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PRODUCTION OF BIO ETHANOL FROM – PINEAPPLE PEEL AND DETERMINATION OF EFFECTIVE HYDROLYSIS METHOD

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***Abstract*:** processing of pineapple generates the 60% of the weight of the original pineapple fruit in the form of peel that can be converted into bioenergy sources like bioethanol, biobutanol, bio hydrogen. Bioethanol is the potential alternate source of energy. New approach for the optimizing of physiochemical and biological pretreatment as well as fermentation process using peel of pineapple as substrate. The effect of type of pretreatment is investigated from the amount of bioethanol produced.

Key points: Bioethanol, pineapple peel, pre-treatment, fermentation.

I. INTRODUCTION:

Bioethanol can be produced from different raw materials which are generally classified in three categories sucrose containing feed stocks (sugarcane, sugar beet, sweet sorghum), starch materials (corn, potato, wheat) and lignocellulose materials (wood, grasses) [3]. The main problem in the production of bioethanol is the availability of the raw materials and the cost of production. This being considered the most promising feed stocks due to availability and low costs although, a successful effective conversion of lignocellulose materials into bioethanol is still limited due to lignocellulose complex. Pineapple waste is rich in simple sugars and complex carbohydrates [cellulose and hemicellulose] that are potentially hydrolysable into fermentable sugars [7, 10, 14]. The residue needs to be pre-treated and scarified before fermentation to increase the production and make large quantity [3]. The use of the pineapple waste as a source of proteolytic enzymes could represent an interesting alternative. Proper waste disposal of pineapples organic residues (peel and crown) is

necessary especially for the use of industrial companies that use pineapple as the main ingredient in their products. The use of their waste has become an interest in the production of biofuels, mainly for bioethanol production. The aim of the study is to discuss the possibility of producing bioethanol from pineapple waste, in separated processes as well as in an integrated one. The present work focuses on the valorization of industrial pineapple waste coming from the juice and canning industries, by obtaining bioethanol and thus offering an alternative to present uses [1]. The objectives of this study were:

1. Collection and pre-treatment of the fruit waste by acidic, enzymatic and acid enzymatic treatment.
2. To determine and estimate the reducing sugar in solution by benedicts test and DNSA method.
3. Allowing the solution for fermentation and separation and quantification of bioethanol by distillation.
4. Confirmatory by gas chromatography

II. MATERIALS AND METHODS:

The basic instrumentations used for this process were pH papers, conical flasks, beakers, screw cap tubes, water bath, spectrophotometer and rotary evaporator.

Collection of samples

Pineapple peel was collected from the juice shop and washed fully. The washed peel was dried and chopped into small pieces.

Pre-treatment of sample

Pre-treatment is required to alter the structures of cellulosic biomass to convert the carbohydrate polymers into fermentable sugars. There are different process to increase the digestibility of cellulose before it is exposed to microbial conversion. Mechanical, physical chemical or biological pre-treatment, as well as the combination of these methods [4,13]. The first step in the treatment was milling and grinding. The size of the waste materials is usually 10-30 mm after chopping.

Acid treatment

A weight of 10g pine apple peelings were added to 100ml of 5% sulphuric acid. The utilization of 5% sulphuric acid was based on the study of [11]. The sample was heated at 90°C for 120 minutes. After heating the sample was allowed to cool at room temperature and the substrate is filtered using the filter paper.

Enzymatic treatment

The cellulose degrading enzyme are used to break down the cellulose of plant cell walls into simple sugars that can be transformed by microbes to fuels, primarily ethanol, hydrogen. The cellulose degrading bacteria (*bacillus sp.*) is inoculated into the Luria Bertani (LB) broth medium and incubated at 37°C for 24hrs. The incubated medium is centrifuged at 12,000 rpm for 15mins [4]. The supernatant (enzyme) is taken and pellet is discarded. The supernatant(enzyme) is added into the conical flask containing 10g pineapple peel with 100ml distilled water, and is kept for 1-2 weeks.

Acid and enzymatic treatment

10g of the pineapple peel is taken and carried out with the acidic treatment. pH is neutralized to 5.5 - 6.5. After that the supernatant (enzyme) is added along with it and kept for 1-2 weeks.

pH adjustment

The pH of the hydrolyzed solution of pineapple peelings may affect the ethanol production during the fermentation process. So, the pH was adjusted by adding the 1M sodium hydroxide solution. The pH value of the solution was varied from 5.5 to 6.5 to determine the highest yield [1].

Benedict's test

Benedict's test is used to indicate the presence of reducing sugars. Some sugars such as glucose are called reducing sugars because they are easy to transfer the hydrogen to other compounds, a process is called reduction. 1ml of sample is added in clean test tube. 2ml of benedict's reagent ($CuSO_4$) is added into the tube. Tubes were heated in the boiling water bath for 4-5 minutes. Observe the tubes for the color change.

DNSA method

3,5-Dinitrosalicylic acid method test is performed to identify the presence and to quantify the reducing sugars. Prepare 20ml of 2N NaOH. Weigh 30 g of sodium potassium tartrate and dissolve in 50mL dH_2O.Slowly pour sodium potassium tartrate solution in the DNSA and NAOH solution and made the volume up to 100mL. Take eight clean dry tubes and label as 1 to 7 and last as blank. Make dilutions of glucose standards. Add 3 ml of the DNSA reagent to 1 to 7 and the blank also. Mix well and keep

the tubes in boiling water bath at 100°C for 15 minutes. After that keep the tubes in ice water until it comes to room temperature, record the absorbance with a spectrophotometer at 540nm [9].

Yeast

A large variety of yeasts and yeast strains are utilized for bioethanol production, such as *Saccharomyces cerevisiae*, *Endomicopsis burtonii*, *Scwanniomyces castelli* etc. Among these, *S. cerevisiae* is well known, not expensive and available in the market. The microorganisms were obtained from the commercial supermarket (commercial dry yeast). The concentration of yeast was used as 1, 3 and 5 g/l. For the activation of the yeast 10% of water was added to it and it underwent warming in water bath at 40°C for 15 min.

Fermentation process

The process of sterilization and fermentation was conducted to produce mixture that contains high content of carbohydrates. The starter and substrate were placed in autoclave to obtain sterile condition by preventing decomposition of microorganisms other than yeasts [8]. Next, 3 g of yeast Saccharomyces cerevisiae was mixed into the starter solution and stirred until the solution dissolved homogeneously. Microorganisms such as yeasts play an essential role in bioethanol production by fermenting wide range of sugars to ethanol [12]. After that, pour the starter solution into the substrate in a conical flask. Keep the flask closed to maintain the anaerobic condition and keep the flask in the shaker at 90 rpm for 84 hours. Yeast growth rate and its metabolism increase as the temperature increase until it reaches the optimum value. During the process of fermentation, yeast needed to be at optimum condition to operate efficiently. Therefore, it is important to ensure constant temperature rate of 25-28 ºC throughout the fermentation process. The fermentation of the sugar to ethanol is based on

Equation 1 as follows:

$$C6H12O6 \rightarrow 2C2H5OH + 2CO2$$

Distillation Process

Distillation was performed to separate the alcohol from the sample prepared after the fermentation process to get the pure bioethanol without any impurities and residues. The fermented sample was heated using fractional distillation equipment to produce gas that later will be converted to liquid (bioethanol). The process of distillation was conducted at constant temperature of 78.37 ºC until all the bioethanol was properly separated from the sample.

Gas chromatography Analysis

The bioethanol sample was further analyzed by using gas chromatography test. The compounds present in the bioethanol sample were identified by observing the peak of the chromatogram.

III. RESULTS AND DISCUSSION

Benedict's Test

Positive results: colour change green- traces of sugar, orange- moderate amount of sugar, brick red- more amount of sugar

Negative results: no colour change remains blue in colour absence of reducing sugars.

Colour changes for the test are shown in figure 1.

Acid Hydrolysis | Enzymatic Hydrolysis | Acid + Enzymatic Hydrolysis

Figure 1 Benedict's test

Acid hydrolysis - light orange colour – moderate amount of reducing sugar.

Enzymatic hydrolysis- greenish colour - traces of reducing sugar.

Acid +Enzymatic hydrolysis- bright orange – moderate amount of reducing sugar.

Estimation of amount of sugars:

Table 1

TUBES	1	2	3	4	5	6	7	8	Sample 1	Sample 2	Sample 3
Dextrose concentration	0.04 mg/ ml	0.08 mg/ml	0.12 mg/ml	0.16mg/ ml	0.2mg/ ml	0.24 mg/ml	0.28 mg/ml	0.32 mg/ml	-	-	-
OD values	1.68	1.69	1.72	1.75	1.76	1.78	1.81	1.84	1.75	1.70	1.77

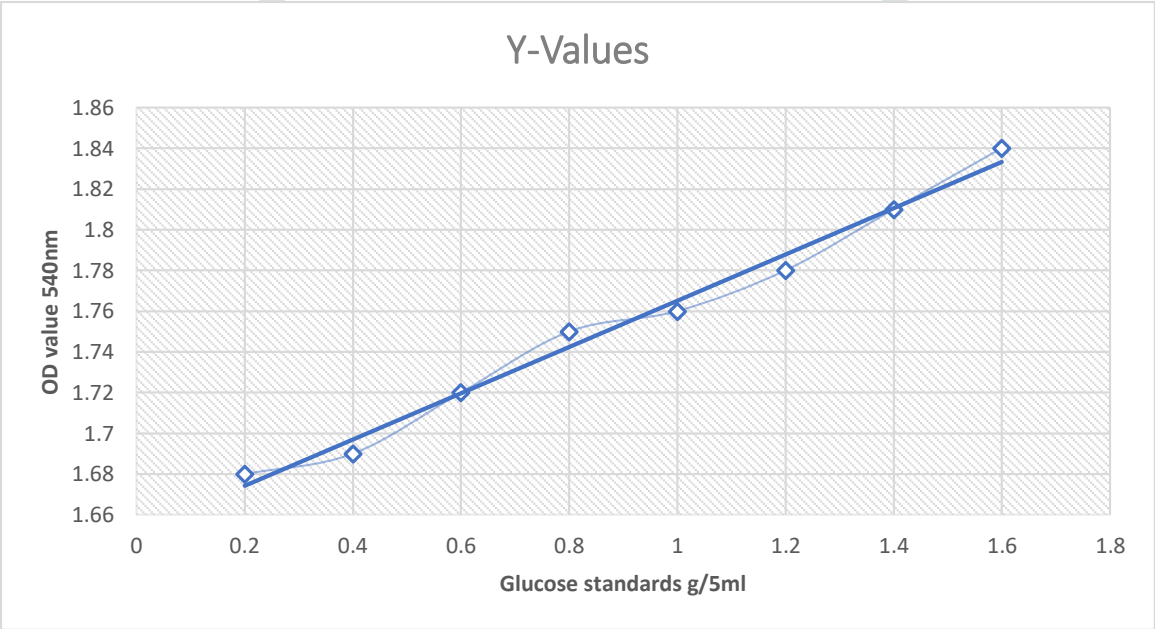


Figure 2 graph

From Table 1 & Figure 2 the amount of reducing sugar determined in the solutions by plotting the standard graph are

Sample 1 – 0.16g/ml = 16 grams in 100ml of sample

Sample 2 – 0.1g/ml = 10 grams in 100ml of sample

Sample 3 – 0.2g/ml = 20grams in 100ml of sample

Distillation

The fermented product after 84 hours is carried out for the distillation process to isolate the bio ethanol produced during the fermentation process. Water bath is kept at the temperature of 78°C for ethanol evaporation [6]. The vapour is again cooled and converted into liquid as shown in Figure 3. The amount of ethanol produced from the fermentation process are

Sample 1 – 7ml of solution extracted from sample

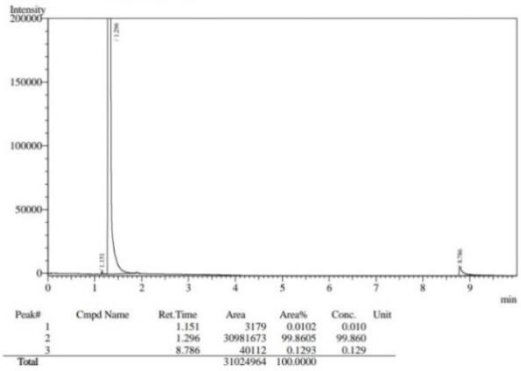
Sample 2 – 4ml of solution extracted from sample

Sample 3 – 9ml of solution extracted from sample


Figure 3 Distillation

Gas chromatography

The distillate was sent for the confirmation of ethanol. It is confirmed that the distillate was ethanol by the graph in the gas chromatography analysis. Figure 4 shows the percentage of the ethanol present in the solution.



Peak#	Cmpd Name	Ret.Time	Area	Area%	Conc.	Unit
1		1.151	3179	0.0102	0.010	
2		1.296	30981673	99.8605	99.860	
3		8.786	40112	0.1293	0.129	
Total			31024964	100.0000		

Figure 4 chromatogram graph

IV. SUMMARY AND CONCLUSION:

Bioethanol can be produced from pineapple peels through the process of pre-treatment, sterilization, fermentation and distillation. pineapple peels have shown great ability to produce bioethanol. The hydrolysis process is to achieve an efficient process for depolymerization of cellulose and hemicellulose to the fermentable simple sugars with high concentration. The hydrolysis process positively enhances the production of reducing sugars by Acid + enzymatic hydrolysis than the other two process. The gas chromatography analysis confirmed the presence of bioethanol in the distillate as shown by the peak that have similar retention time of 1.29 minute and 99% peak area to the pure ethanol. The overall results obtained in this study conclusively reveal that pineapple peels could produce 45% ethanol per gram of sugar (20g of sugar produce 9ml of ethanol) that can be used as an alternative source.

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