PRODUCTION OF BIOETHANOL FROM KUNDUR [BENINCISA HISPIDA (THUNB) COGN]

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SITI SALBIAH MUTALIB BACHELOR OF CHEMICAL ENGINEERING (BIOTECHNOLOGY) 2013 UMP

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Thesis submitted to the Faculty of Chemical and Natural Resource Engineering in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering in Biotechnology

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

SUPERVISOR DECLARATION

I hereby declare that I have checked this thesis and in my opinion this thesis is adequate in terms of scope and quality for the award of Degree of Bachelor of Chemical Engineering (Biotechnology)

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STUDENT DECLARATION

I hereby declare that the work in this thesis is my own except for the quotation and summaries which have been daily acknowledge. This thesis has not been accepted for any degree and is not concurrently submitted for award of any other degree.

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ACKNOWLEDGEMENT

First of all, I would like to express my biggest appreciation to my supervisor, Dr. Azilah binti Ajit @ Abd Aziz for her contribution, support and guidance throughout this research. Without her help, this project would be hard to met a successful end. I am also very thankful to lecturers and laboratory assistant's staff for their support, motivation and guidance for me to accomplish this project.

In particular, I wish to express my sincere appreciation to En. Shaikh Ismail bin Abdullah for his guidance, information, support and motivation for me to carry out this project until the end. I am also very grateful to my parents, my family and my colleagues for their motivation and supports.

Great deals appreciated go to the contribution of my faculty and my University, Faculty of Chemical Engineering and Natural Resources and University Malaysia Pahang for funding my undergraduate project and in providing good facilities and equipments. Last but not less, I would like to thank everyone who helping me in completing this projects.

PRODUCTION OF BIOETHANOL FROM KUNDUR [BENINCASA HISPIDA (THUNB.) COGN.]

ABSTRACT

Bioethanol is a clean burning, renewable resources which has become one of the alternative biofuels which are environmentally friend. Bioethanol has been proved that it can be produced by using agro-industrial and food wastes. Kundur (Benincasa hispida) also known as fuzzy melon is a vegetable crop that popular in Asian for their nutritional and medical attributes such as anti-obesity, anti-inflammatory, anti-diarrheal, antioxidant and etc. The aim of the study is to investigate the production of bioethanol from Kundur. This study was conducted by using different parts of Kundur. The moisture contents in Kundur seeds, pulps and fleshes are 77, 86 and 97 % (w/w) respectively. Kundur fleshes have the highest amount of reducing sugar (glucose) and carbohydrate contents which is 2.909 and 79.885 g/L. Meanwhile the amount of reducing sugar and carbohydrate are 0.924 and 33.333 g/L for Kundur seeds and 0.796 and 13.103 g/L for Kundur pulps.

Kundur juices were selected for the batch fermentation in the shake flasks to investigate the suitability of Kundur juice to produce ethanol by using *Saccharomyces Cerevisae* as yeast. The fermentation process was carried out in duplicate at 30°C, pH 5.5 and 200rpm for 72hours. According to the results obtained, the ethanol yield for the fermentation process without nutrient supplement is in the range of -0.192~ -0.194 (g ethanol/g biomass) while the specific growth rate is in the range of -0.0148~ -0.015h^{-1}. As for the fermentation process with nutrient supplement, the ethanol yield and specific growth rate is in the range of -0.136~ -0.1442 (g ethanol/g biomass) and -0.017h^{-1}. Hence, we can conclude that Kundur juice is not suitable for ethanol production

PENGHASILAN ETHANOL DARIPADA KUNDUR [BENINCASA HISPIDA (THUNB.) COGN.]

ABSTRAK

Bioethanol adalah pembakaran bersih, salah satu sumber alternatif biofuel yang boleh diperbaharui dan mesra alam. Bioethanol telah dibuktikan bahawa ia boleh dihasilkan dengan menggunakan bahan buangan agro-industri dan makanan. Kundur (Benincasa hispida) juga dikenali sebagai tembikai kabur adalah tanaman sayur-sayuran yang popular di Asia dari sudut pemakanan dan perubatan seperti anti-obesiti, anti-radang, anti-cirit-birit antioksidan, dan lain-lain Tujuan kajian ini adalah untuk menkaji penghasilan bioetanol dari Kundur. Kajian ini telah dijalankan dengan menggunakan bahagian-bahagian Kundur. Kandungan lembapan dalam benih, kulit dan isi Kundur adalah 77, 86 dan 97% (w / w). Isi Kundur mempunyai jumlah tertinggi pegurangan gula (glukosa) dan karbohidrat iaitu 2.909 dan 79.885 g/L. Sementara itu, jumlah pengurangan gula (glukosa) dan karbohidrat dalam biji Kundur adalah 0.924 and 33.333 mg/L manakala 0.796 dan 13.103 g /L untuk kulit Kundur.

Jus Kundur telah dipilih dalam fermentasi untuk menyiasat kesesuaian jus Kundur bagi menghasilkan etanol dengan menggunakan Saccharomyces Cerevisae sebagai yis. Proses fermentasi telah dijalankan dalam dua salinan pada 30 ° C, pH 5.5 dan 200rpm selama 72jam. Menurut keputusan yang diperolehi, hasil etanol untuk proses fermenatsi tanpa tambahan nutrien adalah dalam julat -0,192 ~ -0,194 (g etanol / g biojisim) manakala kadar pertumbuhan spesifik adalah dalam julat -0,0148 ~-0.015h-1. Manakala untuk proses fermentasi dengan tambahan nutrien, hasil etanol dan kadar pertumbuhan spesifik adalah dalam julat -0,136 ~ -0,1442 (g etanol / g biojisim) dan-0.017h-1. Oleh itu, kita boleh menyimpulkan bahawa jus Kundur tidak sesuai untuk pengeluaran etanol.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

CO_2	-Carbon Dioxide
CO	-Carbon Monoxide
HCl	-Hydrochloric Acid
RPM	- Rotation per Minutes
KJ	-Kilo Joules
%	- Percent
%w/w	- Percent Gram over Gram
g	- Gram
Tg	-Tera Gram
mg	-Milli Gram
L	- Liter
GL	-Giga liter
mL	-Milli liter
OD	- Optical Density
nm	-nano Meter

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bioethanol is an ethanol (ethyl alcohol) produced from plants such as soybeans, sugarcane, grass and wood. Several researchers have successfully produced ethanol employing juice or pulp from various fruits such as guava (Bardiya et al., 1974; Dhawan et al., 1983), pineapple, blackberry, orange, tamarind, cashew fruit (Maldonado *et al.*, 1975), banana (Kundu et al., 1976; Srivastava, 1984) and chashew apple (Modi, 1984; Sandhu and Joshi, 1994). Nowadays, bioethanol along with biodiesel has become one of

the most promising biofuels and is considered as the alternative medium to fossil transport fuels in Europe and in the wider world.

Bioethanol is seen as a good alternative fuel due to the source crops can be renewably and inexpensive. The economics of ethanol production are significantly influenced by the cost of the raw materials, which account for more than half of the production costs (Classen et al., 1999). This is important to achieve a lower production cost. Therefore, in order to achieve a lower production cost, the raw materials supply must be cheap. Moreover, the use of bioethanol is generally CO_2 neutral. This is because in the growing phase of the source crop, CO_2 is absorbed by the plant and oxygen is released in the same volume that CO_2 is produced in the combustion of the fuel (Wyman, 1990; Chandel et al., 2006). In addition, the emission and toxicity of ethanol are lower than those of fossil fuels such as petroleum (Wayman and Hinman, 1990).

1.2 Problem Statement

In recent years, most country around the world seeking a solution for the uncertain fuel supply and efforts to reduce carbon dioxide, CO_2 emission. This is because the availability of fossil fuels sources nowadays can only survive for another 20 to 30 years. Besides that, the use of fossil fuels such as coal, gasoline and natural gas has increased the concentration of carbon dioxide in the environment due to combustion

process. The increase of carbon dioxide concentration in the environment will lead to the increase in the earth's temperature which then will cause global warming. So that, to encounter this problems the solution should be developed.

Recently several resources had succeed produce ethanol from various fruits and plants such as rice straw, corn stalk (Kuhad and Singh, 1993), pine, aspen (Olsson and Hagerdal, 1996) and etc as an alternative fuel. Therefore, the present study was undertaken to produce ethanol by using Kundur fruit.

1.3 Research Objectives

The main objectives are:

(i) To investigate the properties of Kundur.

(ii) To identify the suitability of Kundur in ethanol production.

(iii) To identify the kinetic parameter of fermentation process.

1.4 Research Scopes

According to the objectives stated, the scope of research is to investigate the properties of Kundur parts consists of seed, flesh and pulp. The properties that will be

investigates are moisture contents, carbohydrates and reducing sugars. Apart from that, the purpose of this study is to identify the suitability of Kundur as a raw material for producing ethanol. In addition, this study will also investigate the kinetic parameter of the fermentation process which is biomass generation rate, glucose consumption rate and ethanol production rate

CHAPTER 2

LITERATURE REVIEW

2.1 Ethanol

Ethanol having a formula C_2H_5OH (or empirically C_2H_6O) is a second member of the aliphatic alcohol series which is a group of chemical compounds, whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom. Ethanol also known as ethyl alcohol, ethyl hydroxide and etc is a clear, colorless, and volatile liquid with a characteristic of agreeable odor (Pradyot Patnaink, 2007). The main physical properties of ethanol are as shown in **Table 2.1**. In a dilute aqueous solution, ethanol has

a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Other physical property of ethanol is that it is miscible (mixable) in all proportions with water and with most organic solvents, with quite an affinity for moisture absorption, even from the air.

Table 2.1 Physical Properties of Ethanol

Component	
Formula weight	46.06g/mol
Boiling point	78.5°C
Melting point	-114.1°C
Density	0.789g/ml at 20°C
Vapor pressure	43 torr at 20°C
Latent heat of evaporation	396 BTU/lbm
Gravimetric lower heating value	11,604 BTU/lbm

Source: CRC Handbook of Chemistry and Physics, (1993)

2.2 Applications of Ethanol

The largest use of ethanol is as a motor fuel in the automotive fuel industry. Ethanol is used as an alternative vehicle fuel, for example as E85 which is a mixture of

85% ethanol and 15% of gasoline by volume. For E85 fuel, 100 km driven consumes 2.2L of gasoline and 12L of bioethanol (S. Kim and B.E. Dale, 2004). Therefore 1L of bioethanol could replace 0.72L of gasoline. The market for this sector has highest growth potential. Many countries have attempted to replace some of their gasoline consumption with ethanol by mixing certain percentage of ethanol into gasoline to achieve the resulting product called gasohol. The largest national fuel ethanol industries exist in Brazil. Ethanol for fuel use is further divided into hydrous and anhydrous alcohol, each contained different purity from distillation.

Besides that, ethanol is useful as a solvent for many substances and in making perfumes, paints, lacquer, and explosives (Pradyot Patnaink, 2007). Alcoholic solutions of nonvolatile substances are called tinctures while if the solute is volatile, the solution is called a spirit. Ethanol acts as a drug affecting the central nervous system. Its behavioral effects stem from its effects on the brain and not on the muscles or senses themselves. It is a depressant, and depending on dose which can be a mild tranquilizer or a general anesthetic. It suppresses certain brain functions. At very low doses, it can appear to be a stimulant by suppressing certain inhibitory brain functions. However, as concentration increases, further suppression of brain functions produce the classic symptoms of intoxication which are slurred speech, unsteady walk, disturbed sensory perceptions, and inability to react quickly. Meanwhile, at very high concentrations, ethanol produces general anesthesia which is a highly intoxicated person will be asleep and very difficult to wake, and if awakened, he unable to move voluntarily.

2.3 Alternative Sources of Fuels

Energy demand increase year by years. According to BP statistical review of world energy June 2011, the consumption of energy growth reached 5.6%, the highest rate since 1973 in 2010. The total consumption of energy in 2010 easily surpassed the pre-recession peak reached in 2008. Consumption growth accelerated in 2010 for all regions. China energy consumption grew by 11.2%, and china surpassed the US as the world's larger energy consumption. Globally, energy consumption grew more rapidly than the economy, which means the energy intensity of economic activity increase for a second consecutive year. Therefore, we need an alternatives fuel to sustain the energy demand. Ethanol has already been introduced on a large scale in Brazil, USA, and some European countries, and it is expected to be one of the dominating renewable biofuels in the transport sector within the coming 20 years.

Ethanol can be blended with petrol or used as neat alcohol in dedicated engines that taking advantage of higher oxygen content, higher octane number, higher heat of vaporization and reduction of CO emission (Cardona et al., 2010). Furthermore, it is an excellent fuel for future advance flexfuel hybrid vehicles. Nearly, all fuel ethanol is produced by fermentation of sucrose in Brazil or corn glucose in the USA. However these raw material bases will not be sufficient to satisfy the international demand. Therefore many countries had made many researches to produce bioethanol from other sources such as oat, rice, sorghum and etc. The raw materials for production of bioethanol can be from agriculture residues, virgin biomass, waste paper, organic

fraction of municipal solid waste (MSW) and other materials containing fermentable sugar (McMillian, 1997).

Table 2.2 Quantities of Wasted Crop Potentially Available for Bioethanol Production

	Africa	Asia	Europe	North America	Central America	Oceania	South America	Subtotal
Corn (Tg)	3.12	9.82	1.57	0.30	1.74	0.01	4.13	20.70
Barley (Tg)	0.17	1.23	2.01	0.01	0.01	0.19	0.04	3.66
Oat (Tg)	0.004	0.06	0.43	0.01	0.001	0.001	0.05	0.55
Rice (Tg)	1.08	21.86	0.02	0.96	0.08	0.02	1.41	25.44
Wheat (Tg)	0.83	10.28	4.09	0.02	0.24	0.82	0.91	17.20
Sorghum (Tg)	2.27	0.54	0.004	0.00	0.13	0.001	0.18	3.12
Sugar cane (Tg)	0.46	1.64	0.00	0.00	0.36	0.00	0.74	3.20
Subtotal (Tg)	7.94	45.43	1.30	1.30	2.56	1.05	7.45	73.86

Source: S. Kim, B.E. Dale, (2003)

Table 2.3 Potential of Bioethanol Production from Waste crop

	Africa	Asia	Europe	North America	Central America	Oceania	South America	Subtotal
Corn (GL)	2.17	6.82	1.09	0.21	1.21	0.01	2.87	14.4

Table 2.3 Continue

Barley (GL)	0.12	0.83	1.35	0.005	0.01	0.13	0.03	2.46
Oat (GL)	0.002	0.04	0.30	0.01	0.0004	0.001	0.03	0.38
Rice (GL)	0.71	14.4	0.02	0.63	0.05	0.02	0.93	16.8
Wheat (GL)	0.55	6.78	2.70	0.02	0.16	0.54	0.60	11.3
Sorghum (GL)	1.55	0.37	0.003	-	0.09	0.0004	0.12	2.14
Sugar cane (GL)	0.23	0.82	-	-	0.18	0.0001	0.37	1.59
Subtotal (GL)	5.33	30.1	5.45	0.87	1.70	0.70	4.95	49.1

Source: S. Kim, B.E. Dale, (2003)

2.4 Kundur [Benincasa hispida (Thunb.) Cogn.]

Kundur which is also known as fuzzy or hairy melon is also called Chinese squash or moqua. Mature fruits of some *Benincasa hispida* [syn. *B. cerifera* (Thunb) Cogn.] are commonly called wax gourd, winter gourd or Chinese preserving melon. *Benincasa hispida* can be stored for a long period of time which is for many months. The immature Kundur fruits have a delicious flavor, stronger and distinctive. Flavor can change during storage, with the fuzzy melon taking on an acidic, less agreeable flavor.

Kundur fruit is an important source of water–soluble polysaccharide. Polysaccharides that present in Kundur juice are mainly arabinogalactans (Mazumder, Ray, and Ghosal et al, 2001). Natural sugars that are present in immature and mature Kundur fruit pulp are glucose and fructose. The level of both glucose and fructose are reported to be reduced from 0.9% to 0.5% and 0.8% to 0.5% respectively, as the fruit matured (Wills, Wong, Scriven and Greenfield et al, 1984).

Table 2.4 Nutritive Composition of Kundur per 100 g Edible Portion

Component	*Benincasa hispida*
Edible portion (%)	98
Water (%)	93.8
Energy (KJ)	44
Protein (g)	0.7
Fat (g)	0.1
Carbohydrate (g)	2.0
Dietary fiber (g)	2.1
Organic acids (g)	0.04
Ash (g)	0.7
Minerals	
Ca (mg)	12
K (mg	250
Mg (mg)	15

Fe (mg)	0.3
Na (mg)	2

Table 2.4 Continue

Zn (mg)	0.2
Vitamins	
A (mg)	0.02
thiamin (mg)	0.07
riboflavin (mg)	0.05
Niacin (mg)	0.20
C (mg)	69

Source: Marita Cantwell, Xunli Nie, Ru Jing Zong, and Mas Yamaguchi, (1996)

The diversity and antiquity of cultivars in China suggest that this crop may be indigenous to southern China (Yang and Walters, 1992). Cultivars of *B. hispida* are classified on the basis of wax formation on the mature fruit, shape, fruit size and pubescence of the immature fruits which is from which its common name is derived. Generally, separate cultivars are used for immature vegetable production and for mature fruit production (Yang and Walters, 1992). One vegetable type has fruits which are cylindrical and roundish with many bristle-like trichomes on the epidermis. The other jointed gourd has immature fruits which are narrowed in the center that have dumbbell shaped with their length 2-3 times their width.

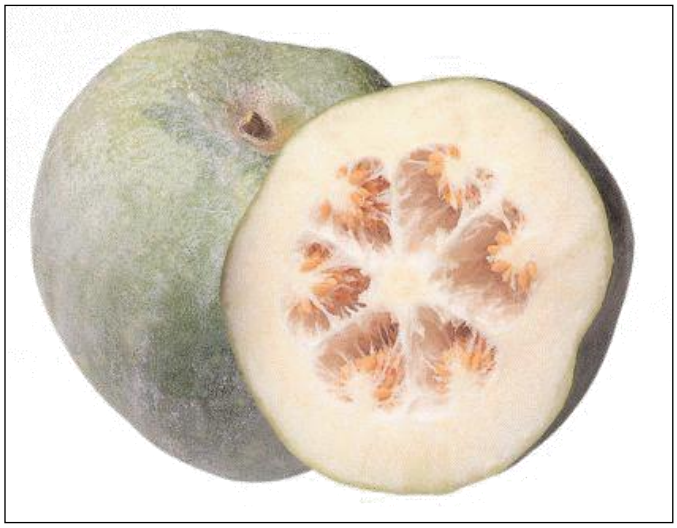

Figure 2.1 Kundur [Benincasa hispida (Thunb.) Cogn.]

2.5 Applications of Kundur [Benincasa hispida (Thunb.) Cogn.]

There are many applications of *B. hispida* such as the fruits and seeds of the *B. hispida* are used for medicinal purpose such as anti-obesity (Kumar & Vimalavathini, 2004), anti-inflammatory (Huang et al., 2004), anti-diarrhoeal (Mathad et al., 2005), anti-pyretic (Qadrie et al., 2009), anti-compulsive (Girdhar et al., 2010), antioxidant (Gill *et al*., 2010), anti ulcer and diuretic (Grover *et al*., 2001; Rachchh and Jain, 2008). Externally, the pulp of fruit is applied on wounds and burns to alleviate the burning sensation while the seeds mashed with water to serve the same purpose. The seed oil can be use to relief headache.

Internally, *B. hispida* can be used in many of diseases. *B. hispida* can help you to relief thirst due to vitiated pitta. The pulp of the fruit along with laxative is an effective remedy for tapeworm infestation. The seed oil facilitates the stools smoothly as well as renders styptic action, hence beneficial in bleeding piles. The fruit juice mixed with sugar ameliorates hyperacidity. In tuberculosis with cavitation and haemoptysis, *B. hispida* is highly recommended because it bestows rejuvenative, styptic and tonic properties. The root powder is given with water to alleviate the bronchospasm in asthma. *B. hispida* inhibits mental instability, agitation and induces sound sleep. The fruit juice of *B. hispida* is said to be an antidote for lead poisoning.

2.6 *Saccharomyces Cerevisiae (S.Cerevisiae)*

Saccharomyces Cerevisiae (*S. Cerevisiae*) is yeast cells that belong to the eukaryotes and are classified as fungi. Yeasts do not require sunlight to grow, but do use sugars as a source of energy. *S. Cerevisiae* cells use three major pathways for growth on glucose.

First, the fermentation of glucose:

$$C_6H_{12}O_6\,(s) \longrightarrow 2CH_3CH_2OH\,(l) + 2CO_2\,(g) \qquad (2.1)$$

Second, the oxidation of glucose:

$$C_6H_{12}O_6\,(s) + 6O_2\,(g) \longrightarrow 6CO_2\,(g) + 6H_2O\,(l) \quad (2.2)$$

Third, the oxidation of ethanol:

$$CH_3CH_2OH\,(l) + 3O_2\,(g) \longrightarrow 2CO_2\,(g) + 3H_2O\,(l) \quad (2.3)$$

The process where there is the presence of oxygen is called as aerobic respiration. When oxygen is not present, the yeast will then go through anaerobic fermentation. During this process the glucose will be converted into ethanol. The net result of this process is two ATP, and two by product which are ethanol and carbon dioxide. *S. cerevisiae* is capable of converting only hexose sugars to ethanol. The most promising yeasts that have the ability to use both C_5 and C_6 sugars are *Pichia stipitis*, *Candida shehatae* and *Pachysolan tannophilus*. However, ethanol production from sugars derived from starch and sucrose has been commercially dominated by the yeast *S. cerevisiae* (Lin and Tanaka, 2006). Ethanol is produced commercially by yeast because it ferment glucose to ethanol a virtually sole product and it is known for its high ethanol tolerance, rapid fermentation rates and insensitivity to temperature and substrate concentration (Linden and Hahn-Hagerdal, 1989). The main use for *S. cerevisiae* is it helps in the rising of bread with the presence of carbon dioxide produce from the process. The gas that is produce inside the dough causes it to rise and expand. Another

importance of *S. cereviseae* is that live yeast supplementation to early lactating dairy goats significantly increased the milk production (Stella, A.V, 2001).

Table 2.5 Bioconversion of Various Biomass Sources into Ethanol by Different Microorganism

Raw Material	**Microorganism**	**Reference**
Sugarcane baggase	*C. shehatae* NCIM3501	Chandel et al., 2006b
Wheat straw	*E. coli FBR5*	Saha et al., 2005
Rice straw	*C. shehatae* NCIM3501	Abbi et al., 1996
Sorghum straw	*Kluyveromyces marxianus* CECT10875	Ballesteros et al., 2004
Corn stover	*S. cereviseae* TMB3400	Ohgren et al., 2006
Barley husk	*S. cereviseae*	Palmarola et al, 2005
Sun flower stalk	*S. cereviseae var ellipsoideus*	Sharma et al., 2002
Sugarcane leaves	*S. cereviseae* NRRL-Y-132	Krishna et al., 2001
Wheat bran	*S. cereviseae*	Palmarola et al, 2005

Ground nut shell	*S. cereviseae*	Akpan et al., 2005
Apple pomace	*S. cerevisiae* ATCC 24702	Ngadi and Correial, 1992
Pine apple canary waste	*S. cereviseae* ATCC 24553	Nigam, 2000

Table 2.5 Continue

Pine	*P. stipitis* NRRL-1724	Qureshi et al., 1991
Poplar	*S. cerevisiae*	Cantarella et al., 2004
Birch	*S. cerevisiae*	Johanssen et al., 2001
Spruce	*S. cerevisiae*	Taherzadeh., 1999
Banana pulp waste	*S. uvarum* NCIM culture 3528	Joshi et al., 2001
Finger Millet (*Eleusine corcana* flour)	*S. cerevisiae*	Reddy and Reddy, 2006
Municipal solid waste (MSW)	*S. cerevisiae*	Mtui and Nakamura, 2005
Industrial waste	*K.marxianus, S. cereviseae*	Kadar et al., 2004

Source: Chandel et al., (2006)

CHAPTER 3

MATERIALS & METHODOLOGY

3.1 Research Design

This research intends to investigate the properties of Kundur parts consists of seed, flesh and pulp and to finding out the suitability of Kundur to produce ethanol by using yeast *Saccharomyces Cerevisiae* (*S. Cerevisiae*). The kinetic parameter which consists of biomass generation rate, glucose consumption rate and ethanol production rate were investigated.

3.2 Flow Chart of Work Process

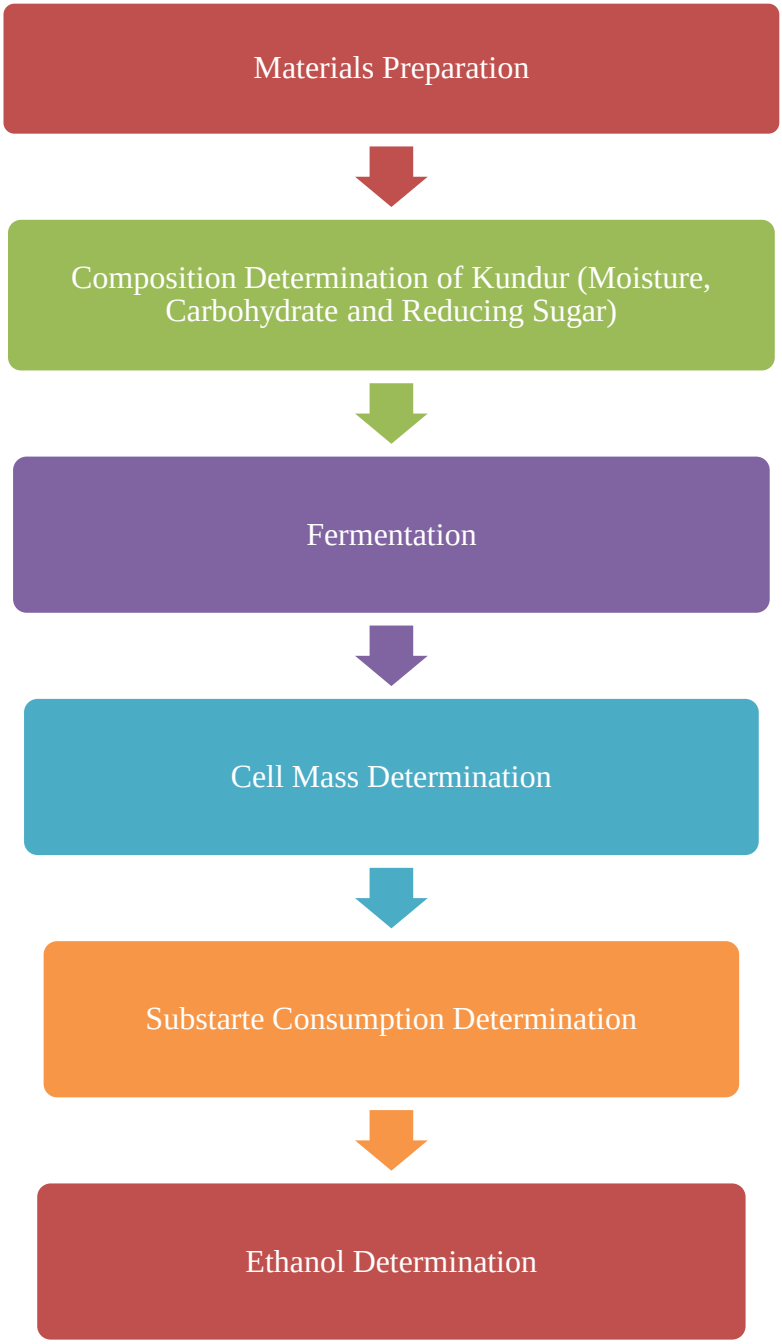

Figure 3.1 Flow Chart of Work Process

3.3 Materials

The materials used in this research are Kundur as a glucose source, yeast *S.Cerevisiae*, nutrient broth for growth of *S.Cerevisiae* and nutrient agar for the cultivation of *S.Cerevisiae.* The *S. Cerevisiae* used in this study was obtained by using the instant yeast (Baker's yeast) for bread, doughnut and etc that were available in the market as in **Figure 3.2.**

Figure 3.2 Baker's Yeast *(S. Cerevisiae)*

3.4 Preparation of Kundur [Benincasa hispida (Thunb.) Cogn.]

Kundur were purchased from Warisan Kundur Resources in Pekan, Pahang. The Kundur obtained is then thoroughly washed. The Kundur were separated into three main parts which is seed, flesh and pulp as in **Figure 3.3**. The fleshes were cut into small pieces in order to ensure a rapid rate of juice extraction. The flashes were grinded by using blender. Then, the juice is squeezed by using cheesecloth to obtain pure juice. As for seeds and pulps, they were dried first in the 60°C oven before grinded to fine powder by using dry blender. The juice samples was filled in the sterilize 2.5L Schoot's bottle while the seeds and pulps powder were placed in the seal bag. All samples were preserved in the 4°C chiller to prevent any possible degradation or spoilage during storage.

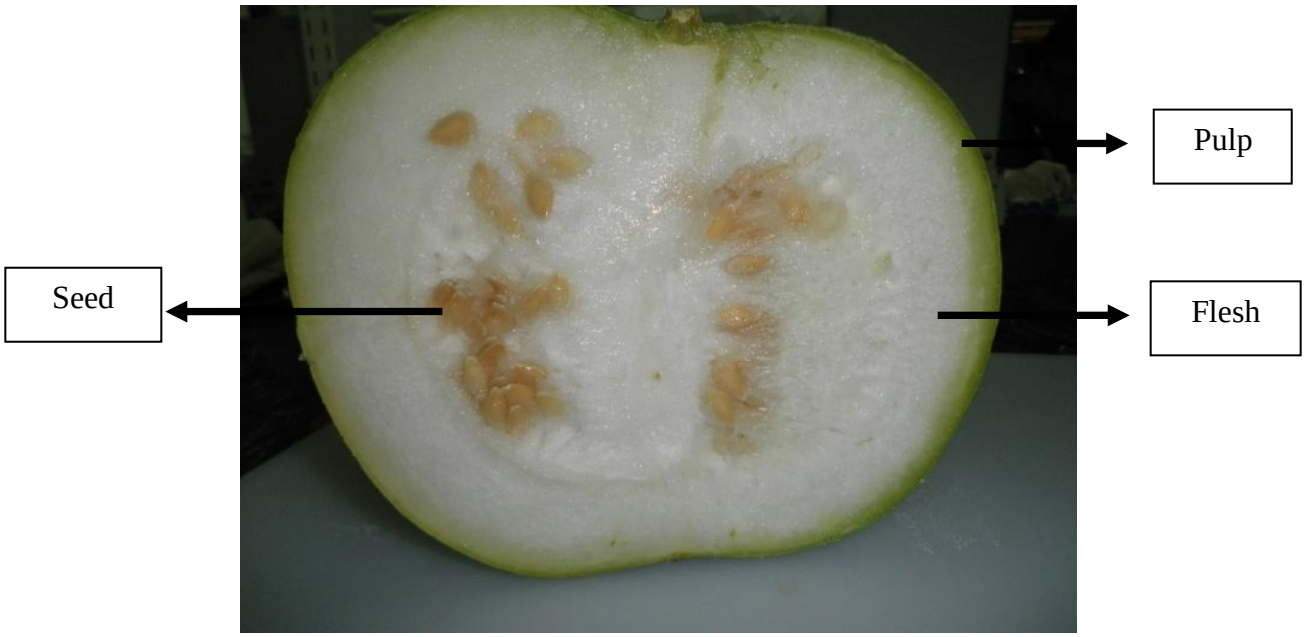

Figure 3.3 Kundur Part.

3.5 Moisture Determination

The moisture content of Kundur is determined by measuring the mass of samples before and after the water is removed by evaporation process as in equation (3.1). The moisture contents in each parts of Kundur can be found by using microwave oven that using a principle of evaporation where the water molecules in the parts will be evaporated because they absorb microwave energy, which causes them to become thermally excited. The weighed samples were placed in a microwave oven at 60°C for about 72 hours. Then, the dried masses were weighted. The major advantages of microwave methods over drying method are that they are simple to use and rapid to carry out.

$$\%\text{Moisture} = \frac{M_{\text{INITIAL}} - M_{\text{DRIED}}}{M_{\text{INITIAL}}} \times 100 \qquad (3.1)$$

Figure 3.4 Microwave Oven

3.6 Preparation of Standard Calibration Curve for Glucose

The purpose of preparing the calibration curve for glucose is to find the concentrations of reducing sugar of the sample tested. A 10mg/L standard glucose solution was prepared by adding 10mg D+ Glucose Anhydrous into 1000mL of distilled water. Then, the solution was stirred until all the glucose dissolves in the water. Next, 10 different concentrations of glucose solution was prepared by using dilution method. The preparation of all the concentrations was shown as in **Table 3.1**.

Table 3.1 Preparations for ten concentrations of standard calibration curve for glucose

Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Concentration (mg/l)	1	2	3	4	5	6	7	8	9	10
Volume of 10mg/mL Glucose Solution (ml)	0.3	0.6	0.9	1.2	1.5	1.8	2.1	2.4	2,7	3
Volume of solvent, Distilled Water (ml)	2.7	2.4	2.1	1.8	1.5	1.2	0.9	0.6	0.3	0
TOTAL (ml)	3	3	3	3	3	3	3	3	3	3

Then, 3mL of each glucose solution was pipette out by using pipette. Next, all the solutions were tested by using DNS calorimetric method. A graph of absorbance versus concentrations was plotted as in **Figure 3.5**.

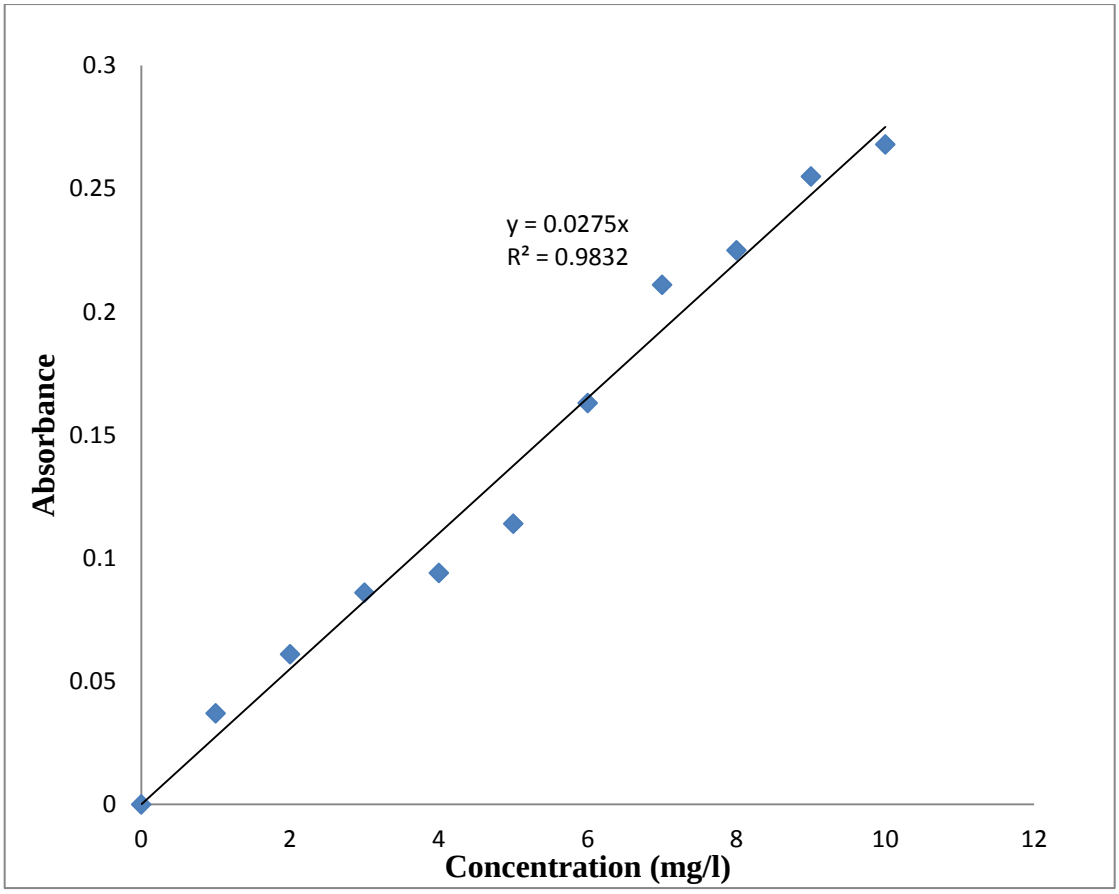

Figure 3.5 Effect of Different Concentration of Standard Glucose on Absorbance at 510nm

3.7 Reducing Sugar (Glucose) Determination

For reducing sugar estimation the method use is by using DNS calorimetric method (Miller, 1959). First of all, for Kundur pulps and seeds, the reducing sugars were extracted first from sample by weighing a 100mg of the sample and extracted with hot

80% ethanol twice (5mL each time). The supernatant were collected and were evaporated by keeping it in a water bath at 80°C. 10mL distilled water was added to dissolve the sugars. Meanwhile, Kundur juice was diluted by transferring 1mL of juice into test tube having 199mL distilled water. Then, all the solution was tested by using DNS Calorimetric method. The amount of reducing sugars present in the sample was calculated by using standard calibration curve of glucose.

3.7.1 DNS Calorimetric Method

DNS calorimetric method is use to tests the presence of reducing sugar (Miller.1959). This method is simple, sensitive and adoptable during handling of a large number of samples at a time.

3.7.1.1 Preparation of DNS Reagent

DNS reagent is prepared by dissolving 10g of Di-Nitro Salicyclic Acid (DNS), 2g of phenol and 0.5g of sodium sulphite (Na_2SO_2) into 500mL 2% NaOH solution. Then, distilled water was added into the solution until the volume is up to 1000mL. The reagent was stored in a dark place due to the sensitivity of the reagent to light.

3.7.1.2 DNS Reagent Test

The DNS reagent test was carried out by taking out 3mL of sample and put in a test tube. Then, 3mL of DNS reagent was added to the test tube. This step was repeated for other samples. All the test tubes were covered by using aluminium foil and were placed in a water bath at temperature 70~90°C for approximately 10 minutes until the solution turn red-brown color. After that, all the tubes were taken out. 1mL of potassium sodium tartrate was put into each test tube when the contents of the tubes are still warm and mix properly. Then, the tubes were let to cool down to room temperature before measured the absorbance by using UV-Vis Spectrometer at 510nm.

Figure 3.6 UV-Vis Spectrometer

3.8 Preparation of Standard Calibration Curve of Carbohydrate

The purpose of preparing the calibration curve of carbohydrate is to find the concentrations of total carbohydrate of the sample tested. A 100mg/l standard glucose solution was prepared by adding 100mg D+ Glucose Anhydrous into 1000mL of distilled water. Then, the solution was stirred until all the glucose dissolves in the water. Next, 10 different concentrations of glucose solution was prepared by using dilution method. The preparation of all the concentrations was shown as in **Table 3.2**.

Table 3.2 Preparations for ten concentrations of standard calibration curve for carbohydrate

Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Concentration (mg/l)	10	20	30	40	50	60	70	80	90	100
Volume of 10mg/mL Glucose Solution (ml)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Volume of solvent, Distilled Water (ml)	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0
TOTAL (ml)		1	1	1	1	1	1	1	1	1

Then, 1mL of each glucose solution was pipette out by using micro pipette. Next, all the solutions were tested by using Phenol-Sulphuric Acid method. A graph of absorbance versus concentrations was plotted as in **Figure 3.7**.

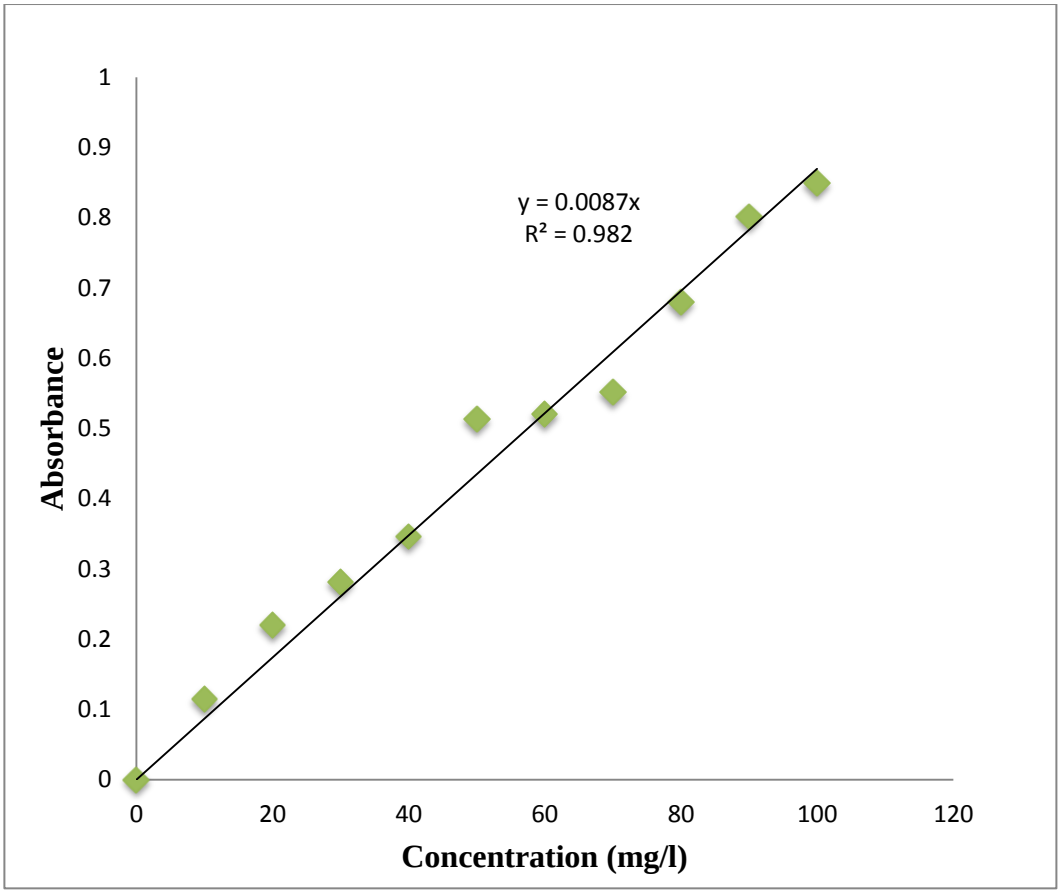

Figure 3.7 Effect of Different Concentration of Standard Glucose on Absorbance at 490nm

3.9 Carbohydrates Determination

For the sample preparation, 100mg of the Kundur pulps and seeds were weighted and put into a boiling tube. Then, the sample is hydrolyzing by keeping it in a boiling water bath for three hours with 5mL of concentrated hydrochloric acid, HCl and was left

to cool to room temperature. The samples were neutralizing by adding solid sodium carbonate until the effervescence ceases. The volume was making up to 100mL and then was centrifuge to obtain the supernatant. Then, 0.1mL of the sample was pipette out and put into test tubes. The volume was making up to 1mL by adding distilled water. Meanwhile for Kundur juice, 1mL of the juice was diluted with 999mL distilled water. Then, all the solution was tested by using Phenol-Sulphuric Acid method. The amount of total carbohydrate present in the sample was calculated by using standard calibration curve of carbohydrate.

Figure 3.8 Refrigerated Centrifuge

3.9.1 Phenol-Sulphuric Acid Method

Total concentration of carbohydrates present in foods is widely determined by using a Phenol-Sulfuric Acid method (Dubois et al, 1956) which is an example of calorimetric method. This method is simple, rapid, accurate, sensitive, specific for carbohydrates and widely applied. The reagent for this method are inexpensive, rapidly available and stable (S.Suzanne, 2009).

3.9.1.1 Phenol-Sulphuric Acid Test

The tests were carried out by pipette out 1mL of each sample into test tube. Then, 1mL of phenol solution was added to each tube. Next, 5mL of 96% sulphuric acid was added to each tube and shake well. After 10minutes the contents in the tube were shaken and were placed in water bath at 25~30°C for about 20 minutes. Then, the tubes were let to cool down to room temperature before measuring the absorbance by using UV-Vis Spectrometer at 490nm. The amount of total carbohydrate present in the sample solution is calculated using the standard graph.

3.10 Fermentation

Based on the glucose determination of Kundur pulp, seed and juice, it is shows that the juice has high glucose concentration rather than pulp and seed. So, the further study on the fermentation for ethanol production was carried out by using Kundur juice as a substrate. The fermentation process was carried out in the incubator shaker at pH 5.5 and at temperature of 30°C with *S. Cerevisiae* as yeast for 72 hours

3.10.1 Aseptic Technique

Aseptic technique is a fundamental and very important laboratory skill in the field of microbiology in order to minimize and eliminate bacteria to prevent contamination. Microbiologists use aseptic technique for a variety of procedures such as transferring cultures, inoculating media, isolation of pure cultures and for performing microbiological tests. Aseptic technique is of utmost importance to maintain pure stock cultures while transferring cultures to new media. Proper aseptic technique prevents contamination of cultures from foreign bacteria inherent in the environment. The aseptic technique including disinfect the working area by using 70% ethanol before and after the working process. As for this study, all aseptic work was carried in the laminar flow

hood. Then, the UV light was switched on for about 30 minutes to eliminate pathogens. Next, the aseptic works were carried near the flame. After all the aseptic works is done, the working area was disinfecting again by using 70%ethanol. Lastly, the UV light was switched on again for about 30 minutes.

3.10.2 Preparation of Nutrient Agar plate

Agar plate is used in the culture of *S. Cerevisiae*. The agar plate was prepared by dissolving the weighed amount of nutrient agar powder according to the specification stated into 1L beaker containing 1000mL of the warm distilled water. After that, the solution was stirred using magnetic stirrer with the heat on until all the nutrient agar powder dissolved. The ready nutrient agar solution was then transferred into 1L Schoot's bottle before sterilize by using autoclave at 121°C for 30 minutes. After that, the sterilized nutrient agar solution was poured into the plate in the laminar flow hood and by using aseptic technique. The nutrient agar solution in the plates was left to cool down to obtain solid agar as in **Figure 3.9**. Next, the plates were closed with the cover and were sealed with parafilm. All the agar plates and the remaining nutrient agar solution were kept in 4°C chiller until used.

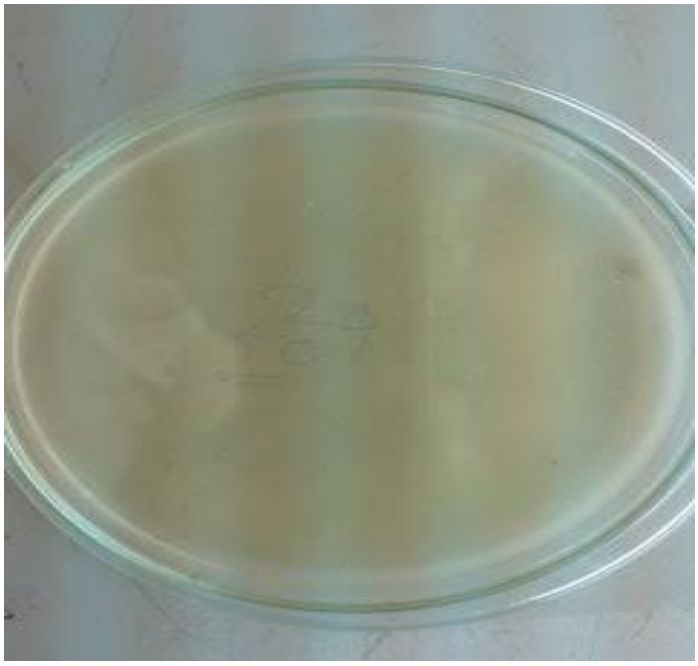

Figure 3.9 Nutrient Agar Plate

3.10.3 Preparation of Nutrient Broth

The nutrient broth used consists of dextrose, 1 g/L; yeast extract, 3 g/L; peptone, 15 g/L; and sodium chloride, 16 g/L. This supplemented nutrient will supply enough nutrients which help *S. Cerevisiae* to growth. The nutrient broth was prepared by weighing the appropriate amount of nutrient broth powder by referring to the specification on the nutrient broth bottle. Then, the weighing nutrient broth powder was put in the beaker containing 1000mL distilled water. The solution was dissolved by using magnetic stirrer until all the nutrient broth powder is dissolve. The ready nutrient

broth solution was transferred into 1L Schoot's bottle sterilized at 121°C for 20 minutes before further uses. The solution was kept in the 4°C chiller until used. This nutrient broth will be use for the inoculums process.

3.14.4 Preparation of *Saccharomyces Cerevisae (S.Cerevisae)*

The *S. Cerevisiae* was prepared by weighing 2.5g of the Baker's yeast and put in the sterilized 500mL conical flask which contains 250mL sterilized nutrient broth by using aseptic technique. Then, the solution was incubated at 30°C and 150rpm for 24hours.

Figure 3.10 Culture of *Saccharomyces Cerevisae*

3.14.5 Transferring *S. Cerevisiae* to Nutrient Agar Plate

S. Cerevisiae was transferred to nutrient agar plate by using streaking method. This objective this method is to obtain a pure culture for the following fermentation process. This method will ensure the culture can be used in a long period of time without losing any nutrient. This method was carried out in the laminar flow hood. First of all, the working area in the laminar flow hood was swab with 70% ethanol to sterilize the working area. Then, UV lamp was switched on for about 30 minutes. After that, the Bunsen burner was light on. The incubated *S. Cerevisiae* and nutrient agar plate was placed near the Bunsen burner to avoid any contamination. Next, the inoculating loop was sterilized by heat the loop until the loops is turn redness. Then, the loop full of the incubated *S. Cerevisiae* was taken and streaked on the nutrient agar plate. Then, the inoculating loop was sterilized again to kill the remaining *S. Cerevisiae* on the loop. Then, the nutrient agar plates was closed with the cover and sealed with parafilm. Then, the agar plate was incubated at 30°C for 24 hours. Make sure the agar plate was incubated in an inverted position to prevent the condensate from smearing the colony.

Figure 3.11 *S. Cerevisiae* Culture on Nutrient Agar Plate

3.14.6 Preparation of Inoculums

Inoculums are a very important process if the fermentation process is running at small scale. This is to ensure a consistent initial cell density for each batch of experiment in order to obtain a consistent reproducibility of product. The inoculum was done by taking two loops full of the prepared culture of *S. Cerevisiae* on the nutrient agar plate and transferring into sterilized 500mL conical flask which contains 200mL nutrient broth using the aseptic technique. Next, the conical flask containing the *S. Cerevisiae*

and nutrient broth was incubated at 30°C and 150rpm for 24 hours. After 24 hours, the optical density (OD) of the cell suspension was found at 660nm. The final cell suspension with OD value of 0.5 will be used as the inoculums for subsequent work (10% of the working solution). If the OD of cell suspension is larger than 0.5, more nutrient broth will be put in the solution. Meanwhile if the OD of the cell suspension is less than 0.5, the solution will be left to incubate further until the OD achieve 0.5.

3.14.7 Fermentation Process

The fermentation will be carried out by using Kundur juice with *S. Cerevisiae* and Kundur juice with *S. Cerevisiae* and yeast extract as a nutrient supplement. These two fermentations were carried out in duplicate (A and B). The working volume for the fermentation process is 200mL. Firstly for the fermentation of Kundur juices with *S. Cerevisiae*, 20mL (10% of the working solution) inoculums of *S. Cerevisiae* having an OD of 0.5 were transferred to 180mL Kundur juice by using aseptic technique. As for the fermentation of Kundur juice with *S. Cerevisiae* and yeast extract as a supplement, 20mL (10% of the working solution) inoculums of *S. Cerevisiae* having an OD of 0.5 and 2g (1%w/v) of yeast extract were transferred into 178mL of Kundur juices. The pH of all solution adjusted until pH 5.5 is achieved by using sodium hydroxide (NaOH) and hydrochloric acid (HCl). The control was prepared by transferring 20mL (10% of the

working solution) inoculums of *S. Cerevisiae* having an OD of 0.5 into 180mL nutrient broth. All the process was carried out by using aseptic technique. After that, all the conical flasks were placed in an incubator shaker at 30°C and 200rpm for 72 hours. The samples will be taken for each 6 hours interval. The samples were kept at 4°C chiller until used. The samples were analyzed for the biomass generation, substrate consumption and ethanol production. Substrate consumption will be analyzed by using DNS calorimetric method.

3.15 **Cell Mass Determination**

The dry cell mass of the sample is determined by using drying method. First of all, the empty centrifuge tubes were weighed. Then, 10mL samples were put in the centrifuge tubes and centrifuged at 10,000rpm for 10minutes. The supernatant were collected for further analysis of glucose and ethanol production. Then, the cell was re-suspended with 10mL distilled water. The solution was mixed thoroughly by using vortex until the entire cell is dissolved. This step was called as a cell washing. After that, the solutions were centrifuged again at the same condition. Then, the supernatant were decanted. The cells were dried by using oven at 60°C for 24 hours. The dried cells were then weighed. The cell mass will be obtained by subtracting the weight of final cell dried with the weight of empty centrifuge tube.

3.16 **Preparation of Standard Calibration Curve for Ethanol**

Standard calibration curve for ethanol is needed in order to find the concentration of ethanol of the sample tested. A 5ml of 15 different ethanol concentrations were prepared from pure ethanol having a purity of 99%. The preparation of all the concentrations was shown as in **Table 3.3**.

Table 3.3 Preparations for ten concentrations of standard calibration curve for ethanol

Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Concentration (mg/ml)100%	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Volume of 99% ethanol solution (ml)	0.05	0.1	015	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75
Volume of solvent, Distilled H_2O (ml)	4.95	4.90	4.85	4.80	4.75	4.70	4.65	4.60	4.55	4.50	4.45	4.40	4.35	4.30	4.25
TOTAL (ml)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

After that, the ethanol were determined the by using Pocket Refractometer. The graph of Refractometer Brix against concentration of ethanol (%) was plotted as in **Figure 3.12.**

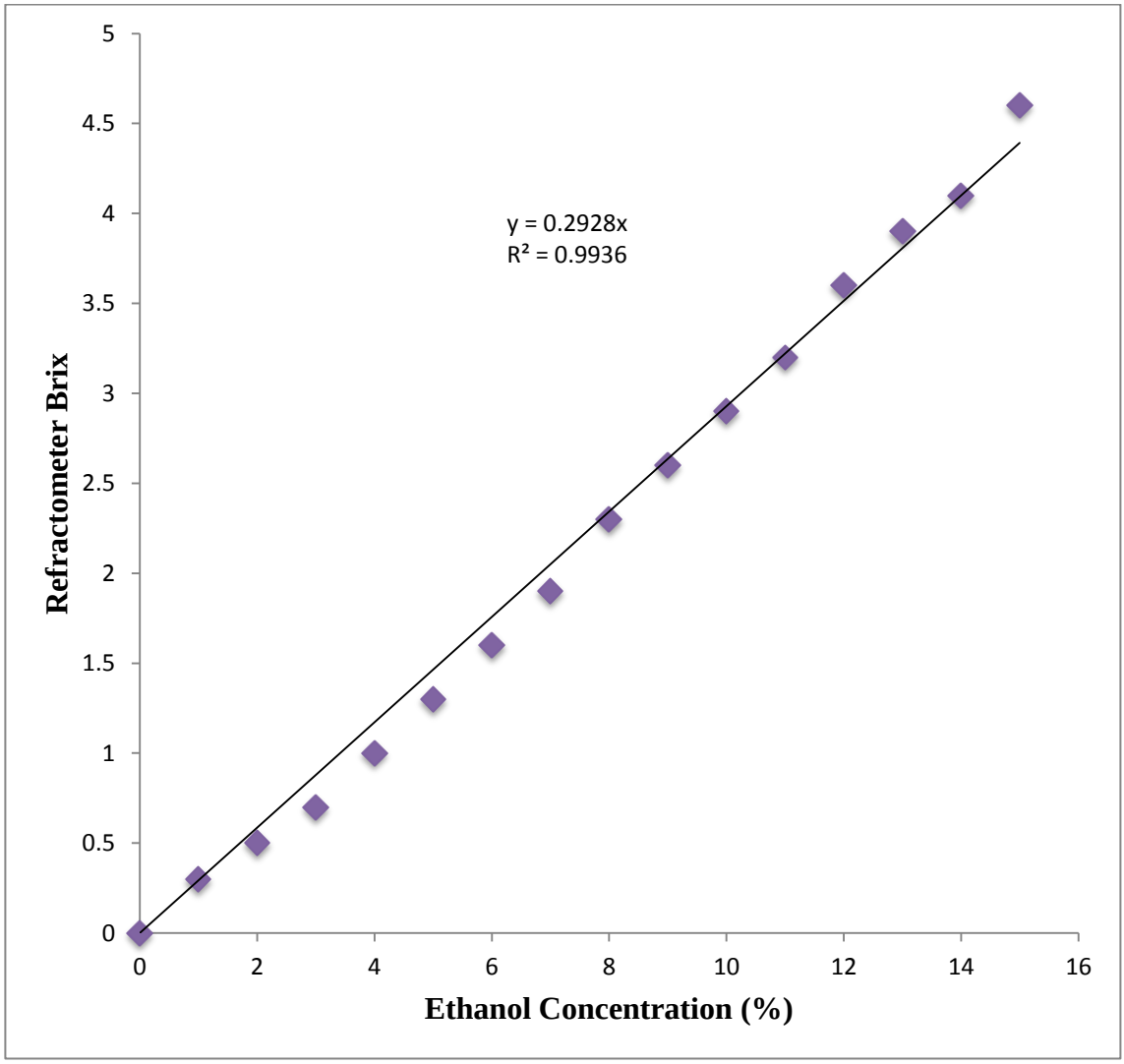

Figure 3.12: Effect of Ethanol concentration (%) on Refractometer Brix

3.17 **Ethanol Determination**

The supernatant collected from the cell mass determination were using for determination of ethanol production by using Pocket Refractometer. The Refractometer was calibrated by adding distilled water on the prism surface until a concave surface is obtained. Then, the start button was pressed and the reading will be obtained. As for the calibration, the RI reading for the distilled water must be 0.0. After that, the distilled water is sucked off from the prism surface by using a dropper. Then, the prism surface was dried by using clean tissue. Ensure that, the prism surface is fully dried before continue for other test. The samples were then added on the prism surface. Then, the reading of the samples were observed and recorded. After that, the sample was sucked off by using dropper and the surface prism was dried by using tissue. Next, the surface prism were cleaned by adding distilled water and the Brix reading that must be achieved was 0.0 before continue for testing another sample in order to obtain accurate reading for concentration of ethanol. These steps were repeated for testing other samples.

Figure 3.13 Pocket Refractometer

3.18 **Kinetic Model**

The model below employs rate of equations for biomass (X), ethanol (P) and glucose (S) to describe the fermentation process. Experimental findings suggest a high degree of dependence of biomass growth on glucose. The dependence of specific growth rate on glucose was assumed to follow the Monod kinetic model, which considers substrate limitation at low concentration. The biomass growth rate can be described as

$$\frac{dX}{dt} = \mu X \tag{3.2}$$

Where the specific growth rate μ (h^{-1}) is given by the Monod type model as

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{3.3}$$

Where

X = biomass concentration (g/L)

μ_{max} = maximum growth rate (h^{-1})

S = substrate concentration (g/L)

K_s = Monod constant. K_s can be obtained when μ is equivalent to half of μ_{max}.

The glucose consumption can be described by

$$\frac{dS}{dt} = -\frac{\mu X}{Y_{X/S}} \tag{3.4}$$

Where $Y_{X/S}$ is the biomass yield (g biomass/g glucose)

$$Y_{X/S} = -\frac{\Delta X}{\Delta S} \tag{3.5}$$

The ethanol formation can be described by

$$\frac{dP}{dT} = q_P X \tag{3.6}$$

Where q_P is the specific product formation rate (h^{-1})

$$q_P = Y_{P/X}\mu \tag{3.7}$$

Where $Y_{P/X}$ is product yield (g product/g biomass)

$$Y_{P/X} = -\frac{\Delta P}{\Delta X} \tag{3.8}$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter discusses the characteristics of Kundur part such as reducing sugar, moisture contents and carbohydrate in order to find out the suitability of Kundur as a raw material for ethanol production. The glucose consumption, specific growth rate and theoretical yield of ethanol were calculated by Monod type model for the whole experiments.

4.2 Composition of Moisture Contents in Kundur

The composition of moisture in Kundur parts were determined by using equation (3.1) as stated in **Chapter 3.5**. **Table 4.1** shows the moisture contents in Kundur seeds, pulps and fleshes after drying in the microwave oven at 60°C for 72 hours. Meanwhile **Figure 4.1** shows the comparison of moisture contents between Kundur parts.

Table 4.1 Moisture Contents of Kundur Parts

Parts	Moisture Contents (% w/w)
Seeds	77
Pulps	86
Fleshes	97

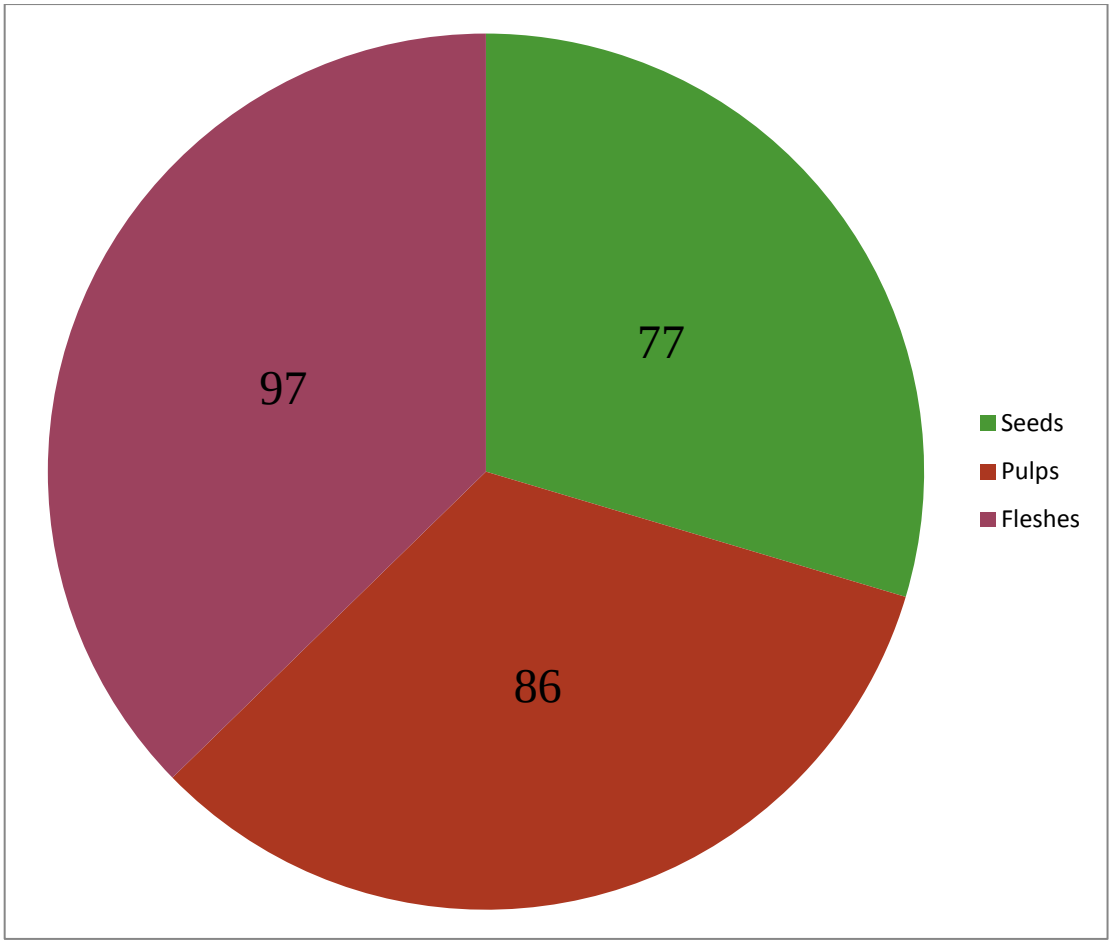

Figure 4.1 Comparisons of Moisture Contents between Kundur Parts

Based on **Figure 4.1**, we can see that fleshes have higher moisture contents which are 97% than pulps and seeds. Seeds have the lowest moisture contents which are 77%. Generally, flesh contains higher moisture contents rather than seed and pulps. Besides, there is no other finding on the moisture contents of Kundur parts that we can compared to. The moisture content of Kundur parts is a new finding.

4.3 Reducing Sugar Determination

The amount of reducing sugar (expressed as glucose) contents in Kundur parts were determined in order to identify which parts contains high amount of reducing sugars. Part that has the highest amount of reducing sugar was used as substrate in the fermentation process for the production of ethanol. The data obtained were tabulated in **Table 4.2** that shows the amount of reducing sugar in Kundur parts while **Figure 4.2** shows the comparison between the reducing sugar contents with Kundur parts.

Table 4.2 Reducing Sugar Contents in Kundur Parts

Parts	Reducing sugar Contents (g/L)
Seeds	0.924
Pulps	0.796
Fleshes	2.909

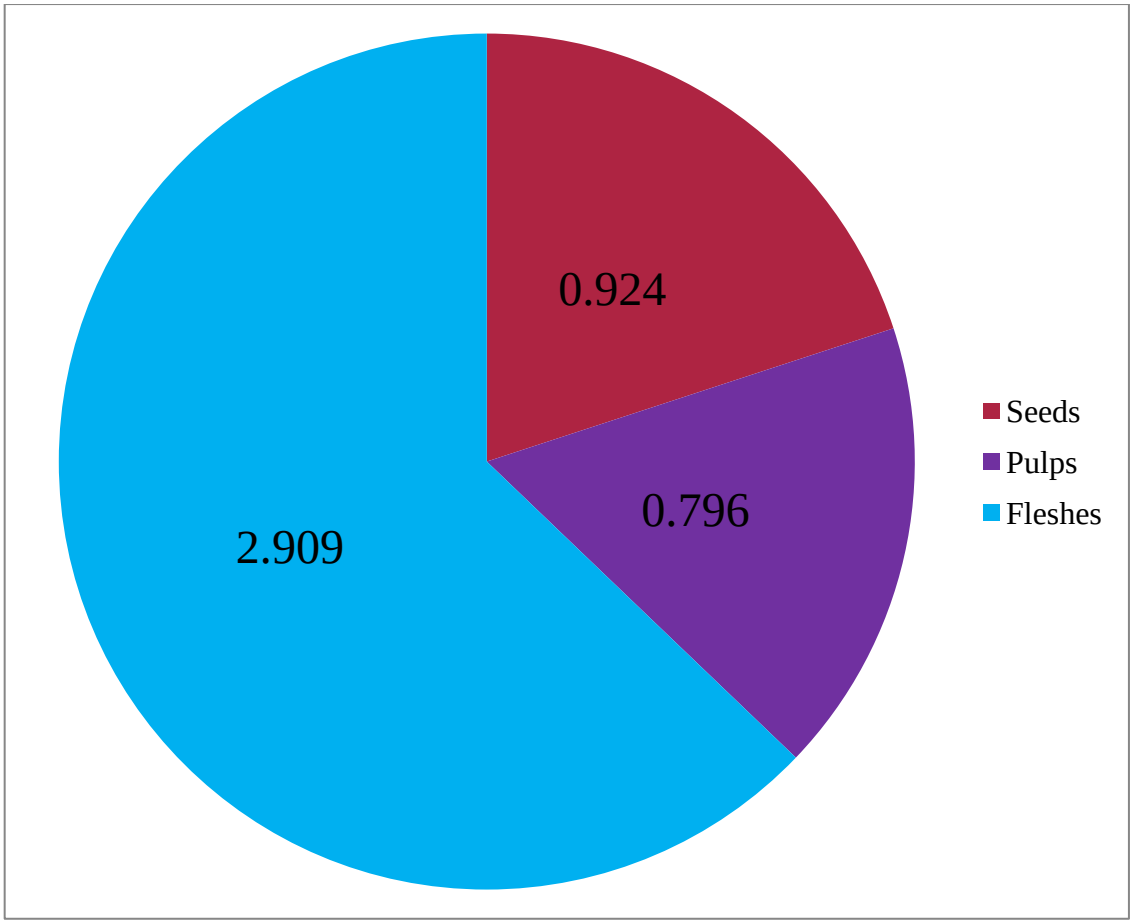

Figure 4.2 Comparisons of Reducing Sugar (Glucose) Contents between Kundur Parts

According to **Figure 4.2**, it is shown that Kundur fleshes contains highest amount of reducing sugar which is 2.909g/L. Meanwhile, the part that has the lowest amount of reducing sugar is Kundur pulps which having 0.796g/L reducing sugar compared to Kundur seeds which having 0.924g/L reducing sugar. Since, the amount of reducing sugar content in the fleshes is the highest; Kundur fleshes are used as a substrate for the fermentation process for producing ethanol in term of juice.

4.4 Composition of Carbohydrate in Kundur

The amount of carbohydrate in Kundur parts were determined by using Phenol-Sulphuric Acid Method. **Table 4.3** shows carbohydrate contents in Kundur parts while **Figure 4.3** represents the comparison between carbohydrate contents with Kundur parts. Carbohydrate is the main source for ethanol production since glucose is contains in the carbohydrate which easily found in various plant-parts. According to Dake et al., (2010), natural resources along with *S. Cerevisiae* are the highest bidders for the commercial production of ethanol.

Table 4.3 Carbohydrate Contents in Kundur parts

Parts	Carbohydrate Contents (g/L)
Seed	33.333
Pulp	13.103
Flesh	79.885

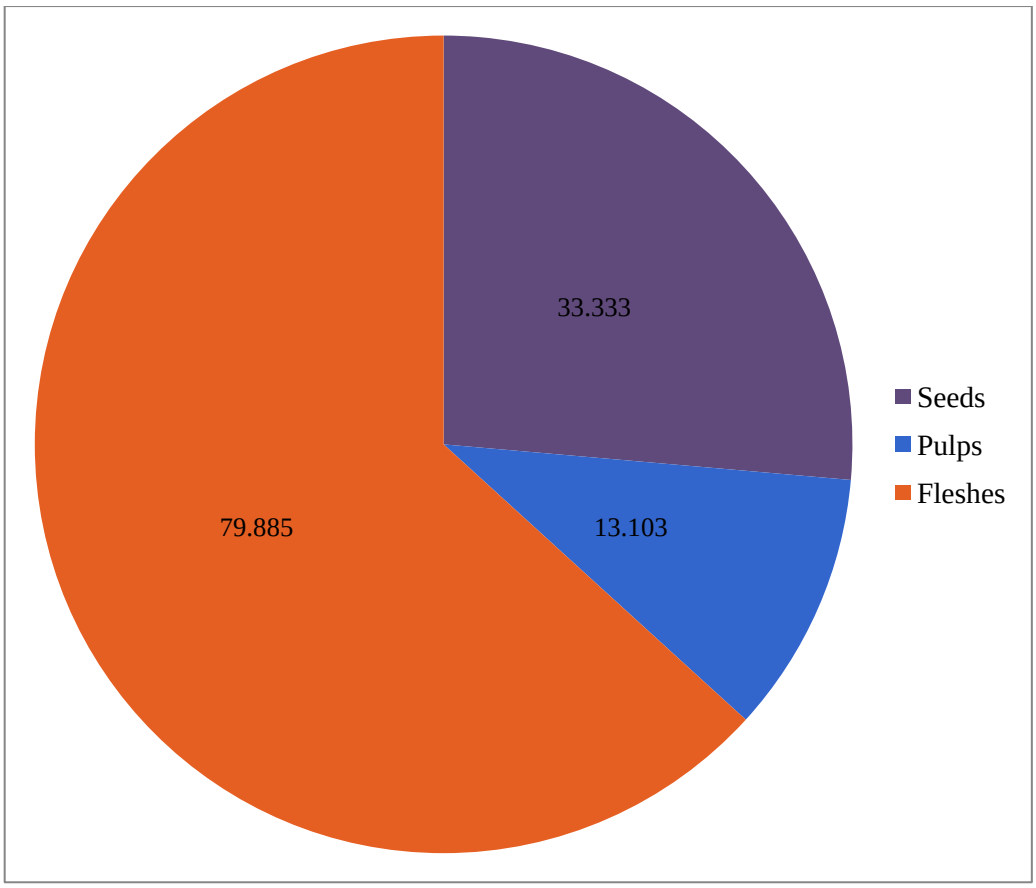

Figure 4.3 Comparisons of Carbohydrate Contents between Kundur Parts

Based on **Figure 4.3**, carbohydrate contents in Kundur fleshes is the highest which is 79.885g/L. The carbohydrates contents in Kundur parts range from fleshes, seeds and pulp. Kundur seed having 33.333g/L carbohydrates higher than Kundur pulp which having the lowest contents of carbohydrates. The amount of carbohydrate in Kundur pulps is 13.103g/L. The determination of carbohydrate contents for Kundur parts is the new finding, hence there are no other previous study that we can compared to.

4.5 Fermentation Performance

Kinetic model for this study is finding to describe the fermentation process. The kinetic model that was used in this study is Monod type model given by Bailey and Ollis, (1986). The fermentation was carried out by using Kundur juice as a raw material and *S. Cerevisiae* as yeast. The batch fermentation in shakes flasks for ethanol production was carried out in duplicate for 72 hours. **Figure 4.4**, **Figure 4.5** and **Figure 4.6** show the biomass production, substrate consumption and ethanol production respectively.

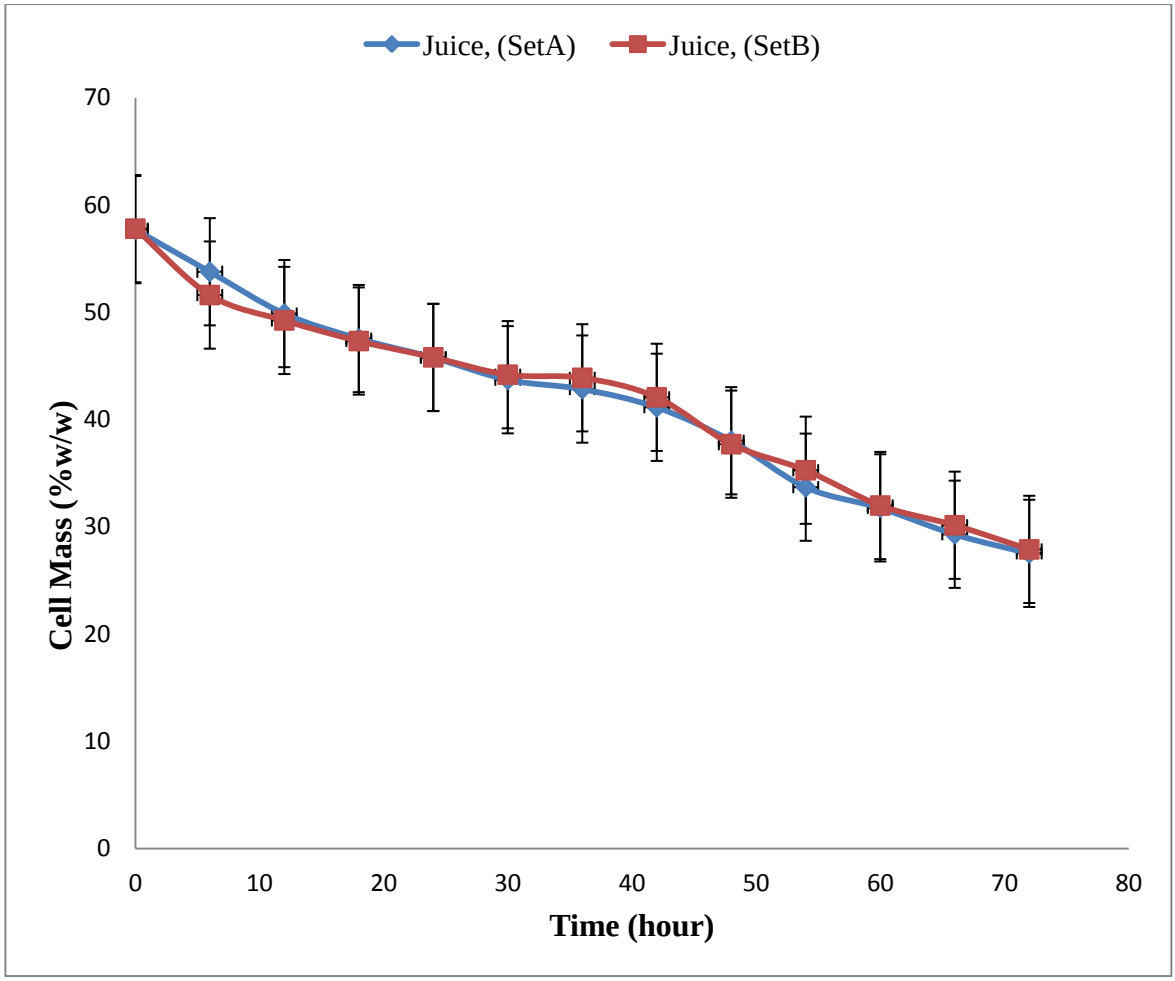

Figure 4.4 Cell Mass Generations for Kundur Juice Fermentation against Time

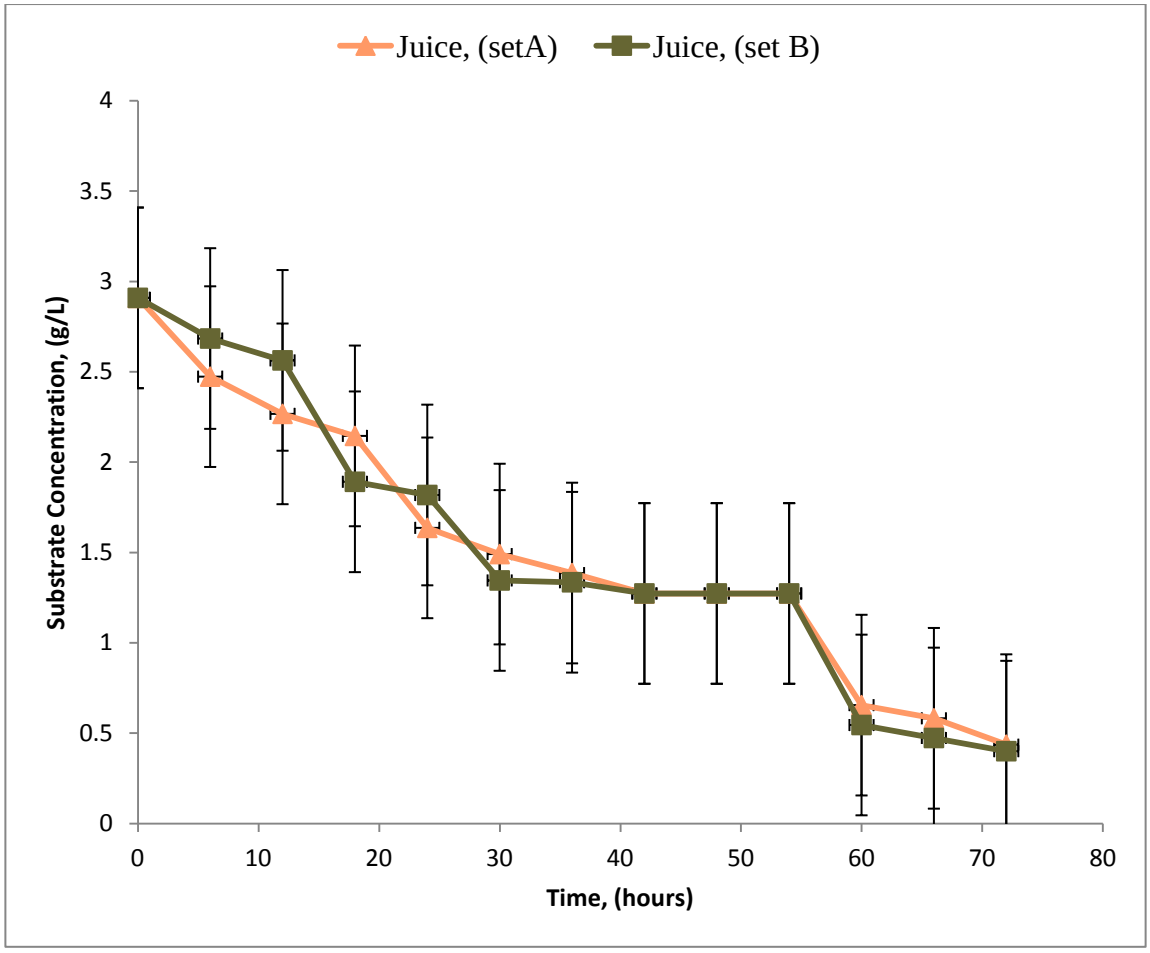

Figure 4.5 Substrate Concentrations for Kundur Juice Fermentation against Time

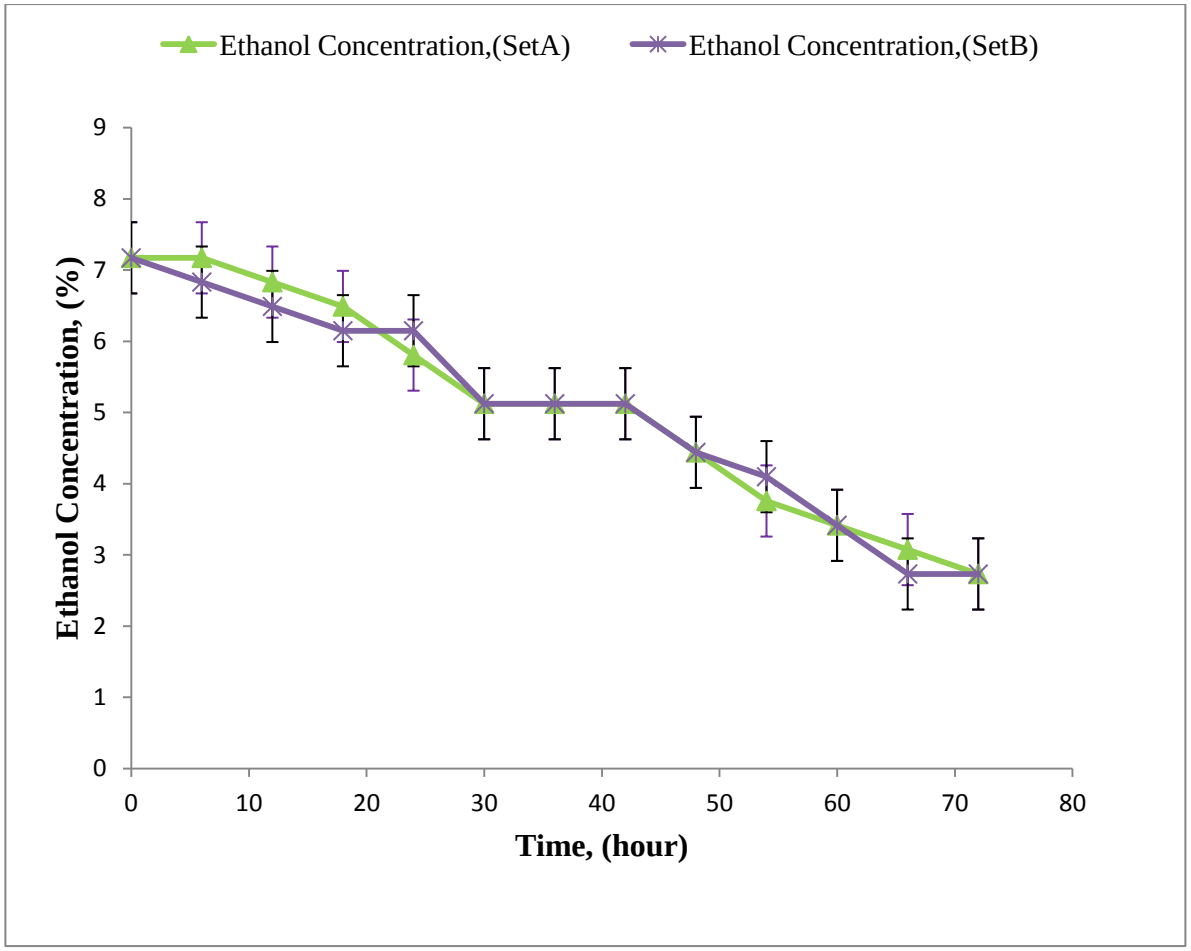

Figure 4.6 Ethanol Productions for Kundur Juice Fermentation against Time.

Figure 4.4 shown that the cell mass generation for both set decreased as a time of fermentation increased. It is expected that the cell mass generation will be increased as a time of fermentation increased until a stationary phase is achieved due to the cell growth during the fermentation period. Unfortunately, the result obtained from the fermentation process is against the expected results. The cell growth is inhibited maybe due to the insufficient amount of nutrient which is the low amount of glucose in the Kundur juice. The cell consume the glucose just to maintain their live but not for growth. The cells were competing between each other to obtain the substrate to survive.

Therefore, the amount of cell will be decreased with time due to the lack of substrate. Although, the result obtained is not satisfied the expected result, the specific growth rate for both processes were still finds. The specific growth rate for fermentation by using Kundur juice without any nutrient supplements for set A is $-0.015h^{-1}$ while for set B is $-0.0148h^{-1}$. The specific growth rate for both processes is approximately differences with an only error of 1.3%. This shows that, fermentation process for both set does not deviate from each other. Another kinetic parameter for both processes can be seen in **Appendix A**.

As we can see from **Figure 4.5**, the substrates were consumed during the fermentation period for both processes. Both sets of the experiments show the substrate concentration of 1.273g/L at 42 hours. This indicates that the substrates were used by the *S. Cerevisiae* as a carbon sources for their growth. It expected that the ethanol produce is increased with time because the substrate is consumed during the process. The ethanol percentage depends on sugars which is present in the juice. In contrast, the results obtained for the cell mass generation shows otherwise. This shows that, the cell growth of *S. Cerevisiae* is inhibited during the process. Since the cell growth is inhibited during the process, the ethanol productivity will be loss. It is proved from **Figure 4.6** which shows that the ethanol produce decreased with time.

As we can see from **Figure 4.6** there are a presence of ethanol at time 0 hours. Generally, there are no ethanol presences at time 0 hours but the results obtained shows otherwise. This indicates that there is a presence of ethanol in Kundur juice even without fermentation process. This is maybe due to the possibility that Kundur juice is contaminated. The contaminate Kundur juice maybe having other bacteria which

produce ethanol. So that, when checking for ethanol concentration at time 0 hours we obtained that there is a presence of ethanol. Besides, as we discussed before the cell growth is depleted during the process due to the lack of nutrient which will causes the ethanol concentration to decrease. This is because the cells consume glucose just to maintain their live instead of converting the glucose to ethanol. Therefore, the ethanol produce will be loss with time. Apart from that, the other factor is the side products produced during the fermentation process inhibits the growth of *S.Cerevisiae* and causes the ethanol to loss.

4.6 Effect of Nutrient Supplement

Despite from the fermentation process by using Kundur juice, another fermentation process was carried out by adding 1% yeast extract as a nutrient supplement for the growth of *S. Cerevisiae*. The fermentation process was also carried out in duplicate by batch fermentation in shake flasks in 72 hours. The results obtained were illustrated in **Figure 4.7, 4.8 and 4.9** which shows the comparison of the cell mass generation, substrate consumption and ethanol production for the fermentation process with and without nutrient supplement.

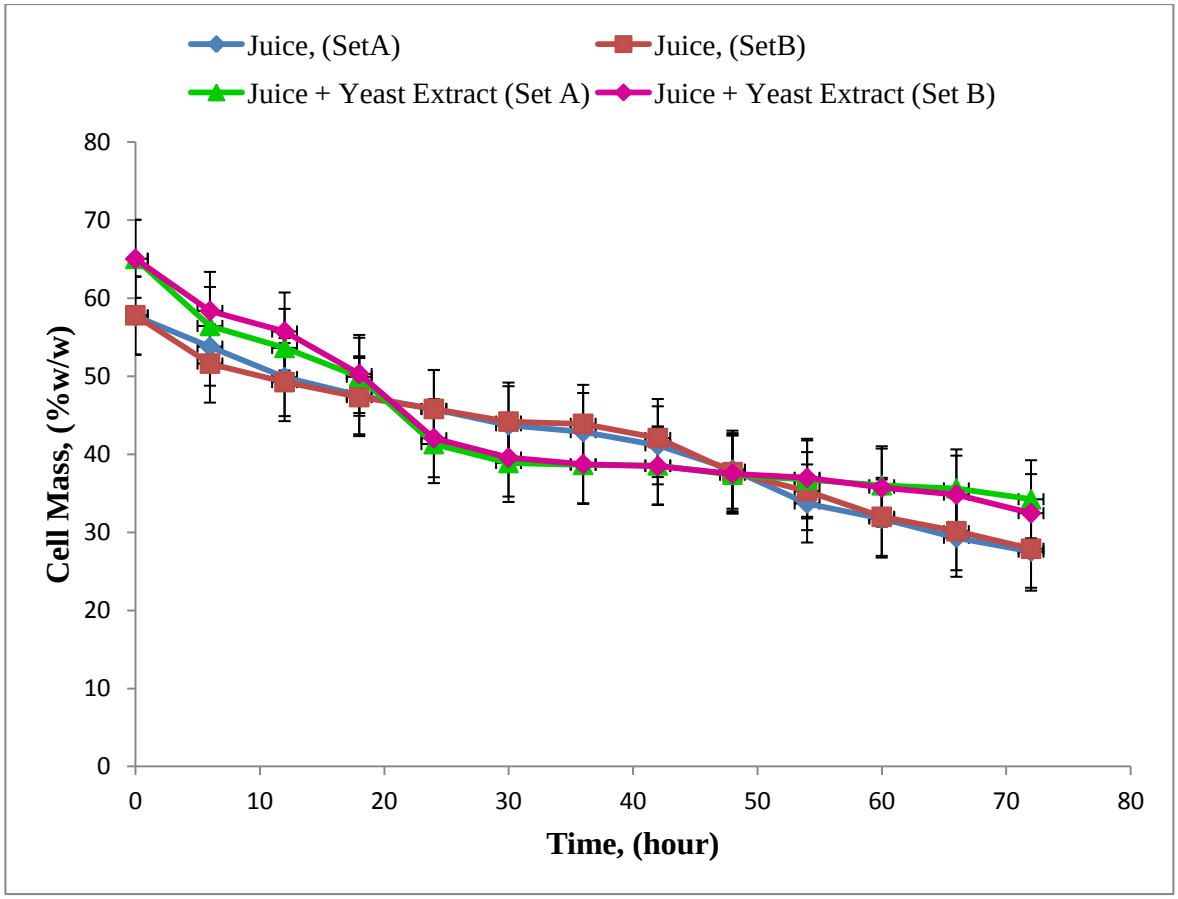

Figure 4.7 Effect of Nutrient Supplement on Cell Mass Generation

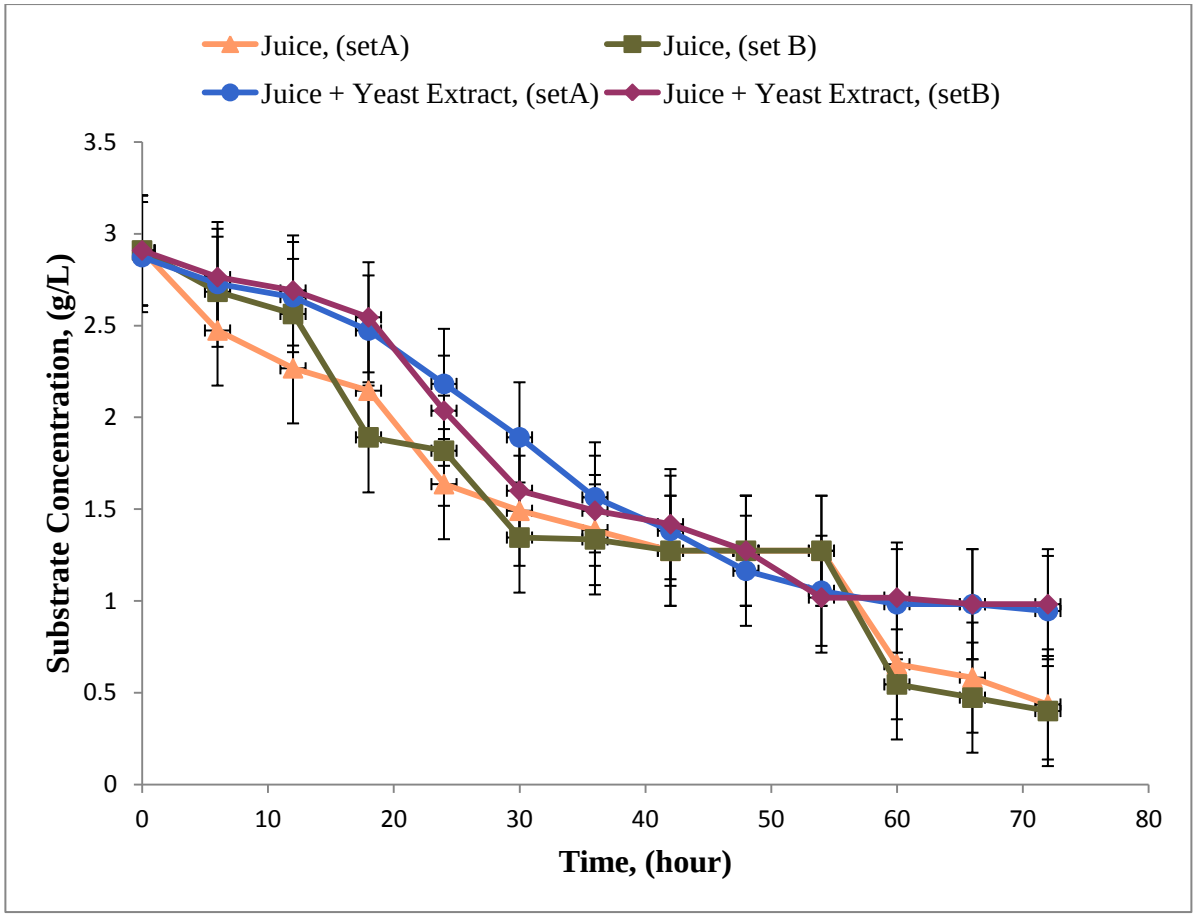

Figure 4.8 Effect of Nutrient Supplement on Substrate Consumption

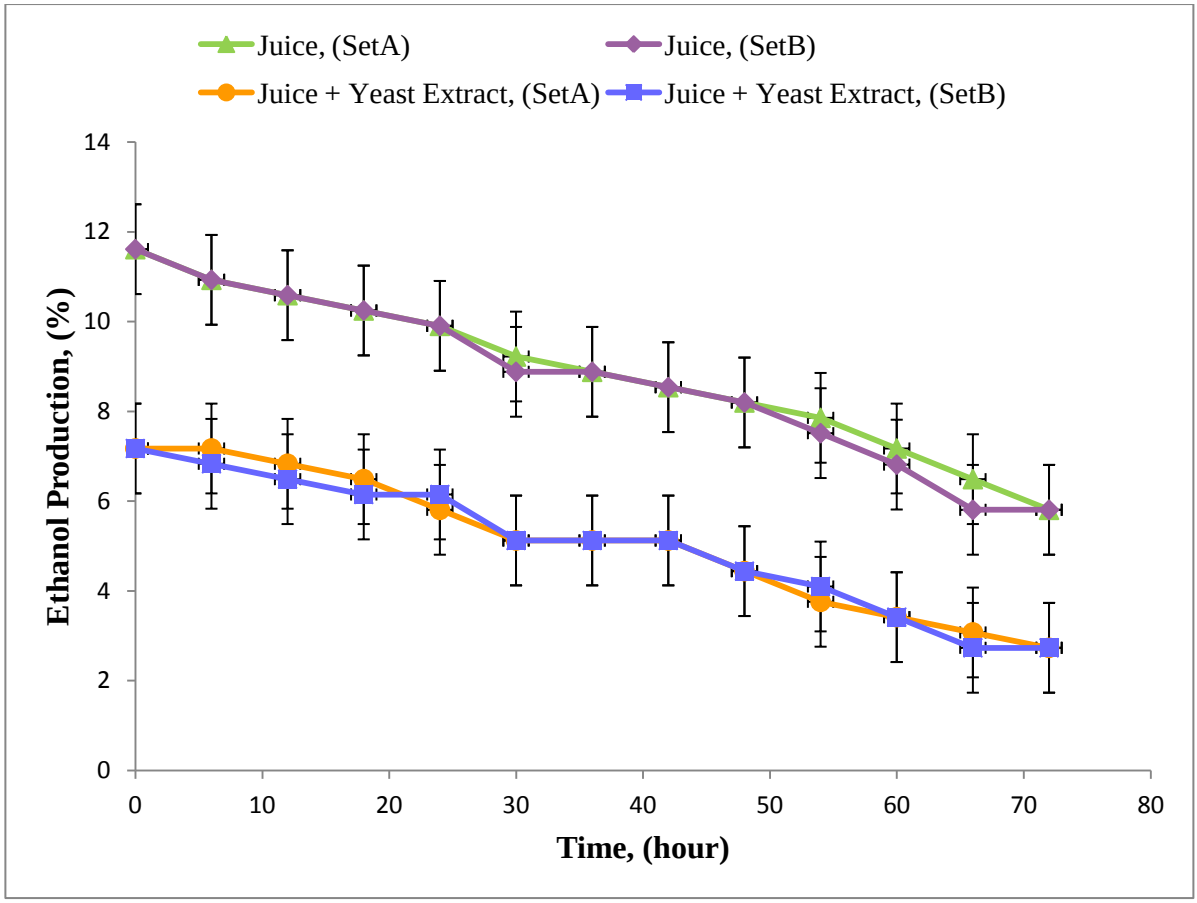

Figure 4.9 Effect of Nutrient Supplement on Ethanol Production

Based on **Figure 4.7, 4.8 and 4.9**, we can see that the supplementation of nutrients significantly increased the ethanol concentration and fermentation rate. The addition of yeast extracts increased the initial concentration of ethanol which is 11.612% instead of 7.172% respectively. The increased of initial ethanol production by addition of yeast extract maybe because of the presence of other bacteria in the contaminated Kundur as discuss before in **Chapter 4.5.** The yeast extract added will be consumed by the bacteria and the bacteria will produce more ethanol. The reaction can be said as fast reaction because the ethanol produce at time 0 hours is increased by the addition of yeast extract. This indicates that the bacteria consume the yeast extract instants after the yeast

extract is added and converting glucose to ethanol. Therefore the concentration of ethanol increased. Although, yeast extract was added in order to supply nutrients for yeast growth, the ethanol produced still loss. The cell mass generation and the ethanol production decreased as the fermentation time increase. The specific growth rate for both fermentation process of Kundur juice with yeast extract as a nutrient supplement is same which is -0.017 h^{-1}. The other kinetic parameter for the fermentation process with nutrient supplement can be seen in **Appendix A**.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The presented results demonstrated that the Kundur fleshes have the highest amount of moisture contents; 97 %(w/w), reducing sugar (glucose); 2.909g/L and carbohydrate; 79.885g/L. Since Kundur fleshes have the highest amount of glucose, Kundur juices were used for the fermentation process. Based on the results obtained, it is shows that ethanol loss corresponding to the fermentation period for the fermentation process with or without nutrient supplement. Despite from the ethanol loss, the additions of yeast extract as nutrient supplement increase the fermentation rate and initial

concentration of ethanol which is 11.612% instead of 7.172%. The ethanol yield for the fermentation process without nutrient supplement is in the range of -0.192~ -0.194(g ethanol/g biomass). As for the fermentation process with nutrient supplement, the ethanol yield is in the range of -0.136~ -0.1442(g ethanol/g biomass). Hence, as the ethanol yield is negative, we can conclude that Kundur juice is not suitable for ethanol production.

5.2 Recommendation

From the research done, there are some recommendations that should be concern and understand to improve the research in the future. The recommendations are as follows:

1) Researched regarding the cause of ethanol loss during the fermentation process by using Kundur juice should be carried out in order to find the reasons of the ethanol loss.
2) Researched on the parameter that can enhanced the ethanol yield for the fermentation process by using Kundur juice should be carry out.
3) Study on the fermentation process for ethanol production by using Kundur waste since the fruits is mainly used in food and medical purpose.

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APPENDIX A KINETIC PARAMETER FOR FERMENTATION PROCESS

i. Fermentation Process without Nutrient Supplement

Table A.1 Kinetic Parameter for Fermentation Process without Nutrient Supplement

Parameter		Set A	Set B
$Y_{x/s}$	(g biomass/g substrate)	-12.21	-11.92
$Y_{p/x}$	(g ethanol/ g biomass)	-0.192	-0.194
q_p	(h^{-1})	0.00289	0.00287

ii. Fermentation Process with Nutrient Supplement

Table A.2 Kinetic Parameter for Fermentation Process with Nutrient Supplement

Parameter		Set A	Set B
$Y_{x/s}$	(g biomass/g substrate)	-15.98	-16.91
$Y_{p/x}$	(g ethanol/ g biomass)	-0.1442	-0.1363
q_p	(h^{-1})	0.00245	0.00232

APPENDIX B STANDARD DEVIATION FOR FERMENTATION PROCESS

1. Cell Mass Generation

i. Fermentation Process without Nutrient Supplement

Table B1 Standard Deviation of Cell Mass Generation for Fermentation Process without Nutrient Supplement

Time (hour)	Juice, (SetA)	Juice, (SetB)	STDEV
0	57.71	57.8	0.06363961
6	53.78	51.61	1.534421715
12	49.88	49.24	0.45254834
18	47.55	47.32	0.16263456
24	45.78	45.8	0.014142136
30	43.71	44.18	0.332340187
36	42.84	43.89	0.74246212
42	41.14	42.07	0.657609307
48	38.02	37.7	0.22627417
54	33.69	35.27	1.117228714
60	31.76	31.98	0.155563492
66	29.3	30.14	0.593969696
72	27.52	27.89	0.261629509
		Average	0.485727966

ii. Fermentation Process with Nutrient Supplement

Table B.2 Standard Deviation of Cell Mass Generation for the Fermentation Process with Nutrient Supplement

Time (hour)	Juice + Yeast Extract (Set A)	Juice + Yeast Extract (Set B)	STDEV
0	65.03	65.03	0
6	56.43	58.36	1.364716
12	53.62	55.72	1.484924
18	49.92	50.28	0.254558
24	41.3	42.05	0.53033
30	38.88	39.57	0.487904
36	38.63	38.72	0.06364
42	38.57	38.49	0.056569
48	37.38	37.5	0.084853
54	36.77	36.98	0.148492
60	36.02	35.72	0.212132
66	35.62	34.82	0.565685
72	34.23	32.45	1.25865
		Average	0.500958

2. Substrate Consumption

i. Fermentation Process without Nutrient Supplement

Table B.3 Standard Deviation of Substrate Consumption for Fermentation Process without Nutrient Supplement

Time (hr)	Juice, (setA)	Juice, (set B)	STDEV
0	2.909	2.909	0
6	2.473	2.684	0.149199531
12	2.267	2.563	0.209303607
18	2.145	1.891	0.179605122
24	1.636	1.818	0.128693434
30	1.491	1.345	0.10323759
36	1.386	1.335	0.036062446
42	1.273	1.273	0
48	1.273	1.273	0
54	1.273	1.273	0
60	0.655	0.545	0.077781746
66	0.582	0.473	0.077074639
72	0.436	0.4	0.025455844
		Average	0.075877997

ii. Fermentation Process with Nutrient Supplement

Table B.4 Standard Deviation of Substrate Consumption for Fermentation Process with Nutrient Supplement

Time (hour)	Juice + Yeast Extract, (setA)	Juice + Yeast Extract, (setB)	STDEV
0	2.873	2.909	0.025455844
6	2.727	2.764	0.026162951
12	2.655	2.691	0.025455844
18	2.473	2.545	0.050911688
24	2.182	2.036	0.10323759
30	1.891	1.6	0.205768073
36	1.564	1.491	0.051618795
42	1.382	1.418	0.025455844
48	1.164	1.273	0.077074639
54	1.055	1.018	0.026162951
60	0.982	1.018	0.025455844
66	0.982	0.982	0
72	0.945	0.982	0.026162951
		Average	0.051455617

3. Ethanol Production

i. Fermentation Process without Nutrient Supplement

Table B.5 Standard Deviation of Ethanol Production for Fermentation Process without Nutrient Supplement

Time (hour)	Juice, (SetA)	Juice, (SetB)	STDEV
0	7.172	7.172	0
6	7.172	6.831	0.241123
12	6.831	6.489	0.241831
18	6.489	6.148	0.241123
24	5.806	6.148	0.241831
30	5.123	5.123	0
36	5.123	5.123	0
42	5.123	5.123	0
48	4.44	4.44	0
54	3.757	4.098	0.241123
60	3.415	3.415	0
66	3.074	2.732	0.241831
72	2.732	2.732	0
		Average	0.111451

ii. Fermentation Process with Nutrient Supplement

Table B.6 Standard Deviation of Ethanol Production for Fermentation Process with Nutrient Supplement

Time (hour)	Juice + Yeast Extract, (SetA)	Juice + Yeast Extract, (SetB)	STDEV
0	11.612	11.612	0
6	10.929	10.929	0
12	10.587	10.587	0
18	10.246	10.246	0
24	9.904	9.904	0
30	9.221	8.88	0.241123412
36	8.88	8.88	0
42	8.538	8.538	0
48	8.197	8.197	0
54	7.855	7.514	0.241123412
60	7.172	6.813	0.253851334
66	6.489	5.806	0.482953932
72	5.806	5.806	0
		Average	0.093773238