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Comparison of Bioethanol Production from Corncob and Sugarcane Bagasse on Fermentation Process by Saccharomyces Cerevisiae (Baker's Yeast)

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Abstract: Bioethanol is a potential alternatives source of energy from renewable materials as compared to non-renewable energy sources from fossil fuels. The purpose of this research is to study the comparison of bioethanol production from corncob and sugarcane bagasse and the effect of pH on the fermentation process by *Saccharomyces cerevisiae* (baker's yeast). The methodology of bioethanol production including the pre-treatment of feedstock, media preparation, fermentation process and distillation. For analytical methods, this involves the bioethanol yield produced, standard curve and substrate efficiency. From the result, it can be found out that the pH that will have the highest production of bioethanol is at pH 4.5 at temperature 37℃. Sugarcane bagasse will have a higher bioethanol conversion and better substrate efficiency than corncob. The sugarcane bagasse has a higher cellulose content, which means that the sugar concentration that can be utilized by yeast is higher. As the result, sugarcane bagasse has higher substrate efficiency compared to corncob at 63.98 %, 99.06% and 81.59 %. For overall substrate efficiency for both feedstocks, the highest efficiency was obtained at pH 4.5, which is 99.06 % for sugarcane bagasse and 75.74 % for corncob. In bioethanol conversion, sugarcane bagasse has a higher percentage than corncob at 66.82 %, 98.65 % and 85.23 %. In conclusion, sugarcane bagasse is more efficient in bioethanol production while the most suitable pH for S.cerevisiae in bioethanol production is pH 4.5. In the future, more research should be conducted to study the effect of the parameter during the fermentation process by yeast to produce a better yield of bioethanol at the highest efficiency.

Keywords: Bioethanol, Sugarcane Bagasse, Corncob, Baker Yeast, *Saccharomyces Cerevisiae*, Substrate Efficiency

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1. Introduction

In the fast-paced growth of the human population, the energy demand had increased due to improvements in industrial activity. According to Khuong, the demand rate for energy sources will be increase to 105 Mb per day in 2030 and the rate will be further increases [1]. The over-increasing energy demand from human activity causes the depletion of natural resources, which is petroleum and fossil fuels. The pollution that coming from the burning of fossil fuels as sources of energy also causes a huge problem. At the same time, the increase in waste products produced from agricultural activity also causes the main problem in the disposal of biomass waste. The costs for disposal of biomass waste is higher as it requires proper waste management.

Therefore, the potential of biomass waste as alternative energy to replace energy from natural resources was studied by many researchers. From the studies, it is found out that ethanol is a substance that can be used as alternative energy to substitute fossil fuels [2]. With the availability of agricultural biomass waste, biofuels from bioethanol and its blends have gained attention in development a greener energy source.

On the other hand, ethanol also can be produced from biomass waste by the process of fermentation by yeast. Bioethanol is expecting to produce from biomass waste such as corncob and sugarcane bagasse. Corncob and sugarcane bagasse are the biomass that can be abundantly and do not require a high cost. Corncob and sugarcane bagasse also having a high carbohydrate content which are good for bioethanol production through fermentation process by yeast as the process require the source of glucose in order for reaction to occur.

However, the production of bioethanol from corncob and sugarcane does not fully succeed with all kinds of the condition such as pH and temperature. Therefore, it is important that to reveal the optimum pH and temperature for the fermentation process of yeast for bioethanol production from corncob and sugarcane bagasse. The purpose of the project is to compare the bioethanol production from the corncob and sugarcane bagasse process and the effect of pH on the fermentation process by Saccharomyces Cerevisiae (yeast). The amount of sugar consumed by corncob and sugarcane bagasse during fermentation process is also determined by phenol sulphuric acid method to identify the substrate efficiency of the biomass waste.

1.1 Objectives

This research was carried out to produce bioethanol from sugarcane bagasse and corncob through the fermentation by using baker yeast. The objectives of this study are:

1. To investigate the optimum pH conditions for bioethanol production by baker's yeast.
2. To compare the yield of bioethanol produced by corncob and sugarcane bagasse.
3. To study the substrate efficiency of corncob and sugarcane bagasse during fermentation process.

1.2 Scope of Study

The scope of the project that being focus are:

1. Bioethanol was produced from corncob and sugarcane bagasse by using fermentation process by yeast.
2. The conversion of bioethanol produced from corncob and sugarcane bagasse will be characterized based on their volume.
3. The samples were fermented at different pH, as well as test the optimum condition for bioethanol production.
4. The substrate efficiency of corncob and sugarcane bagasse during fermentation process were determined from phenol-sulphuric acid method.

2. Materials and Methods

2.1 Materials

Materials that used in the bioethanol production are sugarcane bagasse, corncob and baker's yeast (*Saccharomyces cerevisiae*). Chemical materials in the pretreatment and hydrolysis process using 0.5 mol/L H_2SO_4, 1.0 mol/L NaOH, NH_4Cl, $Na_2HPO_4.7H_2O$, KH_2PO_4, $MgSO_4$, $CaCl_2$, and $Na_3C_6H_5O_7$. The tools used in the production and analysis of bioethanol are: drying oven, grinder, autoclave, analytical balance, pH meter, incubator shaker and rotary evaporator.

2.2 Methods

The purpose of the study is to produce bioethanol from waste agricultural sources, which are corncob and sugarcane bagasse. Corncob and sugarcane bagasse contains sugar which will be used as substrate in the fermentation process by *Saccharomyces cerevisiae* (baker yeast). During the fermentation process, the parameter such as pH will be manipulated to observe the change of parameter on the yield of bioethanol produced. Besides that, the substrate efficiency between two waste feedstocks on the fermentation process by S. cerevisiae will then be compared using the phenol test. Since two feedstocks are used to compare the bioethanol production, the experiment is carried out with one feedstock first then followed by another feedstock. The methodology for both feedstocks is the same because they are the same types of lignocellulosic biomass. Figure 1 shows the overall methodology of the study.

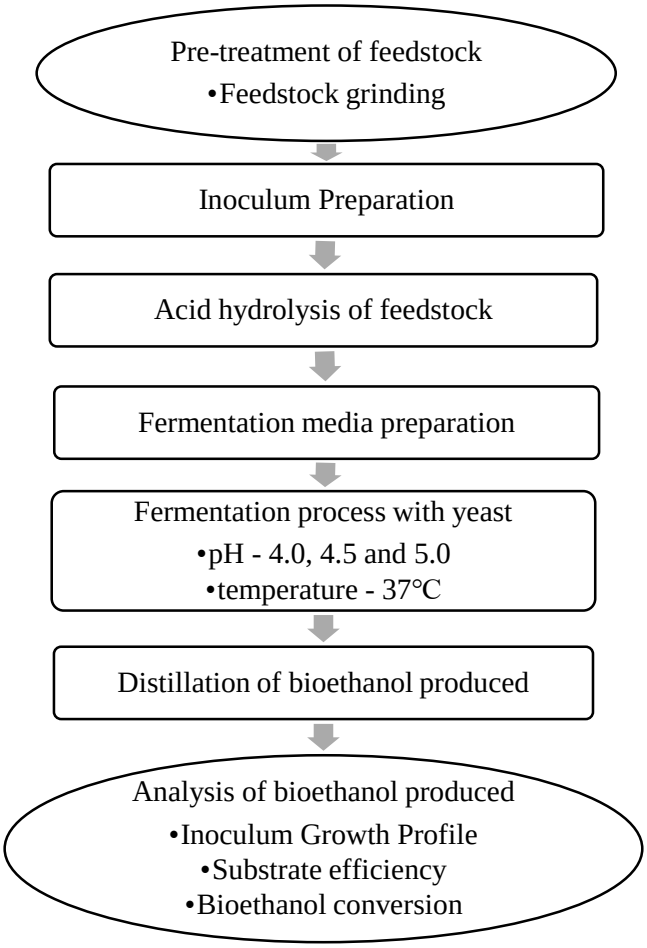

Figure 1: Flowchart of methodology

2.2.1 Feedstock Grinding

Feedstock grinding is the physical method in the pre-treatment process. The physical pre-treatment uses mechanical approaches to ground the feedstock. The two agricultural wastes, the corncob and sugarcane bagasse are purchased from the local hawker center and market at the area around Muar, Johor. After the wastes are collected, the wastes are washed thoroughly with tap water to wash away the other substances such as soil or dirt. The wastes are then cut into smaller pieces. The wastes are then dry in the oven at 60 ℃ for 28 hours. Once dried, the wastes are taken out from the oven and ground using a grinding machine. The weight of grinded waste samples is then weighed using an analytical balance and recorded in the table. The samples are sealed in a sealed bag and placed in a closed container to prevent any contamination. The samples are store in room conditions.

2.2.2 Acid Hydrolysis

Acid hydrolysis is the chemical method in the pre-treatment process. The acid using in acid hydrolysis is diluted sulphuric acid. In preparation for acid hydrolysis, 60 g of feedstock powder is weighted and mixed with 0.6 L of 0.5 mol/L of sulphuric acid (H_2SO_4) solution. The mixture is then heated at 121 ℃ for 20 minutes to break down most of the starch and recovered as dissolved sugars.

2.2.3 Inoculum Preparation

The inoculum is prepared by using commercial baker's yeast incubated with glucose solution for 8 to 10 hours at room temperature. 8 mL of inoculum is added into the flask containing the culture medium, prior to fermentation start up.

2.2.4 Fermentation Media Preparation

In fermentation, culture media is prepared as a nutrient to yeast inoculums. The flask scale fermentation is used in this experiment. The medium culture is prepared at 200 mL. The components of the culture medium are shown in Table 1. The components are dissolved with 200 mL of hydrolysed feedstock culture. The mixed culture media is adjusted to the desired pH using 1 mol/L sodium hydroxide and 0.5 mol/L of sulphuric acid. The medium culture will then sterilize at 121 ℃ for 20 minutes and later cool down to room temperature.

Table 1: Ingredient for culture medium [3]

Ingredient for culture medium	g/L	g/200 mL
Ammonium chloride, NH_4Cl	2.500	0.500
Sodium phosphate dibasic heptahydrate, $Na_2HPO_4.7H_2O$	2.910	0.582
Potassium dihydrogen sulphate, KH_2PO_4	3.000	0.600
Magnesium sulphate, $MgSO_4$	0.250	0.050
Calcium chloride, $CaCl_2$	0.080	0.016
Sodium citrate, $Na_3C_6H_5O_7$	3.000	0.600

2.2.5 Fermentation Process

The lab-scale fermentation is carried out for 48 hours using shake flask fermentation. The flask is incubated at 150 rpm at 37 ℃. The *S. cerevisiae* is used to ferment the feedstock to ethanol and carbon dioxide. Two sets of experiments are carried out to test the effect of pH on bioethanol yield between two types of feedstocks, which are sugarcane bagasse and corncob. To test the effect of pH, the temperature is maintained at 37 ℃ while varying the pH at 4, 4.5 and 5.

2.2.6 Distillation of Bioethanol

The distillation is carried out to determine the ethanol production by two feedstocks. The distillation is carried out to separate ethanol from water fractions. The bioethanol is distilled using a rotary evaporator at water bath temperature of 40 ℃ and at pressure of 58 mbar.

2.3 Analytical method

2.3.1 Inoculum growth profile

The absorbance of inoculum is tested using UV-Visible spectrophotometer at wavelength of 600 nm for every one hour. The growth profile of yeast inoculum is then plotted using the graph of absorbance (OD_{600}) against time.

2.3.2 Substrate Efficiency

The substrate efficiency is measured using the phenol-sulphuric acid method. The method is used to measure the total sugar contents (substrate concentration) in the mixture. The substrate efficiency is carried out by using the initial substrate concentration minus substrate concentration at the end of the process. Therefore, the substrate concentration at 0 hours and 48 hours is measure using the phenol-sulphuric acid method. 0.5 mL of sample is pipette into test tubes with 0.5 mL of water and the blank is set up with 1mL of water. Add another 1 mL of phenol solution and 98.00 % of sulphuric acid into the test tube and shake for 10 minutes. After the shaking, the test tube are placed in water bath at 25 ℃ at 20 minutes. Next, the samples are transferred to cuvette and measure at 490 nm using UV-Visible spectrophotometer. The reading obtained is recorded and calculate the total carbohydrate present in sample. The equations are shown below (Eq. 1 & Eq. 2):

$$Sugar\ concentration(\%) = \left(\frac{OD_{490}}{Vs}\right) x\ m_f \text{ ,where}$$

(*Eq.* 1)

OD_{490} = absorbance at 490 nm

v_s = volume of sample

m_f = mass of feedstock

$$Substrate\ Efficicency\ (\%) = \frac{S_0 - S_1}{S_0}\ X\ 100\% \text{ , where}$$

(*Eq.* 2)

S_0 = initial sugar concentration at 0 hr

S_1 = final sugar concentration at 48 hr

2.3.3 Bioethanol conversion

In the production of bioethanol, the fermentation media solutions with feedstock were prepared. The culture media added with feedstock before adjusting the pH was prepared at 200 mL. After the pH was adjusted to 4.0, 4.5 and 4.5, the volume of the fermentation media solution was changed and was measured using a measuring cylinder. The volume of fermentation media at each pH condition was recorded in the Table 2 below. The fermentation broth is then undergone fermentation and distilled to obtain bioethanol produced. The bioethanol produced is then measured using a measuring cylinder. The

conversion of media for each feedstock at different pH conditions to bioethanol is then calculated using the Eq. 3 below.

$$Conversion\ of\ feedstock\ to\ bioethanol = \frac{V_E}{V_T}\ x\ 100\%\ ,\ \text{where} \quad (Eq.\ 3)$$

V_E = volume of bioethanol produced

V_T = total volume of media solution

Table 2: Final volume of fermentation broth with feedstock after pH adjustment

Feedstock	Target pH	Volume of media before pH adjustment (mL)	Added volume to adjust pH (mL)	Total volume after pH adjustment (mL)	Volume of inoculum added (mL)	Final media volume (mL) (Total volume + volume inoculum)
Sugarcane bagasse	4.0	200	9 mL NaOH	209	8	217
	4.5	200	15mL NaOH	215	8	223
	5.0	200	27mL NaOH	227	8	235
Corncob	4.0	200	10mL NaOH	210	8	218
	4.5	200	16mL NaOH	216	8	224
	5.0	200	25mL NaOH	225	8	233

3. Results and Discussion

3.1 Growth Profile of Yeast Inoculum

The growth profile of yeast inoculum is studied through the reading obtained from the UV-Visible spectrophotometer. The inoculum was incubated at a temperature of 30 ℃ for 8 hours. It is expected that the absorbance reading should be increased from 0 hours to 8 hours. The data obtained in table 3, shows that inoculum for flask 1 has a stable increase in absorbance for OD_{600} while the reading for flask 2 does not meet the expectation. The increase in absorbance represented the growth of yeast in the inoculum. The growth of yeast is very crucial as the inoculum will be added into the culture media to facilitate the fermentation of feedstock to produce bioethanol. Therefore, three flasks were used including one flask as blank to test the absorbance and to ensure the inoculum used is in a good condition.

From the result obtained, the inoculum for flask 1 was chosen to be used in the next step. Figure 2 shows the growth profile of the inoculum for 8 hours. The main purpose of taking the reading for every one hour is to ensure the growth of yeast and to stop the growth of yeast at its exponential phase (phases in which the yeast has the highest growth rate). From the growth profile, it can be analysed that the exponential phase started at the 7th to 8th hours since the graph shows a sudden peak up between the hours. Therefore, the incubation was stopped at the 8th hour and the yeast inoculum was kept in the chiller which the temperature is at 15 ℃. The inoculum was kept at the chiller to stop the growth of yeast growth at exponential phase and save for later use.

Table 3: Absorbance of three flask for every one hours

Time (hours)	Absorbance (OD_{600})		
	Blank	Flask 1	Flask 2
0 h	0.000	0.001	0.000

1 h	0.001	0.013	-0.024
2 h	0.000	0.057	-0.128
3 h	0.001	0.078	-0.376
4 h	0.000	0.143	-0.443
5 h	0.001	0.174	-0.426
6 h	0.001	0.215	-0.422
7 h	0.001	0.324	-0.514
8 h	0.000	0.576	-0.503

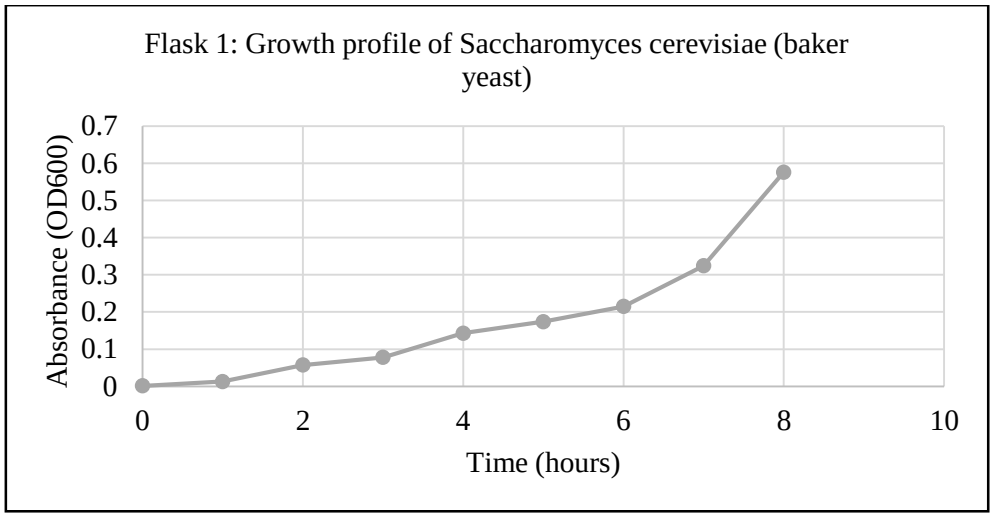

Figure 2: Growth profile of S. Cerevisiae (baker yeast) of flask 1

3.2 Substrate Efficiency

The substrate efficiency is measured using the phenol-sulphuric acid method. The method is used to measure the total sugar contents (substrate concentration) in the mixture. The final sugar concentration residue in the sample mixture is compared with its initial sugar concentration. The absorbance of samples mixtures is measured using a UV-Visible spectrophotometer at wavelength 490 nm (OD_{490}). The sugar concentration and substrate efficiency are calculated using the formula stated (equation 2 and 3) and are recorded in Table 4.

Table 4: The substrate efficiency of two feedstocks at different pH

Feedstock	pH	Absorbance (OD_{490})		Sugar concentration		Substrate efficiency (%)
		0 hr	48 hr	0 hr	48 hr	
Sugarcane bagasse	4.0	0.791	0.284	94.92	34.08	63.98
	4.5	2.043	0.019	245.16	2.28	99.06
	5.0	1.706	0.314	204.72	37.68	81.59
Corncob	4.0	1.342	0.802	161.40	96.24	40.37
	4.5	0.812	0.197	97.44	23.64	75.74
	5.0	0.444	0.124	53.28	14.88	72.07

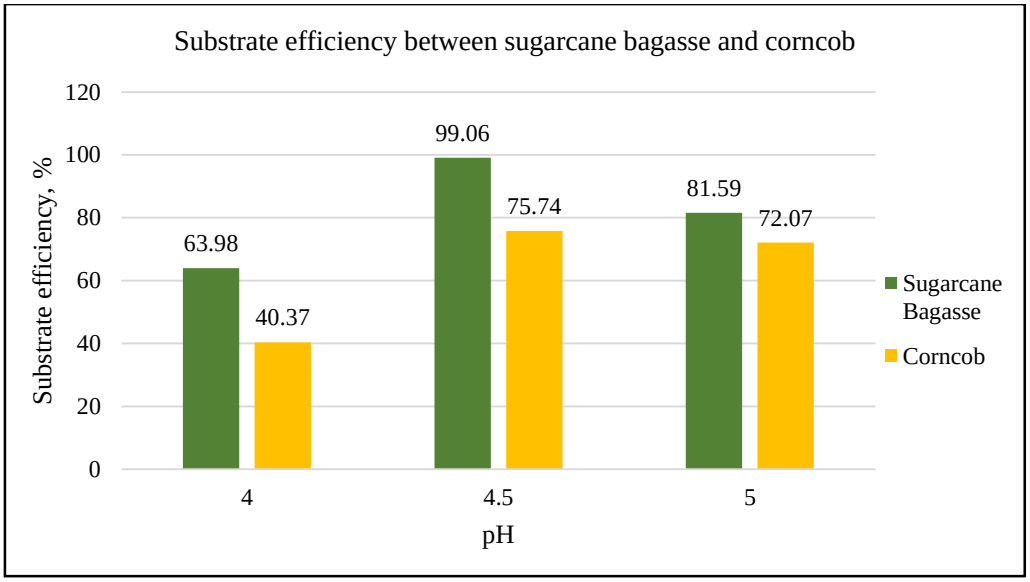

Figure 3: The substrate efficiency between two feedstocks

From Figure 3, it can be seen that the overall substrate efficiency of both feedstocks has the highest percentages for pH 4.5, which are 99.06 % for sugarcane bagasse and 75.74 % for corncob. While at pH 4, the substrate efficiency has the lowest percentages at 63.98 % for sugarcane bagasse and 40.37 % for corncob. Besides that, the substrate efficiency for sugarcane bagasse is higher than corncob at all pH conditions, which are about 10.00 % to 25.00 % different in number at 63.98 %, 99.06 % and 75.74 %.

Figure 3 showed the substrate efficiency between two feedstocks investigated, which are sugarcane bagasse and corncob. The substrate efficiency is used to compare the changes of sugar concentration in the feedstock mixture before and after the fermentation by baker's yeast *S.cerevisiae*. The higher the substrate efficiency, the lower the amount of sugar residue in the sample mixture. Therefore, the fermentation of yeast is said to be successful since the yeast managed to take up the nutrient provided in the production of bioethanol. In other words, the substrate efficiency indicates the sugar consumed during the fermentation by yeast.

Based on the substrate efficiency, it can be concluded that the total sugar content has decreased for 48 hours as sugar is used in fermentation by yeast in anaerobic conditions to produce ethanol. The 48 hours fermentation is carried out on a flask scale without the supply of oxygen. Therefore, yeast fermentation is said to be anaerobic, and glucose (sugar) is broken down into pyruvate. The pyruvate acid produced through glycolysis will break down, produces acetaldehyde and carbon dioxide. Next, with the help of NADH, ethanol was produced. The chemical reaction formula of alcohol fermentation is

$$C_6H_{12}O_6 \rightarrow 2CH_3CHO + 2CO_2 \rightarrow 2C_2H_5OH$$

where the glucose is transformed into acetaldehyde, carbon dioxide and lastly transform into ethanol. For the formula, one molecule of glucose is consumed to produce two molecules of ethanol.

Other than that, the overall substrate efficiency of sugarcane bagasse is higher than corncob. This is because of the difference in cellulose content between the two types of feedstocks. Sugarcane bagasse and corncob are two types of lignocellulosic rich waste which are having a huge potential in bioethanol production. The cell wall of these lignocellulosic-rich waste breaks down, and thus cellulose is released and fermented into alcohol by the use of microbes or enzymes. Sugarcane bagasse has a higher cellulose content, which at 43.60 % while corncob has cellulose contents at 34.00 %. The difference in cellulose

contents makes the substrate efficiency of corncob lower than sugarcane bagasse since the sugar contents for corncob are limited.

According to a study carried out by Dimas, in the production of bioethanol from sugarcane bagasse and corncob, the difference in substrate efficiency obtained was only different at less than 5.00 % between two feedstocks [4]. While for the result obtained from our study, the difference is 10.00 % to 25.00 % between two feedstocks. This is probably due to external reasons such as the treatment that had been done on the feedstock before the feedstock is collected. During the waste feedstocks collection, the sugarcane bagasse is collected right after the juice is extracted and does not undergo any further process, while the corncob is boiled before consuming the corn grain. Therefore, the thermal treatment that happened on the corncob might affect the sugar contents in the corncob since the heat will cause the loss of sugar substrate and lowered the substrate efficiency for corncob. According to the research carried out by Koan et al, the glucose contents is decreasing with the increasing of heat treatment temperature and time [7]. The thermal treatment that happened to corncob decreases the glucose contents and free sugar contents in corncob. Thus, the amount of sugar for the fermentation for corncob by yeast is affected and proved the lower in substrate efficiency.

3.3 Conversion of fermentation media solution to bioethanol

In bioethanol production, the conversion of fermentation media solution to bioethanol were calculated and recorded in Table 5. Table 5 shows the bioethanol produced for two types of feedstocks at different pH conditions.

Table 5: Conversion of media to bioethanol for two feedstocks at different pH condition

Feedstock	pH	Final media volume (mL) (Total volume + volume inoculum)	Bioethanol produced after 48 hours (mL)	Conversion (%)
Sugarcane bagasse	4.0	217	145	66.82
	4.5	223	220	98.65
	5.0	235	201	85.53
Corncob	4.0	218	113	51.83
	4.5	224	174	77.68
	5.0	233	169	72.53

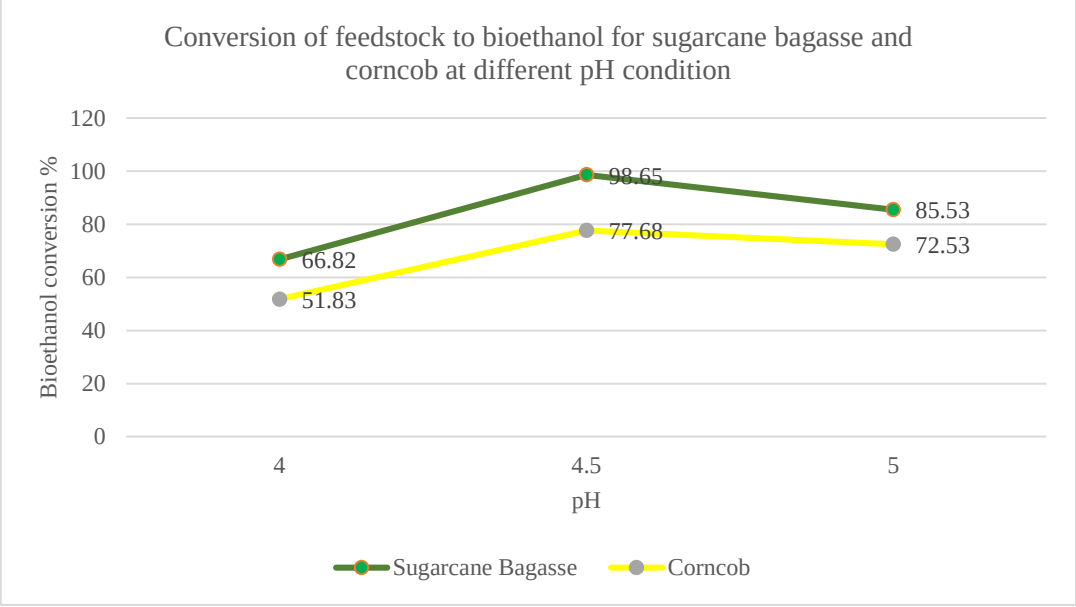

Figure 4: Bioethanol conversion for sugarcane bagasse and corncob at different pH condition

Table 5 and figure 4 shows the conversion of feedstock to bioethanol for sugarcane bagasse and corncob at different pH condition. From the bioethanol conversion, it can be seen that the bioethanol conversion for sugarcane bagasse is higher than corncob at 66.82 %, 98.65 % and 85.23 %. Meanwhile, for corncob, it has lower bioethanol conversion at 51.83 %, 77.68 % and 72.53 %. This shows that sugarcane bagasse has a higher efficiency in the production of bioethanol. It is probably because of the higher substrate efficiency of sugarcane bagasse.

Besides that, for overall bioethanol conversion for two types of feedstocks, the conversion percentage is the highest at pH 4.5, which are 98.65 % for sugarcane bagasse and 77.68 % for corncob. The maximum bioethanol conversion at pH 4.5 reflects the optimum pH environment for yeast *S.cerevisiae* to carry out the reaction. By comparing the bioethanol conversion trend line before and after pH 4.5, it shows that the bioethanol conversion before pH 4.5 is lower than after pH 4.5. The increase in bioethanol conversion between pH 4.5 to pH 5.0 indicates that the yeast is more active reacted in the range and the most efficiently at pH 4.5.

In the enhancement of bioethanol production, there are factors that will affect the yield of production, such as temperature, pH, sugar concentration, time, rate of agitation and inoculum size (Khaled et al, 2018). In this study, the main factor that was investigated was pH condition at a constant temperature, which was at 37 ℃. For yeast incubation, the optimum temperature is between 32 ℃ to 35 ℃. However, in sugar fermentation, raising of temperature from 35 ℃ to 45 ℃ is a common challenge in bioethanol fermentation. Therefore, in the study, the temperature of 37℃ is chosen to study the ability of yeast to overcome the rise in temperature in a shake flask scale fermentation process. The yeast growth rate and metabolism are also affected by the increase in ethanol concentration during fermentation. The increase in ethanol concentration may inhibit the growth of the microorganism and its viability [5]. However, in shake flask scale fermentation, the volume of media used is lower (usually lower than 1 Liter) compared to the bioreactor scale (higher than 1 Liter), thus the increment in ethanol concentration does not have a significant effect on the yeast growth.

The pH value plays a major role in the fermentation process and ethanol production as it is directly affected yeast growth, rate of fermentation, formation of by-products, and media contamination possibility [6]. Different organisms have different optimum pH values. For S. cerevisiae, the optimum pH is between 4.0 to 5.0 [5]. Therefore, three pH values between 4.0 to 5.0 were used in the experiment to find the most optimum pH for yeast S. cerevisiae in bioethanol production from two lignocellulosic waste feedstocks. According to Khaleb, when the pH is lower than 4.0, the time required for incubation should be increased [6]. However, bioethanol production will not have significantly different due to the stress and toxicity which inhibit the growth of yeast cells. Furthermore, microorganisms that are able to survive in the severe conditions of lignocellulosic bioethanol production are potential strains for completing fermentation procedures. These strains can be obtained in one of two methods, which are through the isolation from an environment with ideal conditions for generating desirable traits, or by genetic manipulation using model organisms such as *S. cerevisiae* [6].

4. Conclusion

From the result and discussion, it can be concluded that the bioethanol can be successfully produced from sugarcane bagasse and corncob through a fermentation process using the baker yeast (*Saccharomyces Cerevisiae*). For substrate efficiency, sugarcane bagasse has higher efficiency compared to corncob at 63.98 %, 99.06 % and 75.74 %. For overall substrate efficiency for both feedstocks, the highest efficiency was obtained at pH 4.5, which is 99.06 % for sugarcane bagasse and 75.74 % for corncob. Besides that, in bioethanol conversion, sugarcane bagasse has a higher percentage than corncob at 66.82 %, 98.65 % and 85.23 %. Meanwhile, for corncob, it has lower bioethanol conversion at 51.83 %, 77.68 % and 72.53 %. This shows that sugarcane bagasse has a higher efficiency in the production of bioethanol because of the higher substrate efficiency of sugarcane bagasse. Furthermore, the increase in bioethanol conversion between pH 4.5 to pH 5.0 indicates that the yeast is

more active reacted in the range and the most efficiently at pH 4.5. In conclusion, sugarcane bagasse is more efficient in bioethanol production while the most suitable pH for *S.cerevisiae* in bioethanol production is pH 4.5. In the future, more research should be conducted to study the effect of the parameter during the fermentation process by yeast in order to produce a better yield of bioethanol at the highest efficiency such as wider the temperature or changing the flask scale to bioreactor scale fermentation.

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