International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 4 Issue 05, May-2015

Bioethanol Production from Pineapple (Ananas comosus) Peelings using Saccharomyces cerevisiae as Fermenting Yeast with Focus on Fermentation pH

Rudolph Maynard D.R. Antonio[1], Anna Angelica C. dela Cruz[1], Allan S. Quinto Jr.[1], Paul Rodrigo Cordero[1] and Maria Natalia R. Dimaano[1, 2]
[1] Department of Chemical Engineering, Faculty of Engineering
[2] Research Center for Natural Sciences
University of Santo Tomas
España Street, Manila 1015, Philippines

Abstract. **Pineapple was initially used up only as a fresh fruit. With the help of the developing technology and promising researches, the fruit is now prepared and consumed in different forms. Pineapple peelings are also utilized as a 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 yeast was first standardized. After standardization, the pineapple peelings were subjected to a dilute-acid pre-treatment using 5% (v/v) sulphuric acid for 2 hours at 90°C yielding 0.3% (w/v) reducing sugar. The resulting solutions after hydrolysis are then subjected to fermentation at different pH readings. Values of pH from 4.5 to 5.5 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 pre-treated substrate used. The fermentation broth subjected to pH 5.5 gave the maximum ethanol concentration of 9.13%.**

Keywords. Ananas comosus (pineapple) peelings, bioethanol, dilute-acid hydrolysis, 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].

According to the Worldwatch Institute, between 1850 and 1970, the population is more than tripled and the energy consumed rose more than 12 percent. By the year 2002, the world population had increased by 68 percent and the energy consumption increased another 73 percent. Transportation roughly uses 30 percent energy usage and about 95 percent of the global oil consumption [4].

Because of the energy crisis in 1970, the development in using renewable energy sources evolved and ethanol has been one of the focuses in researches. Ethanol can be derived from different renewable biomass like sugar, starchy material and lignocellulose biomass with the use of microbial fermentation [5]. Biomass is any organic material like fruits, plant parts and crops that is produced by biological process that stores energy of sunlight in the substance of living plants. There are three types of biomass that is used in the bioethanol production namely: sugar-containing biomass, starch-containing biomass, and lignocellulosic biomass. Sugar-containing biomass can be readily fermented without undergoing chemical or biological pre-treatments. Example of this is sugar cane. In order to produce bioethanol from starch containing biomass, it must undergo pre-treatment like hydrolysis before the yeast do fermentation. Lignocellulosic biomass has the most expensive pre-treatment of all the types of biomass. This is due to the difficulty in converting cellulose and hemicellulose into fermentable sugar. Some of the pre-treatments applied in bioethanol production are steam explosion treatment, enzymatic hydrolysis and chemical hydrolysis [6-7].

Pineapple was initially used up only as a fresh fruit. With the help of the ever developing technology and promising researches, the fruit is now prepared and consumed in different forms such as pineapple chunks, slices juices, syrups, jams etc.

In the Philippines, an average of 435,000 metric tons of pineapples are produced annually, therefore making pineapple as one of the country's leading commercial fruit products. On the other hand, there are lots of unused excess parts of the pineapple, specifically the peelings. They are considered as waste and add up to the country's total solid waste count. As a solution to this problem, researchers come up with studies about bioconversion of these waste products. These wastes are now further used as raw material in the manufacturing of other products namely wines, vinegar and paper.

Pineapple peelings are also utilized as a biomass to bioethanol production due to its substantial amount of carbohydrates that can be converted into fermentable sugar. Carbohydrates contained in the biomass are represented as total structural carbohydrates, which constitute 37% of dry pineapple waste and, consequently considered the main component of dry pineapple waste. Ethanol extractive is the component of the pineapple peelings that can be converted to ethanol. Ethanol extractive covers a great amount of dry pineapple waste at 22.2%. Aside from ethanol extractive and total structural carbohydrates, dry pineapple waste is composed of 7.5% Acid-insoluble lignin, 0.96% Acid-soluble lignin, 5.4% Ash, 27.14% Proteins and Acetic Acid. The lignin content of dry pineapple waste is somewhat similar to that of rice straw with lignin content ranging from 5-24%. Unlike wood residue and straw, the low lignin content of pineapple may be related to its soft structure [8].

According to Drapcho *et. al.* (2008), the yeast *Saccharomyces cerevisiae* is the universal organism for fuel ethanol production using starch and sugar feedstocks. The sugars that are metabolizable by the organism include glucose, fructose, mannose, galactose, sucrose, maltose and maltotriose [9]. *S. cerevisiae* is widely used for its capability to highly tolerate ethanol. Some strains can also proceed to fermentation at high concentration of ethanol. *S. cerevisiae* is a facultative anaerobe which has the ability to grow in an environment without oxygen. Due to its limited respiratory system that has the ability of a Crabtree effect, the yield loss during the conversion occurs in little amounts only. Even in the presence of oxygen and some fermentable sugar, Crabtree effect is essential in the fermentation of carbohydrates. This is due to the usage of substrate unaccompanied from respiration. Since fermentation happens under absolute anaerobic environment, the yield loss by aerobic ethanol reassimilation doesn't occur [10].

The aim of this study is to investigate the potential of Pineapple peelings as an alternative source of bioethanol using *Saccharomyces cerevisiae* as yeast. It also aims to investigate the effect of pH in ethanol fermentation.

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 cheesecloth. 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). Nine (9) 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 asceptically transferred to 20 mL 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 0.1 mL 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 pineapple peelings were collected in Manila, Philippines in October 2011. 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.

Pre-treatment of Substrate

A weight of 100 g pineapple peelings were added to 1000 mL of 5% sulphuric acid. The utilization of 5% sulphuric acid was based on the study of Kuhad, R.C et al [11]. The sample was heated for 120 minutes at a temperature of 90^{o}C. After heating, the sample was filtered using cheesecloth. The hydrolysate was neutralized with 1.0 M NaOH.

Investigation of pH Effect on Ethanol Fermentation

The pH of the hydrolyzed solution of 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 1 M NaOH and its pre-treated substrate. After adjusting the pH values, 200 mL of the sample was placed in a glass bottle and autoclaved at 121^{o}C 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 5 mL 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 (HTST) pasteurization after 48 hours for the consequent ethanol purity testing. This procedure was performed at a minimum of 3 trials.

Analytical Methods

The optical density with absorbance value of 1 was determined with the UV/VIS Spectrometer (Perkin Elmer Phil, Lambda 35) using UV Winlab as its software. Sugar analysis was determined following the Munson-Walker analysis as adapted by FAST Laboratory (Appendix). Alcohol was determined using Gas Chromatography. The ethanol concentration was expressed as the ratio of the area under the first peak and the total area under the curve generated based on the chromatogram.

RESULTS AND DISCUSSION

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. Through this method, it was found out that the logarithm of CFU per mL of yeast (*Saccharomyces cerevisiae* #2044) has a relationship of y=2.8x+6.9 with the optical density. Based on this correlation, the amount of cell that was used in fermentation was 5.01 x10^9 cells/mL. This is quite a large number for fermentation, and this could have caused the cell inhibition which may affect the ethanol yield.

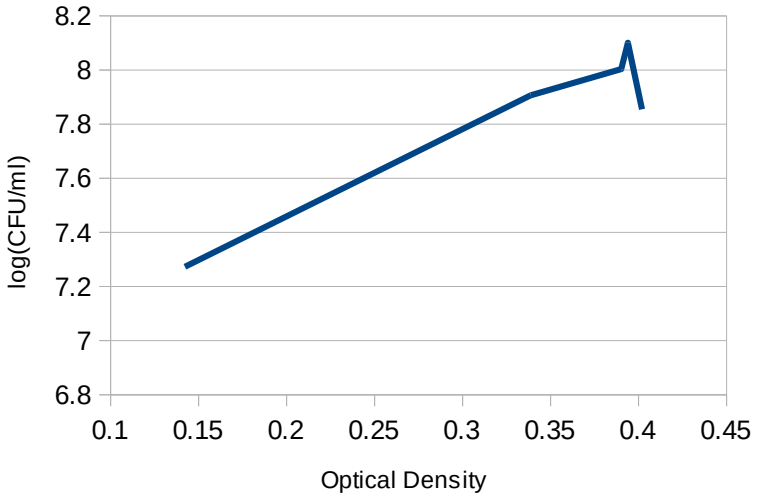

Figure 1. Standardization of Yeast

Acid Pre-Treatment

The dilute acid pre-treatment of the 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. It was done to avoid the formation of furfural and other compounds like lignin which cannot be converted further into reducing sugars. The residence time from dilute-acid pre-treatment up to the neutralization process was observed at 4 hours. And before the end of the residence time the hydrolysate was neutralized. In that way, the formation of inhibitor that may interfere with the fermentation process can be avoided. If inhibitors are present in the fermentation process, competitive inhibition will take place. The yeast would find a hard time fermenting the substrate.

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. Unfortunately, the sample with the dilute-acid treatment resulted to a decrease in the reducing sugar. The results of the unhydrolyzed and hydrolyzed pineapple peelings are presented in Table 1. The unhydrolyzed pineapple peelings contain 1% reducing sugar. Thus, additional acid or enzymatic hydrolysis must be done. The result in the unhydrolyzed substrate was due to the sugar content in the pineapple juice that still remained in the peelings when it was cut. 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.

Table 1. Reducing Sugar Content of Hydrolyzed and Unhydrolyzed Samples

	Reducing Sugar
Unhydrolyzed	1%
Hydrolyzed	0.30%

Effect of pH

The ethanol concentration had been varied at the pH of 4.5 to 5.5 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 4.5 to 5.5 in three trials, the trend shows a detailed effect of the pH. Table 2 shows the averaged ethanol concentrations that were obtained after subjecting it to Gas Chromatography. The ethanol concentration was measured in terms of volume of ethanol to volume of the hydrolyzed sample.

Table 2. Average Ethanol concentration (% vol) in pH from 4.5 to 5.5

pH	% ethanol yield
4.5	3.885
4.75	4.315
5	3.875
5.25	3.8
5.5	9.13

The fermentation pH is one of the parameters that could affect the cell growth during the fermentation process. If the pH does not fit to the environment of the yeast, it could be detrimental to the cell. Figure 2 shows the effect of fermenting pH on the % ethanol yield of reducing sugar from pineapple peelings. The fermenting pH that was used was based on the optimum temperature [1, 5] of *S. cerevisiae* which has a range of pH 4.5 to pH 5.5. At pH values from 4.5 to 4.75 an increase in the yield of ethanol was observed. However, it dropped until it reached pH of 5. Upon reaching pH 5 an abrupt increase on the ethanol yield was observed until it reached the maximum point which corresponds to pH 5.5. The escalation in the graph represents the boost in the activity of the yeast which results to an increase in the %ethanol concentration. Between the pH range of 4.5 and 5.5, pH 5.5 showed the highest % yield of ethanol with an average yield of 9.13%. The trend in the formed ethanol concentration at varied pH may be due to the response of the microorganism affecting the discrepancy in its conversion.

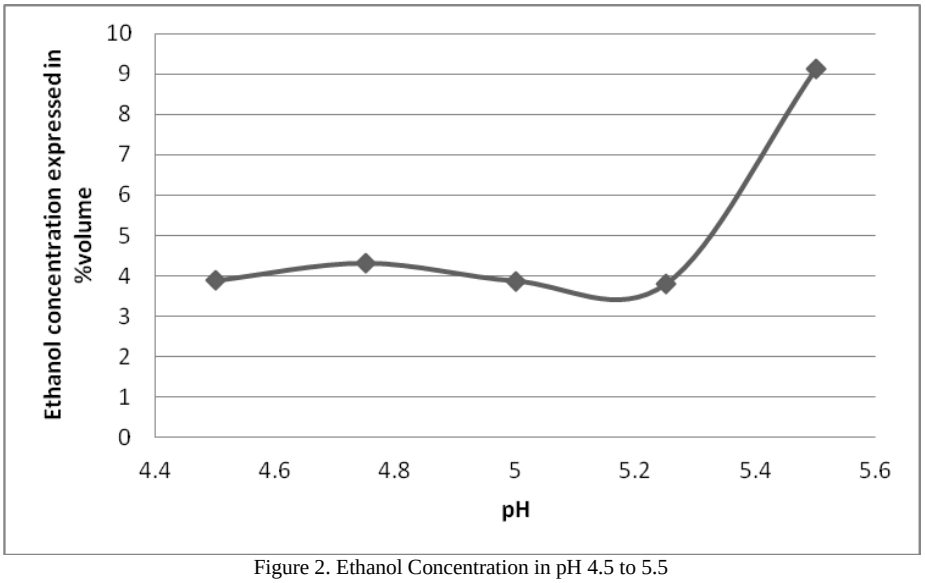

Figure 2. Ethanol Concentration in pH 4.5 to 5.5

CONCLUSION AND RECOMMENDATION

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% to 0.3%. 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 9.13% which is the maximum yield 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 pineapple peelings, it can be an alternative source of bioethanol with the use of *S. cerevisiae* as a fermenting yeast.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support from Thomas Aquians Research Center and Chemical Engineering Department. The authors also thank Dr. Maria Natalia R. Dimaano and Paul Rodrigo Cordero for their patience, motivation and expert technical assistance.

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) Casper, J. K.(2010). *Fossil Fuels and Pollution: The Future of Air Quality*. United States: Infobase Publishing.
(5) Asli, M. S.(2010). A Study on Some Efficient Parameters in Batch Fermentation of Ethanol Using *Saccharomyces cerevesiae* SC1 extracted from Fermented Siahe Sardasht Pomace. *African Journal of Biotechnology*. 9 (20), 2906-2912.
(6) Patterson, W.(1994). *Power from Plants*. Earthscan Publications Ltd: London.
(7) Hiroshi N. and Glazer(1995). A. *Microbial Biotechnology: Fundamentals of Applied Microbiology*. Freeman and Company, New York.
(8) Amago, Melchizedek(2011). *Preliminary Study On The Effect Of Enzyme Loading, pHAnd Dilute Acid Pretreatment On The Sugar Yield Of The Enzymatic Saccharification Of Pineapple Waste Of Del Monte Phils., Inc*. University of Santo Tomas, Manila: Department of Chemical Engineering.
(9) Drapcho, C. M., Nhuan, N. P., and Walker, T. H.(2008). *Biofuels Engineering Process Technology*. Mcgraw-Hill Companies, Inc., United States.
(10) Finkelstein, Picataggio, and Zhang(1994). *Enzymatic Conversion of Biomass for Fuels Production*. American Chemical Society, Washington, D.C.
(11) Chander, R. C., Gupta, R., Khasa, Y. P, Singh, A.(2010) *Bioethanol Production from Lantana Camara (red sage): Pretreatment, Saccharification and Fermentation.* University of Delhi South Campus, New Delhi, India: Department of Microbiology.
(12) Retrieved from http://people.umass.edu/~mcclemen/581Carbohydrates.html

APPENDIX

The Munson and Walker method is an example of a gravimetric method of determining the concentration of reducing sugars in a sample. Carbohydrates are oxidized in the presence of heat and an excess of copper sulfate and alkaline tartrate under carefully controlled conditions which leads to the formation of a copper oxide precipitate:

reducing sugar + $Cu2+$ + base oxidized sugar + $CuO2$ (precipitate)

The amount of precipitate formed is directly related to the concentration of reducing sugars in the initial sample. The concentration of precipitate present can be determined gravimetrically (by filtration, drying and weighing), or titrimetrically (by redissolving the precipitate and titrating with a suitable indicator). This method suffers from the same disadvantages as the Lane-Eynon method, neverthless, it is more reproducible and accurate. [12]