Study on Optimal Bioethanol Production From Corn Seed (*Zee Mays* L.)

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Abstract

The main aim of the present research work is to produce bioethanol from corn seed. The optimal production of bioethanol from corn seed was studied utilizing two different commercial yeasts (I and II). The qualitative tests of reducing sugar in the fermented samples were done by means of Fehling's solution and Benedict's reagent respectively. Moreover, quantitative test of sugar in the fermented sample was determined by Iodometricmethod. In addition, pH values of the fermented substrate were also determined from first day to fifth day during fermentation process. The production of bioethanol was estimated by spectrophotometric method at different substrate concentration and different yeast percent (1% to 5%). Then, bioethanol was separated by distillation process at 78.3ºC. The resulting ethanol was also confirmed by FT-IR spectroscopic method.

Keywords: corn seed, commercial yeasts, fermentation, bioethanol, FT-IR

Introduction

Bioethanol, a natural product, is manufactured by the fermentation of plants including sugar and starch. Bioethanol bears the suffix “bio” as it is produced by the action of microorganisms and enzymes through the fermentation of sugars or starches or cellulose. Bioethanol has been widely used as alcoholic beverages in alcohol industry, as a base chemical for other organic compounds in chemical industry, and as an antiseptic or as a treatment (in medical) for poisoning by other alcohol(Hossain,N. *et al*,2017).

Ethanol from biomass can provide a sustainable, albeit limited alternative to oil to mitigate the global energy problem associated with fossil fuels exhaustion and greenhouse gas emissions.Declining supplies of fossil fuels, production of bioethanol has been attention worldwide as an alternative source of energy. (Farrell et al.,2006)

Ethanol is produced from fermentation of carbohydrate such as starch, provides a renewable energy source that produces a number of additional benefits. The demand for bioethanol has therefore increased.The world bioethanol research is driven by the need to reduce the cost of production.(Nikolic, et al., 2009)

Nowadays, industrial bioethanol production is mainly focused on biomass corn, wheat and sugarcane, as well as on highly abundant agricultural wastes. The use of residual biomass for bioethanol productions has the added advantage of transforming a waste material into a valorized product. The increase in the prices of fuel and possibility of shortfalls has led to an extensive evaluation of alternative sources of energy to meet the global energy demand. (Nigam, J.N., 2000)

In this research work, production of bioethanol from corn seeds was performed as substrate using two kinds of commercial yeast.

Materials And Methods

Sample Collection

The corn seeds were collected from Shan-kalay-kyun village, Amapura Township, Mandalay Region. Fresh maize seeds were allowed to dry well and crushed in blender. They were stored in a well-stoppered bottle and used throughout the experiment.

Figure 1. Corn and corn powder

Preparation of Yeast

Commercial yeast I was purchased from Able Chemical Shop, Mandalay and commercial yeast II was purchased from Local Market, Taunggyi, Shan State.

Commercial yeastI Commercial yeastII

Figure 2. Commercial yeastIand commercial yeastII

Conversion of Glucose to Ethanol

2mL of 1% of commercial yeast I solution and 1 ml of 1 % of glucose solution were added into a test tube. 1ml of Benedict's solution was added into the test tube. Then, it was heated and cooled. The yellow precipitate was obtained. Similary, Commercial yeast II solution were also tested.

Figure 3. Conversion of glucose to ethanol

Fermentation of Corn powder

Firstly, corn powder (50g) was mixed with (20mL) of distilled water in order to make corn plup. And then 400 mL of distilled water was poured to 50 g of corn powder. Similarly five bottles were prepared and the various amounts of commercial yeast I (1%, 2%, 3%, 4%, 5%) were added to each bottles, respectively. Fermentation took place in anaerobic conditions. The fermentation process was taken for 5 days.

Qualitative Testsof Reducing Sugar in Fermented Sample

Fehling's test(Fehling, H., 1849)

1 mL of Fehling's **A** and 1 mL of Fehling's **B** were taken in a test tube and mixed well. 1 mL of the test solution was added and boiled the mixture for a few minutes. The observation was made for this precipitation.

Benedict's test(Benedict,S.R.,1908)

1 mL of the test solution was added to 2 mL of Benedict's reagent and boiled for 5 minutes in a water bath. The solution was cooled. The observation was made for this precipitation.

Determination of pH Values(AOAC,1990)

The determination of pH values of each sample solution (before and after fermentation) was performed.

About 30 ml of each sample solution (before and after fermentation)was placed into a beaker and shaken for 15 minutes. Then, pH values were measured by digital pH meter.

Determination of Reducing Sugar Content in Fermented Solution by Iodometric Method(Browne,C.A.,1912)

1 mLof sample solution was placed in the conical flask. 2 mL of iodine solution and 4.5 mL of 0.1 M sodium hydroxide solution were added into the flask and closed the flask and left the flask in the dark place for 15 min. Then, 0.6 mL of 1 M hydrochloric acid solution was added and titrated with the 0.05 M sodium thiosulphate solution. When the liquid became light-yellow, 1 ml of starch solution was added. The solution became dark again and titrated with the 0.05 M sodium thiosulphate solution until the colorless solution was obtained.

This procedure was repeated three times. The final concentration of glucose can be calculated from the experimental data.

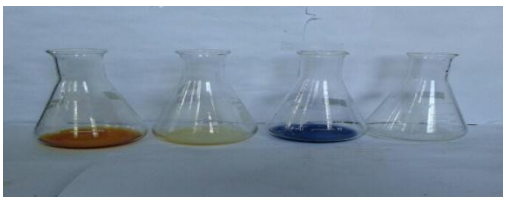

Figure 4. Determination of reducing sugar content

Spectrophotometric Determination of Ethanol(Sumbhate, S., *et al*, 2012)

Preparation of authenticethanol solutions

Standard 5% ethanol solution was prepared by diluting 5 mL of the standard ethanol solution to 100 mL with distilled water and mixed thoroughly. Similarly, standard 10% , 15%, 20%, 25%, 30%, 35% and 40% ethanol solution were prepared by diluting 10 mL, 15 mL, 20 mL, 25 mL, 30 mL, 35 mL and 40 mL of the standard ethanol solution to 100 mL with distilled water and mixed thoroughly.

Construction of calibration curve for authenticethanol solution

For quantitative analysis of a compound by visible spectroscopy, it is firstly necessary to know the wavelength of maximum absorption (λ_{max}). The absorbance of standard ethanol solution was measured at 572nm, 574nm, 576nm, 578nm, 580nm, 582nm and 584nm.

In the test tubes, 1 mL of each standard ethanol solution were made to react with 5 mL of potassium dichromate($K_2Cr_2O_7$)(0.25M) and 2 mL of sulfuric acid (H_2SO_4)(6M). These test tubes were incubated in the water bath at 60˚C for 20min and the absorbances of standard ethanol solutions were determined at 578 nm by UV-Visible spectrophotometer.

Determination of Ethanol Content from the Fermented Sample Solutions

In each test tube, 1 mL of each fermented sample solution was made to react with 5 mL of potassium dichromate ($K_2Cr_2O_7$)(0.25M) and 2 mL of sulfuric acid (H_2SO_4) (6M). These test tubes were incubated in the water bath at 60˚C for 20min and the absorbances of fermented sample solutions were determined at 578 nm by UV-Visible spectrophotometer. The presence of a bluish-green colour indicated that the used fermented sample solution was able to produce bioethanol. Ethanol percent was estimated from standard curve drawn using an authentic ethanol.

Distillation (Carlson, C.S. and Steward,J., 1965)

Determination of ethanol content was performed on the fermentation process and was determined by distillation method. Distillation was made at 78.3°C after the ethanol fermentation process.

Confirmation of Bioethanol

Iodoformtest(Kosaric, N.A., *etal,* 1983)

The extracted bioethanol solution sample was confirmed by iodoform test. 1 mL of bioethanol sample and 1 mL of 10 % sodium hydroxide (NaOH) solution were taken in a test tube. 10% iodine solution was added drop by drop and was heated for 2 minutes. Then yellow precipitates of iodoform were observed.

Infrared Spectroscopy

The prepared bioethanol was also confirmed by Infrared spectroscopy. The functional group determination of bioethanol was performed by FT-IR spectrophotometer at Department of Chemistry, University of Mandalay.

Results and Discussion

Qualitative Tests of Reducing Sugar in Fermented Corn Seed Sample

The qualitative tests of reducing sugar in the fermented samples were done by means of Fehling's solution and Benedict's reagent respectively.

Table 1.Results of qualitative tests of reducing sugar infermented sample

No.	Samples	Experiment	Observation	Reducing Sugar
1.	Fermented sample	Fehling's Test	Brick red ppt	+
2.	Fermented sample	Benedict's Test	Brick redppt	+

The fermented samples gave rise to positive tests for reducing sugar respectively.

Quantitative Determination of Reducing Sugar Content in Fermented Samples

Quantitative tests of reducing sugar in the fermented corn seed sample using different amount of commercial yeast I and II were determined by iodometric method. The results are shown in table 2 and table 3.

Table 2. Reducing sugar content in fermented samplesusing commercial yeast I

Yeast I (%)	Reducing sugar content before adding yeast I (mmol mL^{-1})	Reducing sugar content after adding Yeast I(mmol mL^{-1})				
		After 1 day	After 2 days	After 3 days	After 4 days	After 5 days
1%	0.0527	0.0564	0.0477	0.0402	0.0377	0.0354
2%	0.0527	0.0577	0.0452	0.0402	0.0375	0.0352
3%	0.0527	0.0614	0.0452	0.0421	0.0411	0.0352
4%	0.0527	0.0602	0.0427	0.0367	0.0322	0.0227
5%	0.0527	0.0547	0.0502	0.0462	0.0452	0.0384

It was observed that fermented corn seed samples treated with3% commercial yeast I gave the highest content of reducing sugar after 1 day.

Table 3. Reducing sugar content in fermented samplesusing commercial yeast II

Yeast II (%)	reducing sugar content before adding yeast II ($mmolmL^{-1}$)	Reducing sugar content after adding Yeast II($mmolmL^{-1}$)				
		after 1 day	after 2 days	after 3 days	after 4 days	after 5 days
1%	0.0527	0.0672	0.0652	0.0427	0.0427	0.0322
2%	0.0527	0.0683	0.0664	0.0452	0.0448	0.0377
3%	0.0527	0.0675	0.0652	0.0377	0.0350	0.0352
4%	0.0527	0.6483	0.0665	0.0352	0.0452	0.0322
5%	0.0527	0.686	0.0677	0.0477	0.0427	0.0356

The fermented corn seed samples treated with 2% commercial yeast II gave the highest content of reducing sugar after one day.

pH Values of Fermented Sample Using Different Amount of Commercial Yeast I and II

pH values of the fermented corn seeds samples using different amount of commercial yeast I and II were determined and the results were shown in table 4and table 5.

Table 4. pH Values of fermented corn seeds sample using different amount of commercial yeast I

Yeast I (%)	pH before adding yeast I	pH after adding Yeast I				
		after 1 day	after 2 days	after 3 days	after 4 days	after 5 days
1%	5.30	4.23	4.23	4.11	3.80	3.67
2%	5.30	4.68	4.68	4.23	3.87	3.68
3%	5.30	4.70	4.70	4.36	3.86	3.79
4%	5.30	4.78	4.78	4.42	3.94	3.78
5%	5.30	4.59	4.59	4.21	3.84	3.77

pH values of fermented corn seeds treated with 1% to 5% commercial yeast I were observed from 3.67to 3.79.

Table 5. pH values of fermented corn seeds sample using different amount of commercial Yeast II

Yeast II (%)	pH before adding yeast II	pH after adding Yeast II				
		after 1 day	after 2 days	after 3 days	after 4 days	after 5 days
1%	5.30	4.10	4.02	3.66	3.65	3.54
2%	5.30	4.82	4.42	3.82	3.79	3.53
3%	5.30	4.42	4.12	3.98	3.84	3.71
4%	5.30	4.32	4.10	3.70	3.65	3.55
5%	5.30	4.54	435	3.79	3.78	3.42

pH values of fermented corn seeds treated with 1% to 5% commercial yeast II were observed from 3.42 to 3.71.

Construction of Calibration Curve for Standard Ethanol Solution

The wavelength of maximum absorption (λ_{max}) was determined andthe wavelength of maximum absorption was found at 578nm. The calibration curve for standard ethanol solution was constructed. The results are shown in table 6 and figure 11.

Table 6. Relationship between absorbance and percent of standard ethanol solution

Ethanol (%)	Absorbance	Ethanol(%)	Absorbance
5	0.102	25	0.489
10	0.187	30	0.626
15	0.343	35	0.713
20	0.411	40	0.774

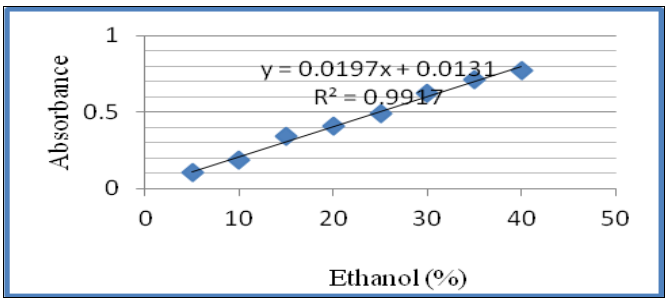

Figure 5. Plot of absorbance as a function of percent of standard ethanol solution

Bioethanol Content of Samples by Spectrophotometric Method

The production of bioethanol was estimated by spectrophotometric method at different yeast percent. The results are shown in table 7.

Table 7.Bioethanol content of corn seedsamples using commercial yeast by spectrophotometric method

Yeast	Yeast I		Yeast II	
(%)	Absorbance	Bioethanol (%)	Absorbance	Bioethanol (%)
1%	0.248	13.6	0.251	13.8
2%	0.318	14.1	0.246	13.5
3%	0.326	14.6	0.320	14.2
4%	0.320	14.2	0.336	15.5
5%	0.251	13.8	0.331	14.9

Bioethanol content of corn seeds samples using 3% commercial yeast I and 4% commercial yeast II were higher than the other treatment.

Confirmation of Biethanol by IodoformTest and FT-IR spectral Data

The production of bioethanol was confirmed by iodoform test. The yellow crystals of iodoform were formed. The preparedbioethanol was also confirmed by FT-IR spectroscopic method.

Table 9. FT- IR spectral data of prepared bioethanol andauthenticethanol

No.	Absorption band (cm^{-1}) wave number		Peak Assignments
	Prepared bioethanol	Authentic ethanol	
1.	3407.37	3352	-OH stretching
2.	2974.33,2928.04,2898.14	2976,2933,2899	C-H stretching (sp^3)
3.	1453.41	1481	O-H bending
4.	1086.92,1046.42	1087,1047	C-O stretching of C-O-H
5	879.57	879	C-C-O bending

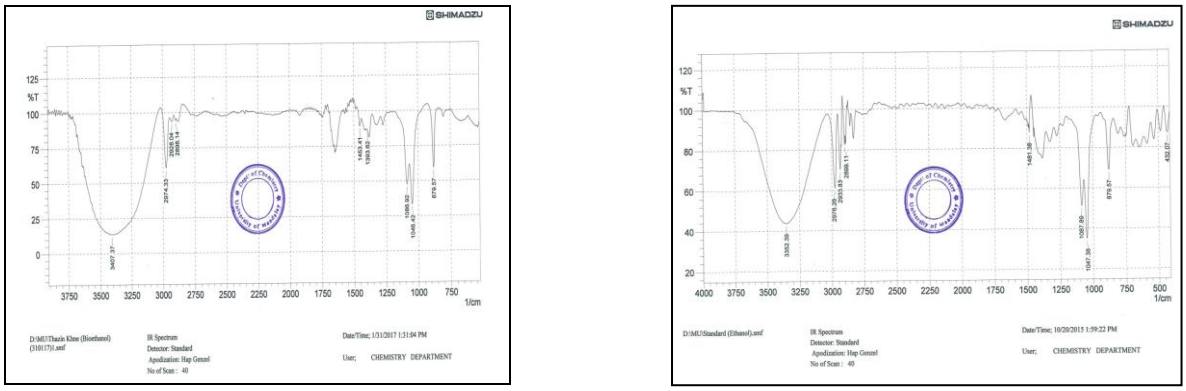

Figure6 FT-IR Spectrum of prepared ethanol and authenticethanol

Conclusion

In this research work, the qualitative tests of reducing sugar in the fermented samples were done by using Fehling's solution and Benedict's reagent respectively. The fermented samples gave rise to positive tests for reducing sugar respectively.

Moreover, quantitative test of reducing sugar in the fermented corn seed sample were determined by iodometric method. Among the treatment, 3% commercial yeast I treatment and2% commercial yeast II treatment also gave the highest content of reducing sugar. The sugar content in the fermented substrate was found to be remaining until the end of the fermentation process. It was showed that all sugar in the substrate was not utilized by the yeast to produce ethanol.

pH values of the end of fermentation of corn seeds treated with 1% to 5% commercial yeast I were observed from 3.67to 3.79 and from 3.42 to 3.71 by 1% to 5% commercial yeast II. The best ethanol production was observed at pH 3.79 and 3.55.

The production of bioethanol was estimated by spectrophotometric method at different yeast percent.The bioethanol content of corn seed samples using 3% commercial yeast I and 4% commercial yeast II were higher than the other treatment.Then, bioethanol was separated by distillation process at 78.3ºC.

The production of bioethanol was confirmed by iodoform test. The yellow crystals of iodoformwere formed. The resulting bioethanol was also confirmed by FT-IR spectroscopic method. According to FT-IR assignments, the functional groups containing in this bioethanol are consistent with authentic ethanol. Therefore, it was found that there are many factors affecting bioethanol production process.

Acknowledgements

We would like to express our heartfelt gratitude to Rector, DrThida Win, University of Mandalay for encouragementson our research work. We deeply thank to Dr Yi YiMyint, Head of Department of Chemistry, University of Mandalay for her guideline and encouragement on our research work.

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