IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Production of Bioethanol by Fermentation of *Zingiber officinale* and *Ananus comosus* peelings using *Saccharomyces cerevisiae*

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Abstract: Bioethanol is a global issue on the effort to be reduced global pollution, contributed significantly by the petroleum or diesel combustion or combination of both. With the help of developing technology and promising researches, the Ginger and the Pineapple peels are utilized as biomass to bioethanol production due to its substantial amount of carbohydrates that can be converted into fermentable sugar. In this study, the effect of fermentation pH was investigated. The concentration of yeasts was first standardized. After standardization, the Ginger and the Pineapple peels were subjected to a dilute-acid pre-treatment using 2.5% (v/v) sulphuric acid for 2 hours at 90°C yielding 0.4% (w/v) reducing sugar. The resulting solutions after hydrolysis are then subjected to fermentation at different pH readings. Values of pH from 4.0 to 6.0 with an increment of 0.25 are used in the investigation of pH. The adjustment of pH was done with 1.0 M Sodium hydroxide solution and pretreated substrate used. The fermentation broth subjected to pH 5.5 gave the maximum ethanol concentration of 8.6% (Ginger) and 8.8% (Pineapple). The optimization method discussed in the present study leading to be relatively high bioethanol production could provide a promising way for production with cellulose content.

Keywords: Bioethanol, *Zingiber officinale*, *Ananus comosus*, *Saccharomyces cerevisiae*

Introduction

In recent years, the increased dependence on fossil fuel has been a huge problem in the society. Due to the excessive usage of this fuel, it resulted to the increase of CO_2 level in the atmosphere resulting to global warming. Several researches and developments are being conducted to promote the commercial production of biofuels from renewable resources. One of these biofuels is the bioethanol. It has been a growing interest for many years because of its alternative green energy sources, consequently minimizing greenhouse gas (GHG) emission and finally help alleviate the rise of fuel prices [1-3]. Bioethanol is ethanol derived from biomass or biological sources, such as corn, straw, arrowroot, sap, sorghum, sugar cane, cassava, sweet potato, and wood. The raw materials for making bioethanol consist of materials containing carbohydrates, glucose and cellulose. However, the use of large amounts of raw materials can interfere with food needs because materials containing carbohydrates, glucose, and cellulose are mostly food ingredients. Therefore, there must be other more effective and efficient raw materials that do not function as food ingredients alone, one of which is pineapple peel [4]. As production increases, so does the waste obtained. Currently, the

utilization of pineapple peel waste has not been optimally used. Pineapple peel waste is generally used as feed material for livestock. To increase the economic value of pineapple peel waste, it can be utilized as raw material for making ethanol by fermentation method using yeast and purification by distillation. Consuming pineapple fruit can produce pineapple peel waste of 34.61% by weight, which contains carbohydrate content of about 10.54%. From research on ethanol production with pineapple peel juice, it is known that the glucose content of pineapple peel juice is 17% [5].

Ethanol is used for numerous purposes which include but not limited to fuel cell for stationary power and also as an automobile fuel by itself or mixed with gasoline which forms gasohol, manufacture of alcoholic drink, ethanoic acid, solvent for paint, preservation of specimen, fluid in thermometer and lots more. Ethanol can be obtained through a process called fermentation. Fermentation is the breakdown of complex forms of carbohydrate into glucose and conversion of

glucose to ethanol and carbon (iv) oxide (CO_2), [6]. In fermentation, numerous microorganisms such as yeast, fungi and bacteria are used to achieve maximal results. However, the most successful and commonly used for this process are *Saccharomyces cerevisiae, Penicillium sp, Aspergillus niger, Echerichia coli, Zynomonas mobilis*. They are essential in the fermentation process as they serve as catalyst in the reaction. *Saccharomyces cerevisiae,* commonly called Baker's yeast is a single celled eukaryote that is frequently used in scientific research especially fermentation. It is considered to be the top fermenting yeast because as yeast flocculate or clump together they attach to the carbon dioxide that is produced. It is also called the top-fermenting yeast as it is characterized by a warm temperature of 59 to 60^{o}F required for optimal fermentation [6]. The objective of the study was to analyze the efficiency of Ginger and Pineapple peels using baker's yeast separately for Bioethanol production through fermentation process.

Materials and Methods

Preparation of Culture Media

The Potato Dextrose Broth (PDB) was prepared by boiling 200g of chopped potatoes with 1L of distilled water for 30 minutes. The extract was filtered in a 1L beaker using Muslin cloth. Water was added until the 1L mark. Dextrose (10g/L) and 5g/L of peptone were added in the PDB and the solution was transferred to smaller glass containers. Agar-agar (15g/L) was added in the PDB. Cotton plugs and aluminium foils were put in the containers and autoclaved at 121^{o}C for 15 minutes. The Potato Dextrose Agar (PDA) was cooled and then was plated in the petri dish using the aseptic technique.

Standardization of Yeast

Two hard glass test tubes with 20mL PDB each were prepared, together with 0.85% by weight of NaCl solution or Natural Saline Solution (NSS). 9.0 mL of NSS was transferred into each of the 10 test tubes. These test tubes were autoclaved at 121^{o}C for 15 minutes. One hard glass test tube was used for the standardization and the other served as the control. The yeast was aseptically transferred to 20mL potato dextrose broth and placed in a shaker at a rate of 30rpm for 18 hours. After inoculation, the broth containing the yeast was serially diluted with NSS up to its 10th dilution. Each dilution was subjected to

the analysis of its optical density and about 0.1mL was plated into the culture media. After 48 hours, cell count was determined by manual counting of each cell colony. This was done to comply with a standard that will consequently referred for determining the cell number per mL.

Collection of Substrate

The Ginger and Pineapple peels were collected in local market. It was washed and chopped into smaller pieces and then subjected to size reduction using a blender. Peelings were stored in a beaker and refrigerated. These peelings were also collected for determination of the sugar content. A weight of 50g Ginger and Pineapple peelings were added to 100mL of 2.5% Sulphuric acid. The sample was heated for 120 minutes at

a temperature of 90ºC. After heating, the sample was filtered using muslin cloth. The hydrolysate was neutralized with 1.0M NaOH.

Effect of pH on Ethanol Fermentation

The pH of the hydrolyzed solution of Ginger and Pineapple peelings may affect the ethanol production during fermentation. The pH value of the substrate was varied from 4.5 to 5.5 at an increment of 0.25 to determine the highest ethanol yield. The adjustment of pH in the substrate was done by regulating the concentration with 1M NaOH and its pre-treated substrate. After adjusting the pH values, 200mL of the sample was placed in a glass bottle and autoclaved at 121ºC for 15 minutes. The calcium hydroxide was also simultaneously autoclaved at the same conditions. The bottles were cooled to room temperature to prevent the yeast degradation. About 5mL of the yeast were added to the fermentation broth. The set-up was a conventional fermentation set-up where the two bottles each, with the fermentation broth and calcium hydroxide, were connected by rubber tubing. These set-ups were placed in a shaker at a rate of 90rpm for 48 hours. These samples were subjected to high temperature short time pasteurization after 48 hours for the consequent ethanol purity testing.

Simulation Studies

For Simulation Flow Sheet is drawn is aspen plus version 8.0. In methods UNIFAC and UNIQUAC are usually chosen but these are based on temperature parameter. The flow sheet is drawn with an extraction column. The feed and solvents were specified from the library. The components like water, ethyl acetate, methyl tertiary butyl ether, acetic acid is chosen from the library as in Figure 1.1. After assuming, by clicking ternary map analysis can able to see the relation between the extractions of those three components.

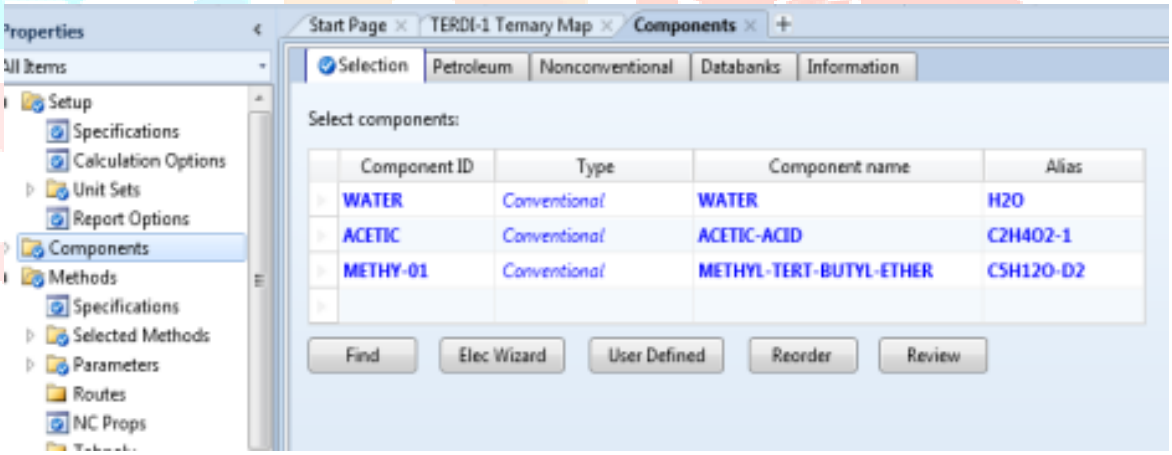

Figure 1.1 Components for Simulation

Ternary Map Analysis

By clicking the ternary map, the ternary equilibrium diagram for water, acetic acid, ethyl acetate system can be obtained. This is given in Figure 1.2, 1.3 and 1.4.

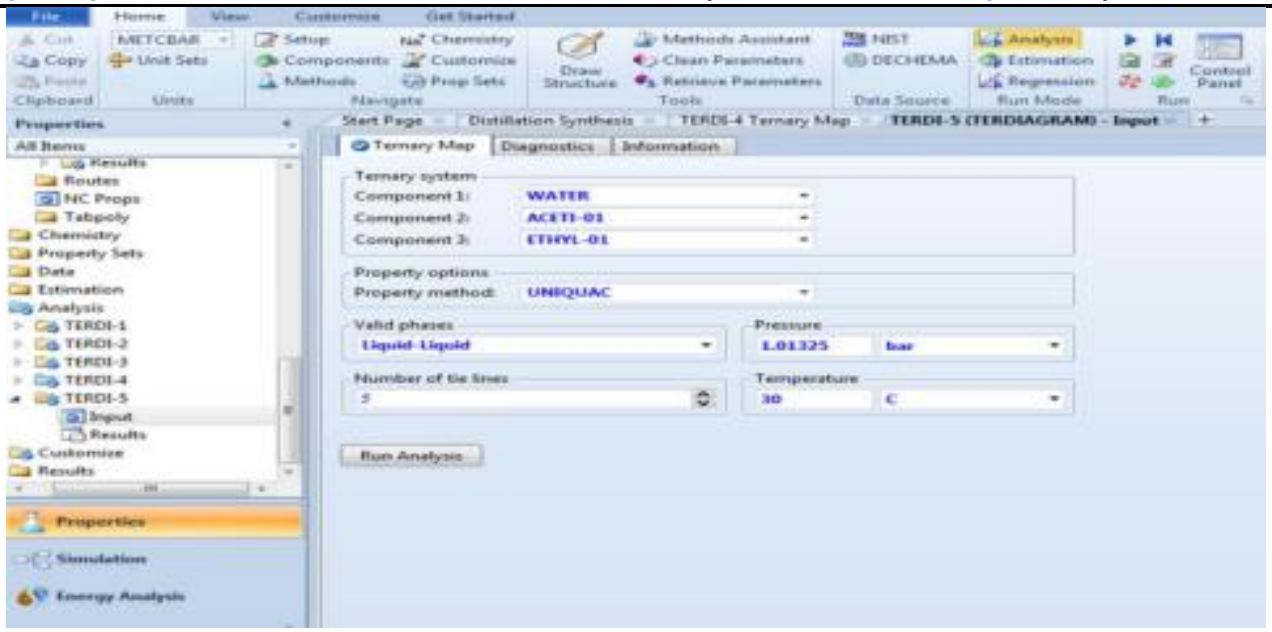

Figure 1.2 Block Column Extraction

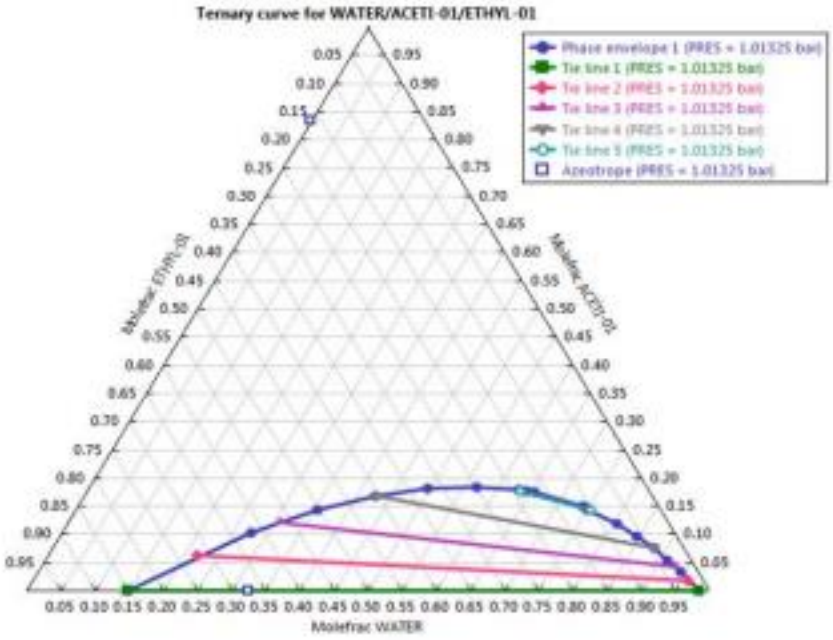

Figure 1.3 Ternary Curves for Ethyl Acetate

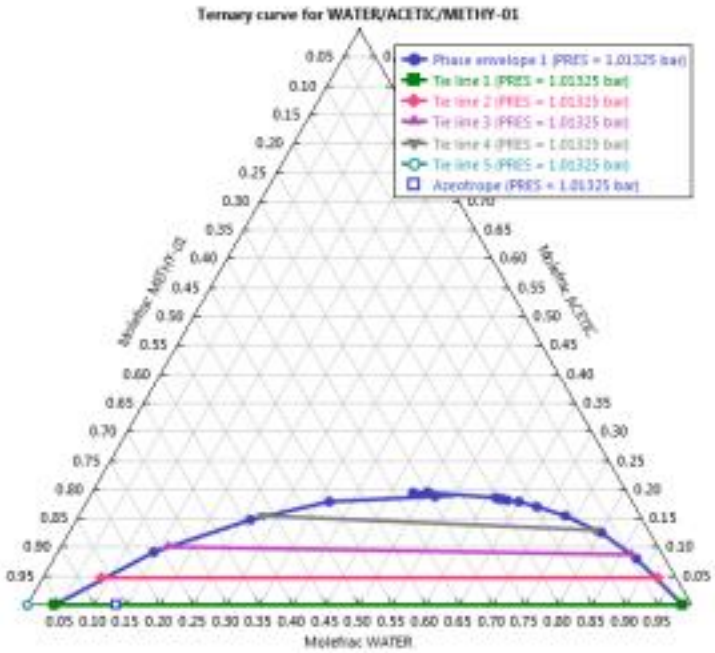

Figure 1.4 Ternary Curves for MTBE and MTVE

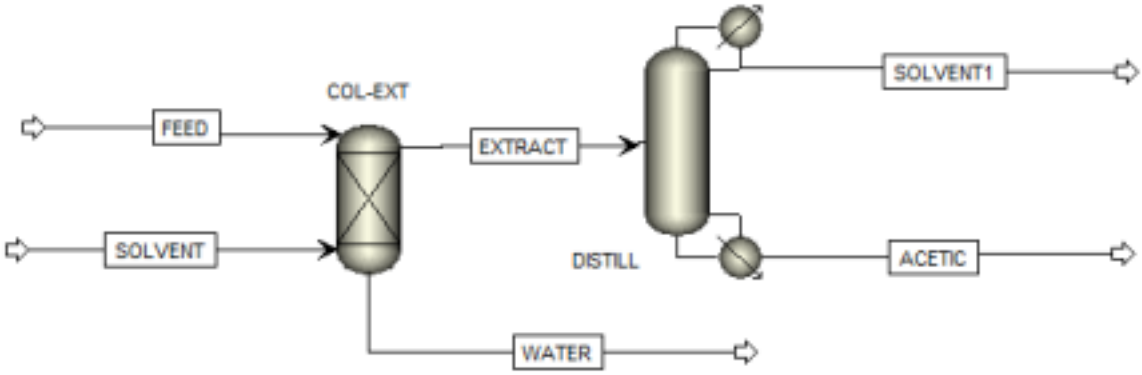

Figure 1.5 Flow Sheet for Block Column Extraction

In simulation tab the flow sheet is drawn using extraction column. The feed stream, solvent, extraction stream were connected to the column as shown in flow sheet in Figure 1.5. In specifications tab the number of stages were induced as 16. In key component tab first the liquid phase is water and second liquid phase is methyl tertiary butyl ether as given in Figure 1.6.

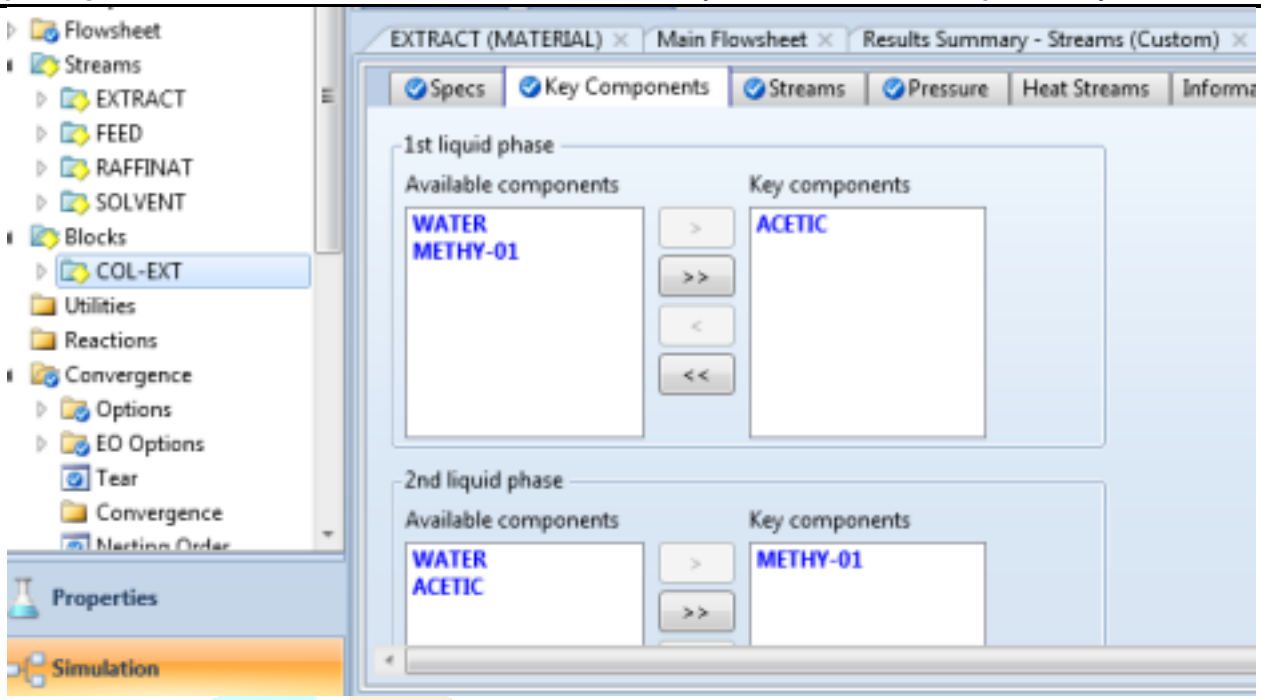

Figure 1.6 Simulation of Block Column Extraction

Results and Discussion

The quality of Bio-ethanol can be characterized by its alcohol content which is the chief component found in all types of wine. It is worthy to note that final sugar concentration has inverse relationship with ethanol concentration. Therefore, in the current study ginger and pineapple peelings was optimized for wine production for above factors. The different parameters were studied after 21 days of Incubation.

Standardization of Yeast

The growth rate of yeast varies from one another depending on its type and strain number. In order to obtain the growth rate of specific yeast, standardization must be done. This is done by plotting the logarithm of CFU per mL against the optical density of the diluted sample. The result of standardization is presented as Figure 1.7. Through this method, it was found out that the logarithm of CFU per mL of yeast (*Saccharomyces cerevisiae*) has a relationship of y=2.4x+5.8 with the optical density. Based on this correlation, the amount of cell that was used in fermentation was 5.01 x109 cells/mL. This is quite a large number for fermentation, and this could have caused the cell inhibition which may affect the ethanol yield.

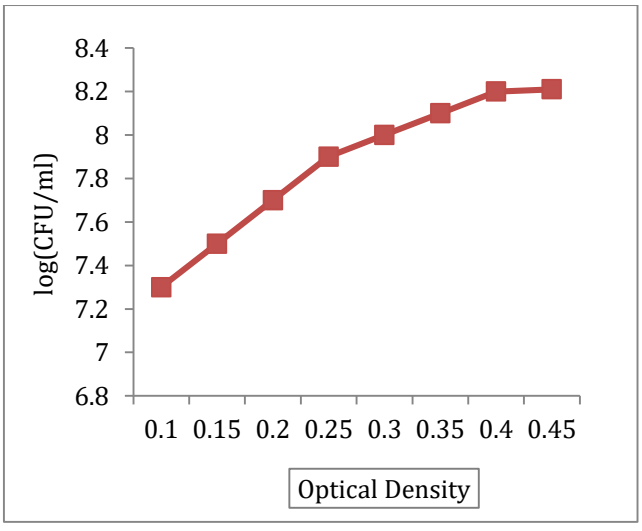

Figure 1.7 Growth rate of Yeast

Acid Pre-Treatment

The dilute acid pre-treatment of the Ginger and Pineapple peelings was used to extract and further convert the cellulose and hemicellulose content of the peelings into glucose and xylose. These latter two products are the reducing sugars that can be converted into ethanol. It was performed at a temperature of 90^{o}C for 1 hour. We proceeded to hydrolysis since the hydrolysis of the substrate is necessary for the conversion of the lignocellulosic biomass to its sugar formation. The process will break down the cellulose into its monomer unit such as glucose and xylose. The results of the unhydrolyzed and hydrolyzed Ginger and Pineapple peelings are presented in Figure 1.8. The unhydrolyzed peelings contain 1.2% and1.4% reducing sugar. The dilute-acid pre-treatment resulted to further extraction of cellulose from the peelings and only a few of its amount was able to be converted to sugar. The pre-treatment of the substrate with sulphuric acid caused the decrease in the amount of the sugar in the broth.

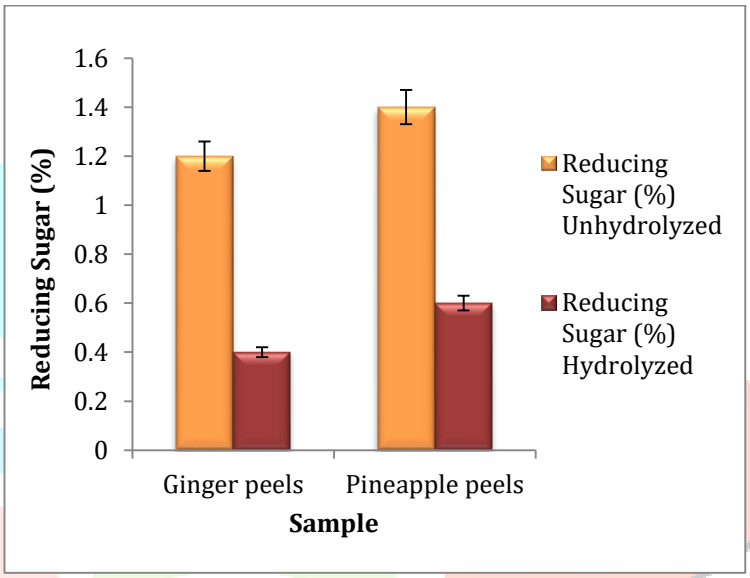

Figure1.8 Reducing Sugar Content of Unhydrolyzed and Hydrolyzed Samples

Effect of pH

The ethanol concentration had been varied at the pH of 4.0 to 6.0 for in a *S. cerevisiae* system. The effect of pH to the ethanol yield was relatively constant. However, ethanol yield was observed to be greater at pH 5.5. Taking the average ethanol concentration for each pH values from 4.0 to 6.0 in three trials, the trend shows a detailed effect of the pH (Figure 1.9). The ethanol concentration was measured in terms of volume of ethanol to volume of the hydrolyzed sample. There is a significant variation between residual Sugar % (w/v) and alcohol % in different pH levels. With increase in pH the ethanol content was reduced gradually, because yeast produce acid rather than alcohol with increase in pH 7. The maximum alcohol production was found to be at pH 5.5 with alcohol content 8.6% v/v (Ginger peels) and 8.8 % v/v (Pineapple peels).

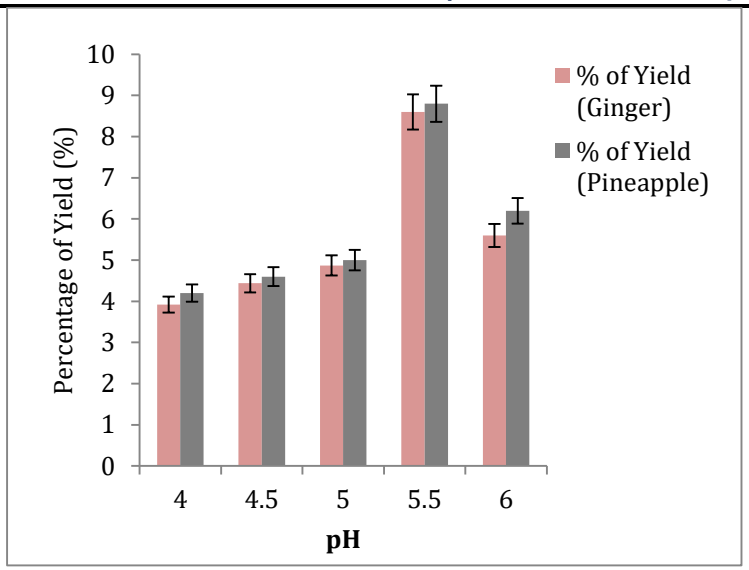

Figure 1.9 Effect of pH on Ethanol yield (%)

However prior study on screening of ginger and pineapple for wine production (Matapathi*et al.*, 2004) was found to be at pH 2.9-3.4 with maximum alcohol content 9.6% v/v.

Stimulation studies

Simulations are studied using different solvents Ethyl Acetate and Methyl Tertiary Butyl Ether is shown in Table 5.4.

Table 1.1 Mole Fraction of Bio-ethanol, Ethyl Acetate solvent used

Ethyl Acetate Solvent rate (L/min)	Feed rate (L/min)	Bio-ethanol Extract
0.2	0.2	0.180689
0.2	0.4	0.213521
0.2	0.8	0.270689
0.2	1.2	0.329632
0.2	1.6	0.399161
0.2	2	0.477561
0.2	2.4	0.516478
0.2	2.8	0.549584
0.2	3.2	0.592468
0.2	3.6	0.618426
0.2	4	0.633812
0.2	4.4	0.677126

0.2	4.8	0.714735
0.2	5.2	0.742124

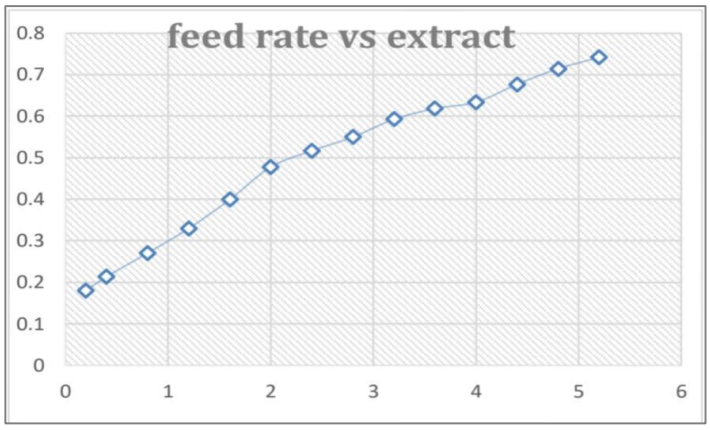

Figure 1.10 Feed rate vs Extract of Ethyl acetate solvent

Table 1.2 Mole Fraction of Bio-ethanol, Methyl tertiary butyl ether solvent used

Methyl tertiary butyl ether (MTBE) Solvent rate (L/min)	Feed rate (L/min)	Bioethanol Extract
0.2	0.2	0.570649
0.2	0.4	0.678138
0.2	0.8	0.741732
0.2	1.2	0.762512
0.2	1.6	0.772512
0.2	2	0.798088
0.2	2.4	0.836152
0.2	2.8	0.855158
0.2	3.2	0.869781
0.2	3.6	0.890313
0.2	4	0.919427
0.2	4.4	0.934518
0.2	4.8	0.943487
0.2	5.2	0.969412

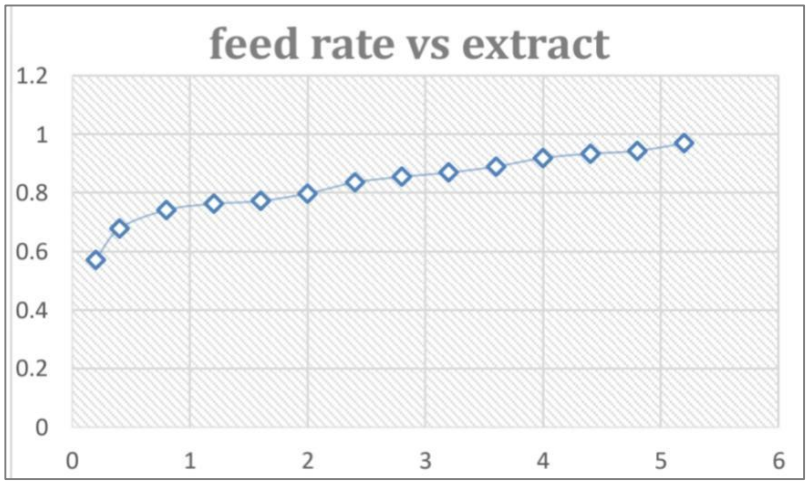

Figure 1.11 Feed rate vs Extract for MTBE solvent

Study 1: If Feed Rate is 0.2 L/min and Solvent Rate is 0.2 L/min then the Maximum Mole fraction of 18.06% of Acetic Acid attained at 0.2 L/min.
Study 2: If Feed Flowrate is 2 L/min then it is observed that the Mole fraction of 47.75% of Acetic Acid attained at 0.2 L/min.
Study 3: If Feed Flowrate is 4 L/min then it is observed that Mole fraction of 63.38% of Acetic Acid attained at 0.2 L/min.
Study 4: If Feed Flowrate is 5.2 L/min then it is observed that Mole fraction of 74.21% of Acetic Acid attained at 0.2 L/min.
Study 1: If Feed Rate is 0.2 L/min and Solvent Rate is 0.2 L/min then the Maximum Mole fraction of 57.06% of Acetic Acid attained at 0.2 L/min.
Study 2: If Feed Flowrate is 2 L/min then it is observed that the Mole fraction of 79.80% of Acetic Acid attained at 0.2 L/min.
Study 3: If Feed Flowrate is 4 L/min then it is observed that Mole fraction of 91.94% of Acetic Acid attained at 0.2 L/min.
Study 4: If Feed Flowrate is 5.2 L/min then it is observed that Mole fraction of 96.94% of Acetic Acid attained at 0.2 L/min.

Summary and Conclusion

Bioethanol from agricultural and biodegradable wastes provides a viable solution to multiple environmental problems by simultaneously creating sink for waste and renewable energy production as well. Using ethanol blended fuel for automobiles can significantly reduce petroleum use and greenhouse gas emissions. The choice of newer substrate for the production of ethanol is being a non-seasonal plant available throughout the year. The waste from the plant can be efficiently utilized based on overall economics and energy. Production of bioethanol from agricultural waste residues using indigenous yeast isolates is very economical, especially when the fermentation conditions are optimized.

The amount of *S. cerevisiae* has caused variation in the amount of ethanol concentration. Further study has to be conducted to determine the optimum amount of *S. cerevisiae* for a more effective fermentation process. The use of sulphuric acid in the pre-treatment caused a decrease in the reducing sugar of the sample from 1.2% to 0.4% (Ginger) and 1.4% to 0.6% (Pineapple). The result of dilute-acid pre-treatment can be improved by supplementary method of acid pre-treatment or an enzymatic hydrolysis that may further break down the cellulose into its reducing sugar. The effect of pH was investigated as the parametric condition for

fermentation. Results showed that at a pH value of 5.5, the ethanol concentration obtained was at an average of 8.6% (Ginger) and 8.8% (Pineapple) in which the maximum yield is obtained among the different pH readings. An increase in the pH range could determine the optimum temperature of *S. Cerevisiae* system and can give a clearer view to the effect of fermenting pH to ethanol yield concentration. Since reducing sugar can be found and produced from the Ginger and Pineapple peelings, it can be an alternative source of bioethanol with the use of *S. cerevisiae* as fermenting yeast.

References

1. Culea, R., Jurcoane, S., Popa, N., Rosu, A. and Tamba-Berehoiu, R. (2009). Researches Regarding Bioethanol from Renewable Sources such as Sweet Potato, as an Ecotoxic Alternative to Fossil Fuels. Romanian Biotechnological Letters. 14 (5), 4673-4680.
2. Nikolic, S., Rakin, M. and Vukasinovic, M.(2006). Production of Bioethanol from Corn Meal Hydrolyzates. Fuel. 85, 1750-1755.
3. Mazmanci, M. A.(2011) Ethanol Production from Washingtonia robusta fruits by Using Commercial Yeast. African Journal of Biotechnology. 10 (1), 48-53
4. Author 1, A.; Author 2, B. Title of the chapter. In Book Title, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196.
5. Azevedo, A., Mario, J., and Felix, C. (2000). Characterization of Amylase Produced By A Trichodermaharzianumisolate With Antagonistic Activity Against Crinopelliperniciosa, The Causative Agent of Witch Broom Of Cocoa. FEMS Microbiology Letters. 188, 171-175.
6. David, T. (2015). Saccharomyces cerevisiae. Use and Function in Alcoholic Production. Microbe Wiki. 7, 15-20.