maximum yield of citric acid is obtained in pineapple. From DNS test, it is observed that high sugar reduction is from banana peel. Till now banana peels and pineapple peels were considered as a waste and thrown without any use. These peels are available throughout the year and very useful for the production of valuable fermented products. It is also a eco friendly process.

IV. ACKNOWLEDGEMENT

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V. REFERENCE

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