



E85 is Produced by Pretreating of Musa Peels

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Abstract

Musa Peels is a lignocellulosic agricultural waste that has the potential to produce E85 as a renewable energy form. Musa peel is pretreated, saccharified and fermented to make bioethanol from Musa peel. Our results showed that acid pretreatment of musa peels increased the concentration of reducing sugars. It was used to convert reducing sugars to bio-fuel.

Keywords: Musa Peel, Reducing sugar, Lignocellulosic

INTRODUCTION

Fossil fuels are the most important energy source in the world. Fossil fuel use is linked to global warming, climate change, and various energy and security issues. Furthermore, fossil fuels are not evenly distributed across the country and are also non-renewable. Bio-fuel, an ethanol liquid known as a clean fuel for internal combustion engines, is a readily available alternative as it is derived from plant-based materials (Navarrete-Perea J et al., 2017). The total consumption of Bio-fuel in 2008 was more than 65 billion liters, and in 2013 it has already replaced 5.4% of gasoline consumption, so consumption is growing rapidly. Using Bio-fuel as an alternative, either as an octane booster or as a primary fuel, tends to alleviate the problems associated with fossil fuels (Stryński R 2020). However, obtaining Bio-fuel from food sources is not a viable alternative as it requires a choice between food and ethanol (Chang SL 1978).

Alternative liquid fuels from various sources have been sought for many years. The cost of raw materials, which can account for up to 50% of the total cost of production, is one of the most important factors influencing the economics of alcohol, so efforts continue. The emphasis is on using cheap and abundant raw materials. There are many different types of biomass resources (starch or sugar crops, weeds, oil crops, agriculture, forestry, and municipal waste), but of all biomass resources, cellulose resources represent the most abundant global resource (Schwab SJ 1987). It represents. There is no other sustainable option for producing transportation fuels.

It has the potential to match lignocellulosic biomass ethanol with dramatic environmental, economic and infrastructure benefits. Lignocellulosic materials include agricultural waste, municipal solid waste (MSW), pulp mill waste, switchgrass and lawn, and garden waste (Schuster FL 2004).

Musa, a tropical fruit, is widely cultivated around the world (Fig 1). Globally, its products represent the second largest volume compared to other fruits. Enabling fermentable sugars in the digestate and especially not creating elements that inhibit the fermentation process is one of the major challenges in Bio-fuel production. The use of chemical pretreatment with acids or bases followed by enzymatic hydrolysis is considered one of the very important techniques and goals for obtaining high yields of sugars from lignocellulose (Berlana D 2005).

Bio-fuel is produced from musells (Fig 2). First, the



Figure 1. Banana Peels.



Figure 2. Dried form of banana peels through hot oven or sunlight.



Figure 3. Powder form of banana peels by grinding of dried peels.

mussels were treated with acids (oxalic and sulfuric). Various concentrations of acid were used to find out which concentration of acid produced the highest amount of reducing sugars. Samples showing the highest amount of reducing sugars were selected for further processing. The samples were then treated with cellulase enzymes for saccharification (**Fig 3**). some samples were not treated with acid and were treated with cellulase enzyme only. Nearly all samples were fermented by his *Saccharomyces cerevisiae*. Bio-fuel was separated from the remaining substrate using a rotary evaporator.

Methods (Acid Treatment)

Production in Plants

Musa peels were collected from QC-6 (Campus-11 girls' dormitory) and dried in an oven at 40 °C.

After drying, the shells were ground into a powder using a mortar and pestle for greater availability for pretreatment and subsequent processing (**Pier GB 2008**).

13 bottles were taken. Six of his were used for oxaling, and the remaining six of him were used for sulphating. Her one remaining flask was used as a control. Approximately 10 grams of Musa peels was added to each flask (**Hansen LS 1974**).

As a control, only 10g of sample was dissolved in distilled water.

Prepare 3 different concentrations of oxalic acid solution (2 bottles - 1%, 2 bottles - 2%, 2 bottles - 5%) and add Musa peels powder I was.

Three different concentrations of sulfuric acid solution were prepared (1% in 2 flasks, 2% in 2 flasks, 5% in 2 flasks) and added to the musa peels powder (**Savka MA 1990**).

All flasks were autoclaved. After autoclaving, all samples were carefully washed with distilled water.

Glucose content was estimated using the Miller method.

Miller Method

Glucose standards were prepared in 5 tubes and another 13 tubes were prepared for acid-treated samples and controls.

1 mL of DNAA reagent was added to all tubes.

The mixture was heated in a water bath for 10 minutes to develop a reddish brown color.

0.5 ml of 40% potassium sodium tartrate was added to each tube to stabilize the color.

Absorbance at 575 nm was measured after cooling the tubes to room temperature. A standard plot was generated and the concentration of each sample was determined.

Method Crude Enzyme Extraction

Five samples of *Bacillus* species (S1, S7, S8, S10, and S11) were obtained using glucose stocks from the Environment Biotechnology Lab at the KIIT School of Biotechnology.

A 50 µl sample of each *Bacillus* species was taken and placed in a Falcon containing 5 ml of LB broth and labeled accordingly.

To check the enzymatic activity of this sample, 5 CMC plates were prepared, a hole was punched in the center using a microtip, and 50 µl of sample was poured onto the plate.

The plates and peregrine falcons were placed in the incubator for 24 hours. The next day, a 1 mL sample was taken from each hawk and placed in a flask containing 250 mL of LB broth and incubated for 36 hours. A sample from the flask was placed in another flask and centrifuged at 4°C.

After centrifugation, we retained both the supernatant and the pellet because we did not know whether the enzyme was extracellular or intracellular.

Collected pellets were treated with equal volumes of 10% TRITON-X and PBS buffers.

Samples were centrifuged, pellets discarded, and supernatants containing intracellular enzymes collected (**Agudelo Higuita NI 2016**).

After 24 hours, cellulolytic enzymes were detected by treating the CMC plates with gram iodine. Upon processing, halos were visible in all marked plates except S10.

Methods Enzymatic Treatment and Acid +Enzymatic Treatment

Samples were pretreated with acid

We found the concentration of reducing sugars in the pretreated samples according to the Miller method.

As a result, the sample treated with 2% oxalic acid and 1% sulfuric acid showed the highest absorbance and the highest amount of reducing sugars.

Replicates of 2% oxalic acid and 1% sulfuric acid were used as controls. The remaining 2% oxalic acid and 1% sulfuric acid treated samples were removed for further enzymatic treatment.

Four cellulase enzyme samples were extracted from a given four S7, S7, S8, and S11 Bacillus strains.

Each of the four crude cellulase enzymes S1, S7, S8, and S11 was added to four additional separate samples (musa peels powder) store in an incubator at 37°C for 3 days.

Crude enzymes extracted from strains S1 and S8 were added to preselected samples containing 2% oxalic acid and 1% sulfuric acid, respectively. After Saccharomyces cerevisiae was activated, an equal volume of Cerevisiae solution was added to all samples (Fig 4).

Activation of Saccharomyces Cerevisiae

Inactivated Saccharomyces cerevisiae in Lab 206 (KIIT School of Biotechnology)

A sucrose solution (1 M) was made to activate it.

A pinch of inactivated Saccharomyces cerevisiae was added to the sucrose solution in the test bottle and incubated at room temperature for 24 hours.

All samples were incubated at 37°C for 7 days.

After 7 days, bioethanol was separated from the samples using a rotary evaporator.

Bioethanol was recovered after sample separation.

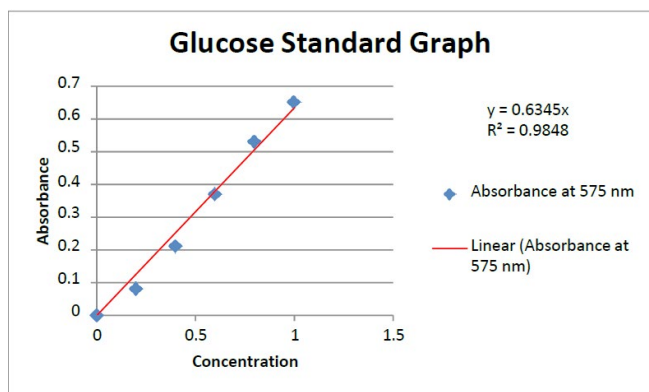


Figure 4. Concentration of glucose proportional to the absorbance.

Observation

DNSA METHOD (Acid Treatment)

Stock concentration - 10 mg/ml working concentration - 1 mg/ml

Colorimeter Readings (Glucose Standard) (Fig 5 and 6)

CONCLUSION

Maximum yields of ethanol are obtained in Musa and maximum yields of oxalic acid are obtained in pineapple. It has been observed from DNAA tests that high sugar content reduction can be obtained from Musa peels. Until now, Musa and pineapple peels were considered waste and wasted. Available all year round, these peels are very

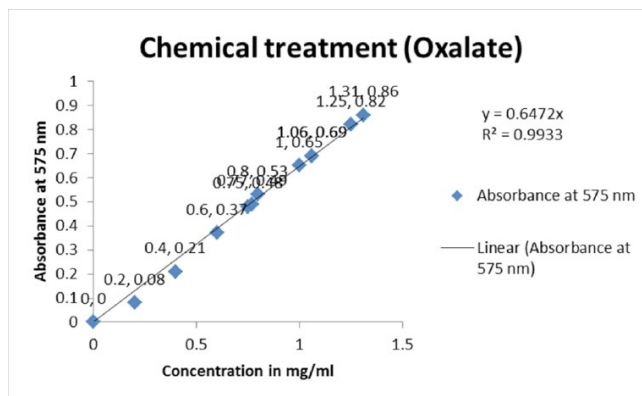


Figure 5. Chemical treatment of Oxalic acid according to proportional of concentration and absorbance.

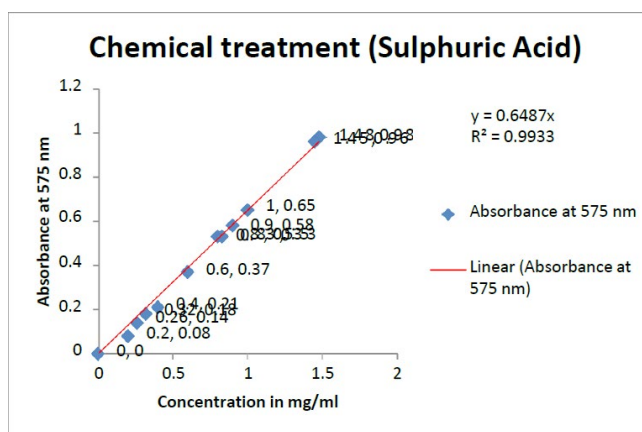


Figure 6. Chemical treatment of Sulphuric acid according to proportional of concentration and absorbance.

Table 1. Glucose Concentration and Absorbance.

Cocntration of Glucose	Absorbance at 575 nm
0	0
0.4	0.21
0.6	0.37
0.8	0.53
1	0.65
1.2	0.78

Table 2. Colorimeter Reading (Oxalic Acid).

Oxalic Acid 2% (1)	0.89
Unknown Concentration (mg/ml)	1.26
Oxalic Acid 2% (2)	0.9
Unknown Concentration (mg/ml)	1.28
Oxalic Acid 5% (1)	0.49
Unknown Concentration (mg/ml)	0.76
Oxalic Acid 5% (2)	0.47
Unknown Concentration (mg/ml)	0.79

Table 3. Colorimeter Reading (Sulfuric Acid).

Sulphuric Acid 1% (1)	0.99
Unknown Concentration (mg/ml)	1.45
Sulphuric Acid 1% (2)	0.96
Unknown Concentration (mg/ml)	1.48
Sulphuric Acid 2% (1)	0.59
Unknown Concentration (mg/ml)	0.96
Sulphuric Acid 2% (2)	0.53
Unknown Concentration (mg/ml)	0.88
Sulphuric Acid 5% (1)	0.14
Unknown Concentration (mg/ml)	0.26
Sulphuric Acid 5% (2)	0.17
Unknown Concentration (mg/ml)	0.39

useful for making valuable fermented products. It is also an environmentally friendly process.

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