Optimization of Ethanol Production from Mango Peels using Response Surface Methodology

by

Muhammad Ridzuan bin Abdul Ghani
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Dissertation submitted in partial fulfillment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

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Universiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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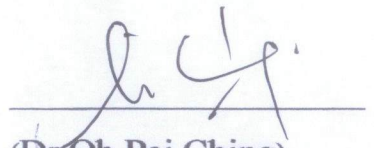
Approved by,



(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.

MUHAMMAD RIDZUAN BIN ABDUL GHANI

ABSTRACT

Environmental concern as well as escalating of fossil fuel price and demanding for renewable energy is the main reasons why bioethanol technology being developed. Bioethanol can act as alternatives energy and reduce dependencies on fossil fuel alone. Recently, research has been done to produce bioethanol by utilization of agricultural waste. Production of bioethanol from mango peels is examples of utilization of agricultural waste. Mango fruit is grown naturally in over 90 countries worldwide and is known to be the second largest produced tropical fruit crop in the world. This shows that the amount of waste that will produce will be significant. The focal point of this project is to optimize production of bioethanol from mango peels using Response Surface Methodology (RSM) and to studies the effects of temperature, yeast concentration and fermentation time towards production of bioethanol. Mango peels will undergo hydrolysis and fermentation in order to produce bioethanol. To achieve these objectives, experiment was conducted which consist of four stages; preparation of mango peels, hydrolysis of mango peels, preparation of yeast and optimization using RSM. The first part of experiment, mango peels is manually peeling off using knife and the underlying pulp was removed. Then, the mango peels is treated with sulphuric acid with concentration of 0.25 % to 1 % (w/v). At acid concentration of 0.25 % (w/v) was the highest amount of glucose yield. Fermentation is conduct according to experimental design layout that is generated by Design Expert software and the range for temperature, yeast concentration and fermentation time is 25-40°C, 6-14 g/ml and 48-96 hours respectively. RSM using three factors and two level central composite designs was employed to optimize production of ethanol from mango peels. Based on the results obtained, the concentration of ethanol yield ranged from 5.28-7.34 g/ml. The optimize condition to produce 7.34 g/ml is 38 °C, 6 g/ml yeast and 48 hours fermentation time. Therefore, this finding portray that the production of ethanol from mango peels can be scale up for large production industry.

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LIST OF ABBREVIATIONS

RSM	Response Surface Methodology
CCD	Central Composite Design
GYE	Glucose Yeast Extract
RI	Refractive Index
HMF	Hydroxymethylfurfurals
ANOVA	Analysis of Variance
HPLC	High Performance Liquid Chromatography

CHAPTER 1

INTRODUCTION

1.1 Background of Project

Biofuels are fuels that are derived and made from living organisms. Gasoline and diesel are actually ancient biofuels. But they are known as fossil fuels because they are made from decomposed plants and animals that have been buried in the ground for millions of years. Biofuels are similar, except that they are made from plants grown today. The high price escalation of fossil fuel as well as green house gas emission from fossil fuel are the reason why biofuels technology is being developed and research has been done to optimize production and utilizing biofuels energy. Today, bioethanol contribute around 3% of total road transport fuel globally and considerably higher shares are achieved in certain countries (International Energy Agency, 2010). That also the reason studies are being done to find other renewable energy that are useful to be alternative energy. There are various ways of deriving and creating biofuels such as chemical reactions, fermentation, and heat to break down the starches, sugars, and other molecules in plants.

The focal point on this project is the production of ethanol as a biofuels from mango peels. Ethanol is an alcohol made by fermenting the sugar components of biomass. In the present study, researcher has attempted to optimize the production of bioethanol from agricultural waste which is mango. These wastes are rich in sugars due to their organic nature and are very useful in production of bioethanol. The method is cheaper and much more environmental friendly as compared to the current available method to produce bioethanol. Enzyme is used to breakdown mango peels into sugars in this particular method. Then the sugar will further be fermented into ethanol. This project involves experiments with series of multiple tests in order to study the optimization of bioethanol production from mango peels, focusing on the parameters such as temperature, yeast

concentration and fermentation period. The Response Surface Methodology is used in order to optimize the selected parameters.

1.2 Problem Statement

The food and agricultural industries produce large volume of wastes annually worldwide, causing serious disposal problems. This is more in countries where the economy is largely based on agriculture and farming practice. Currently, these agriculture wastes are either allowed to decay naturally on the fields or are burnt. The utilization of biological wastes is of great interest due to legislation and environmental reasons; the industry is increasingly being pressured to find an alternative use for its residual matter (Rodrı´guez-Couto, 2008). Few studies were conducted to produce bioethanol from agricultural waste such as mango peels. The conversion of mango peels to bioethanol is one of the solutions to avoid disposal residual matter as well as its low in cost and provide alternative source of energy instead of dependencies on fossil fuel alone.

Besides, environment also concern as the wastes from processed mango is just left over after juice extraction and commonly is burnt. High energy is required in order to burn the mango wastes. According to Natural Resources Defense Council (2010), the conventional corn based ethanol production contains high concentration of greenhouse gases which are not environmental friendly. In addition, the existing bioethanol production method uses food-based feedstock instead of using agricultural waste. This type of feedstock is highly demand in other industries for example, potato, corn, and sugar cane that are more demanding in food processing industries. As a result, escalation of food price will happen. Therefore, a study should be carried out in order to produce bioethanol from mango waste as this method is cheaper and more environmental friendly. The optimum temperature and yeast concentration as well as fermentation time need to be studied with the use of Response Surface Methodology to optimize production of bioethanol.

1.3 Objectives

i) To produce bioethanol from mango peels via hydrolysis and fermentation process.

ii) To study the effects of temperature, yeast concentration and fermentation period towards ethanol production from mango peels.

iii) To optimize the production of ethanol by using Response Surface Methodology method.

1.4 Scope of Study

There are many factors which affect the production of ethanol from mango peels. For the first objective, experiment is conducted to produce ethanol from mango using hydrolysis and fermentation process. Hydrolysis is done in order to convert cellulose into fermentable sugar, which will be further analyzed for the amount of sugar content in the sample. Based on the second objective, studies are done in order to identify parameters such as temperature, yeast concentration and fermentation period in optimizing the production of ethanol from mango peels. Moreover, in order to optimize the production of ethanol, design software expert by Stat-Ease is utilized to achieve the third objective.

1.5 Relevancy of Project

The project is important to study effects of temperature, yeast concentration and fermentation period towards ethanol production from mango peels besides optimize ethanol production using Response Surface Methodology. This project is compatible with the author's field of study which is chemical engineering. In order to come up with the procedure to conduct the experiment required basic engineering and chemical knowledge, as well as common sense in safety and environment practices and most importantly is to have high quality work ethics. Generally, to complete this project, it require knowledge learnt during lectures and apply the knowledge as well as uses the tools given by university and supervisor to self-learn to solve problems that will be arise.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The agriculture sector plays an important role in Malaysia's economic development. Hence agricultural waste becomes significant as alternative sources for production of bioethanol. The estimated availability of the biomass and its potential energy generated in Malaysia are 50,919 dry kton/year and 13,343 kton/year, respectively while the estimated energy generated from biomass can contribute to approximately 21.5% of the national energy requirement (Tye et al, 2011). In addition, there is a much larger portion of vehicles in Malaysia which run on gasoline compared to biodiesel showing potential bioethanol market in Malaysia.

2.2 Bioethanol as Alternatives Fuel

Rising fossil fuel prices as well as growing demand for energy, and environment concerns is a key factor driving strong interest in renewable energy sources, particularly in biofuel. Biofuel refers to any type of fuel whose energy is derived from plant materials. Recently, biofuel which includes solid biomass, liquid fuels and various biogases is among the most rapidly growing renewable energy technologies. According to International Energy Agency (2011), biofuel can be divided into two categories which are conventional and advanced technology. Advanced technology includes hydrotreated vegetable oil (HVO), which is based on animal fat and plant oil, as well as bioethanol based on lignocellulosic biomass, such as cellulosic-ethanol. Although there are wide varieties of advanced biofuels conversion technologies in existence, they are not commercially available yet. Nevertheless, the most commercializable technology and most used biofuel on the global market is bioethanol. Bioethanol are not composed of hydrocarbons and the combustion of bioethanol produces much lower level greenhouse

gases. Moreover, bioethanol is carbon neutral in which the carbon dioxide released during bioethanol production is almost the same amount as the amount the crops previously absorbed during photosynthesis. Thus, bioethanol production is studied in this research.

2.3 Mango Peels as a Source of Biomass

Mango is one of the most important fruits marketed in the world with a global production exceeding 26 million tons in 2004 (FAOSTAT, 2004). It is grown naturally in over 90 countries worldwide mainly in the tropical and subtropical regions and is known to be the second largest produced tropical fruit crop in the world (Joseph and Abolaji, 1997). In addition, mango peels as a byproduct of mango processing industry could be a rich source of bioactive compounds and enzymes such as protease, peroxidase, polyphenol oxidase, carotenoids, and vitamins C and E (Ajila et al., 2007). However, as mentioned by Berardini et al. (2005), mango peels which consist of 20–25% of total fruit weight during mango processing are not currently being utilized commercially thus, contributing to pollution. Therefore, mango peels is now being studied by researcher as a potential revolutionary biomass resource.

2.4 Bioethanol Production

Bioethanol is chemically known as ethyl alcohol (C_2H_5OH) and is made from carbohydrate-rich crops such as corn, sugar beet wheat, potatoes and variety of other starch crops. Besides, bioethanol also can be derived from cellulose found in common vegetation. Bioethanol can be produced from fermentation of fermentable sugars from plant sources using micro-organism. In order to produce sugars from the biomass, the biomass is pre-treated with acids or enzymes to reduce the size of the feedstock and to open up the plant structure. According to Saravanan et al. (2012), the pretreatment process decreases the crystallinity of mango while removing lignin and other inhibitors by enabling its enzymatic hydrolysis. Hence, the cellulose portions are hydrolysed by enzymes or dilute acids into sucrose sugar which is then fermented into ethanol.

2.4.1 Dilute Acid Hydrolysis

Dilute acid hydrolysis of biomass is the oldest technology for converting biomass to ethanol. Hydrolysis usually occurs in two stages in order to maximize sugar yields from the hemicellulose and cellulose fractions of biomass. The second step is more severe with the aim to hydrolyze most of the cellulose (Galbe and Zacchi, 2002). The first stage is operated under milder conditions to hydrolyze hemicellulose, while the second stage is optimized to hydrolyze more resistant cellulose fraction. The liquid hydrolyze are recovered from each stage and fermented to alcohol. In bioethanol processing plant, the residual cellulose and lignin left over in the solids from the hydrolysis process will then be serve as boiler fuel for electricity or steam production. Since dilute acid is utilized, it is less hazardous as compared to concentrated acid hydrolysis method. Besides, it is low in terms of cost and high in efficiency when converting biomass into fermentable sugar.

2.4.2 Enzymatic Hydrolysis

Enzymes function based upon their specific structure with unique active sites where substrates can bind for bio-chemical reactions. Enzymatic hydrolysis has contributed significantly for biological conversion of cellulosic biomass to ethanol. Inhibition compound present in sample, prevent the cellulase system from reaching near complete conversion of biomass to glucose. The production of inhibitors cause longer incubation time that is needed for hydrolysis. Inhibitory compounds alter the enzyme structure, leading to deactivation of the enzymatic proteins. Enzymatic hydrolysis of cellulose is not only influenced by structural features of the cellulosic substrate but also by enzyme-related factors. This method is less attractive because the presence of complexity of both the cellulose substrate and enzyme as well as the mechanism of cellulose hydrolysis is still not completely understood. The challenge faced in enzymatic hydrolysis is to reduce enzyme usage which will then impact cost production of ethanol. Besides, other challenge is to discover new enzyme sources that will enhance more production of bioethanol with desirable features.

2.4.3 Fermentation Process

Fermentation is a metabolic process in which a microorganism converts a carbohydrate, such as starch or a sugar, into an alcohol or an acid. It is a conventional process that is practiced since prehistoric time. Nowadays, large number of chemicals produced by fermentation technology is playing major a role in industrial production. The microorganisms can be yeast, bacteria or even molds. There are many microorganisms which can be used in fermentation process and each one of them works in different way to achieve a different objective. The most commercially used yeast for ethanol production is *Saccharomyces cerevisiae* (Jefferies, 2006). *Saccharomyces cerevisiae* is able to break down their food through both aerobic respiration and anaerobic fermentation. Besides, they can survive in an oxygen defiency environment for a period of time. The chemical reaction that happens is shown below:

$$C_6H_{12}O_6 \xrightarrow{\text{Zymase}} 2C_2H_5OH + 2CO_2$$

Glucose Catalyst Ethanol Carbon Dioxide

In order to optimize production of ethanol, parameter such as temperature, yeast concentration and fermentation period and interaction between variable is very important.

2.5 Response Surface Methodology (RSM)

Originally, Response surface methodology (RSM) was developed to model experimental responses (Box and Draper, 1987), and then migrated into the modeling of numerical experiments. The difference is in the type of error generated by the response. RSM is a collection of mathematical and statistical techniques for empirical model building. Main objective is to optimize a response which is an output variable that is influenced by several independent variables which is the input variables. Production of ethanol can be optimized by using this software by varying parameters such as temperature, yeast concentration and fermentation period.

Jawad et al. (2012) stated that, RSM has been successfully employed for the optimization of production of the lactic acid from mango peels waste. Besides, according to Saravanan et al. (2012), the central composite design (CCD) is used in the experimental design for the optimization production of cellulase by microorganism called Trichoderma reesei using mango peels.

In designing an experiment, RSM promotes low cost in terms of experimentation because it allows statistical model to estimate lesser number of runs that need to be done to get optimal result. Moreover, optimal design is estimated without any bias. Besides, Noordin et al. (2004) reported that, in order to determine the relationship between factors and the response variables investigated, the analysis of the data collected must be done in a statistical manner using regression. A regression is performed based on a functional relationship between the estimated variable, Y and one or more regressor input variable, x_1, and $x_2 \ldots x_i$. The regression coefficients included in the statistical model are estimated by minimizing the sum of squares of the errors. Therefore RSM is use in this research to optimize production of ethanol from mango peels.

CHAPTER 3
METHODOLOGY

3.1 Experimental Design

This project is aimed to study the effects of temperature, yeast concentration and fermentation period on ethanol production from mango peels thus; this can be done by using Central Composite Design (CCD) by RSM. A three factor and two level CCD consisting of 15 experimental runs for ethanol production is employed. The experimental design is generated by Design Expert Software. The range for temperature, yeast concentration and fermentation period are set at 25-40°C, 6-14 g/ml and 48-96 hours respectively. Table 3.1 shows the tabulated value for variables generated by Design Expert software.

Table 3.1 : Experimental Design Layout

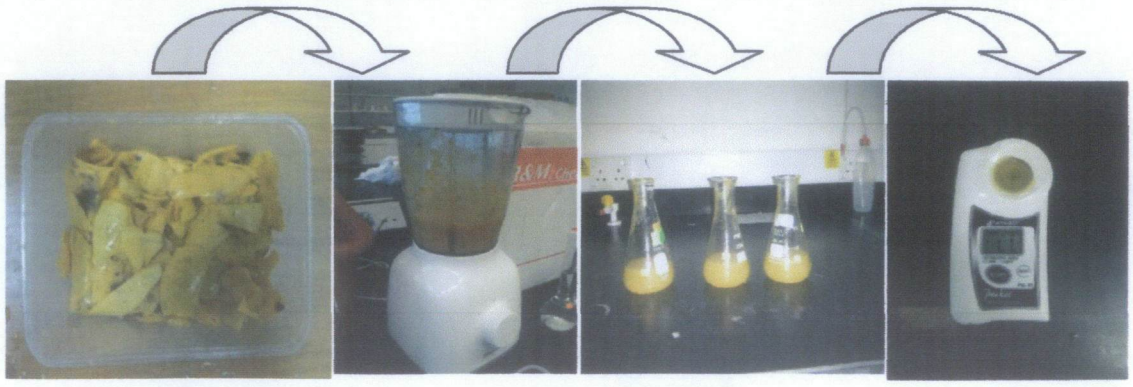
Run	Temperature (°C)	Yeast Concentration (g/ml)	Fermentation Period (hours)
1	33	10	72
2	25	14	96
3	33	10	72
4	33	10	106
5	33	10	72
6	40	14	48
7	22	10	72
8	33	16	72
9	33	4	72
10	33	10	72
11	40	6	96
12	43	10	72
13	33	10	38
14	25	6	48
15	33	10	72

3.2 Preparation of Mango Peels

1. Mango fruits are selected at random and manually peeling off the peels.
2. The underlying pulp on the peels is removed.
3. The peels were chopped into small pieces and blend it using blender as in Figure 3.1 (a) and Figure 3.1 (b).
4. The sample is filter using muslin cloth and the extract is collected in beaker and store for further analysis.

3.3 Hydrolysis of Mango

1. 10gm of mango peels is weighed before being transferred into separate 15 polycarbonated baffle flasks.
2. Polycarbonated flask containing mango peels is added with 90mL of deionized water as shown in Figure 3.1 (c)
3. Sulphuric acid is added at 0.25 M, 0.5 M, 0.75 M and 1.0 M to the sample.
4. The sample is then sterilized by putting it in an autoclave at 121°C for 15 min.
5. The sample is cooled to room temperature then is filtered using vacuum filtration using coarse filter paper.
6. The hydrolysate sample is collected in receiver flask.
7. The sugars contain in the sample are analyzed using refractometer as shown in Figure 3.1 (d).
8. The treatment sample that shows highest amount of sugar is selected for fermentation.



(a) (b) (c) (d)

Figure 3.1: Hydrolysis of mango peels procedure

3.4 Preparation and Propagation of Yeast Cells

1. Sterilized 150 ml Erlenmeyer flask containing 50 ml glucose yeast extract is added with dried yeast powder and put in the incubator at 30°C for 48hr at 100 rpm.
2. The samples are transferred into 250 ml Erlenmeyer flask which contains 100 ml glucose yeast extract broth.
3. 500 ml of sterilized glucose yeast extract broth in 1 L flask is then added with 50 ml of prepared culture.
4. The flask is incubated in the incubator at 30°C for 24 hr and 100 rpm.
5. The sterilized 50 ml centrifuge tube is added with the cells and centrifuged for 10 min at 4°C in centrifuge.

3.5 Fermentation Process

1. Hydrolysate is neutralized and supplemented with a concentrated nutrient solution, to have final concentration of 0.1% weight per volume of yeast extract.
2. The residual pretreated biomass is collected in a sterile bag and frozened.
3. The hydrolysate containing fermenter is agitated at 250 rpm and heated to 80°C for 30 min.
4. The fermentation is performed at temperature, yeast concentration and time according to the runs obtained from Design Expert software as shown in Figure 3.2 (a) and Figure 3.2 (b).
5. The fermenter is inoculated with 120 mL of yeast inoculums at concentration of 1×10^9cells/mL.
6. The samples are drawn at the end of the experiment and analyze for sugar and ethanol concentration.

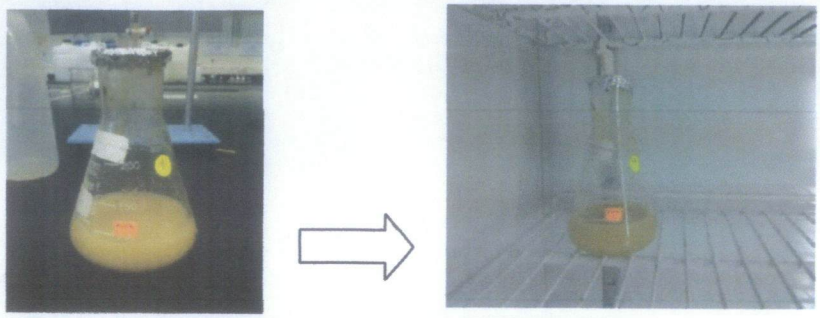


Figure 3.2: Fermentation procedure

3.6 Chemicals, Tools and Materials Required

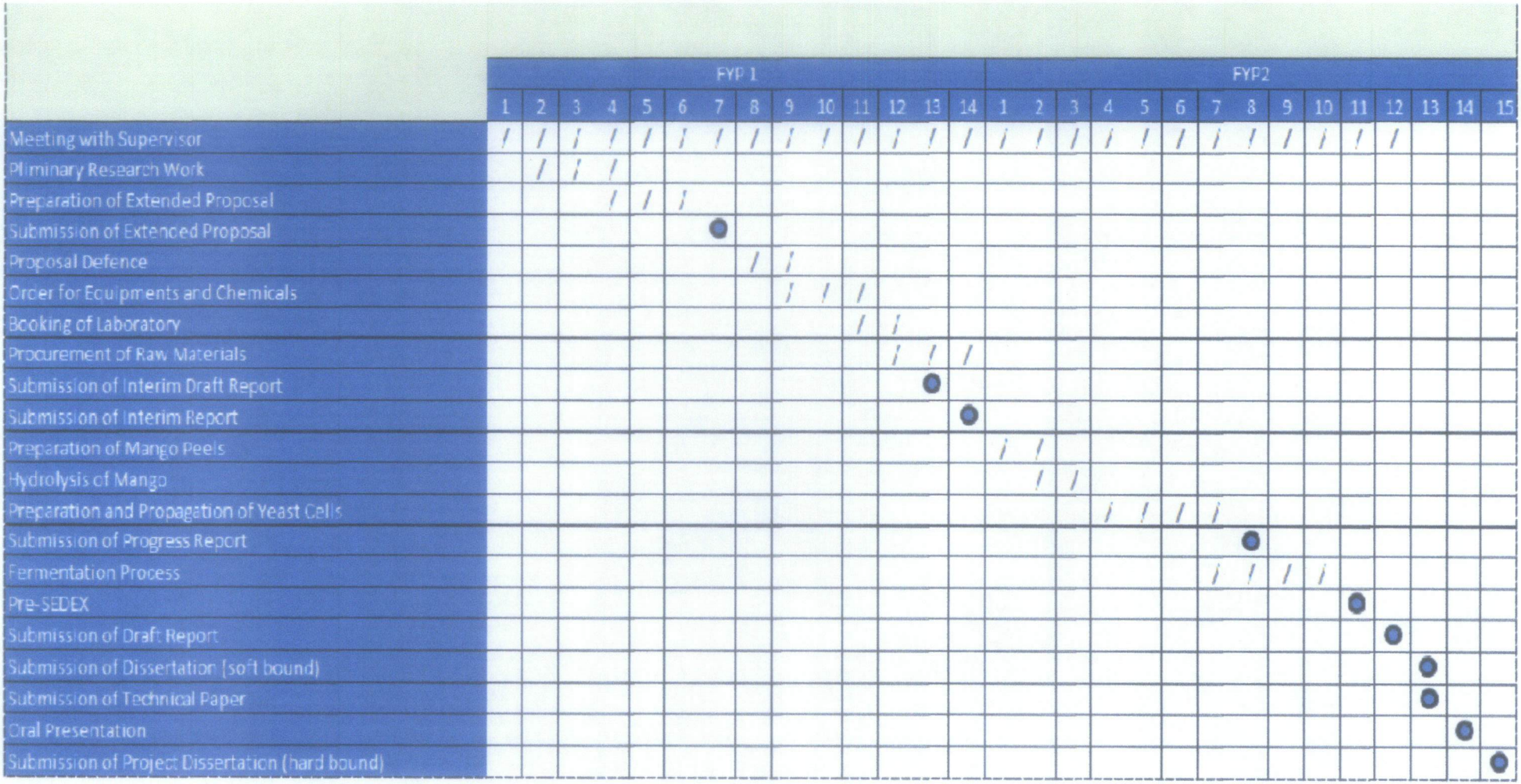
The chemical, tools and materials required for this project are listed in Table 3.2 below:

Table 3.2 : Chemical and Equipment required

Type	No.	Name	Supplier
Chemical	1	Deionized Water	UTP
	2	Sulphuric Acid (0.25M,0.5M,0.75M ,1.0 M)	UTP
	3	Dried Yeast Powder *(Saccharomyces cerevisiae)*	
Glasswares & Equipments	4	Test tube	UTP
	5	Stopwatch	UTP
	6	Autoclave	UTP
	7	Refrigerator	UTP
	8	Refractometer	UTP
	9	Spatula	UTP
	10	Beaker	UTP
	11	Weighing Scale	UTP
	12	Knife	UTP
	13	Oven	UTP
	14	Blender	
	15	Polycarbonated Baffle Flasks	UTP
	16	Coarse Filter Paper	UTP
	17	Dryer	UTP
	18	Erlenmeyer Flask (50mL, 100mL, 150mL, 1000mL)	UTP
	19	Incubator	UTP
	20	Centrifuge Tube (50mL)	UTP
	21	Sterile Bag	UTP
Software	22	Design Expert Software	Stat-Ease

3.7 Gantt Chart

Table 3.3 : Gantt Chart for FYP I and FYP I



CHAPTER 4
RESULT AND DISCUSSION

4.1 Chemical Analysis of Mango Peels

For the chemical analysis of mango peels, the amount of sugar content in the pretreatment process is analyzed. The resulted amount of sugar content for five samples after hydrolysis is tabulated in Table 4.1 and is visualized in Figure 4.1:

Table 4.1 : Sugar content after hydrolysis

Sample (% H_2SO_4)	Refractive Index	Conc (% glucose in water)
0	1.3332	0.4534
0.25	1.3341	0.4600
0.5	1.3328	0.4505
0.75	1.3326	0.4490
1	1.3324	0.4475

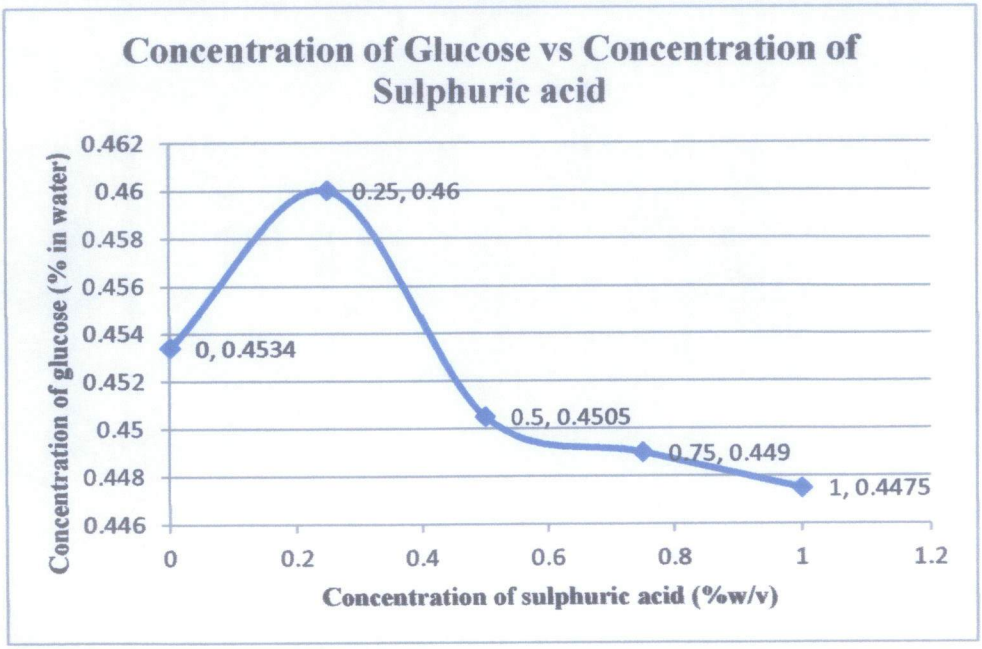


Figure 4.1 : Graph of sugar content after hydrolysis

Chemical analysis of mango peels need to be carried out in order to analyze amount of sugar release during pretreatment process. A pretreatment process is required for the hydrolysis of cellulosic to release sugars for fermentation. Besides, the successful of bioethanol production is depending on removal of lignin through pretreatment process.

4.2 Hydrolysis

As previously stated in methodology section, the hydrolysis of mango peels is done by pretreated using sulphuric acid (H_2SO_4) at different concentration which is 0.25, 0.5, 0.75 and 1.0% (w/v). The sample first being diluted with dionized water before pretreated with sulphuric acid. Then, the samples undergone sterilization pretreatment at 121 °C for 15 min.

Figure 4.1 shows the amount of glucose yield increase rapidly when treated with 0.25%(w/v) sulphuric acid. At this particular point the amount of glucose yield is at peak. Since then, the concentration of glucose decline tremendously as the concentration of acid increase from 0.5 to 1.0% (w/v). As mentioned by Oberoi H.S et al. (2010), as the concentration of acid increases, the glucose will degrades and become Hydroxymethylfurfurals (HMFs). Hence, this is the reason why the amount of glucose concentration declines enormously as the concentration of sulphuric acid increases.

Therefore, from the result obtained the author conclude that at the concentration of acid 0.5% (w/v) and above the effectiveness of sugar yield from mango peels biomass is low. The amount of glucose yield increase tremendously and become peak at 0.25% (w/v) of acid, hence hydrolysis at acid level of 0.25% (w/v) is selected for further analysis. The result portray that the presence of glucose as shown in Table 4.1 and Figure 4.1 in a significant amount promotes mango peels as a good potential becoming revolutionary substrate in ethanol production industry. Thus, the finding proof that the mango peels is a good potential of biomass for ethanol production.

4.3 Propagation of Yeast Cells

The propagation of yeast cells have been cultured successfully. Yeast propagation serves to rehydrate and increase yeast populations using its natural reproduction capabilities as living organism. Glucose Yeast Extract (GYE) is prepare first as a supplement for the propagation of yeast cells. The prepared GYE is shown in Figure 4.2 below :

Figure 4.2: Glucose Yeast Extract

Optimal condition is the key point in determine the successful of yeast propagation. Yeast is cultured for 3 days and the solution is centrifuged at 10000g at 4°C for 10 minutes. The cloudy texture and colors as well as pungent smell indicate the presence of yeast in the culture solution.

4.4 Response Surface Methodology Results

4.4.1 Model Fitting and Analysis of Variance

Response Surface Methodology (RSM) is use as analysis method to determine the production of ethanol. In this project there is three parameter being study which are temperature, yeast concentration and fermentation period. The design layout in Table 4.3 is generated by Design Expert Software :

Table 4.2 : Ethanol concentration yield according to experimental design

Run	Temperature (°C)	Yeast Concentration (g/ml)	Fermentation Period (hours)	Ethanol Concentration (g/ml)
1	33	10	72	6.46
2	25	14	96	6.46
3	33	10	72	6.38
4	33	10	106	5.28
5	33	10	72	6.43
6	40	14	48	6.62
7	22	10	72	6.46
8	33	16	72	7.19
9	33	4	72	7.04
10	33	10	72	6.46
11	40	6	96	7.34
12	43	10	72	7.34
13	33	10	38	6.31
14	25	6	48	6.74
15	33	10	72	6.41

Table 4.3 shows the result for all 15 runs. This result is crucial for fully analyze production of ethanol using RSM. RSM software analyzes the data and fit the data to various model such as linear, two-factorial and quadratic. The analysis of variance (ANOVA) is the most suitable to associate with this kind of interaction. The resulted

from ANOVA suggested quadratic is the best model to describe the interaction between all three parameters.

Table 4.3 : ANOVA for synthesis variables to response percent yield

Source	Sum of squares	Mean square	F value	Prob > F
Model	3.67	0.37	313.68	< 0.0001
x_1-Temperature	0.39	0.39	330.94	< 0.0001
x_2-Yeast concentration	0.011	0.011	9.62	0.0362
x_3-Fermentation time	0.53	0.53	453.38	< 0.0001
x_1x_2	0.45	0.45	384.32	< 0.0001
x_1x_3	0.18	0.18	156.97	0.0002
x_2x_3	0.029	0.029	25.08	0.0074
x_1^2	0.32	0.32	272.02	< 0.0001
x_2^2	0.67	0.67	576.27	< 0.0001
x_3^2	0.57	0.57	489.24	< 0.0001
Lack of Fit	0.015	0.015	12.41	0.0244
Pure Error	0.0047	0.0012		

Based on ANOVA table in Table 4.3, it shows that the model F-value is 313.68 which imply that the model is significant and there is only 0.01% chance of error could occur because of noise. Values of Prob > F less than 0.05 indicate model terms are significant. In this type of interaction all model in Table 4.3 is significant model terms. This indicates that all interaction have bigger impact on production of ethanol yields. The significant model terms contributed to the quadratic equation shown below :

$$y\left(\frac{g}{ml}\right) = 12.698 - 0.293x_1 - 0.315x_2 - 0.048x_3 - 0.0059x_1x_2 + 0.0031x_1x_3 + 0.0057x_2x_3 + 0.0042x_1^2 + 0.021x_2^2 - 0.00055x_3^2 - 0.00014x_1x_2x_3$$

The probability > F for the lack of fit is very low which is 0.0244. It shows that a good reproducibility of experimental data. Reliability of the regression model to sufficiently represent the actual relationship between response and the significant variable is shown in Table 4.4.

Table 4.4 : Summary of ANOVA and regression analysis for ethanol yield

Model	Significant Model Term	Standard Deviation	R^2	Adj-R^2	Adequate Precision
Linear	x_1	0.034	0.9987	0.9955	70.327
Linear	x_2	0.034	0.9987	0.9955	70.327
Linear	x_3	0.034	0.9987	0.9955	70.327
Quadratic	x_1x_2	0.034	0.9987	0.9955	70.327
Quadratic	x_1x_3	0.034	0.9987	0.9955	70.327
Quadratic	x_2x_3	0.034	0.9987	0.9955	70.327
Quadratic	x_1^2	0.034	0.9987	0.9955	70.327
Quadratic	x_2^2	0.034	0.9987	0.9955	70.327
Quadratic	x_3^2	0.034	0.9987	0.9955	70.327

The value for R^2 which is very close to one indicates that the graph plot of the model is lies close enough to straight line. While the adequate precision shows the signal of noise to ratio and for this type of interaction the adequate precision is 70.327 which are desirable.

4.4.2 Mutual Effects of Parameters

According to mathematical analysis of the experimental data, the interaction between independent process factor and response is portrayed graphically. The graphical plot of three-dimensional surface counter plot and contour plot of ethanol yield are shows in Figure 4.3, 4.4, 4.5, 4.6, 4.7 and Figure 4.8.

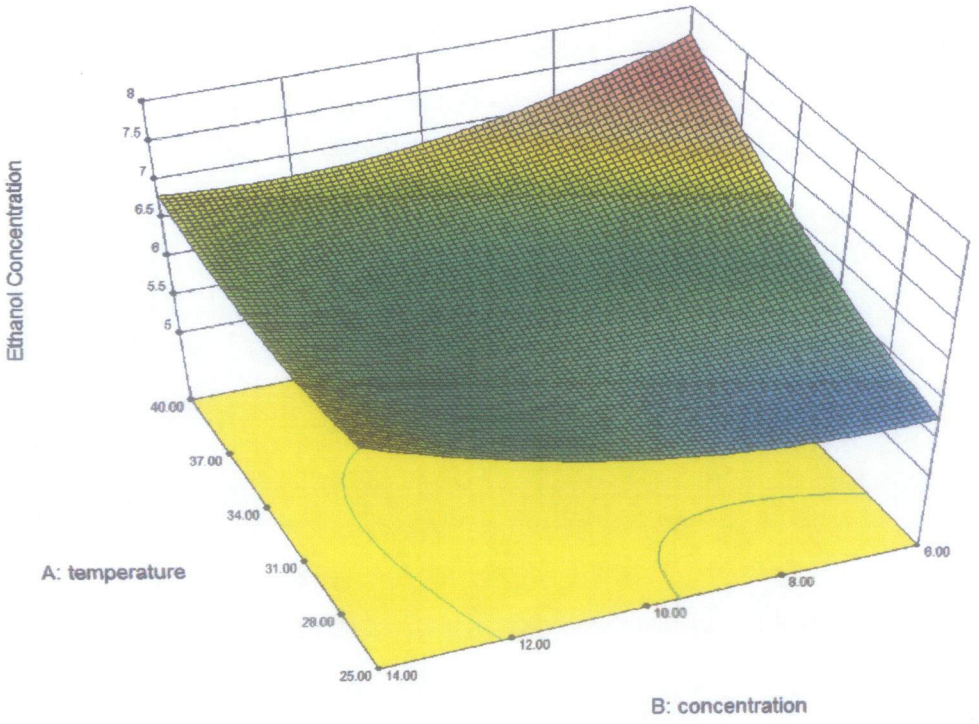


Figure 4.3: Response surface three dimensional plot of temperature, yeast concentration and ethanol yield

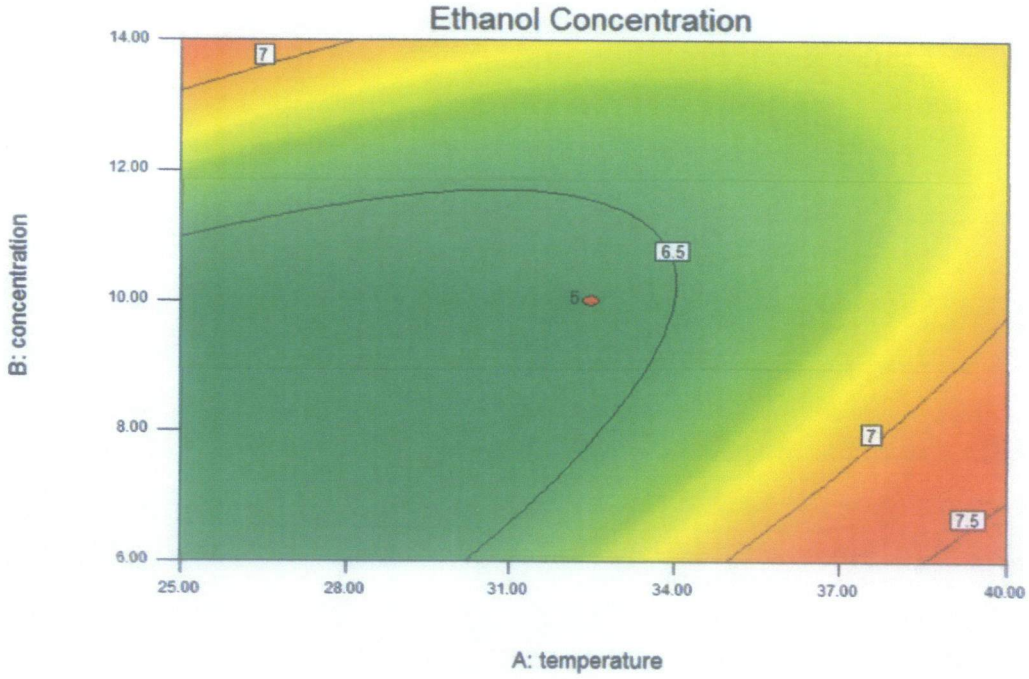


Figure 4.4: Response surface contour plot of temperature, yeast concentration and ethanol yield

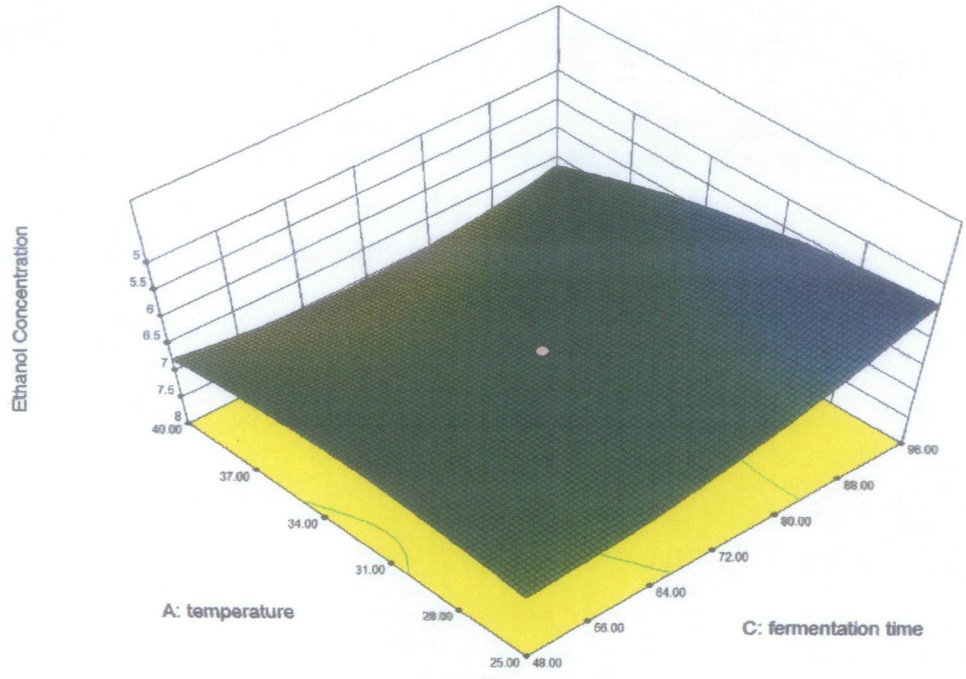


Figure 4.5: Response surface three dimensional plot of temperature, fermentation time and ethanol yield

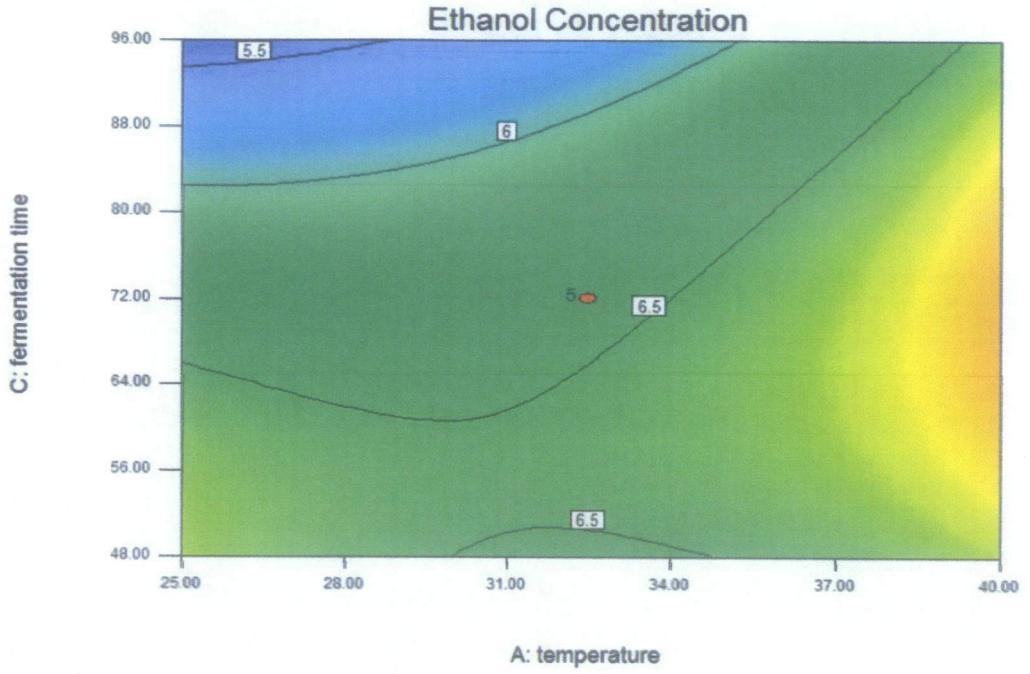


Figure 4.6: Response surface contour plot of temperature, fermentation time and ethanol yield

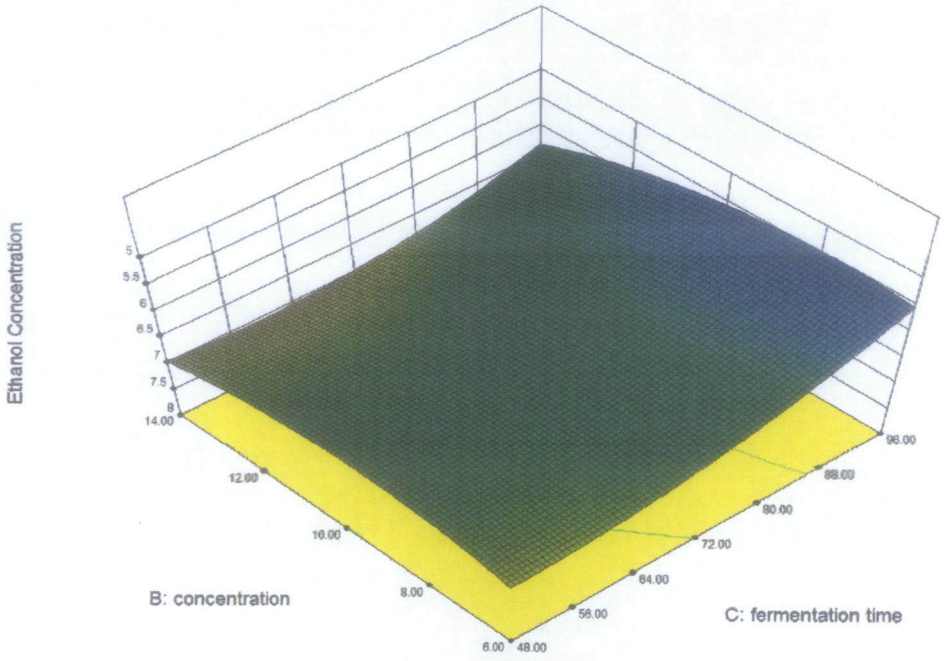


Figure 4.7: Response surface three dimensional plot of yeast concentration, fermentation time and ethanol yield

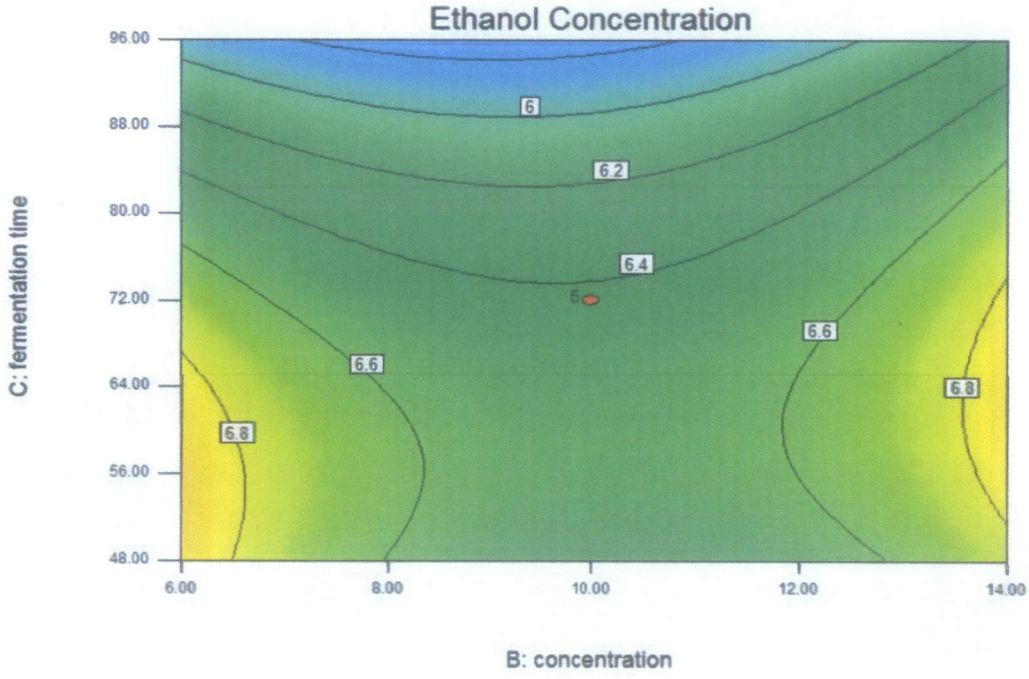


Figure 4.8: Response surface contour plot of yeast concentration, fermentation time and ethanol yield

The graph shown in Figure 4.3, 4.4, 4.5, 4.6, 4.7 and Figure 4.8 is based on the model in which the parameters are temperature, yeast concentration and fermentation time range between 25-40 °C, 6-14 g/ml and 48-96 hours respectively. From the Figures 4.3 and Figure 4.4 it shows that the maximum production of ethanol is in the red region which range from 5.28-7.34 g/ml. Figure 4.4 shows there is two region of red contour. The first region, consist of temperature at higher value while the yeast concentration is at lower value. Mean while the second red contour, is vice versa. According to Peggy (2012), the optimum condition for yeast growth is between 37-46 °C and yeast will start degrading at temperature 49 °C. The range of temperature at the first red contour is between 37-40 °C and at this particular temperature it promotes growth of yeast and as a result higher production of ethanol yield. However, providing higher amount of yeast concentration at low temperature it also help to produce higher ethanol concentration.

Based on the graphs shown in Figure 4.5 and Figure 4.6, it shows that the maximum production of ethanol occur at higher temperature and fermentation time between 64-72 hours. According to R.Arumugam and M.Manikandan (2011) the fermentation time for mango peels to yield maximum production of ethanol range from 42-48 hours. Inhibitory product presence in the sample may cause longer time for high production of ethanol. Based on the graph shown in Figure 4.7 and Figure 4.8, there is no obvious red region in the graph. This may happen because of very high temperature that causes the yeast to start degrading and there is no significant amount of ethanol produce.

4.4.3 Validation of Statistical Model

RSM also can observe the interaction between two independent variables. Figure 4.9 shows the interaction between temperature and yeast concentration. The figure shows that there is interaction between temperature and yeast concentration until certain point where the line graph start to intersect each other. This means that the interaction between temperature and yeast concentration only happen at low temperature while, at higher temperature there is no interaction happen between this two independent parameters. Same things happen for interaction between temperature and fermentation time as shown in Figure 4.11. The interactions also happen at low temperature and low fermentation time. This situation may happen because at high temperature yeast will start degrading and production of ethanol will become low. Figure 4.10 shows the interaction between yeast concentration and fermentation time. The line graph for this type of interaction seems to be parallel which means that interaction happen from the lower yeast concentration and fermentation time to higher yeast concentration and fermentation time.

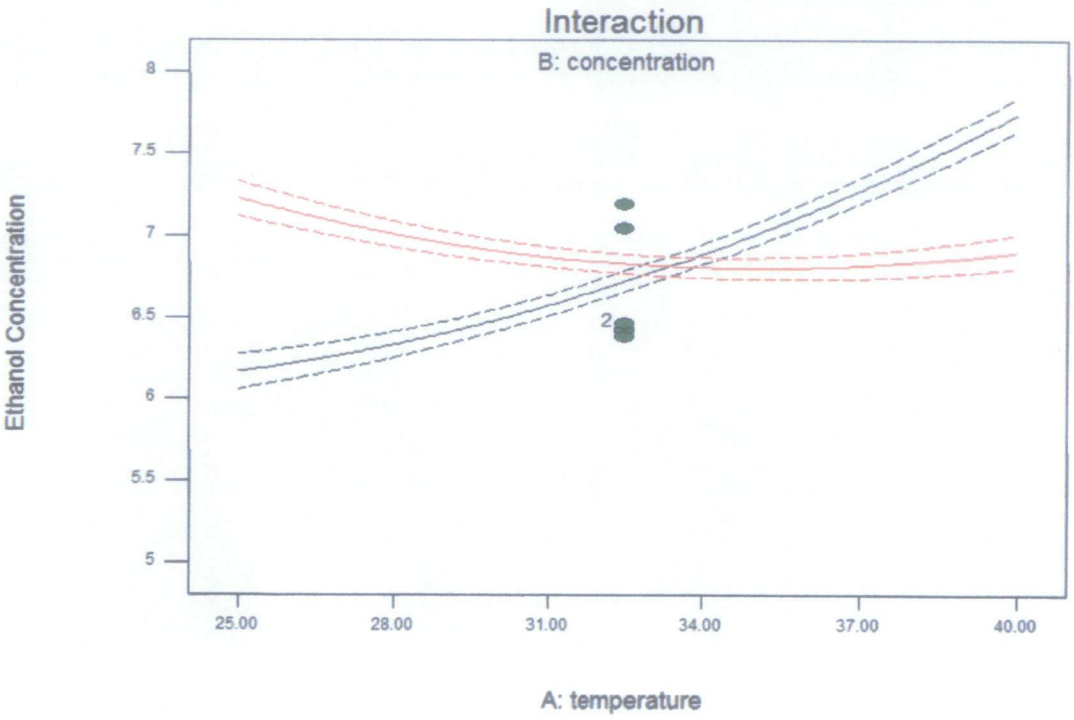


Figure 4.9 Interaction plot of temperature and yeast concentration

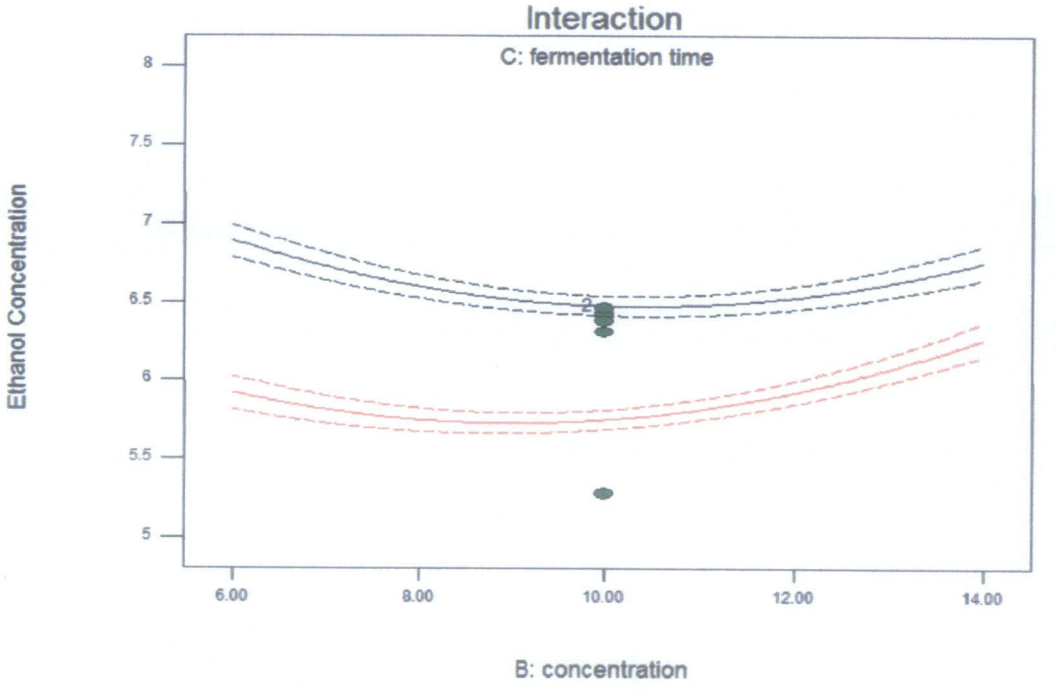


Figure 4.10 Interaction plot of yeast concentration and fermentation time

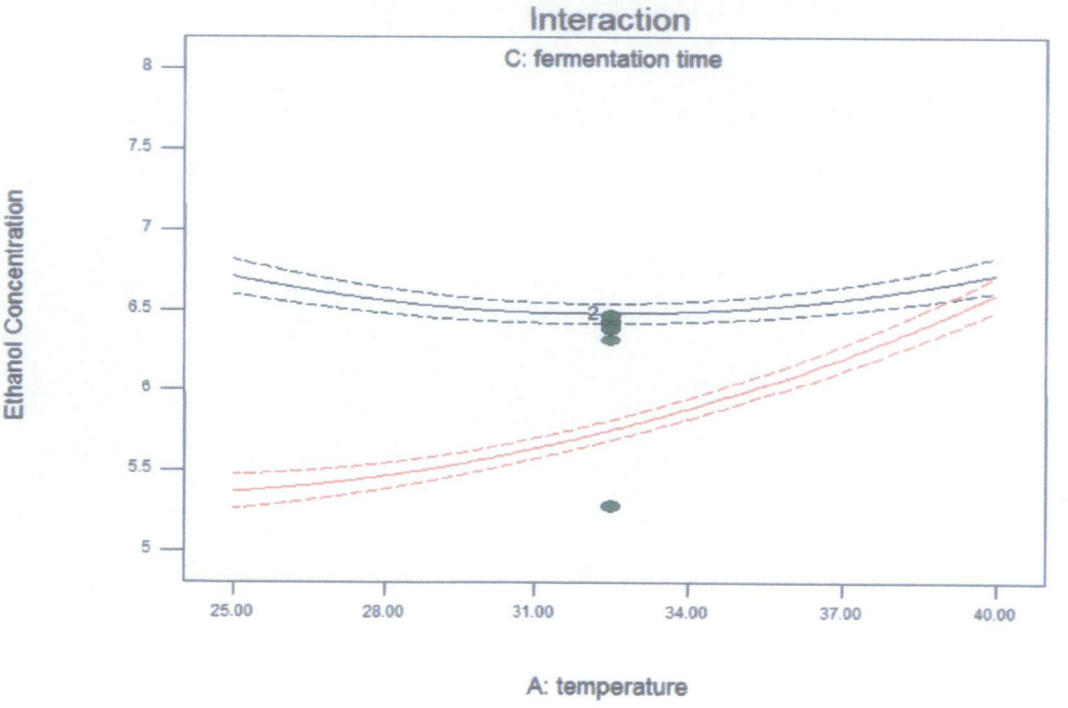


Figure 4.11 Interaction plot of temperature and fermentation time

The diagnostic plot is done to ensure the statistical assumptions are tally with analysis data for ANOVA. Figure 4.12 shows the normal probability plot in term of percentage of studentized residuals for ethanol yield. The graph is created based on theoretical percentage of residual analysis of the response surface design. The figure indicates the standard deviation between actual and predicted response, follow the normal distribution as the residual distributed close to straight line which means there is no abnormalities happen in the experimental results. Figure 4.13 shows the graph plot of residuals versus predicted response for ethanol yield. It shows that the points are lies within area ± 3.00 and it means that the assumption of constant variance was confirmed and the suggested model is adequate. Figure 4.14 shows a graph plot of predicted versus actual values of ethanol yield. From the figure, it shows that the point lies close to straight line and it indicates that the model equation predicted by the software and the actual results obtain from experiment are close enough. Most of the points lie on the straight line and only several points are above the diagonal line. Points that are above diagonal line are representing those over-estimated. This may happen because of failure of equipment as refractometer was used to calculate the refractive index for further analyze and calculate ethanol concentration using formula. Refractometer is less accurate compare to High Performance Liquid Chromatography (HPLC).

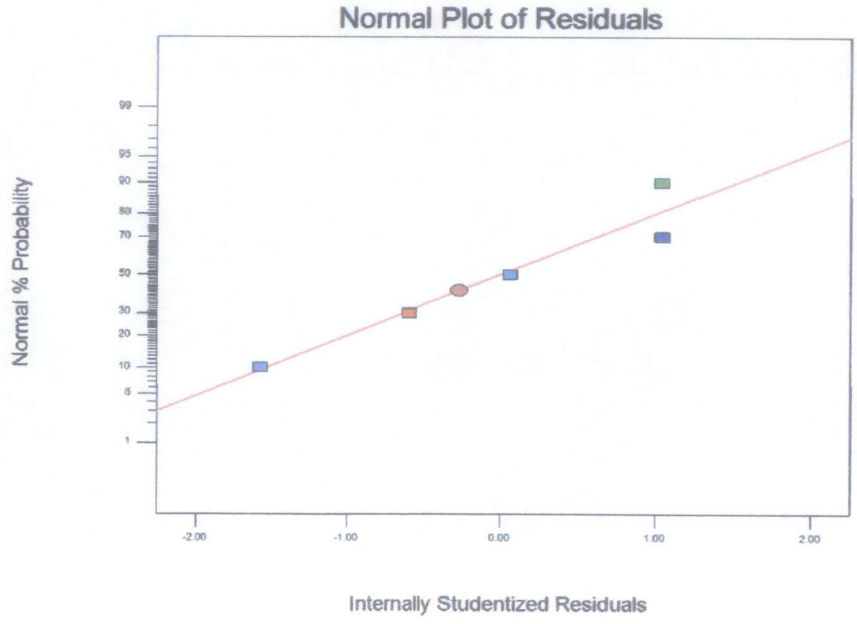


Figure 4.12 Normal probability plots of studentized residuals for ethanol yield

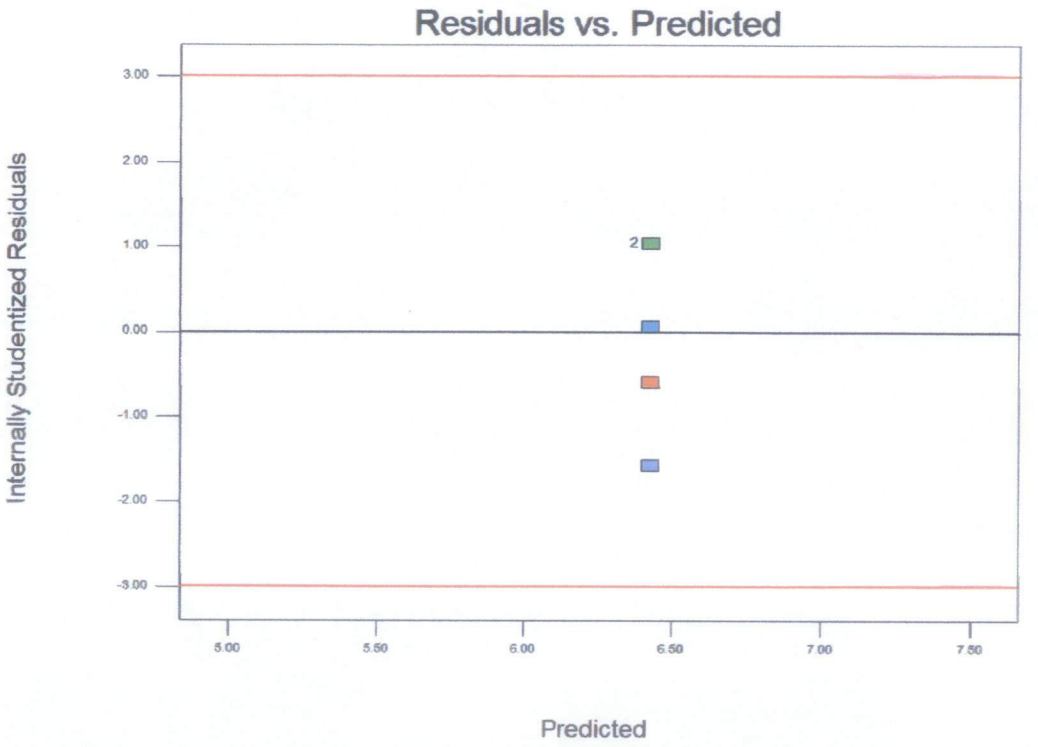


Figure 4.13 Plot of residuals versus predicted response for ethanol yield

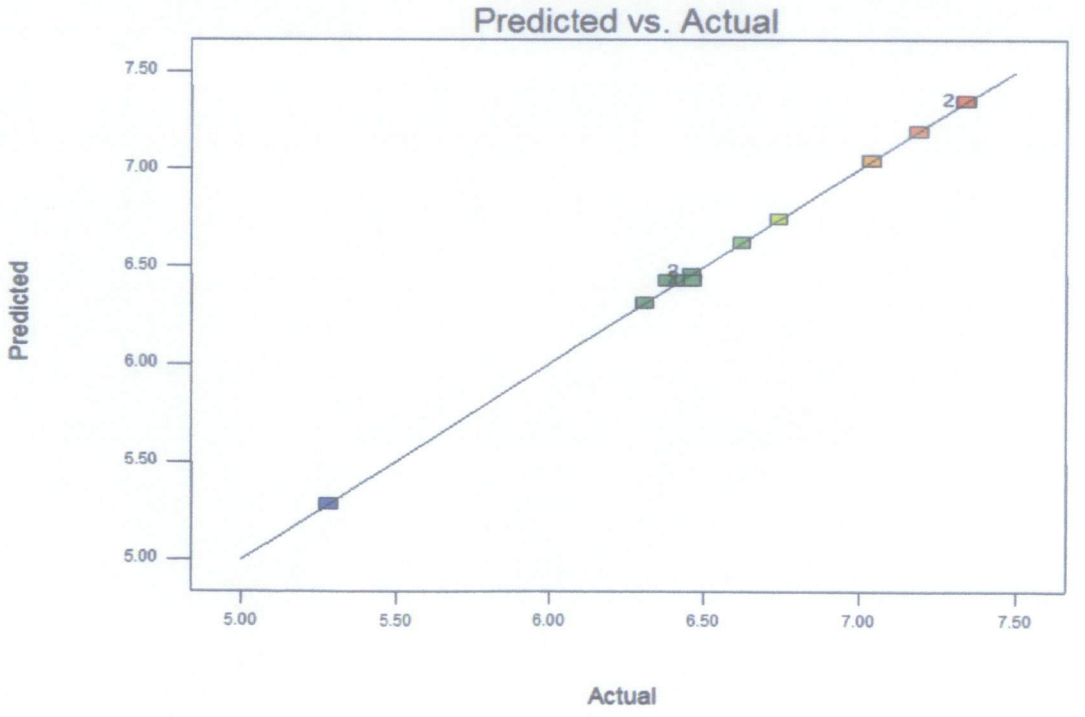


Figure 4.14 Plot of predicted versus actual values for ethanol yield

4.4.4 Response surface optimization and verification

The main objective of this project is to optimize the production of ethanol using RSM and to achieve this objective, numerical optimization was used. Numerical optimization gives highest desirability which indicates highest ethanol yield at the optimum temperature, yeast concentration and fermentation time. Table 4.5 showst hemost desirable optimum conditions were at 38 °C, 6 g/ml yeast and 48 hours fermentation time.

Table 4.5: Numerical optimization for RSM

Reaction condition	Temperature (°C)	Yeast concentration (g/ml)	Fermentation time (hours)	Predicted yield (g/ml)	Desirability
1	38	6.00	48	7.3401	0.974
2	25	13.30	48	7.1916	0.963
3	37	6.00	48	7.1604	0.955
4	25	13.20	48	7.1556	0.954
5	28	14.00	48	7.0288	0.921

The best five is selected according to amount of predicted yield and desirability. Three additional repeated experiments are conducted to verify the optimal points generate by the numerical optimization. Table 4.6 shows the result for production of ethanol yield in which the different between predicted yield and actual yield is less than 5%.

Table 4.6: Validation of the experimental model

n	Temperature (°C)	Yeast concentration (g/ml)	Fermentation time (hours)	Actual Yield (g/ml)	Average yield (g/ml)	Predicted yield (g/ml)	Error (%)
	38	6.00	48	7.25 7.29 7.23	7.2567	7.3401	1.1362

From table 4.6, the average ethanol yield from three repeated experiment is 7.2567 g/ml and is very close to predicted ethanol yield by statistical model which is 7.3401 g/ml. Percentage of error is 1.1362% and is acceptable as it is less than 5%. The result indicates that the numerical optimization is validate and reliable in order to produce high production of ethanol.

CHAPTER 5
RECOMMENDATION

Error can be divided into two types which are systematic error and random error. Error is something than cannot be avoided in experimental project but can be reduces. Types of errors that affect the effectiveness production of ethanol is random error. Random error is error happen due to fluctuation of reading. This type of error cannot be avoided but can be reduce by taking average reading during analyzing ethanol concentration instead of only one reading. Moreover, for future work it is recommend that the sample is drawn and analyze for ethanol concentration for every six hours interval.

Besides, other type of error is systematic error which is occurs because of instrument failed to function properly and the result is deviate from actual value. In this project, refractometer is used to analyze the production of ethanol in the samples. Even though average reading is taken for every sample but still accuracy of refractometer is low. To increase accuracy of analyzing ethanol concentration in sample it is suggest for future work to use HPLC. HPLC can determine a wide range of chemical presence in the sample and it is more accurate compare to refractometer.

Moreover, human error also classify under systematic error. Example of human error that did happen in this project is during taking measurement. To avoid this type of error happen, it is recommend that during taking measurement of distilled water, sulphuric acid solution and yeast solution, the eye level of individual that taking the measurement must be parallel with solution. This is to make sure only exact amount of particular solution is added into the sample so that it will not affect the amount of ethanol yield at the end of the experiment. Other recommendation that need to be implement in future work is by introducing more parameter such as pH to get more reliable result.

CHAPTER 6
CONCLUSION

This research is about maximization of ethanol production from mango peels using Response Surface Methodology. The mango peels is selected as source of biomass because high sugar contains which is preferable for production of bioethanol. Three variables is manipulated which are temperature, yeast concentration and fermentation time to study the effect of it towards production of bioethanol. RSM is use to further analyze and optimize the production of bioethanol.

The experimental works for this project is successfully done in laboratory. This research proved that the fermentation also depends on the temperature, yeast concentration and fermentation time. RSM successfully optimize the production of bioethanol from mango peels and from the numerical optimization the optimum condition to produce 7.34 g/ml ethanol from 10 gm mango peels is 38 °C, 6 g/ml yeast and 48 hours fermentation time.

This project will indeed benefit most importantly to the environment as well as economy. In economical point of view, it will benefit as the main substrate is only mango peels which commonly not utilize and usually being burn. Utilization of mango peels for production of bioethanol helps to reduce pollution and reduce dependencies of fossil fuel. This research also helps in finding potential revolutionary biomass resource as alternative energy.

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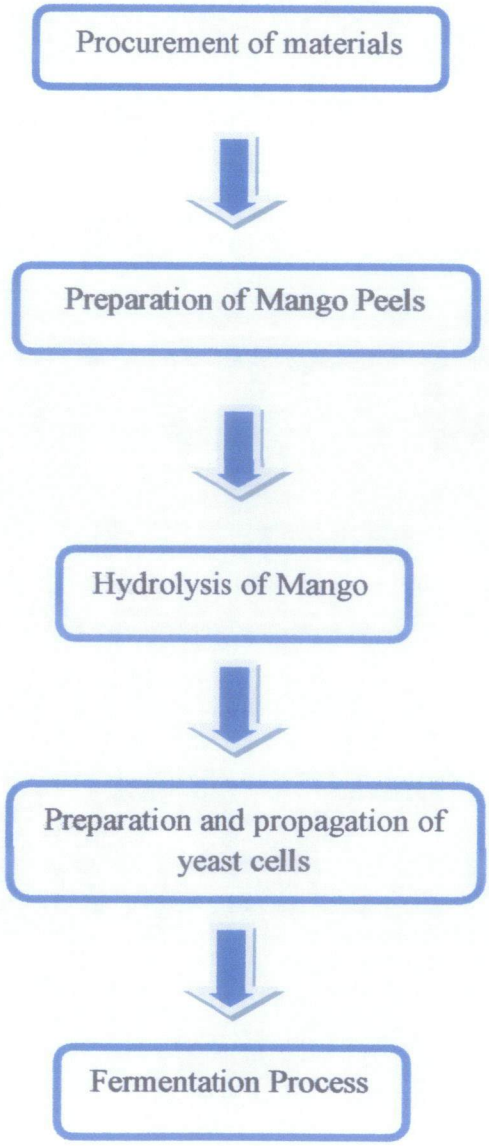
APPENDICES

Appendix I : Overall process flowchart



Appendix II: Equation given by Marker T.L et al (n.d)

$$n = 0.1363x + 1.2714$$

Where:

n = refractive index (RI)

x = concentration of glucose (% in water)