Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2014, Vol. 30, No. (2):
Pg. 507-513

Bioethanol Production from Sugarcane Bagasse using Fermentation Process

Y. C. WONG* and V. SANGGARI

Faculty of Agro Based Industry, University Malaysia Kelantan,
Jeli Campus, Locked Bag 100, 17600 Jeli, Kelantan, Malaysia.
*Corresponding author E-mail: yeeching@umk.edu.my

http://dx.doi.org/10.13005/ojc/300214

(Received: April 10, 2014; Accepted: May 05, 2014)

ABSTRACT

The aim of this study is to produce bioethanol from sugarcane bagasse using fermentation process and to determine the effect of pH and temperature on bioethanol yield. Enzymes such as alpha- amylase and glucoamylase were used to breakdown the cellulose in sugarcane bagasse. *Saccharomyces cerevisie*a, (yeast) also was used in the experiment for fermentation. Five samples were prepared at different pH was varied to determine the effects of pH on ethanol yield at 37^0 C and another five samples were prepared to determine the effect of temperature on ethanol yield, the pH was kept constant at 4.5. The ethanol concentrations were determined by running the samples in High Performance Liquid Chromatography (HPLC). The results showed that at highest ethanol concentration was obtained pH 4.5 and temperature 35^0C. This indicated that pH 4.5 and 35^0C was the optimum parameter for the yeast to produce ethanol.

Key words: Bioethanol, Sugarcane, fermentation, Enzymes.

INTRODUCTION

Bioethanol is simply ethanol is a renewable energy source which is made by fermenting the sugar and starch components of plant. It is produced from the agricultural product such as corn, sugarcane, potatoes, rice, beetroot and recently using grapes, banana, dates and other wastes.This is due to the decreasing amount of fossil fuels, alternative energy sources need to be renewable, sustainable, efficient, cost effective, convenient and safe[2].

The demand for oil is expected to increase to 57% from 2002 to 2030. The average price of gasoline in 2005 was $2.56 per gallon, which was $0.67 higher than the average price of gasoline in the previous year. Yet in June 2008, the average price of gasoline in the US reached $4.10 per gallon[6]. Rise in energy demand in worldwide and the progressive demising of oil reserves motivate the search for alternative energy resources, especially for those derived from renewable materials such as biomass[18].

As a result, they come up ethanol production as substitutes to fossil fuel. The lower cost to produce bioethanol is come from biomass waste because the raw materials are available in abundance.

The main objective of this thesis is to successfully produce bioethanol from sugarcane bagasse. Sugarcane bagasse is an agricultural waste which used to produce bioethanol using fermentation process. Next is to determine the yield of bioethanol produced from sugarcane bagasse. The bioethanol will be determined using HPLC. The highest peck shows the ethanol content. Last objective is to study the effects of temperature and pH of fermentation process in bioethanol production.

MATERIAL AND METHOD

Preparation of Sugarcane Bagasse

The sugarcane bagasse was obtained from area around Tanah Merah and Bazzar Ramadan in Ayer Lanas, Kelantan. The sugarcane bagasse was thoroughly washed with tap water and cut into smaller pieces. Then sugarcane bagasse was dried in oven at 60^{0}C for 3 days. It was treated at 60^{0}C because if the temperature was higher, it will affect the enzymes in the sugarcane bagasse. Once dried, the sugarcane bagasse was grinded using the grinding machine. The sugarcane bagasse sample was sealed in the seal bag or poly bag and stored in room conditions.

Buffer preparation

Buffer was used to dilute the enzyme alpha-amylase and glucoamylase. Enzyme reaction will be more effective if dilute with buffer compared to distilled water. There were two types of buffers prepared which were phosphate buffer for α-enzymes and acetic acid with sodium acetate buffer for glocoamylase. The prepared buffer was covered with aluminum foil and kept at room temperature for further use.

Liquefaction of sugarcane

10g of sugarcane was weighed. The weighed sample was placed into conical flask and 200ml of distilled water was added to the sample. 0.5 ml of NaOH was prepared to be added at this step to adjust the pH of the slurry to 4.5. Next 0.2 microliters of enzyme alpha- amylase was added to the mixture. The alpha-amylase was diluted with phosphate buffer before added to the slurry. The mixture was heated until 50^{0}C. Alpha- amylases will breakdown the cellulose into smaller size called dextrin.

Saccharifaction of sugarcane bagasse

The mixture was cooled down to 40^{0}C. Then, 0.2 microliters of secondary enzyme, glucoamylase was added to the mixture. Glucoamylase was diluted with acetic acid with sodium acetate buffer before added to the slurry.The mixture was maintained at 50^{0}C as the glucoamylase hydrolyzed the dextrin to fermentable glucose. The mixture was cooled down to 32^{0}C and 10 ml of *Saccharomyces cerevisiea* (baker yeast) was added to the sample before transferred to conical flask.

Fermentation of sugarcane

In fermentation process, *Saccharomyces cerevisiea* (baker yeast) was used to ferment the simple sugar to ethanol and carbon dioxide. To determine the effects of pH on ethanol yield, the temperature was kept constant at 37^{0}C while the pH was varied from 3, 3.5, 4, 4.5 and 5. To determine the effect of temperature on ethanol yield, the pH was kept constant at 4.5. The fermentation process continued for 48 hours.

Distillation of ethanol

After 48 hours, the sample was filtered using Whatman Filter Paper to separate the ethanol from the residue. The bioethanol was distilled using rotary evaporator. The sample was heated at 80^{0}C to get the bioethanol.

Determine bioethanol yield

Bioethanol produced was analyzed by high performance liquid chromatography(HPLC). 20 µL of the sample was injected into HPLC system to determine the bioethanol yield. Fatty acid composition in virgin coconut oil was analyzed using HPLC method. The HPLC analysis parameters were determined using the following conditions: column, C18 RP (53 x 7mm); injector temperature was 30^{o}C, 20 µL of the sample was injected into the HPLC system. The mobile phase was phosphoric acid and

the flow rate was 1.5mL/min; and detection was set at a wavelength of 210 nm.

Statistical analyses

The data obtained was subjected to One Way ANOVA using statistical package for social science (SPSS) computer program to find out the significance at $p < 0.01$.

RESULTS AND DISCUSSION

Calibration curve

In this study calibration cure was drawn to determine total ethanol concentration in water from sugarcane samples. A linear graph of standardization of ethanol was drawn using 95% ethanol as a standard. The standard was prepared at different concentrations to such as 25%, 50%, 75% and 100%. The amount of ethanol added to each vial showed in the Table 1. The calibration equation of ethanol standard was determined to be $y = 367.94x - 2853$ ($R^2 = 0.9515$) where y is the peak area of ethanol and x is the concentration of ethanol. The Ethanol standard curve is shown in the Figure 1.

Effects of pH of fermentation process on ethanol concentration in water

The study was carried out to determine the significant influences of pH on fermentation due to its effect on yeast growth and fermentation rate. The sample was fermented at different pH values from 3, 3.5, 4, 4.5 and 5 while the temperature was kept constant at 37^0C to obtain maximum yield of bioethanol. The total bioethanol content in each sample was determined and recorded in the Table 2 and Figure 2.

Table 1: Standard calculation

Concentration (%)	Volume of ethanol Standard (ml)	Volume of mobile phase (ml)	Peak Area
25	0.5	1.5	5229
50	1.0	1.0	15330
75	1.5	0.5	28520
100	2.0	0	31494

Table 2: The Effects of pH on Ethanol Concentration (%) in water

pH	Ethanol concentration in water (%)
3.0	10.7
3.5	11.6
4.0	11.9
4.5	14.8
5.0	0.0

Table 3: Effects of temperature on ethanol concentration in water (%)

Temperature (°C)	Ethanol concentration in water with water (%)
25	0.0
30	0.0
35	13.7
40]	12.3
45	11.1

Based on the results obtained, pH 4.5 showed the highest ethanol content in water which is 14.8 %, followed by pH 4.0 which is 11.9 %, then pH 3.5 at 11.6 % and pH 3.0 at 10.7 %. The lowest ethanol concentration in water with water was achieved at pH 5.0. Figure 4.1 show that the ethanol concentration in water gradually increases along with the increases in pH and reaches a maximum percentage of ethanol production when pH is equals to 4.5 and later it start to declining.

The maximum ethanol concentration in water at pH 4.5 reflects enzyme function in an environment[1] while the lower ethanol concentration in water at pH reflects lesser yeast activity. The maximum ethanol productivity was observed at pH

of 4.2 to 4.5 [3,12]. Furthermore, the increase in ethanol concentration in water is more efficient with the increase in pH from 4.0-4.5 and also found that the optimum pH range for *S.cerevisiae* to be pH 4.5 [22].

In general, yeast is an acidophilic organism and as such, grows better under acidic condition. The optimum pH range for yeast growth can vary from pH 4.0 to 6.0, depending on the temperature, the presence of oxygen and strain of yeast. Optimum pH values are required for the activity of plasma membrane bound proteins, including enzymes and transport proteins[10]. During growth, it is important for the yeast to maintain a constant intercellular pH.

There are many enzymes functioning during within yeast cell during growth and its metabolism. Each enzyme works best at its optimal pH, which is acidic because of the acidophilic nature of the yeast itself. When the extracellular enzymes pH changes from the optimal level, the yeast cell required using energy to either pump in or pump out the hydrogen ions in order to maintain the optimum intercellular pH[11].

If the extracellular pH changes too much from the optimum pH range, it may too difficult for the cell to maintain constant intracellular pH and the enzyme may not function normally. Furthermore, if the enzymes are deactivated, the yeast cell will not be able to grow and make ethanol efficiently[10]. This is the most likely explanation for the observed reduction in ethanol production when the initial medium pH was at 3.0. There were also low carbon dioxide productions at pH3 because the low pH encourages the production of acid instead of alcohol[5].

However this study shows lowest ethanol concentration in water at pH 5. This may be due to the disability of the yeast strain to tolerate at pH 5. Different yeast strain has different pH range to activate and produce ethanol. There are other possibilities; the yeast that was used to conduct the experiment may be old. Old yeast will not carry out fermentation process efficiently compared to new yeast. According to Misono*et al*. (1990) [9], there is increased rate of ethanol production at pH 5. This statement is not applicable for this study since at pH 5 there is no ethanol production.

Effects of temperature of fermentation process on ethanol concentration in water

Temperature is one of the major factors that determine the ethanol production. Table 3 and Figure 3 showed the ethanol concentration in water (%) that obtained at different temperature. Based on the result obtain, no ethanol concentration in water was observed at 25 and 30^{0}C.

However, as the temperature increases beyond 30^{0}C it showed increase in production of

Table 4: Summary for one way ANOVA table for effect of pH and ethanol concentration

Ethanol concentration	ANOVA				
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	447.149	4	111.787	84.051	.000
Within Groups	13.300	10	1.330		
Total	460.449	14			

Table 5: Summary for ANOVA table for effect of temperature and ethanol concentration

Ethanol concentration	ANOVA				
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	487.620	4	121.905	14.384	.000
Within Groups	84.753	10	8.475		
Total	572.373	14			

ethanol. At 35^{0}C ethanol concentration in water were maximum and turned out to be 13.7% followed by 40^{0}C where 12.3% ethanol was obtained. Fermentation process required a suitable temperature for the yeast to react[16]. Temperature that is too high kills yeast, and low temperature slows down yeast activity. Thus, to keep a specific range of temperature were required.

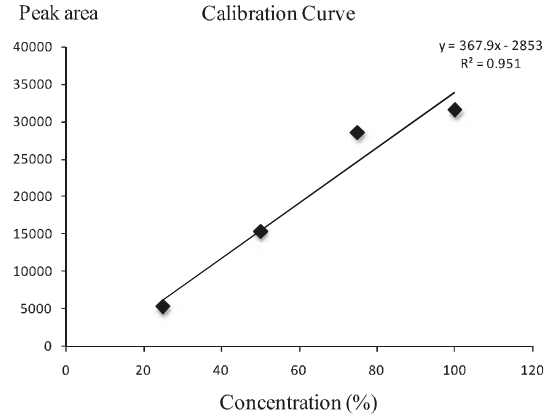

Fig. 1: Ethanol Standard Curve

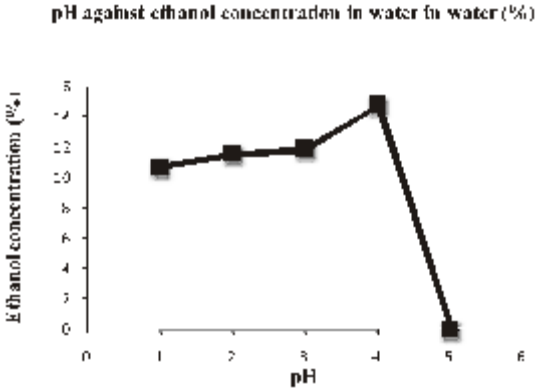

Fig. 2: Effect of pH against ethanol concentration in water

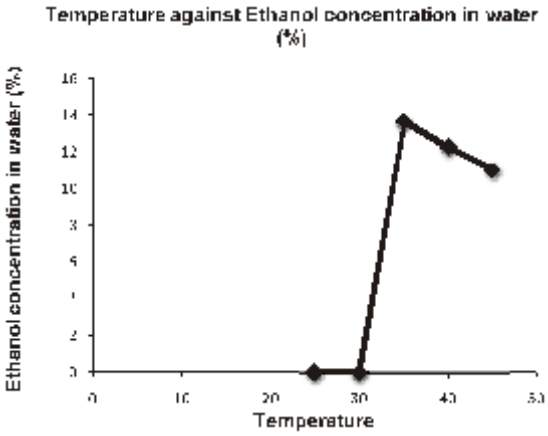

Fig. 3: Effect of temperature against ethanol concentration in water

However the ethanol concentration in water was decreased at 45^{0}C. This indicates that 35^{0}C were the optimum temperature for ethanol production. This finding is in agreeable with last studies about temperature on ethanol concentration in water[14-15,17]. This studies result also denied the study of Yah *et al*., (2010) [23], who found optimum temperature of ethanol production to be 25^{0}C.
From the result we can conclude that higher the temperature, lower the ethanol concentration. The rate of enzyme catalyzed reaction increases with temperature up to a certain temperature and then the enzymes begins to denature. Higher temperature inhibits the growth of the cells and fermentation significantly decreases. In this study, ethanol concentration in water declined considerably at 40^{0}C, which showed the inhibition effects on the cell growth at higher temperature.

This statement supported by study from[8,13]. Based on the high temperature might denature the ribosome and enzymes. Furthermore, higher temperature would alter the structure of the membrane and decreases its functionality[7]. Above the optimum temperature, the enzyme reaction drops precipitously as the enzyme denatures[19].

Enzymes are sensitive to temperature changes. At temperature above 40^{0}C the rate of respiration slows down and drops. This was because all the enzymes are made up of the protein chains of amino acid. It exists in the form of a helix structure with hydrogen bonds holding them together. When heat was applied to the enzyme, energy was given off. The active enzyme cell deforms and the hydrogen bonds break, denature the yeast enzyme. This process called as denaturizing. The optimum temperature in which yeast enzyme work best is around 35^{0}C, below this temperature the rate of reaction was slow and above 45^{0}C the yeast enzyme would denature.

At low temperature the cells showed no ethanol concentration. This may be due to enzymes low tolerance to produce ethanol at lower

temperature[4,21]. Furthermore, at low temperature the enzyme deactivated and reaction slow down or stop altogether[20]. At lower temperature, the molecules move slower than at higher temperature. These explain that the enzyme may not have enough energy to cause chemical reaction. Overall we can conclude that temperature 35^{0}C was the optimum temperature for ethanol production.

Statistical analysis for effects of pH and temperature on fermentation process

One way analysis of variance (ANOVA) was conducted to evaluate the relationship between effects of pH on ethanol concentration. The Independent values were pH while the dependent values were ethanol concentration. AVOVA used determine whether there was a significance difference in ethanol concentration. Table 4 shows the ANOVA table for the effect of pH on ethanol concentration. The one way ANOVA results indicate that there was a significant difference in pH at (P<0.01) level for three conditions [F (4, 10) = 84.051, p = 0.000].

One way analysis of variance (ANOVA) was conducted to evaluate the relationship between effects of temperature on ethanol concentration. The independent values were temperature while the dependent values were ethanol concentration. AVOVA used determine whether there was a significance difference in ethanol concentration. Table 5 shows the one way ANOVA table for the effect of temperature on ethanol concentration. The one way ANOVA results indicate that there was a significant difference in temperature at (P<0.01) level for three conditions [F (4, 10) = 14.384, p = 0.000].

CONCLUSION

This study shows that pH 4.5 showed the highest ethanol content in water which is 14.8 %, followed by pH 4.0 which is 11.9 %, then pH 3.5 at 11.6 % and pH 3.0 at 10.7 %. The lowest ethanol concentration in water with water was achieved at pH 5.0. The study also shows that at 35^{0}C ethanol concentration in water were maximum and turned out to be 13.7% followed by 40^{0}C where 12.3% ethanol. However there is no ethanol production at temperature 25 and 30^{0}C.Statistical analysis one way ANOVA results indicate that there was a significant difference in pH at (P<0.01) level for three conditions [F (4, 10) = 84.051, p = 0.000]. The one way ANOVA results for temperature indicate that there was a significant difference in temperature at (P<0.01) level for three conditions [F (4, 10) = 14.384, p = 0.000].Further study should conduct on more parameter that effect the fermentation process on ethanol production. There are other parameter such as amount of subtract, time and glucose concentration which affects ethanol production during fermentation. This will gives the overall view how ethanol production affected. In conclusion, pH 4.5 and 35^{0}C is the optimum condition for ethanol production.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to University Malaysia Kelantan (UMK) for the financial support and providing the necessary facilities for the successful completion of this research work.

REFERENCES

1. Berg, J.;*Biochemistry, sixth ed. Freemantle and Company, London,* **2007.**
2. Chum, H. L.; Overend, R. P.;*Fuel Processing Technology*, **2001**, *71*, 187-195.
3. De Vasconcelos, J. N.; Lopes, C. E.; de França, F. P.;*International Sugar Journal*,**1998**,*100*, 73-75.
4. Gao, C.; Fleet, G. H;*Journal of Applied Bacteriology,* **1988**,*65*, 405– 410.
5. Jennings, D.H.; *The physiology of Fungal Nutrition. Cambridge University Press,Cambridge*,**1995**, 392-394.
6. Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P.;*Industrial and Engineering Chemistry Research*,**2009**, *48*, 3713–3729.
7. Lucero, P.; Pen˜alver, E.; Moreno, E.; Lagunas, R.;*Applied and Environmental Microbiology*,**2000**, *66*, 4456– 4461.

8. McMeckin, T. A.;Olley, J.;Ratkwsky, D. A.; Ross, T; *International Journal Food Microbiology*, **2002**, *73*, 395-407.
9. Misono, H. M.; Yamaguchi, Y.;*Journal of Fermentation Technology,***1990**,*8*, 210-218.
10. Narendranath, N. V.,;Power, R.;*Applied and Environmental Microbiology*, **2005**, *71*, 2239-2243.
11. Narendranath, N. V.; Thomas, K. C.; Ingledew, W. M.;*Journal of the American Society of Brewing Chemists*, **2001**,*59*, 187-194.
12. Nigam, J. N.;*Journal of Biotechnology*,**1999**, *72*, 197-202.
13. Phisalaphong, M.; Srirattana, N.;Tanthapanichakoon, W.; *Biochemistry Engineering Journal*, **2006**, *28*, 36-43.
14. Pramanik, K.;*Institution Chemistry Engineers*, **2003**,*34*, 487-492.
15. Redzepovic, S.; Orlic, S.; Sikora, S.; Majdak, A.; Pretorius, I. S.; *WhileyInterscience Journals*,**2002**, 350.
16. Rivera, M.; Cardona, C. A.;*Ingenier1´a y Competitividad*, **2006**,*6*, 17–25.
17. Roehr, M.; *The Biotechnology of Ethanol: Classical and Future Applications. Chichester: Wiley-VCH.*, **2001**, 232.
18. Saxena, R. C.;Adhikari, D.K.; Goyal, H.B.;*Renewable and Sustainable Energy Reviews*, **2009**,*13*, 167–178.
19. Southerland, W. M.; *Foundations of Medicine: Biochemistry, Churchill Livingstone. Inc., New York,* **1990.**
20. Togarepi, E.; Mapiye, C.; Muchanyereyi, N.; Dzomba, P.; *International Journal of Biochemistry Research & Review*, **2012**,*2(2),* 60-69.
21. Torija, M.J.; Roze's, N.; Poblet, M.;Guillamo´n, J. M.; Mas, A.;*Antonie van Leeuwenhoek*, **2003**,*79*, 345– 352.
22. Yadav, B. S.;Sheoran, A.; Rani, U.; Singh, D.;*Indian Journal Microbiology*,**1997**,*37*, 65-67.