Extraction of Bio-ethanol from Waste Pineapple Peelings

by

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CERTIFICATION OF APPROVAL

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Approval by:

____

(Dr Oh Pei Ching)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

September 2013

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

NOORAFENDDEY BIN MOHAMAD ROBI

ABSTRACT

The factor of high market price for oil, the need to secure the energy reservoir and green house emission from fossil fuel has opened a new source of energy research on bio-ethanol. Recently researchers have looked into the utilization of renewable feedstock for production of bio-ethanol. One of the researched renewable feedstock is pineapple peelings. On average, more than 11,000 hectares of land in Malaysia is currently planted with pineapples which generate 40 to 65 tons of waste per hectare ultimately contribute to the country's waste disposal problem. There is also global oil crisis, as demand for petroleum increases each year while our supplies are rapidly being depleted. Bio-ethanol, a principal bio-fuel, is a natural alternative to gasoline. One solution to both dilemmas is to produce bio-ethanol from pineapple plant peelings. Bio-ethanol from pineapple (*Ananas comosus*) peel extract will be carried out by controlling fermentation without any treatment. *Saccharomyces ellipsoides* was used as inoculum in this fermentation process as it is naturally found at the pineapple skin. In this study, the capability of Response Surface Methodology (RSM) for optimization of ethanol production from pineapple peel extract using *Saccharomyces ellipsoideus* in batch fermentation process was investigated. Effect of three test variables in a defined range of yeast concentration 6-14% (v/v), temperature (25-40°C) and fermentation time (48-96 hrs) on the ethanol production were evaluated. Data obtained from experiment were analyzed with RSM.

ACKNOWLEDGEMENT

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Lastly, I want to thank my beloved family and friends for giving me such a big support from the start of my final year project to the end. They were the one who always there beside me and help me out in every way they could in the times of trouble.

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CHAPTER 1

INTRODUCTION

1.1 Background Study

Bio-fuels are liquid or gaseous fuels primarily produced from biomass, and can be used to replace some fossil fuels. According to NREL (May 18, 2012), the two most common types of bio-fuel in use today are ethanol and biodiesel. Crops used to make bio-fuels are generally high in sugar, starch or oils. Ethanol and biodiesel are bio-fuels commonly used in transportation. Ethanol is produced from sugar and starch crops that are processed by yeast or bacteria-mediated fermentation, or from cellulose. Biodiesel fuels are extracted from oil crops with suitable solvents or through mechanical processing and conversion of oil into diesel fuel by a transesterification process. Other less commonly used bio-fuels include other alcohols and ethers, such as bio-butanol, methanol, methyl tertiary butyl ether (MTBE), or ethyl tertiary butyl ether (ETBE).

Ethanol the oldest product obtained through traditional biotechnology fermentation using utilization of organic material as bio-fuel. Agricultural waste as substrate in fermentation has been vastly discussed as alternative to replace edible food. Pineapple peel, a potential source of substrate is a by-product of the pineapple processing industry. The concept of producing bio-ethanol from pineapple came from the notion that pineapple peelings have considerable amount of glucose Nickolic S. *et al*. (2009). In this research, bio-ethanol production from pineapple (*Ananas comusus*) peel extract will be carried out by controlling fermentation without any treatment. This study will employ Response Surface Methodology (RSM) for optimization of ethanol production from pineapple peel extract using yeast- *Saccharomyces ellipsoideus* in batch fermentation process.

1.2 Problem Statement

At present, the transportation sector is almost entirely dependent on petroleum-based fuel, it being responsible for around 60% of the world oil consumption. Bio-fuels represent an alternative to petroleum-based fuel; in particular, bio-ethanol is the most widely used bio-fuel for transportation (Balat, 2010).

It is also reported that the existing production feedstock uses food-based feedstock instead of conversion from waste. For example, the production of ethanol from corn, cassava, sugar cane and potato utilizes the whole part of the feedstock, which has a high demand in other relevant industries.

Numerous study and research have been conducted to produce ethanol from pineapple peel because of low cost renewable agricultural residue. This will be a good solution for domestic energy supply demand and also solved the country's disposal problem of agricultural waste.

Therefore, this study should be carried out in order to produce ethanol from pineapple peels as this method is much greener and less expensive. The optimum temperature, yeast concentration and fermentation time need to be studied through fermentation method Bries *et al*. (2010) with the use of Response Surface Methodology for optimization to improve the bio-ethanol yield.

1.3 Objective and Scope of Study

The objectives of this study are:

- To produce ethanol from pineapple peel using fermentation process.
- To study the effects of yeast concentration, temperature and time towards ethanol production from pineapple peel.
- To optimize the yeast concentration, temperature and time using Response Surface Methodology (RSM) method.

The Scope of Study Includes:

- Conducting experiment to produce ethanol from pineapple peel using fermentation process. The fermentation media consisted of solely pineapple peel extracted.
- Analyzed the effect of yeast concentration, temperature and time will from a series of batch experiment.
- Optimize the selected parameters which are yeast concentration, temperature and time using design expert software by Stat-Ease. The method used for the optimization study is the Response Surface Methodology Method (RSM).

1.4 Feasibility of the Project within Scope and Time Frame

This project is divided to three main parts which is data gathering, experimentation on parameters and optimization of the parameters. For the first part, data gathering will involved the understanding on the process of the extraction of bio-ethanol generally and using pineapple peels as substrate in details. This part must be completed on FYP 1 time frame. For the second part, experimental study will be done on to produce the bio-ethanol using fermentation process with 3 variables; yeast concentration, temperature and time. The estimated time frame for this process is about 188 hours. For the third part which is optimization of the parameters, this will be done by using Response Surface Methodology (RSM) from Design Expert software. This study will be finish according to the time frame given.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The current world of bio-ethanol research is driven by the need to reduce the cost of production. Inexpensive waste products from the forestry industry as well as agricultural residues can be utilized as raw material for bio-fuels. More than 11,000 hectares of land in Malaysia currently planted with pineapples which generate 40 to 65 tons of waste per hectare. As an economical fermentation medium, Pineapple waste is a material rich in sugars and lignocellulosic components which led to higher fermentation conversion to ethanol.

2.2 Bio-ethanol as an Alternative Replacement for Fossil Fuels

Petroleum, natural gas and coal accounted for nearly 85% of world energy production; hydro and nuclear resources made up the bulk of the remaining 15%, with renewable resources contributing a nominal amount (IEA, 2009). These figures are consistent with consumptive use pattern, clearly indicating that fossil fuels are relied upon across the globe more or so than any other energy resources. More than two third of the primary fuels currently consumed in the world are used to produce electricity (Gluskoter, H., 2010). This is only one of the sources of energy demand by the world apart from for internal combustion for vehicles and other sources. Although technologies advances, new discoveries an conservation may tend to make the resources last longer, population growth and development will likely have a more influencing impact on consumption thereby making the fossil fuels reserves will near an end between 2050 and 2075 (Walsh, 2007). One of the major alternatives to these fossil fuels dilemmas is bio-ethanol. Bio-ethanol is a renewable energy sources unlike fossil fuels. Bio-ethanol fuel is mainly produce by the sugar fermentation process.

2.3 Fermentation process of Bio-ethanol

Fermentation process of bio-ethanol production is one of the popular subjects in the world with regards to the economic challenge and biological environment. Bio-ethanol fermentation process is usually done by yeast *Saccharomyces*, whereby the sugars in the fruit juice are converted into alcohol and organic acid, that later react to form aldehydes, esters and other chemical components (Smith, A. 2009). Sugar and starch based feedstocks are currently predominant at the industry level and they are economically favourable. Considerable attempts have been made by several researchers to propose a methodology based on mathematical models. Major problems of fermentation process are that they need a large number of experiments and often the models are very complicated to describe the experimental observation.

2.4 Pineapple as a source of feedstock

Technically, ethanol can be produce from wide variety of renewable feedstock, which can be roughly classified into three main group which are those containing considerable amounts of readily fermented sugars (sugar cane, sweet sorghum, sugar beets), starches and fructosans (corn, potatoes, rice) and cellulosics (stover, grasses, corn cobs). Sugar cane, beet and sweet sorghum provide the simple sugars, as sucrose, glucose and fructose that can be readily fermented by yeasts Amorim et al. (2009).This differs from ethanol production processes based on starchy or lignocellulosic feedstock, where prior hydrolysis of polysaccharides is necessary with increased ethanol production costs (Dien and Bothast, 2009). Pineapple peel, highly potential sources as feedstock is a by-product of the pineapple processing industry. Bio-ethanol from pineapple (Ananas comosus) peel extract was carried out by controlling fermentation without any treatment. Saccharomyces ellipsoides was used as inoculum in this fermentation process as it is naturally found at the pineapple skin Nadya Hajar *et al*. (2012)

2.5 Optimization of Process using Response Surface Methodology (RSM)

Optimization of process condition is one of the most critical stages in the development of an efficient and economic bioprocess. The classical method of studying on variable at a time can be effective in some cases, but it is useful consider the combined effects of the entire factor involved. The conventional one-factor-at-a-time approach of optimization is not only tiresome but also ignores to merge interaction of each factor. Venter et al. (1996) have discussed the advantages of using RSM for design optimization applications.

Originally, RSM was developed to model experimental responses (Box and Draper, 1997), and then migrated into the modelling of numerical experiments. RSM is a powerful mathematical model with a collection of statistical techniques by which interaction between multiple processes variables can be identified with fewer experimental trials Gilbert et al. (2008). It is widely used to examine and optimize the operational variables for experimental design, model developing, and test variable and condition optimization (Biles & Swain, 2007). There are various advantages in using statistical methodologies in terms of rapid and reliable short listing of process conditions, understanding interaction among them, and a tremendous reduction in total number of experiments, resulting in saving time, glassware, chemicals and manpower. In spite of various advantages, statistical designs have been applied to only limited number of aerobic submerged and solid state fermentation and anaerobic submerged fermentation processes deal with a large number of variables, and there are several reports on the application of RSM for the production of primary and secondary metabolites through microbial fermentation Nadya Hajar *et al* (2012).

RSM was used for optimization of ethanol fermentation as a function yeast concentration, temperature and time of fermentation in a batch fermentation using limited experimental runs. The accuracy of the estimated data was defined and the overall prediction ability of this technique was assessed.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This project will involve the laboratory experimental study and optimization of the parameters using the Design Expert software. Details regarding the methodology for each part are discussed in the next section. Figure 3.1 shows the work sequence for this project:

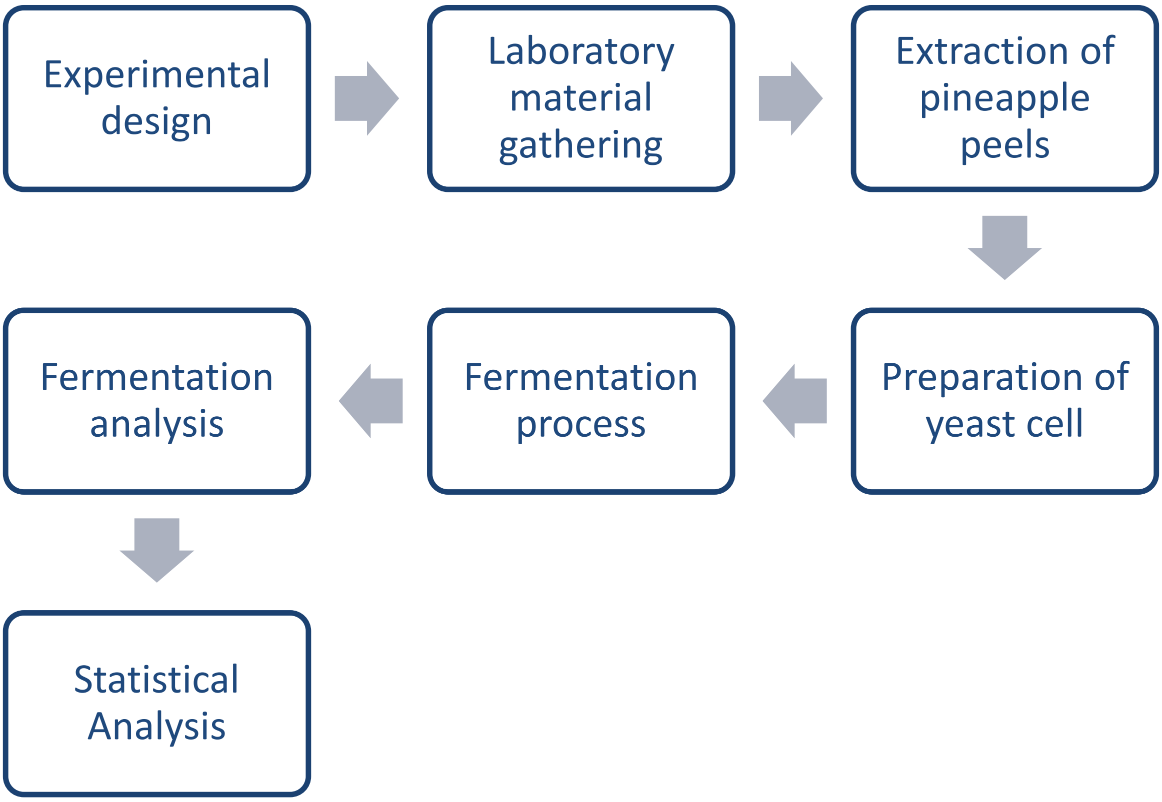

Figure 1 Sequence of Work

3.2 Experimental Design

As this project is aimed to study the effects of yeast concentration, temperature and time of fermentation on ethanol production from pineapple peel, so the value of parameters need to be obtain. A central composite design (CCD) by RSM was employed to study the response ethanol concentration (%). The settings for the test or test variables were concentration of Yeast (6-14% v/v), temperature (25-40C) and fermentation time (48-96 hours) Nadya Hajar *et al* (2012).

Table 1 Experimental Design using RSM

Run	Temperature (°C)	Yeast Concentration (g/ml)	Fermentation Period (hours)
1	40	6	96
2	33	10	38
3	25	14	96
4	22	10	72
5	33	10	72
6	33	10	72
7	43	10	72
8	25	6	48
9	33	10	106
10	33	10	72
11	40	14	48
12	33	13	72
13	33	4	72
14	33	10	72
15	33	10	72

3.3 Laboratory Materials Gathering

Chemical, tools and Equipments required:-

Table 2 Chemicals, tools and Equipments Required

Type	No.	Name	Amount	Supplier
Chemical	1	Deionized Water		UTP
	2	Peptone water		UTP
	3	Dried Yeast extract		
	4	Malt extract		UTP
	5	Distilled water		
	6	Test tube	5	UTP
	7	Stopwatch	2	UTP
Glasswares & Equipments	8	Autoclave	1	UTP
	9	Refrigerator	1	UTP
	10	High Performance Liquid Chromatography	1	UTP
	11	Spatula	1	UTP
	12	Beaker	9	UTP
	13	Weighing Scale	1	UTP
	14	Knife	1	UTP
	15	Oven	1	UTP
	16	Blender	1	UTP
	17	Porcelain	9	UTP
	28	Polycarbonated Baffle Flasks	8	UTP
	19	Coarse Filter Paper	20	UTP
	20	Spectrophotometer	1	UTP
	21	Conical flask (50mL, 100mL, 250mL)	5	UTP
	22	Incubator	1	UTP
	23	Centrifuge Tube (50mL)	12	UTP
	24	Muslin Cloth	10	
	25	Filter Paper	20	
	26	Sterile Bag	12	UTP
Software		Design Expert Software	1	Stat-Ease

3.4 Extraction of Pineapple Peels

1. The crowns of the pineapples were first removed and then peeled.

2. The peel was crushed in a blender (Waring, 2008).

3. The pineapple peel extract was keep frozen at -4 °C

4. Then the pineapple peel extract was autoclaved prior used.

Figure 2 Pineapple peels extraction

3.5 Preparation and Propagation of Yeast Cells

***Saccharomyces ellipsoideus* Yeast**

1. The yeast was maintained on glucose Yeast Extract which consisted of glucose extract (3g/L), yeast extract (3g/L), peptone water (5g/L) and distilled water (top up to 1L)

2. The media was autoclaved at 121°C for 15 minutes and added aseptically prior to fermentation.

3. Before use as inocolum for the fermentation, the culture was aerobically propagated in 100ml flask in an incubator shaker at 24°C for 24 hours.

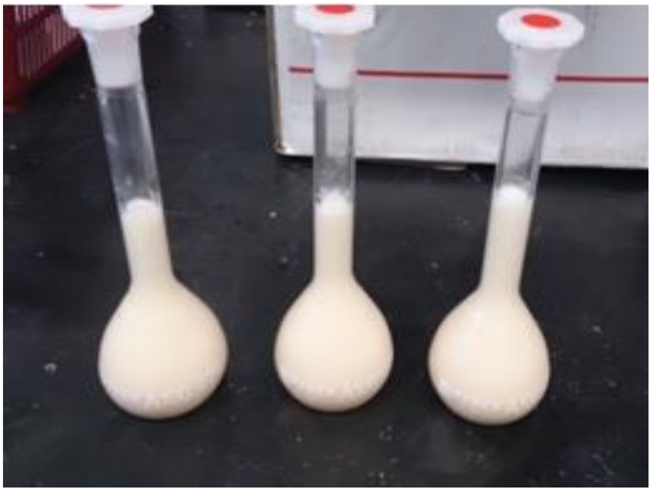

Figure 3 Glucose Yeast Extract

3.6 Performing Fermentation Process

When the yeast cells are prepared, the fermentation process will be carried out according to the procedures below:

Batch Fermentation Experiments

1. The batch fermentation was done with 100 ml Erlenmeyer flask.

2. The parameters were concentration of Yeast (6-14% v/v), temperature (25-40°C) and fermentation time (48-96 hours) Nadya Hajar *et al* (2012).

3. The flask were closed with gauze and aluminium foil.

4. Then, the flask will be put inside the incubator shaker with appropriate temperature and agitated at 200rpm for respective time.

5. All samples were stored at -4°C for further analysis.

3.7 Fermentation and Statically Analysis

The concentration of ethanol yield is determined by using refractometer. Three concentrations of standards are prepared and the refractive index (RI) for each sample is analyzed. Then the RI for samples are checked and compared with standard to find the concentration of samples. The concentration yield is then tabulated into the RSM design for further optimization.

Figure 4 Fermentation media

Figure 5 Refractive Index Analysis

3.8 Gantt Chart & Key Milestone

Table 3 Proposed Gantt chart for the project implementation for both FYP I and FYP II.

TOPIC	PROJECT ACTIVITIES	WEEKS																											
		Final Year Project 1														Final Year Project 2													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Project Scope Validation																												
	Project Introduction																												
	Submission of Extended Proposal																												
	Identify material and equipment																												
	Training on how to conduct experiment																												
	Proposal Defence																												
	Detail Study																												
	Submission of Interim Draft Report																												
	Finalized Procedure																												
	Conducting Experiment																												
	Result analysis and discussion																												
	Submission of progress report																												
	Preparation for Pre-SEDEX																												
	Pre-SEDEX																												
	Submission of draft report																												
	Submission of technical paper and dissertation																												
	Oral presentation																												
	Submission of project dissertation																												

Proposed Gant chart for the project implementation for both FYP I and FYP II. Based on the Gant Chart, the project is feasible to be completed within the given amoun of time.

CHAPTER 4
RESULTS

4.1 Refractive Index Table

The refractive index before and after fermentation is recorded for determining the concentration of ethanol produce after the fermentation.

Table 4 refractive Index Table

Run	Temperature (°C)	Yeast Concentration (g/ml)	Fermentation Period (hours)	Refractive Index Before Fermentation	Refractive Index After Fermentation
1	40	6	96	1.3365	1.3324
2	33	10	38	1.3371	1.3321
3	25	14	96	1.3371	1.3326
4	22	10	72	1.3356	1.3312
5	33	10	72	1.3364	1.3314
6	33	10	72	1.3373	1.3322
7	43	10	72	1.3368	1.3319
8	25	6	48	1.3365	1.3323
9	33	10	106	1.3364	1.3321
10	33	10	72	1.3369	1.3321
11	40	14	48	1.3374	1.3315
12	33	13	72	1.3376	1.3318
13	33	4	72	1.3358	1.3316
14	33	10	72	1.3372	1.3323
15	33	10	72	1.3367	1.3316

4.2 Ethanol Concentration Analysis

Table 5 Ethanol Concentration Table

Run	Temperature °C	yeast concentration (g/ml)	Time (hours)	ethanol concentration (g/ml)
1	40	6	96	6.08
2	33	10	38	6.11
3	25	14	96	6.61
4	22	10	72	6.32
5	33	10	72	6.89
6	33	10	72	7.01
7	43	10	72	7.19
8	25	6	48	6.22
9	33	10	106	6.37
10	33	10	72	6.98
11	40	14	48	7.66
12	33	16	72	8.52
13	33	4	72	5.89
14	33	10	72	6.92
15	33	10	72	6.85

4.3 Response Surface Methodology (RSM) Result

4.3.1 Model Fitting and ANOVA

The analysis of ethanol concentration is the most important part for this project as the optimization of the yeast concentration and temperature is based on the result from Table 5 that shows the ethanol concentration yield according to experimental design.

The RSM software has various models for analyzing data such as linear, two-factorial and quadratic. For this study two-factorial model is chosen as it is most suitable to describe this kind of interaction in the analysis of variance (ANOVA). The model obtains from statistical analysis of RSM using Design Expert Software is significant as show in Table 6.

Table 6 ANOVA for synthesis variables pertaining to response percent yield

Source	Sum of Squares	Mean Square	*F*-Value	Prob >*F*
Model	6.54	0.65	153.77	0.0001
x_1	0.38	0.38	89.05	0.0007
x_2	0.034	0.034	7.95	0.0478
x_3	3.46	3.46	813.75	<0.0001
x_1x_2	0.38	0.38	90.01	0.0007
x_1x_3	0.30	0.30	71.37	0.0011
x_2x_3	0.013	0.013	3.02	0.1573
${x_1}^2$	0.044	0.044	10.29	0.0326
${x_2}^2$	0.68	0.6	160.03	0.0002
${x_3}^2$	0.11	0.11	25.42	0.0073
Lack of fit	8.333×10^{-5}	8.333×10^{-5}	0.02	0.8954
Pure error	0.017	4.25×10^{-3}		

Significant at “Prob >*F*” less than 0.05,

Insignificant at “Prob >*F*” more than 0.05

The values of Prob >F indicate that the model terms were significant. In this case only x_2x_3 and Lack of fit are Insignificant. So the significant term contribute to the model as given in the Equation 1 below

$$y\left(\frac{g}{ml}\right) = 9.85672 - 0.17012X_1 - 6.013 \times 10^{-4}X_2 - 0.44573X_3 + 2.534 \times 10^{-3}X_1X_2 + 0.013731X_1X_3 - 1.556 \times 10^{-3}{X_1}^2 - 5.98 \times 10^{-4}{X_2}^2 + 8.59 \times 10^{-3}{X_3}^2$$

Equation 1

The Prob >F obtain from the data was low for most of the variables. This indicates a good reproducibility of experimental data. Besides, the reliability of regression model to sufficiently represent the actual relationship between the response and the significant variables is confirmed by the high values of coefficient of determination R^2(0.9974) and adj-R^2(0.9909) as shown in table 7.

Table 7 Summary of ANOVA and regression for ethanol yeild

Std. Dev	Mean	R^2	Adj-R^2	Adequate precision
0.065	6.77	0.9974	0.9909	47.110

4.3.2 Mutual Effect of Parameters

Based on mathematical analysis of the experiment data, the interaction between independent process factors and their respective response was plot graphically. The three-dimensional surface counter plots for ethanol yield are show in figure 6 below.

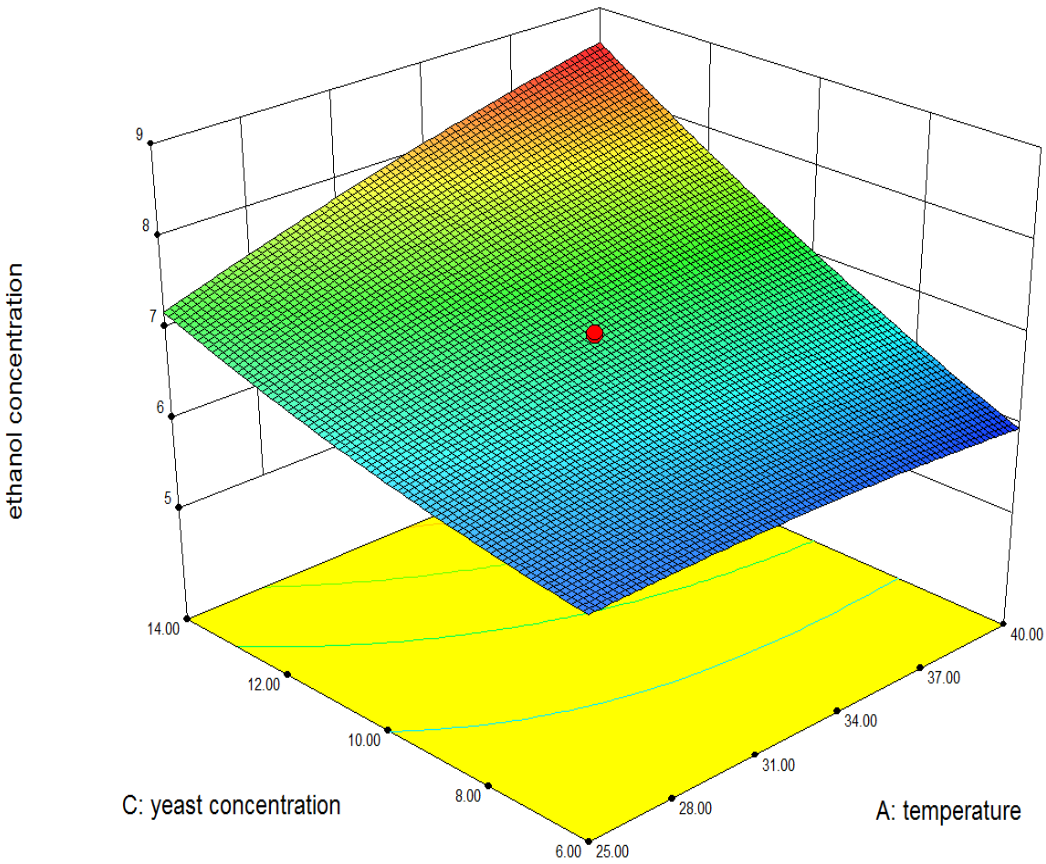

Figure 6 Response of surface plot of temperature, yeast concentration and ethanol yield

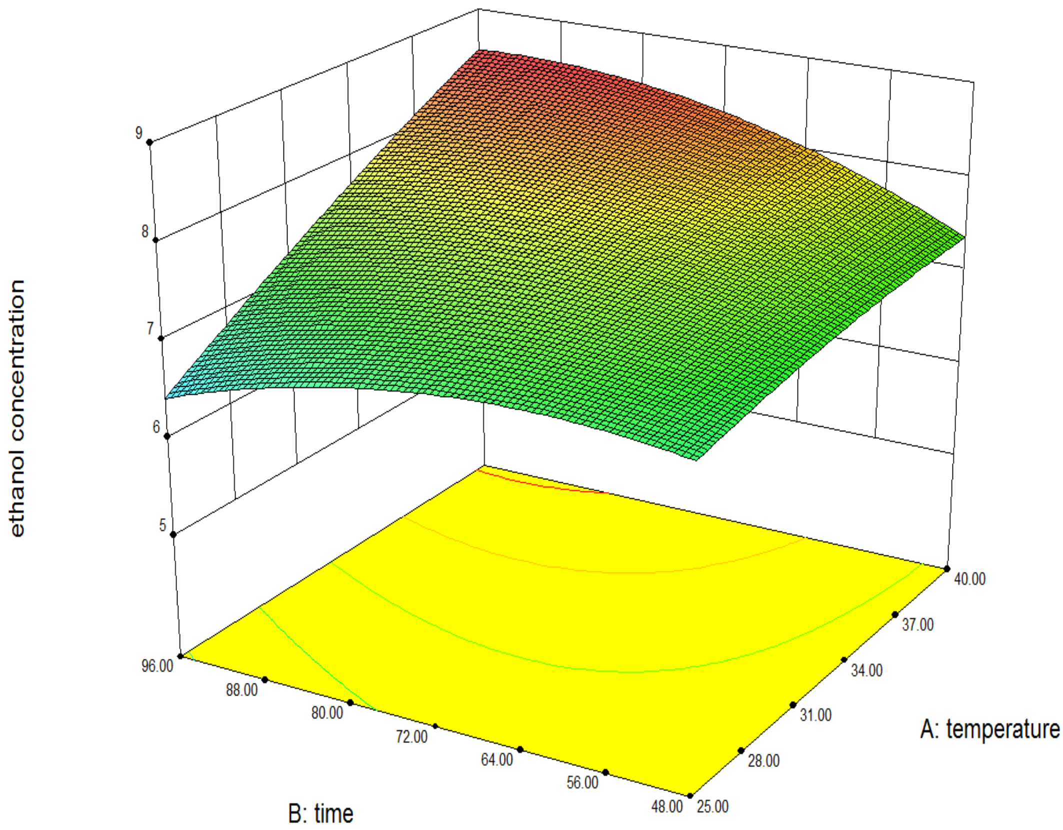

Figure 7 Response surface plot of temperature, time and ethanol yield

The response surface shows in Figure 6 and Figure 7 are based on 3 variables which are temperature, yeast concentration and time. All the variables are has varied range of concentration of Yeast (6-14% v/v), temperature (25-40^{o}C) and fermentation time (48-96 hours) Nadya Hajar *et al* (2012). From Figure 6, it shows that the ethanol concentration goes higher as the colour of contour goes from blue to red, which is from 6.08 g/ml to 8.52 g/ml as clearly shows the highest point is at the red contour, which is at temperature of 33^{o}C to 40^{o}C while the yeast concentration is at 13 g/ml to 14 g/ml. From Figure 7 it shows that the time range between 60 hours to 90 hours gives high ethanol yield with temperature is between 33^{o}C to 40^{o}C.

From Figure 6, we can see that the ethanol start to produced vigorously around temperature of 37 - 40^{o}C with ethanol production around 8g/ml. However, at the temperature around 34 - 36^{o}C, the ethanol production nearly reaches the red contour, but with high yeast concentration. This shows that to produce ethanol at lower temperature, we need to use higher concentration of yeast. From Figure 7,the ethanol start to produced vigorously starting from 60 hours but with high enough temperature which around 37 - 40^{o}C. This shows that to produce ethanol at shorter time we need high optimum temperature.

4.3.3 Validation of Statistical Model and Diagnostic

RSM can also shows the interaction effects among independent variables. Figure 8 shows the interaction plot of temperature and yeast concentration. The effect of temperature to ethanol concentration is not significant without yeast. As the yeast concentration increase with increase of temperature the effect to yeast concentration is clearly can be seen.

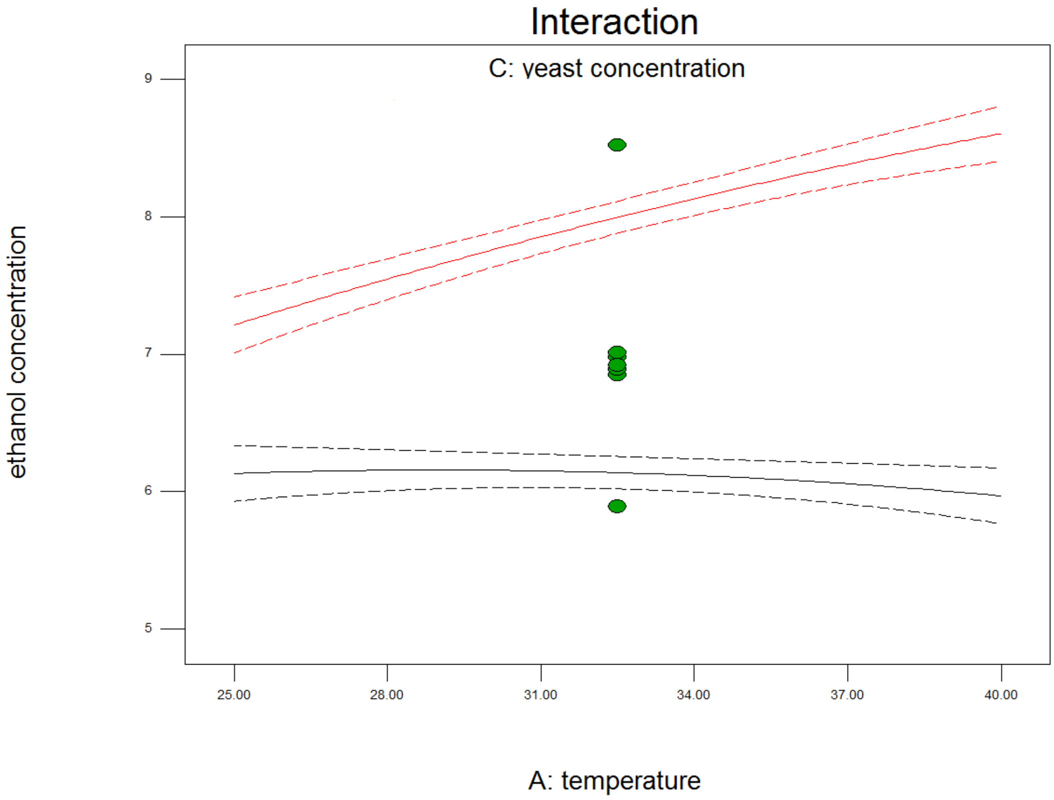

Figure 8 Interaction plot of temperature and yeast concentration

Figure 9 shows the interaction plot of temperature and time. From this figure, the spread of points at the left side of the figure where the temperature is low is lower than the spread of points at the right side of the figure where the temperature is high. This shows that the interaction on the right side of the figure gives higher ethanol production which higher temperature and higher time.

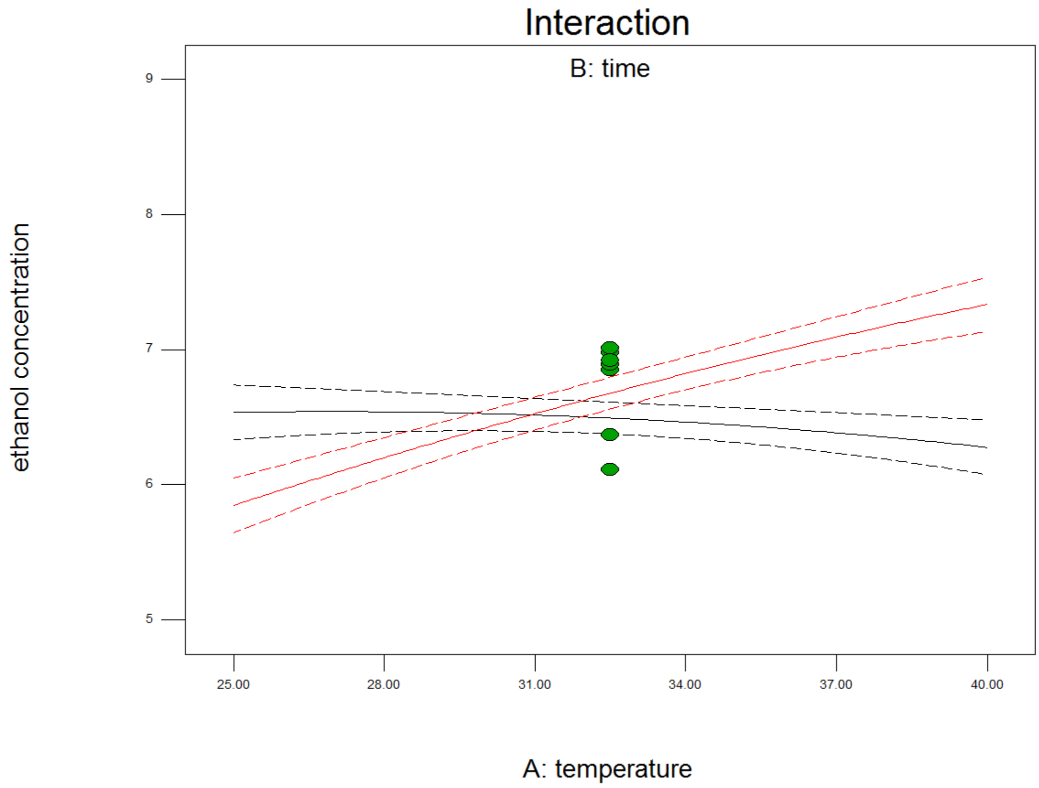

Figure 9 Interaction plot of temperature and time

Figure 10 shows the normal probability plot of studentized residual for ethanol yield. The diagnostic plot was employed by a scatter plot with the theoretical percentiles for residual analysis of the response surface design to ensure that the statistical assumptions fit to the analysis data for ANOVA. The normal probability in percentage is used to clarify whether the standard deviations between actual and predicted response values follow a normal distribution or not. The straight line from the plot proved that there are no abnormalities with the experimental results.

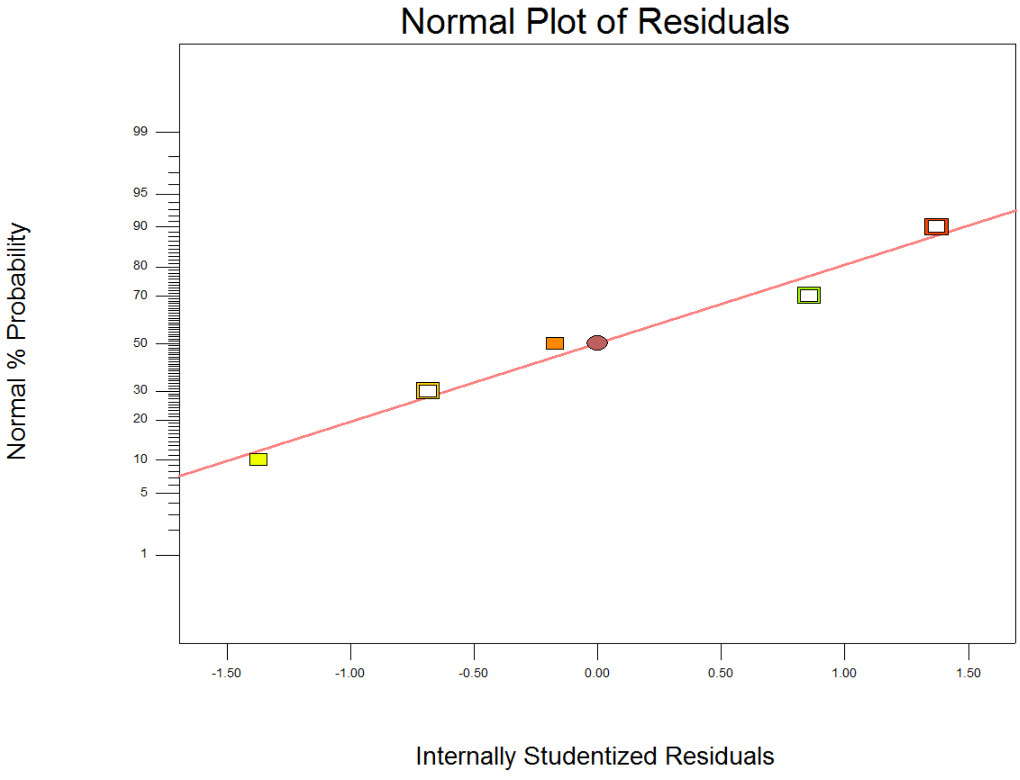

Figure 10 The normal probability plot of studentized residual for ethanol yield

To find whether the suggested model was tolerable and the assumption of constant variance is acceptable, the plot of residuals versus predicted response for ethanol yield is shown in Figure 11. The point scatter between ±3.00 area ranges, therefore we can say the suggested model was tolerable and the assumption of constant variable was acceptable

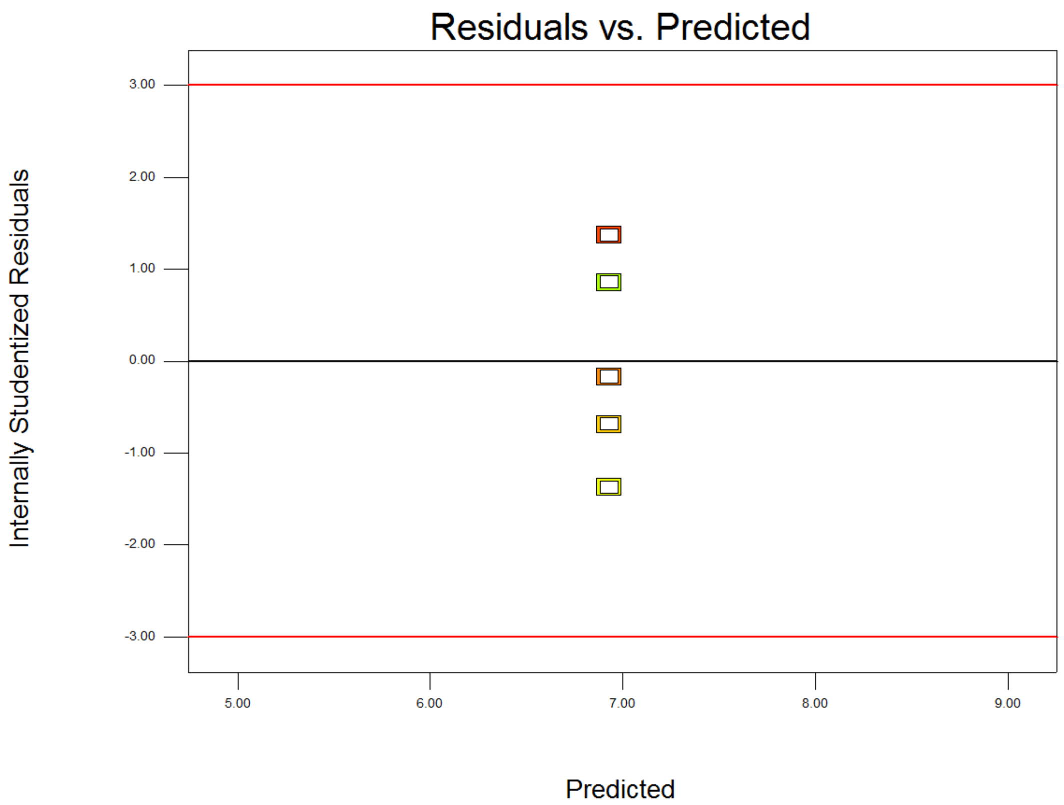

Figure 11 the plot of residuals versus predicted response for ethanol yield

Figure 12 shows the plot of predicted versus actual values for ethanol yield. The predicted result is calculated by the equation model and the actual results are obtained from the experiment conducted. As we can see from the graph most of the points are within the straight line which shows that the model was closed to the actual experimented result. If the point are above the line it is called over-estimated and if the point below the line it is called under-estimated.

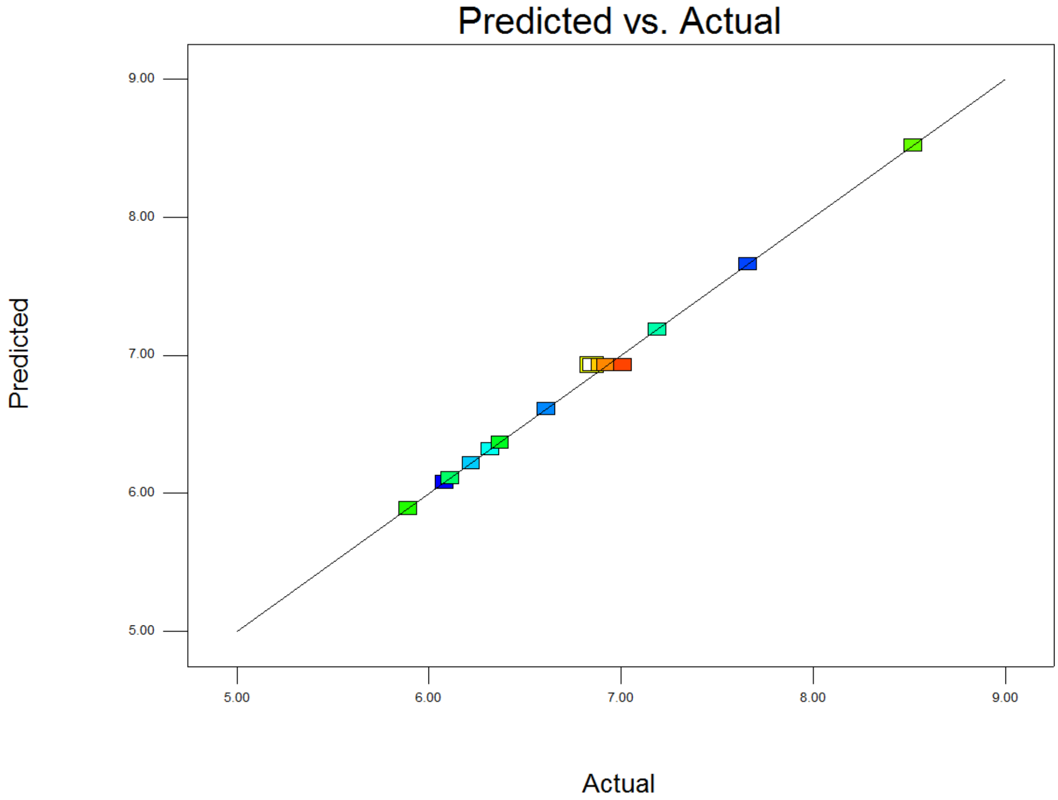

Figure 12 the plot of predicted versus actual values for ethanol yield

4.3.4 Response Surface Optimization and Verification

Numerical optimization is used to maximize the production of ethanol at optimum temperature, yeast concentration and time. As this is the third objective of this project which to optimize the production of ethanol by using response surface methodology (RSM). The numerical optimization gives the optimum variables at the highest desirability which measure the likelihood of the experimentation to success. Table 8 shows the most desirable operating condition which at 40^{o}C temperature, 14 g/ml yeast concentration and with 54hours fermentation time.

Table 8 Numerical optimization of RSM

Reaction condition	Temperature (°C)	Yeast Concentration (g/ml)	Fermentation Time (hours)	Predicted yield (g/ml)	Desirability
1	40	14.00	54	7.92	0.830
2	38	14.00	53	7.80	0.810
3	34	14.00	50	7.64	0.795
4	34	13.00	48	7.43	0.767
5	26	14.00	48	7.19	0.699

To verify the optimization data of the RSM, another experimental run is conducted with operating condition at 40°C temperature, 14 g/ml yeast concentration and with 54hours fermentation time. Table 9 shows the ethanol concentration yield from the experiment.

Table 9 the ethanol concentration yield from the experiment.

Run	**Temperature °C**	**Yeast Concentration (g/ml)**	**Fermentation Time (Hours)**	**Actual Yield (g/ml)**	**Average Yield (g/ml)**	**Predicted Yield (g/ml)**	**Error (%)**
1				7.87			
2	40	14	54	7.76	7.84	7.920	1.01%
3				7.89			

The experiment is conducted 3 times to minimize the error. The average yield from the experiment is 7.84g/ml which gives 1.01% of error. The error is small and can be tolerated. Therefore, the numerical optimization by RSM is proven to optimize the production of ethanol.

CHAPTER 5
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study investigated ethanol production using *Saccharomyces ellipsoideus* from pineapple peel extract. The pineapple peel extract was used as main substrate for ethanol production, and it was very suitable for ethanol fermentation because of the high content of fermentable sugars and *Saccharomyces ellipsoideus* can be used as solely inoculum for ethanol fermentation. Different variations were manipulated to determine whether or not several factors in the fermentation process have a significant effect on the bioethanol yield which are yeast concentration 6-14% (v/v), temperature (25-40°C) and fermentation time (48-96 hrs) on the ethanol production were evaluated.

Data obtained from experiment were analyzed with RSM. The ethanol was successfully produce from the experiment that has been conducted, varied from concentration range of 6.2 g/ml and 8.7 g/ml This experiment was able to shows the relation between the three variables with production of ethanol. The numerical optimization also is proven can be used to optimized the production of ethanol.

This project entitled “**Extraction of Bio-ethanol from Waste Pineapple Peelings”** will indeed be a great help to the economy and the environment if ever this research becomes successful. It will benefit the economy as this will use only wasted pineapple peelings; it will not be in any way detrimental to the environment-friendly energy source and this will consequently lessen the pollution worldwide. This research will also answer the crisis of looking for a clean, alternative source of energy.

5.2 Recommendation for Future Work

For this project to get more accurate result, the concentration of ethanol produced should be measure using High Performance Liquid Chromatography (HPLC). Another important variable also should be added which is the pH which can be fixed or manipulated since pH are also the factor of yeast behaviour. This will gives better and more accurate result.

REFERENCE

Nikolic´S., Mojovic´ L., Rakin M., and Pejin D, "Bioethanol production from pineapple by simultaneous enzymatic saccharification and fermentation with immobilized cells of Saccharomyces cerevisiae var. ellipsoideus," Fuel, vol. 88, pp. 1602–1607, 2009.

Karuppaiya M, Sasikumar E., Viruthagiri T., Vijayagopal V., "Optimization of process conditions using Response Surface Methodology (RSM) for ethanol production from waste cashew apple juice by Zymomonas mobilis," Chemical Engineering Communications, vol. 196, pp. 1425-1435, 2009.

Balat T, (2010). Biofuels: Advantages and trade barriers, United Nations Conference on Trade and Development.

Bries M. Parker S., Sumeru S., Klein L. (2010). Comparative Study of Ethanol Production Using Fermentation Method Retrieved June 14, 2013 from http://www.acedemic journals.org/JYFR

US Department of Energy, International Enegy Annual (IEA 2009): World Energy Overview

Glukoter, H. (2010), Increase in Fossil Fuel Utilization in the 21st Century, USGS

Walsh, J.H., (2007), Resources and Enviroment in the 21st century: Seeing past the phantoms: World Energy Council Journal, July 2007

Smith, A. (2009). Project report on “Biodiesel production from sugarcane, cassava, and maize”. Crops Research Centre, Oak Park, Carlow.

Amorim, H.V.; Basso, L.C. Lopes, M.L. (2009). Evolution of ethanol production in Brazil, in Distiled Spirits – Tradition and Innovation, ed. Bryce, J.H. e Stewart, G;G., Nottingham Univ. Press (UK), v. 1, n. 1, p. 143-148.

Dien, BS and Bothast, RJ. (2009). A primer for lignocellulose biochemical conversion to fuel ethanol. In: The Alcohol Text Book (5th edition), Ingledew, WM, Kelsall, DR, Austin, GD and Kluhspies, C (ed.), Nottingham University Press, Nottingham, 541p

Nadya Hajar, Zainal., Atikah, O. and Tengku Elida, T. Z. M. (2011) Optimization of Ethanol Fermentation from Pineapple Peel Extract Using Response Surface Methodology (RSM), Retrieved June 10, 2013 from http://www.waset.org/journals/waset/v72/v72-114/php

Venter S., Giudici P., Gallo, C.R., & Gomes, F.S.(1996). Advantages of using RSM for design optimization applications. 394-404

Box, A. & Draper, G. (1997). Response Surface Methodology as Means for Experimental Design. Mathematical Model Design, 188-214

Gilbert, J., Olivares, F., Thomas E., Jones, A. (2008). A response surface method for experimental optimization of multi-response processes, Industrial Chemical Process, Design, and Development 14, 188-201

Biles W.E. and Swain J.J., (1977) Strategies for optimization of multiple-response simulation models, Winter Simulation Conference Proceeding (North-Holland, 1977).

Waring M. (2008), "Optimization of process conditions using Response Surface Methodology (RSM) for ethanol production from waste cashew apple juice by Zymomonas mobilis," Chemical Engineering Communications, vol. 196, pp. 1425-1435, 2008

NREL. (2012) . *Biofuels Basic* . Retrieved June 19, 2012 from http://www.nrel.gov/ learning/re_biofuels.html

Avriel R. (2008), The Extraction of Bioethanol from Poneapple Peelings through Simultenous Saccharification and Fermentation using Yeast Saccharomyces Cerevisiae.

D'Amato D., Corbo M. R., Nobile D, and Sinigaglia M, "Effects of temperature, ammonium and glucose concentrations on yeast growth in a model wine system," International Journal of Food Science and Technology, vol. 41, pp. 1152-1157, 2006.

APPENDIX

The concentration of glucose was calculated by using Formula:-

$$x = \frac{refractive\ index - 1.2714}{0.1363}$$

Since 1 mole of glucose produce 2 mol of ethanol,

The concentration of ethanol produce was calculated using formula:-

$$C = (concetration\ of\ glucose\ before\ fermentation - concentration\ of\ glucose\ after\ fermentation) \times 2$$