A Saccharification Study on Various Non-Food Biomasses for Fermentation to Ethanol

by

Nur ‘Amirah Binti Hassan

Dissertation submitted in partial fulfilment of

the requirements for the

Bachelor of Engineering (Hons)

(Chemical Engineering)

SEPTEMBER 2012

Universiti Teknologi PETRONAS

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CERTIFICATION OF APPROVAL

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Approved by,

_____

(Dr Shuhaimi Bin Mahadzir)

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TRONOH, PERAK

September 2012

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.

_____

NUR ‘AMIRAH BINTI HASSAN

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim

Praise is to Allah, The Most Gracious and The Most Merciful for His endless blessings throughout life and the success He granted along this project.

Author's grateful thanks go to her parents and friends that give her endlessly support during the learning process and progression of the project. Their supports in believing the author to complete the project are very much appreciated.

The special thank goes to her supervisor, Dr Shuhaimi b Mahadzir (Chemical Engineering Department) who found time in a very busy schedule to supervise, assist and monitor the author's progress. His passion inspired me. The author also deeply grates for his advice, opinion, encouragement and patience throughout the duration of this project. The support he has given as an expatriate in a predominantly while doing this project.

The appreciation is also extended to Mr Asnizam (Technologist, Block 3), Mr Khairul Hafizan (Technologist, Block 3) and technichians of Chemical Engineering Department for their attention and time. The author have very much benefited from their professional assistance and advices.

Last but not least, endless thanks to those who helped author directly or indirectly for their guidance, sharing and support throughout the entire two semesters. Their help are highly appreciated.

Hopefully this project will be a great contribution to the industry and the society.

ABSTRACT

Nowadays, biomass is an increasingly popular source for alternative energy. Ethanol is one of the alternative renewable fuels made from various plant materials. It can be made out of feedstock through a chemical process known as fermentation which is either from starch-based crop or cellulosic materials. This study is conducted to ferment cellulosic material which is quite challenging in term of structural wall compared to starch-based material. Glutinous rice is starch-based material while thatch, palm oil frond, and banana branch are used as cellulosic materials. The cell structure of starch-based and cellulosic materials are different which cellulosic material composed of molecule called lignocelluloses (cellulose, hemicelluloses and lignin). Due to this structure, several pre-treatment processes are investigated to break down lignocellulose into glucose chains. The processes used are thermal composition, microwave, and ultrasound. Refractometer is used to test the concentration of ethanol from the fermentation process. The sample of fermentation contains ethanol when its refractive index is in range of 1.3357 to 1.3642. Glutinous rice undergoes rapid fermentation day by day and produces 57.6% and 82.0% ethanol by using thermal and ultrasound, respectively. It shows that banana branch and palm oil frond have potential beside glutinous rice because it gives out 18.9% ethanol by thermal composition and 20.7% by ultrasound technique, respectively. Furthermore, ultrasound technique should be further studied as it shows good performance of fermentation compares to other techniques.

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CHAPTER 1
INTRODUCTION

1.1 Background

Ethanol, C_2H_6O or C_2H_5OH also called ethyl alcohol or grain alcohol is one of alcohol group and one of the most popular biofuels currently. It can be made out of feedstock through a chemical process known as ethanol fermentation. Any sugars which it has three types; glucose, fructose and sucrose convert into cellular energy can produce ethanol. Ethanol fermentation is anaerobic reaction because yeast performs the conversion in the absence of oxygen.

Fermentation is a natural process and the oldest chemical process that human used to make products such as wine, several type of food; cheese and *tapai*, flavoring, pharmaceuticals, and chemicals. It can also produce fuels. Therefore, fermentation is a metabolic process in which an organism converts a carbohydrate into an alcohol or an acid. There are three types of raw materials that can be used to make ethanol via fermentation; sugars, starches (need to be hydrolyzed to fermentable sugars by enzyme) and cellulose. Once simple sugars are formed, yeast enzymes can easily ferment them to ethanol.

Almost any plant-based material can be ethanol feedstock. All plants contain sugars, and these sugars can be fermented to make ethanol in a process called biochemical conversion. Even plant-based wastes can be made into ethanol for example thatch, banana branch and palm oil frond. Furthermore, nearly all ethanol derived from starch and sugar-based feedstock. Corn is the leading U.S. crop and serves as the feedstock for most domestic ethanol production. Small amounts of wheat, coco and sugarcane are used although the economics of these are not as favorable as corn. The Renewable Fuel Standard limits to 15 billion gallons production of ethanol from starch-based feedstock to ensure sufficient feedstock to meet demand in livestock feed, human food and export market. Therefore, we move on to cellulosic feedstock which are non-food based feedstock.

Cellulosic ethanol is a biofuel produced from wood, grasses, waste, or the inedible parts of plants. This biofuel derived from lignocelluloses, a structural material in plant which consists mainly of cellulose, hemicelluloses and lignin. Example of the major biomass materials being studied today are *Panicum virgatum* (switchgras), *Miscanthus*, corn stover, and wood chips.

Making ethanol from cellulosic feedstock is more challenging than using starch-based crops. One of the main pathways to produce cellulosic ethanol is biochemical. The biochemical process involves a pretreatment to release hemicelluloses sugars followed by hydrolysis to break cellulose into sugars. Sugars are fermented into ethanol and lignin is recovered and used to produce energy. Therefore, a further study needed to investigate the possible technique to break the cell wall of cellulosic materials.

Ethanol produced from non-food based feedstock is expected to improve energy balance of ethanol. This is due to non-food-based feedstock are anticipated to require less fossil fuel energy to produce ethanol. Biomass used to power the process of converting non-food-based feedstock into cellulosic ethanol is also expected to reduce the amount of fossil fuel energy used in production. Another advantage of cellulosic ethanol is it produces lower levels of greenhouse gas emissions. Thus, recently we are looking forward to use ethanol as the world's primary fuel before gasoline.

Equipment is used to test the product of reaction. Present of ethanol is tested by using refractometer which to check the present of ethanol and ethanol concentration produced.

1.2 Problem Statement

Recently, the fermentation of ethanol is widely used food-based as feedstock. Due to food is one of the main consumption of human, there is a need to use non-food material or cellulosic material as a feedstock. By finding other alternative of the feedstock, people do not depend on food to produce biofuel which prevent any crisis of insufficient food due to proactively producing fuel from food-based. Furthermore, a suitable technique needs to investigate when dealing with cellulosic material due to its structural material of

cell wall. Breaking down of cell wall or lignocelluloses is required to have high conversion of ethanol from the sugar inside cellulose.

1.3 Objectives

The objectives in this project are:

- To investigate different type of biomass feedstock for fermentation to ethanol
- To investigate the effect of different techniques to break the cell wall of cellulosic material on the yield of ethanol

1.4 Scope of Study

In order to complete this project, several scope of study is in need to achieve. This project will emphasize on the laboratory experiment on fermentation process of different non-food biomass. The experiment needs to be conducted successfully to obtain useful information regarding suitable technique of pre-treatment and which non-food biomass can achieve a high ethanol production. Suitable method and approach is designed and applied to conduct the experiment.

Apart from that, the project also covers the analysis on the finding of the experiments. The study will perform calculation on percentage ethanol concentration produced by their refractive index.

CHAPTER 2

LITERATURE REVIEW

2.1 Ethanol fermentation

Ethanol has been used since the dawn of civilization by the Arabs and Romans for industrially used in perfurmes, cosmetics and medicine (Miller,1975). However, nowadays ethanol is drastically produced to meet the demand of energy. Basically, ethanol is produced by spontaneous fermentation of sugar. There are two main process to produce ethanol which are fermentation and chemical synthesis. Fermentation is a chemical process in which an organism converts carbohydrate (starch or sugar) to alcohol or acid. On the other hand, the product of chemical synthesis is synthetic alcohol which is produced from ethylene, obtained from petroleum and natural gas.

Ethanol production is directly related to the starch or sugars in the raw material. Therefore, the conversion of starch and sugar to ethanol as showed below;

Equation1:
Carbohydrate -> simple sugar + glucose

Equation 2:
$C_6H_{12}O_6$ (glucose) -> C_2H_5OH (ethanol) + CO_2 (carbon dioxide) + H_2O(water)

Equation 1 is called pretreatment where the cell wall of materials needs to break down to extract simple sugar and glucose inside. Equation 2 is fermentation of glucose to ethanol and carbon dioxide and water are byproducts. Ethanol fermentation occurs in yeast (*Saccharomyces cerevisiae*) cell and it is an anaerobic process because it performs in the absence of oxygen. *Saccharomyces cerevisiae* acts as an enzyme to convert glucose to ethanol. A process to break complex carbohydrate into simple sugar also known as saccharification.

The fermentation occurs after 24 hours due to the bacterial population increases exponentially or having rapid growth on log phase (H.L. Smith). Graph below shows the bacteria growth curve of four different phases which is *Saccharomyces cerevisiae* acts as bacteria.

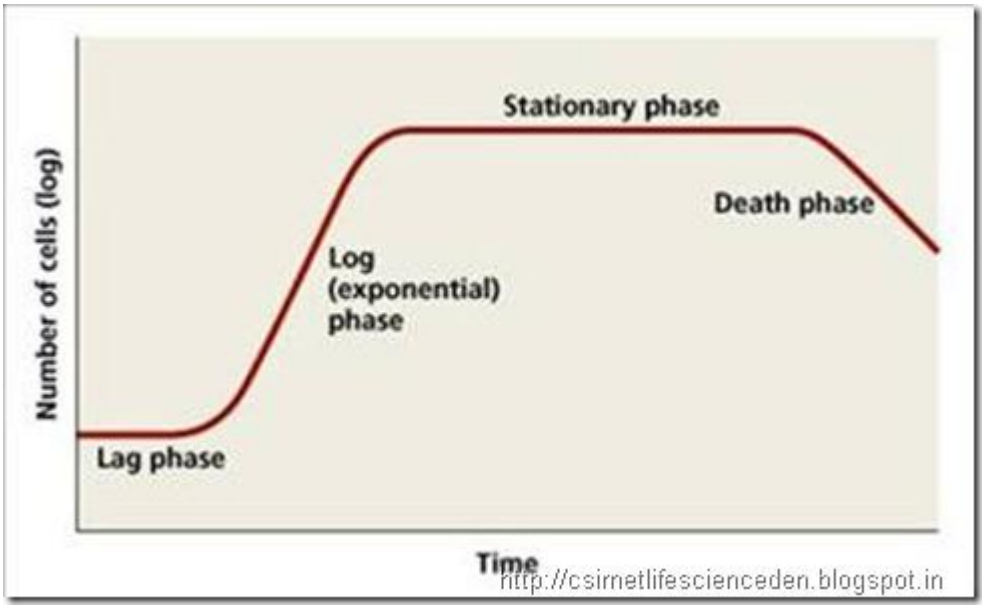

Figure 2.1: Bacterial growth curve

*(*Source: *http://csirnetlifescienceden.blogspot.com/2012/08/bacterial-growth.html#.UL_y2YNthOQ)*

In lag phase, it is an initial period with no growth of increasing cell number but it is metabolically active to repair cell damage and synthesize enzyme. The log phase is where rapid growth of bacterial population occurs. Next phase is stationary which no new growth of bacteria occurs. Lastly, is decline phase or also called death phase where all-microbial cell going to die.

2.2 Fermentation from biomass

In United States, about 90% of industrial ethanol and beverage is produced from cereal grains or from petroleum-based raw materials. Then, until 1929 all ethanol practically prepared from grains, molasses and materials high in starch or sugar. Due to increment of petroleum with much higher prices caused in interest for fermentation of natural product for industrial ethanol (Miller, 1975).

Cellulosic ethanol, a fuel produced from the stalks and stems of plants is starting to take root in the United States. Celunol, based in Cambridge, MA, broke ground on an ethanol

plant in Louisiana that will be able to produce 1.4million gallons of the fuel each year starting in 2008. A research done on profitable investments in cellulosic biofuels in US and EU due to emergence of a cellulosic of a cellulosic ethanol industry is unlikely without costly government subsidies, in part because of strong competition from conventional ethanol and limits on ethanol blending (Bruce et al., 2010).

Much of the existing literature on the future of cellulosic biofuels has focused on whether feedstock supplies will be sufficient to meet a given production target (Perlak et al., 2005), or the relative attractiveness of alternative feedstock supplies (Khanna et al., 2008). Industrial ethanol production has been reported using various starchy materials such as corn, wheat, starch and potatoes, cassava root (Lindeman and Rocchiciolo 1979), corn stover (Kadam and McMillan 2003; Wilke et al. 1981), and starch (Maisch et al. 1979). Among many starchy materials, cassava starch is an expensive fermentable source. It is a tropical root crop produced in more than 80 countries (Sasson 1990). Meyer (April 2010) found that by using sucrose more ethanol is produced compared to using glcose but essentially more compared to using fructose and yeast does not work on lactose.

2.3 Increasing ethanol production

Lazaros et al (1994) reported that ethanol yield can be increased by the delignified cellulosic (DC) material which prepared from sawdust after lignin removal. Ethanol production promoted by delignified cellulosic material is higher compare to absence of DC material by using glucose or molasses (Lazaros, 1995).In order to correlate the rate or extent of hydrolysis, it suggests a basic parameter which is the pore size distribution of wet substrate and the associated surface area available to the cellulose that is the major factor in determining the effectiveness of a pretreatment method. In addition, an environmental factor such as temperature has been shown to influence the amount of cell wall polysaccharides secreted into the fermenting medium (Guillioux-Benatier et al., 1995; Rosi and Giovani, 2003). Mannoproteins derived from yeast cell walls have attracted much attention in the winemaking world because of their reported contribution to wine quality. Yeast cell wall polysaccharides also adsorb myotoxins, thus decreasing their toxic effects and mediating their removal from the medium (Caridi, 2007; Kogan

and Kocher, 2007; Moruno et al., 2006). Al-Judaibi (2011) studied factors effect on decreasing of ethanol production from beet Molasses by Saccharomyces cerevisae CAIM13 and it showed four parameters involved which are cell concentration (inoculums size) of yeast below 3.6x105 cells/100ml, ethanol tolerance on yeast up to 10% concentration, utilizing of bench-scale fermenter and a immobilized cell technique can increase ethanol productivity.

While Helle (1991) showed alcohol effect on the respiration and fermentation of aerated suspensions of baker's yeast by using stopped-flow membrane inlet mass spectrometry. Alkanol group increasing inhibition of anaerobic fermentation but was correlated with increased partition coefficients into a hydrophobic milieu and it is due to alkanols act as respiratory substrate as well as giving inhibitory effects.

2.4 Breaking of lignocellulose

Fundamental biological research was held by US Department of Energy on 2006 to study breaking biological barriers to cellulosic ethanol by using genomics (John, 2006) and the research still on going. Production of ethanol by fermentation from raw corn starch by using the yeast *Saccharomyes cerevisiae codisplaying Rhizopus oryzae glucoamylase* and *Streptococces bovis* α-amylase can be directly and efficiently obtained using the C-terminal half region of α-agglutinin and the functional domain of Flolp as the respective anchor protein (Hisayori, 2004). While on an industrial strain of *S.cerevisiae* engineered for fermentation of lignocellulosic biomass to ethanol by introduction of the genes encoding secreted cellulases into the yeast genome (Nikolai et al., 2011).

Michelle (2011) studied a process to break down lignocellulose whereby all the intermediate processes were conducted simultaneously while undergoing microbial yeast fermentation under optimal condition which is 35^{o}C, 40 g/L of glucose and a pH of 4.5. The fermentation process was followed at a constant temperature of 35^{o}C which was found to be most suitable by Slaa, Gnode and Else (October 2009). Regarding type of process, continuous fermentation employed for commercial ethanol production from cane sugar and corn have higher volumetric productivity, reduced labor costs, reduced

vessel down time for cleaning and filling, reduce cost of overcoming the recalcitrance of cellulosic biomass and can adapt fermentative organisms to inhibitors instead of using batch process (Simone, 2009).

2.5 By using ultrasound wave and microwave

An ultrasound wave is a wave with frequency more than 20 kHz or above the range of human hearing. Ultrasonic energy of high intensity is transmitted through liquid but ineffectively through air (Sobberman, 2012).

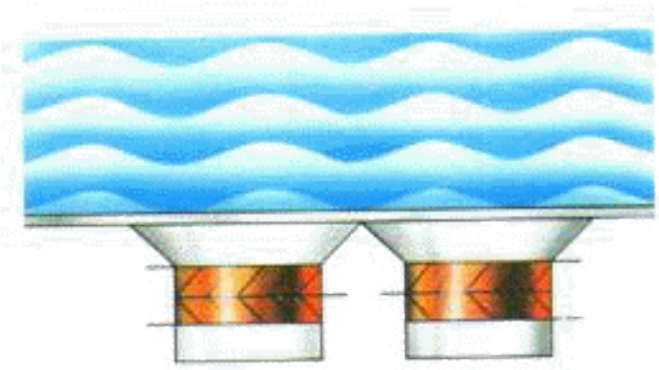

Figure 2.2: Ultrasound wave is transferred through liquid.

(Source*: http://www.elmulab.co.za/ultrasonic_bath.htm*)

Figure 2.2 above shows how ultrasound wave works. Ultrasound wave uses principle of mechanical vibration. Then, the oscillating system produces intense ultrasonic wave or intense vibration. This condition is able to penetrate blind hole, crack, and cell wall of cellulosic material as well.

Fermentation by ultrasound wave is applied at specific frequency, power level and time interval to stimulate organism growth through cell division and protein synthesis. It shows the increase in yield is due to ultrasound wave and is not due to mixing. PLUSwave ; a type of technology use ultrasound wave, increases the ability of the yeast *Saccharomyces cerevisiae* to ferment sugars to ethanol by 20% (Edmonton, 2012). While W. Klomkieng and A.Ziad (2011) studied using ultrasound to assist fermentation and enhance bioethanol productivity. Ethanol fermentation from molasses is enhanced by using low-power ultrasonic in the range of 20-30 kHz which also reduces

fermentation time by 6-9 hours compares to control bioreactor (W. Klomkieng, 2011). Moreover, intermittent sonication with 20kHz increases ethanol production in *K. marxianus* at intensity 11.8W cm^{-2} within 10 to 25 hours (A.Ziad. 2011).

Microwave is an electromagnetic wave with frequency about 2.45 GHz. It is related to basic properties of water molecules and microwaves itself. Water molecules are dipole and in rotation when they expose to alternating electric field of electromagnetic wave. Heat energy is produced when the agitated water molecules rub off with adjacent molecules. The body will heat up faster if the water molecules are well distributed (Villanueva J.C., 2009). Figure 2.3 below shows comparison of convection and microwave heating.

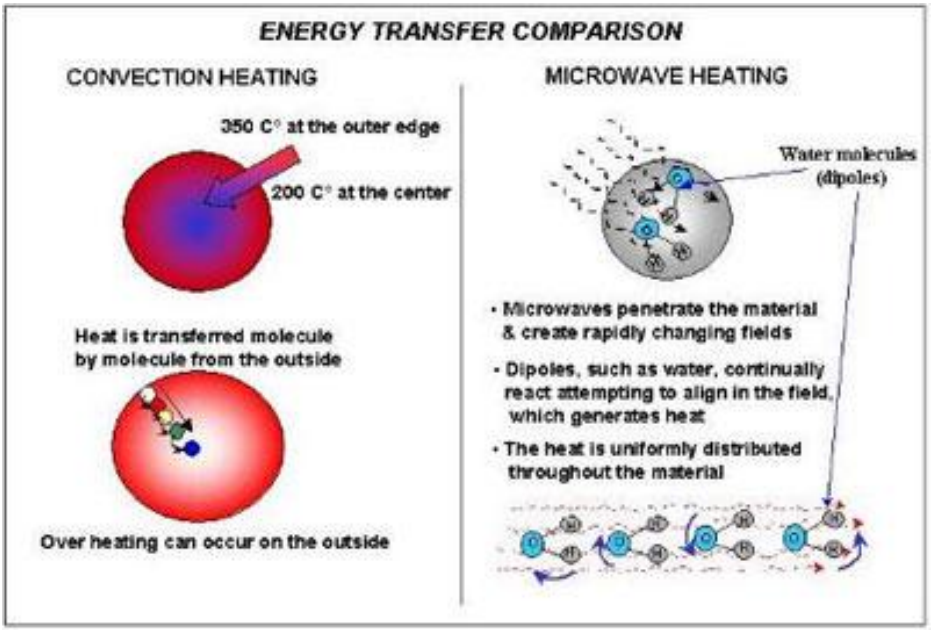

Figure 2.3: Principle of microwave and convection heating

(Source: *http://chetoket.net/microwaves-and-how-they-work/)*

Lj Mojoviv (2008) conducted a microwave-assisted liquefaction as a pretreatment of corn meal using *Saccharomyces cerevisae* to increase maximum ethanol concentration. His experiment showed that an optimal power of microwave should be used is 80W within 5 minutes.

2.6 Potential in economics

Andrew (2000) examines the plant-scale economic viability of the anaerobic fermentation of crude glycerol to ethanol by a hypothetical wild strain of *Eschericia coli* which appear exceedingly favorable with IRR of 32.24% on 2009 and has little risk of being unprofitable. An economic model is proposed by Robert to predict the cost of producing ethanol from cellulosic biomass using technology of prehydrolysis, simultaneous saccharification and co-fermentation, and cellulase enzyme production if a plant were to be built in the next few years (Robert, 2009).

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, methodology used in the project will be discussed. This experimental work is conducted at Reaction Engineering Laboratory. There are four samples used which are glutinous rice, thatch, palm oil frond and banana branch. Techniques of pre-treatment used are thermal transition, ultrasound and microwave. Furthermore, add precursor (fermented glutinous rice) to the sample is also proposed to observe the rate of fermentation. Then, refractometer is used to test the samples. Finally is Gantt chart to show a systematic progress of this experiment.

3.2 Tools and equipment:

- Plastic container
- Beaker
- Conical flask
- Test tube
- Spatula
- Pipette
- Aluminum foil
- Thermometer
- Electronic Weight Balance
- Grinder
- Centrifuge
- Refractometer
- Oven
- Hot plate
- Ultrasound bath
- Microwave

3.3 Chemical and materials:

- Ethanol 96% V/v
- Distilled water
- *Saccharomyces cerevisiae*
- Glutinous rice
- Thatch
- Banana branch
- Palm oil frond

3.4 Calibration of ethanol concentration

A calibration experiment of ethanol concentration is conducted with referring to their refractive index. This calibration is as a reference to calculate the concentration of ethanol in the sample.

3.4.1 Procedure

i. Measure 8ml of 96% EtOH in measuring cylinder.
ii. Measure 2ml of distilled water in other measuring cylinder.
iii. Mix EtOH and distilled water into test tube.
iv. Take the reading of refractive index of the sample by using refractometer.
v. Repeat the experiment by using different ratio of EtOH and distilled water.

Table 3.1: Ratio on ethanol and distilled water for calibration

EtOH (*ml*)	**Distilled water (*ml*)**
10 (pure EtOH)	0
8	2
5	5
3	7
1	9
0	10 (pure distilled water)

3.5 Experiment and procedures

This section explains the experimental procedures which involve pretreatment technique, fermentation and adding precursor. Figure 3.1 summarizes the procedure for pretreatment and fermentation.

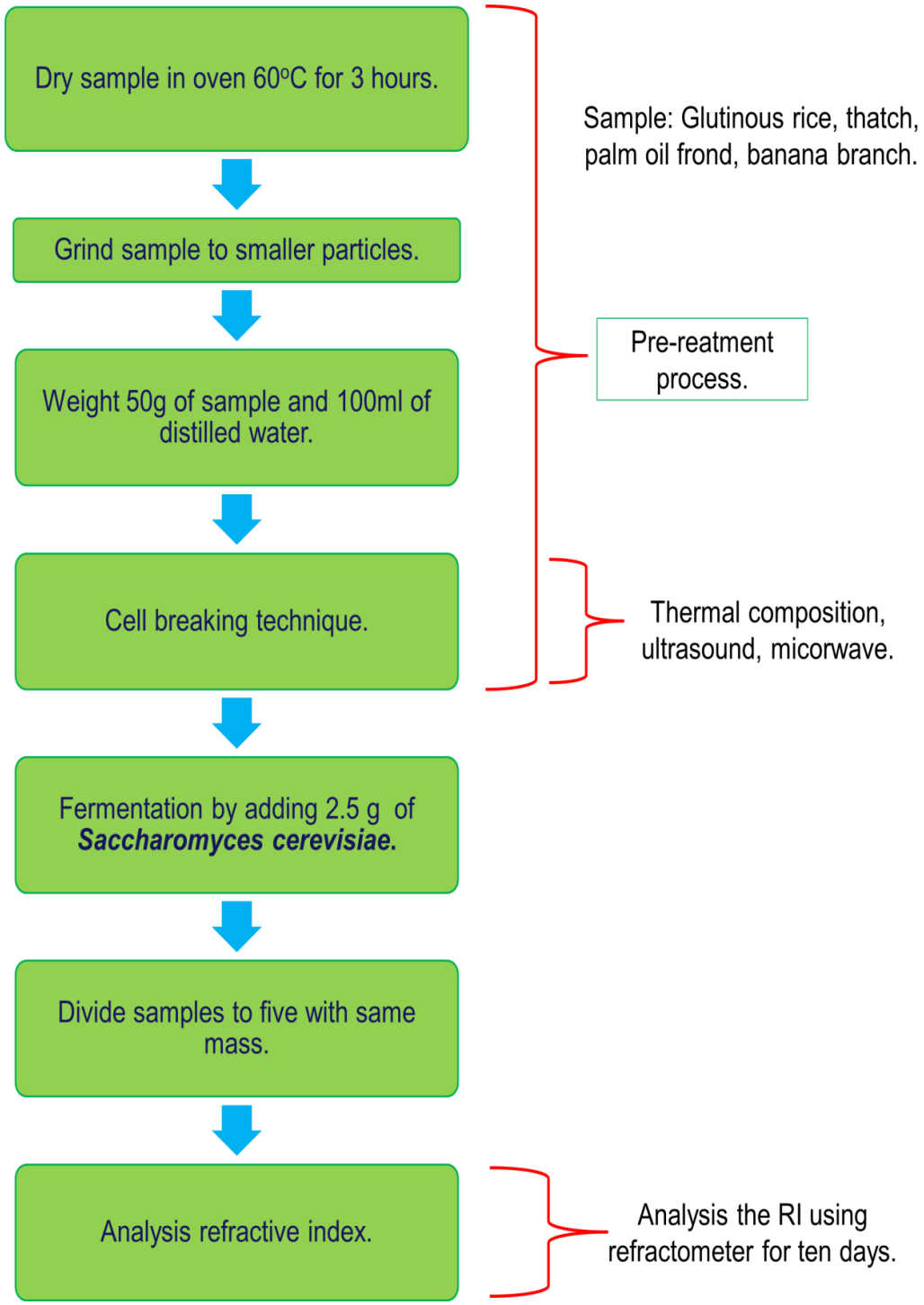

Figure 3.1: Procedure of pretreatment and fermentation.

3.5.1 Pretreament technique

Pretreatment technique is a technique proposed to break the lignocellulose. Three techniques used are thermal composition, ultrasound and microwave.

Procedure (thermal composition)

i. Weight samples; glutinous rice, thatch, banana branch and palm oil frond.
ii. Put them in oven at temperature 60^{o}C for three hours for drying.
iii. The samples are grinded to smaller size by using grinder. (Figure 3.2)
iv. Weight 50g each of the samples (Figure 3.3) and measure 100ml distilled water. The ratio of sample to distilled water is 1 to 2.
v. Mix the sample and distilled water respectively in the beaker. Wrapped the beaker with aluminum foil.
vi. Place the beaker on hot plate. Warm it at temperature 85 ^{o}C to 95^{o}C for 25minutes. No stirring is allowed. (Figure 3.4)
vii. Then, leave and cool at room temperature for one night.

Repeat the procedures except step (vi) for ultrasound and microwave.

Step (vi) for ultrasound.

- The sample is heated in ultrasound bath at 40-50 ^{o}C for 1 hour with frequency 100Hz. This temperature is suitable with vibration from ultrasound wave at this frequency and power level.

Step (vi) for microwave.

- The sample is put in microwave for 5minutes with medium low heating.

3.5.2 Fermentation

i. Weight 2.5g of *Saccharomyces cerevisiae*. (Figure 3.5)
ii. Mix *Saccharomyces cerevisiae* with the sample. Stir it completely.
iii. Weight samples of five equal weights and put in closed container. (Figure 3.6)
iv. Label each container with date (every two days) to test with refractometer.

v. Centrifuge the sample for 10 minutes with speed 4500 rpm before test with refractometer. (Figure 3.7)

vi. Use pipette to take only liquid for refractive index test by using refractometer. (Figure 3.8)

vii. Record the data.

3.5.3 Adding precursor (fermented glutinous rice)

i. Weight samples; glutinous rice,thatch, banana branch and palm oil frond.

ii. Put them in oven at temperature 60^{o}C for three hours for drying.

iii. The samples are grinded to smaller size by using grinder. (Figure 3.2)

iv. Weight 50g each of the samples (Figure 3.3) and measure 100ml distilled water. The ratio of sample to distilled water is 1 to 2.

v. Mix the sample and distilled water respectively in the beaker. Wrapped the beaker with aluminum foil.

vi. Put the beaker on hot plate. Warm it at temperature 85 ^{o}C to 95^{o}C for 25minutes. No stirring is allowed. (Figure 3.4)

vii. 2.5 g fermented glutinous rice and *Saccharomyces cerevisiae* is prepared.

viii. Mix fermented glutinous rice and *Saccharomyces cerevisiae* with the sample. Stir it completely.

ix. Weight samples of five equal weights and put in closed container. (Figure 3.6)

x. Label each container with date (every two days) to test with refractometer.

xi. Centrifuge the sample for 10 minutes with speed 4500 rpm before test with refractometer. (Figure 3.7)

viii. Use pipette to take only liquid for refractive index test by using refractometer. (Figure 3.8)

xii. Record the data.

Figure 3.2: Grinded sample.

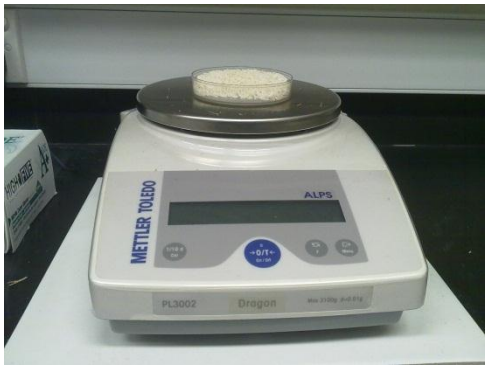

Figure 3.3: Weight the sample.

Figure 3.4: Heat the sample on hot plate.

Figure 3.5: Fermentation by adding 2.5g of yeast.

Figure 3.6: The sample equally distributed to five containers.

Figure 3.7: Centrifuges the sample.

Figure 3.8: Analyse the sample with refractometer.

3.6 Gant Chart

No	Detail/Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	**Selection of Project Topic:** A Saccharification Study on Various Non-Food Biomass for							Mid sem break													Mid sem break								
2	**Preliminary Research Work:** Research on literatures related to the topic																												
3	Submission of Preliminary Report																												
4	Proposal Defense (Oral Presentation)																												
5	**Project work continues:** Further investigation on the project and do modification if necessary																												
6	Experiment : Calibration of ethanol																												
7	Submission of Interim Draft Report																												
8	Submission of Interim Report																												
9	Experiment 1: Fermentation by thermal composition																												
10	Experiment 2: Fermentation by ultrasound																												
11	Submission of Progress Report																												
12	Experiment 3: Fermentation by microwave																												
13	Experiment 4: Fermentation by adding precursor																												
14	Pre-SEDEX																												
15	Analysis the result																												
16	Submission of Draft Report																												
17	Submission of Technical Report																												
18	Submission of Dissertation																												
19	Oral Presentation																												

Figure 3.9: Gantt chart of activity

CHAPTER 4
RESULTS AND DISCUSSION

This section explains the result obtained from experiments. There are five sections of results which are i)calibration of ethanol ii)thermal composition technique iii)ultrasound technique iv)microwave technique and vi)adding precursor. Each technique has two graphs for value of refractive index and ethanol concentration.

4.1 Calibration of ethanol concentration

Table 4.1 shows the refractive index from different ethanol concentration. The sample used is in ratio of volume ethanol to distilled water (v/V).

Table 4.1 : Refractive index of ethanol

Sample (% v/V)	RI
0%	1.3357
1%	1.3376
29%	1.3465
50%	1.3531
77%	1.3616
96%	1.3642

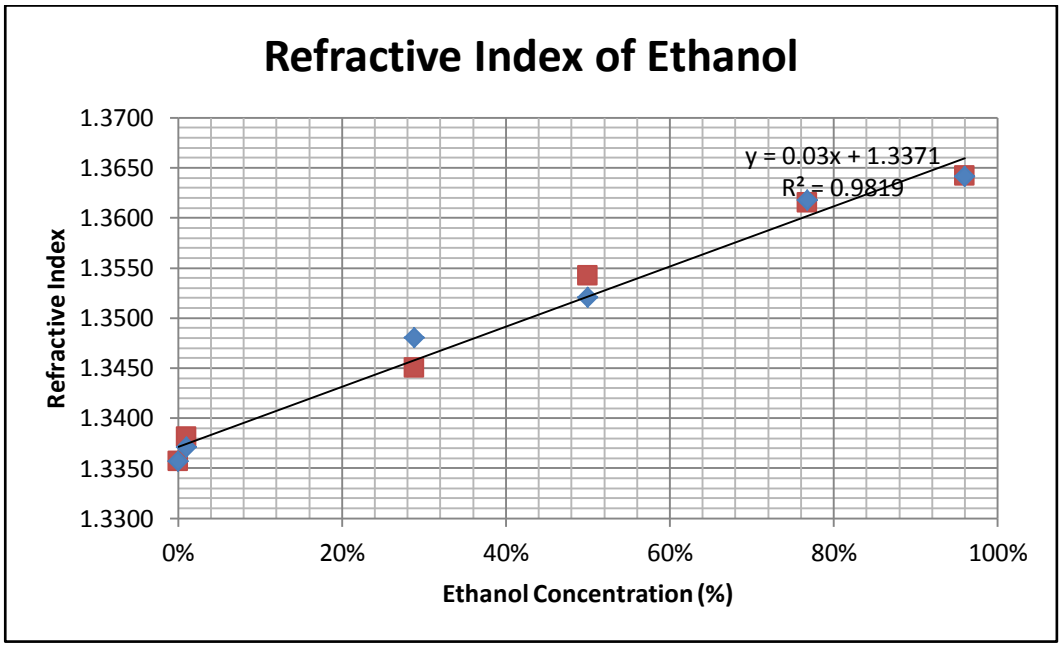

Figure 4.1: Refractive index of ethanol

As showed in Figure 4.1, the refractive index is linear to ethanol concentration with y=0.03x + 1.3371. This calibration is used to indicate the present of ethanol molecule on the sample by calculating ethanol concentration (%). By having the refractive index of the samples, the equation will calculate the ethanol concentration in percentage. At y-intercept which is 1.3357 is pure distilled water and no ethanol present. The maximum ethanol concentration (96%) is on 1.3642.Therefore, the samples will have ethanol if the refractive index is between 1.3357 and 1.3642.

4.2 Thermal Composition

Thermal composition is where the sample is heated on hot plate at temperature 85^{o}C to 90^{o}C within 25 minutes. Table 4.2 shows the observation on their refractive index. The data analysis is converted on two graphs which Figure 4.3 shows refractive index versus days and Figure 4.4 shows the percentage of ethanol versus days after calibrating.

Table 4.2 : Thermal composition technique.

	Banana Branch		Thatch		Palm Oil Fronds		Glutinous rice	
Day	RI	% EtOH	RI	% EtOH	RI	% EtOH	RI	% EtOH
3	N/A Sample is moist.	N/A	N/A Sample is moist.	N/A	N/A	Dried, no effect on yeast.	1.349	38.69%
4	N/A Sample is moist.	N/A	N/A Sample is moist.	N/A	N/A	Dried, no effect on yeast.	1.34901	38.72%
5	1.34185	17.89%	N/A Sample is moist.	N/A	N/A	Dried, no effect on yeast.	1.3539	52.95%
7	1.3421	18.62%	1.3354	0.00%	N/A	Dried, no effect on yeast.	1.3542	53.82%
10	1.3422	18.91%	1.3353	0.00%	N/A	Dried, no effect on yeast.	1.3555	57.60%

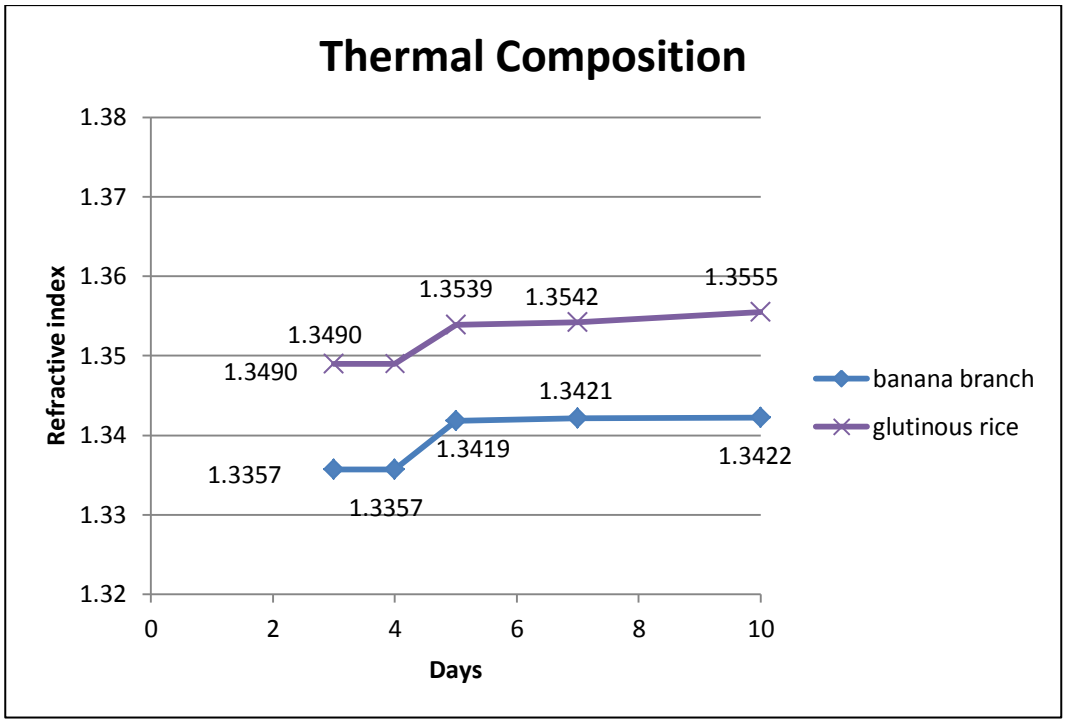

Figure 4.2: Refractive index for thermal composition.

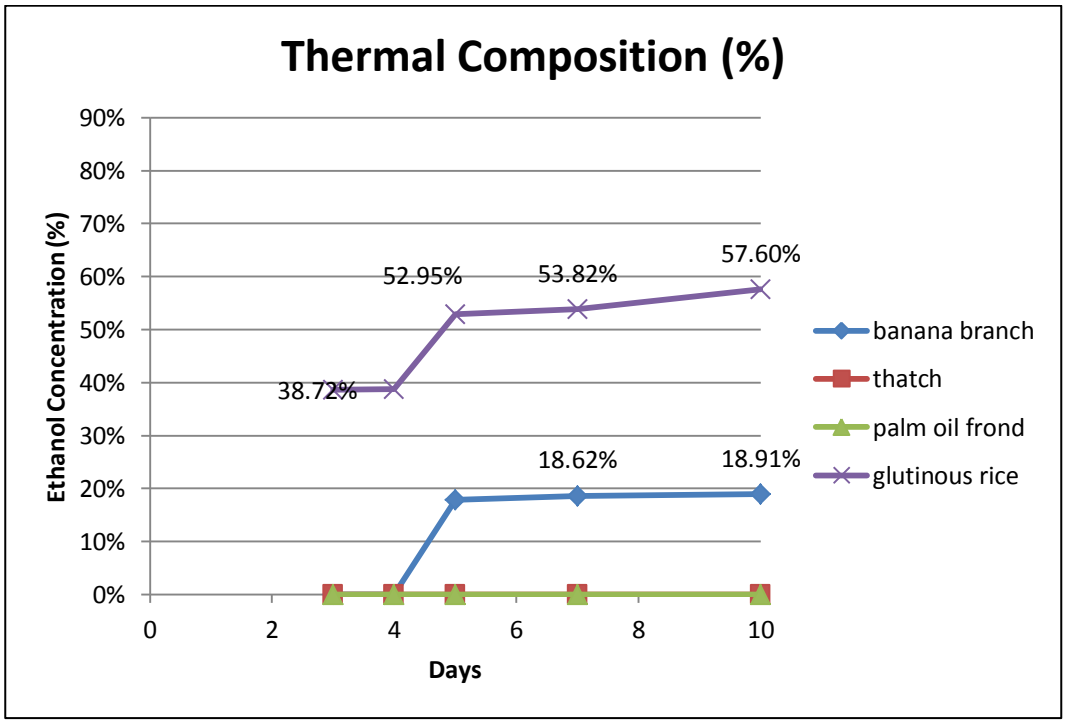

Figure 4.3: Percentage of ethanol concentration for thermal composition.

From the Figure 4.2 and 4.3, it showed that thatch and palm oil frond does not affect or react with *Saccharomyces cerevisiae* (yeast).The thatch is moist but it insufficient to test its refractive index. However, on the 7th day the thatch is tested but no sign present of ethanol due to its refractive index out of range. The palm oil frond dried and no fermentation occurs and evens no effect on yeast. The fermentation of banana branch gave out a small amount of ethanol a little bit late, on 5th day with 17.89% till 18.91%. It proves that thermal composition is able to break lignocellulose of banana branch and extract glucose for fermentation. Yeast accelerates the fermentation of banana branch to produce ethanol on last days. For glutinous rice as expected this traditional technique able to ferment it to ethanol. Real fermentation happened after 48hours with initial 38.72% and at last it had 57.6% of ethanol.

In thermal composition, heat is transferred molecule by molecule from the outside. Heating is well distributed into glutinous rice and a little on banana branch which cause extraction of glucose for fermentation to occur. That is why these feedstocks give reading on ethanol concentration at last. For thatch, thermal composition does not able to penetrate the cell wall successfully, only moist. Their moist do not mean no fermentation occurs, but it might have potential for fermentation because one of the products of fermentation is water. Overheating can occur on the outside of molecule. Therefore, palm oil frond undergoes overheating causes they dried and no effect on yeast.

Figure 4.4 showed the samples during fermentation when undergoes thermal composition technique.

Figure 4.4: Samples on thermal technique (from left; glutinous rice, banana leaf, thatch, palm oil)

4.3 Ultrasound

For ultrasound technique, the sample is heated in ultrasound bath at 40-50 ^{o}C for 1 hour with frequency 100Hz. Table 4.3 shows the observation on their refractive index of ultrasound technique. The data analysis is converted on two graphs which Figure 4.5 shows refractive index versus days and Figure 4.6 shows the percentage of ethanol versus days after calibrating.

Table 4.3: Ultrasound technique.

Day	Banana branch		Thatch		Palm oil frond		Glutinous rice	
	RI	% EtOH	RI	% EtOH	RI	% EtOH	RI	% EtOH
2	N/A Sample is moist.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Sample is moist.	0.00%
4	N/A Sample is moist.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	1.3479	36.86%
6	N/A Sample is moist.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	1.3492	40.57%
8	1.3454	29.71%	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	1.3521	48.86%
10	1.337	5.71%	N/A Dried, no effect on yeast.	N/A	1.3481	20.67%	1.3637	82.00%

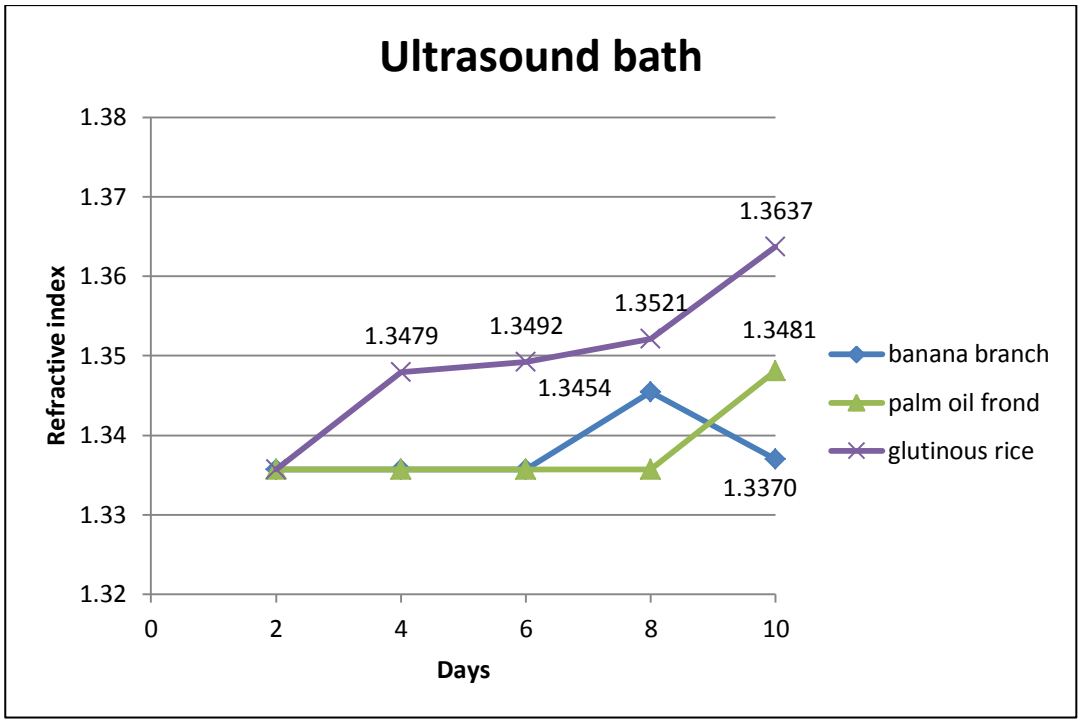

Figure 4.5 : Refractive index for ultrasound.

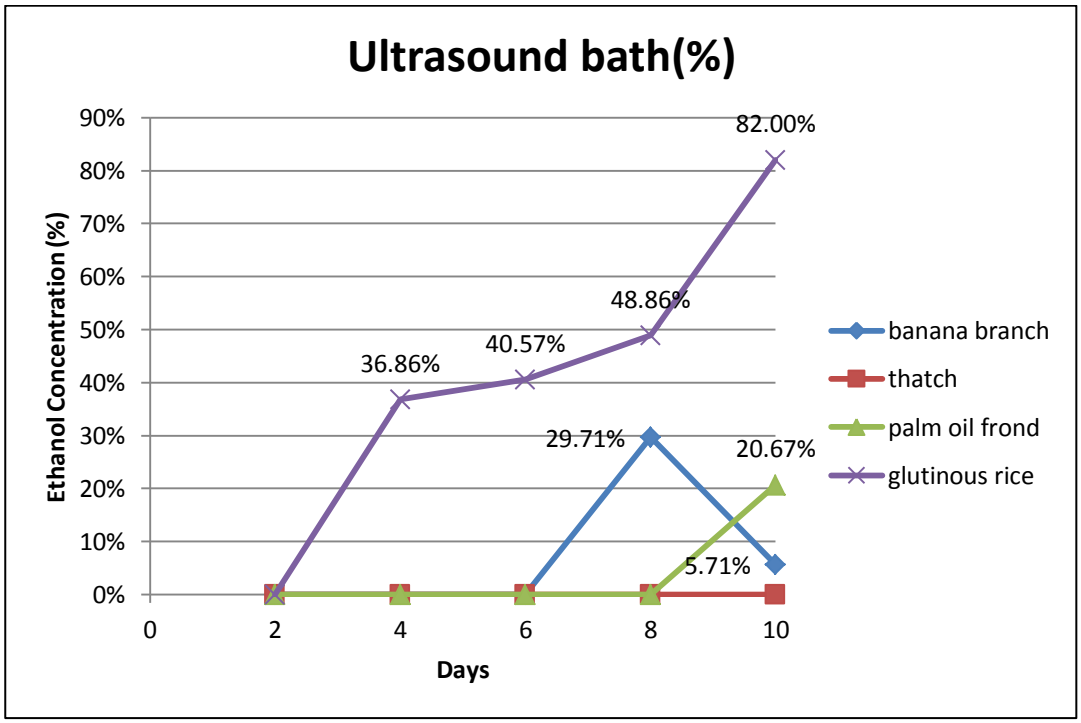

Figure 4.6 : Percentage of ethanol concentration for ultrasound.

From Figure 4.5 and 4.6, it showed that thatch dried which did not affect either by this technique or the *Saccharomyces cerevisiae*. However, banana branch and palm oil frond did show the present of ethanol although not much as glutinous rice. The performance of banana branch's fermentation unstable due to the ethanol concentration does not show increasingly day by day. At first the sample is moist but on day 8, it showed 29.71% of ethanol and 5.71% of ethanol on day 10. While, for palm oil frond on day 10 indicated 20.67% of ethanol production. Ten days earlier the sample just dried which no fermentation occur. Glutinous rice gave the highest production which is 82% ethanol compares to other samples. The fermentation on glutinous rice is linearly increased by time.

It can conclude that, ultrasound technique has potential to break down the lignocellulose of banana branch and palm oil frond in order to have glucose inside. Moist on banana branch show the potential for fermentation to occur because one of the product of this process is water. For glutinous rice, it showed efficiently producing of ethanol in ten days. In ultrasound wave, the oscillating system causes intense vibration and penetrates the lignocellulose of banana branch, palm oil frond and glutinous rice with this range of frequency.

Figure 4.7 showed the sample during ultrasound technique is applied.

Figure 4.7 : Samples on ultrasound technique. (From left glutinous rice, thatch, banana branch, palm oil frond)

4.4 Microwave

The samples are put in microwave for 5 minutes with medium low heating. Table 4.4 shows the observation on their refractive index of microwave technique. The data analysis is converted on two graphs which Figure 4.8 shows refractive index versus days and Figure 4.9 shows the percentage of ethanol versus days after calibrating.

Table 4.4: Microwave technique.

Day	Banana branch		Thatch		Palm oil frond		Glutinous rice	
	RI	% EtOH	RI	% EtOH	RI	% EtOH	RI	% EtOH
2	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Sample is moist.	N/A	Sample is moist.	N/A
4	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Sample is moist.	N/A	1.3440	23.00%
6	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Sample is moist.	N/A	1.3461	30.00%
8	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Sample is moist.	N/A	1.3501	43.30%
10	N/A Dried, no effect on yeast.	N/A	N/A Dried, no effect on yeast.	N/A	N/A Sample is moist.	N/A	1.3602	77.00%

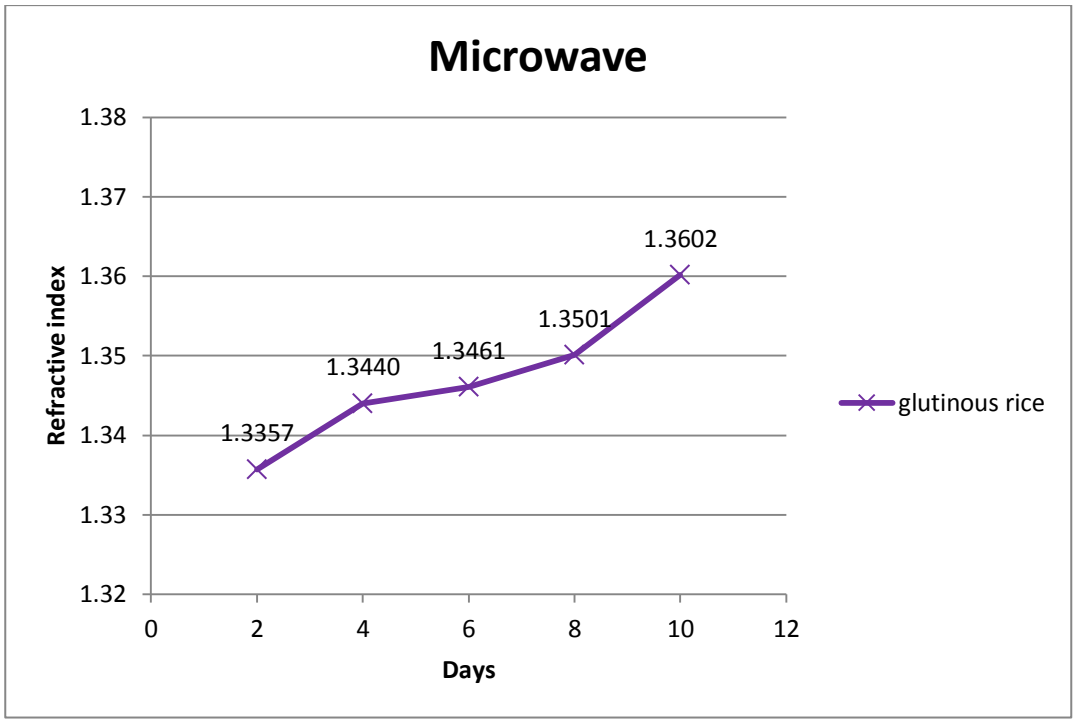

Figure 4.8 : Refractive index of samples when undergo microwave.

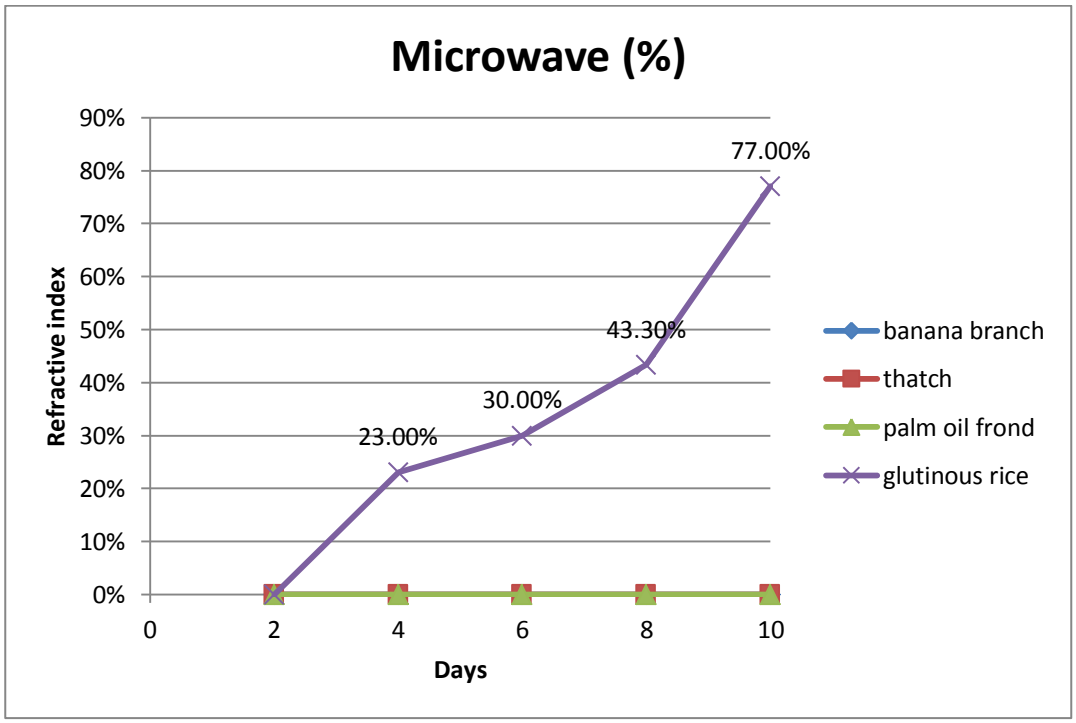

Figure 4.9 : Percentage of ethanol concentration for microwave.

Figure 4.8 and 4.9 showed all banana branches and thatch has no effect either in term of moisture or texture. The samples are dried till on the last days. However, palm oil frond showed a little of moist which indicate fermentation might occur because water is produced during fermentation. Unfortunately, the refractometer is not able read on this moisture. On other hands, glutinous rice has positive feedback ethanol reading linearly increased from 23% to 77% of ethanol from this fermentation.

It can conclude that, microwave technique is not success to break the lignocellulose wall except for glutinous rice. Then, fermentation does not occur. Microwave technique causes banana branch and thatch dried but moist for palm oil frond. Heat energy produced in microwave is due to agitated water molecules rub off each other's, thus the samples dried out. The samples heat up faster when the water molecules are well distributed. Then, due to dried samples, yeast does not react with them on dry medium.

Figure 4.10 showed the samples when undergo microwave technique.

Figure 4.10 : Samples on microwave technique. (from left; glutinous rice, banana branch, thatch, palm oil frond)

4.5 Adding precursor

A precursor is a chemical transformed into another compound and precedes the chemical reaction. Fermented glutinous rice acts as a precursor for this experiment and it is introduced to the sample. The present of precursor is to examine the rate of fermentation of each sample. Pretreatment used is thermal composition where the sample is heated on hot plate at temperature 85^{o}C to 90^{o}C within 25 minutes. Table 4.5 shows the observation on their refractive index of microwave technique. The data analysis is converted on two graphs which Figure 4.11 shows refractive index versus days and Figure 4.12 shows the percentage of ethanol versus days after calibrating.

Table 4.5 : Adding precursor technique.

Day	Banana branch		Thatch		Palm oil frond		Glutinous rice	
	RI	% EtOH	RI	% EtOH	RI	% EtOH	RI	% EtOH
2	1.3357	0.00%	N/A Sample is moist.	N/A	N/A Dried, no effect on yeast.	N/A	1.3491	38.98%
4	1.3357	0.00%	N/A Sample is moist.	N/A	N/A Dried, no effect on yeast.	N/A	1.3521	47.71%
6	1.3417	17.45%	N/A Sample is moist.	N/A	N/A Dried, no effect on yeast.	N/A	1.3539	52.95%
8	1.3419	18.04%	1.3357	0.00%	N/A Dried, no effect on yeast.	N/A	1.3583	70.60%
10	1.3423	19.20%	1.3357	0.00%	N/A Dried, no effect on yeast.	N/A	1.3592	73.67%

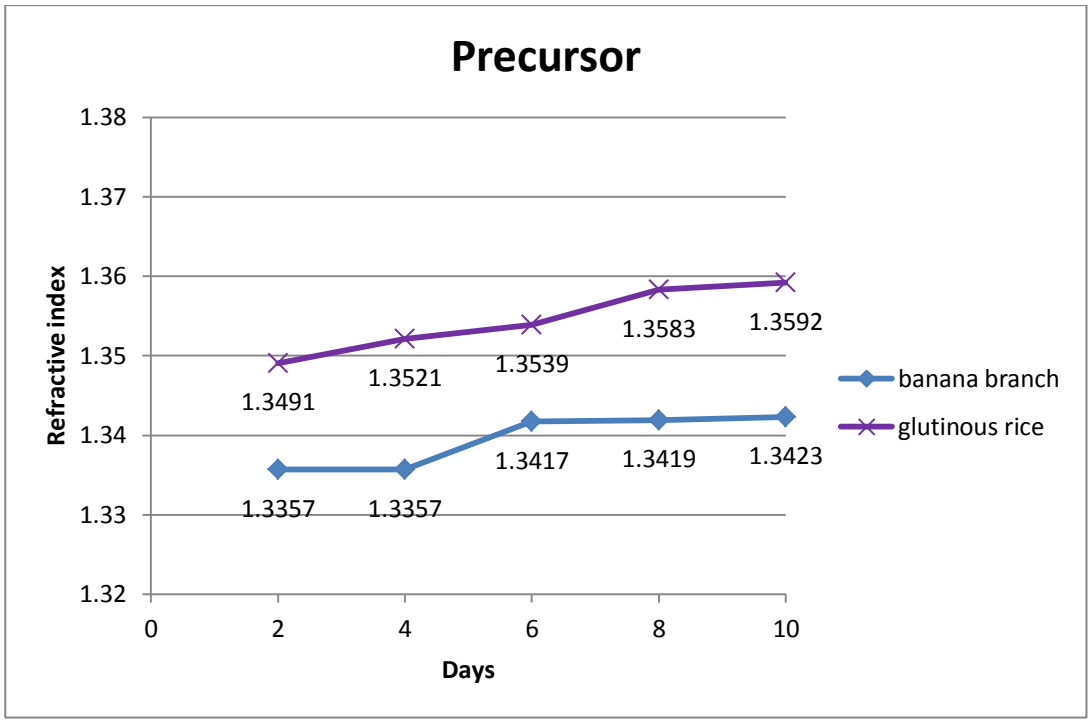

Figure 4.11 : Refractive index when adding precursor.

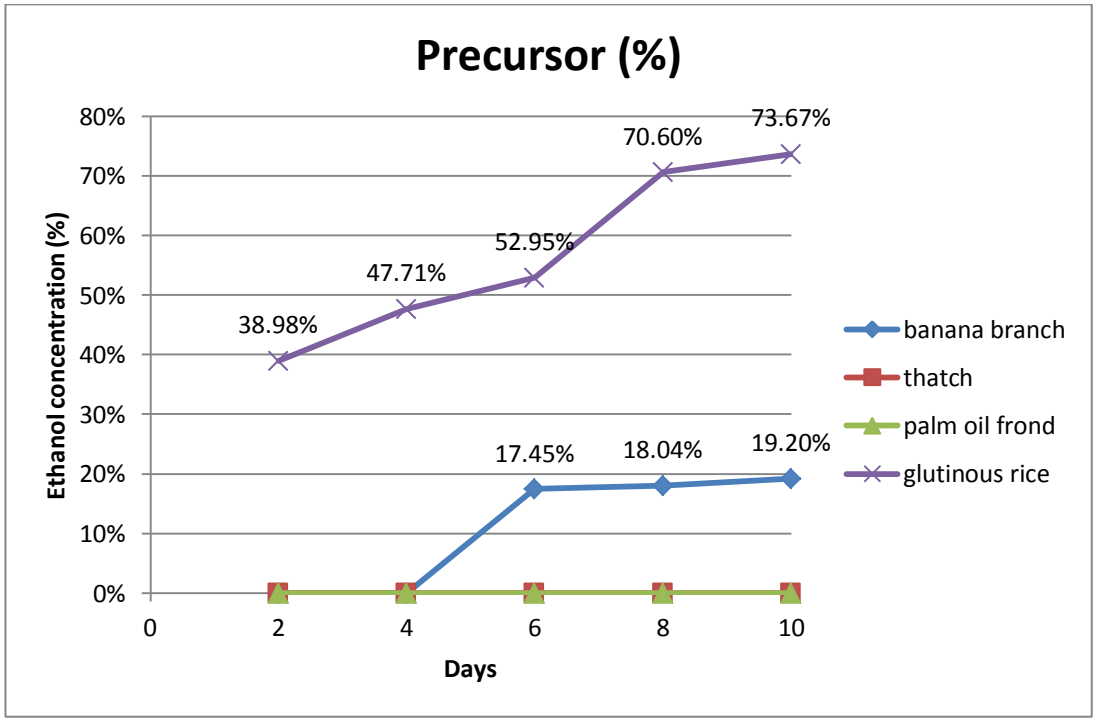

Figure 4.12: Percentage of ethanol concentration when adding precursor.

Figure 4.11 and 4.12 showed thatch and palm oil frond did not affected on fermentation. However, banana branch gives a little bit potential by producing 19.2% ethanol in ten days but not much improve by adding precursor. It showed 1.5% increases compare if not add precursor (18.91% ethanol refer to Figure 4.3). For glutinous rice, 73.67% ethanol is produced when adding precursor compares to 57.6% when precursor is not added. It showed increment of 21.8% ethanol. The adding precursor manipulates and initiates faster growth of yeast, thus increases performance of yeast. It occurs only in glutinous rice.

Figure 4.13 show the samples when undergo experiment of adding precursor.

Figure 4.13: Samples on adding precursor technique (from left; glutinous rice, banana branch, thatch, palm oil)

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The fermented sample has potential in producing ethanol if the refractive index is between 1.3357 and 1.3642 which is in range of distilled water and 96% ethanol. Based on four pretreatments proposed, ultrasound bath gives more positive feedback because it able to treat banana branch and palm oil frond to initiate ethanol production. By using ultrasound bath, 29% and 20% of ethanol is produced from fermentation of banana branch and palm oil frond respectively. Moreover, banana branch also show positively production of ethanol by thermal composition because it gives out 19% of ethanol. While, the best pretreatment for glutinous rice is using ultrasound bath because it produces 82% ethanol compare to thermal composition (57%) and microwave (77%).

5.2 Recommendation

From this study, banana branch and palm oil frond are recommended for next potential to replace glutinous rice as feedstock by studied various parameter of pretreatment. In addition, further study needs to be focused on technique using ultrasound bath as it capable to initiate good production of ethanol.

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APPENDICES

i) Sample

Banana branch

Thatch

Palm oil frond

Glutinous rice

ii) Microwave

Model : PMW-20A

Voltage : 240 a.c. 50Hz

Rated power Input (Microwave) : 1250 W

Rated power Output (Microwave) : 800 W

Microwave frequency : 2450 MHz

Serial No. : A 9135 4659

Ultrasound bath

iii) Material Safety Datasheet of Ethanol

Material Safety Data Sheet
Ethanol, Absolute

MSDS Number: M1004
Effective Date: 8/23/2004

Section 1 - Chemical Product and Company Identification

MSDS Name: Ethanol, Absolute
Synonyms: Ethyl Alcohol; Ethyl Alcohol Anhydrous; Ethyl Hydrate; Ethyl Hydroxide; Fermentation Alcohol; Grain Alcohol
Company Identification:
VEE GEE Scientific, Inc.
13600 NE 126th Pl Ste A
Kirkland, WA 98034
For information in North America, call: 425-823-4518

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
64-17-5	Ethanol	ca. 100	200-578-6

Hazard Symbols: F
Risk Phrases: 11

Section 3 - Hazards Identification

Emergency Overview
Appearance: Colorless clear liquid. Flash Point: 16.6 deg C. **Warning!** Flammable liquid and vapor. Causes respiratory tract irritation. May cause central nervous system depression. Causes severe eye irritation. This substance has caused adverse reproductive and fetal effects in humans. Causes moderate skin irritation. May cause liver, kidney and heart damage.
Target Organs: Kidneys, heart, central nervous system, liver.
Potential Health Effects
Eye Contact: Causes severe eye irritation. May cause painful sensitization to light. May cause chemical conjunctivitis and corneal damage.
Skin Contact: Causes moderate skin irritation. May cause cyanosis of the extremities.
Ingestion: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. May cause systemic toxicity with acidosis. May cause central nervous system depression, characterized by excitement, followed by headache, dizziness, drowsiness, and nausea. Advanced stages may cause collapse, unconsciousness, coma and possible death due to respiratory failure.
Inhalation: Inhalation of high concentrations may cause central nervous system effects characterized by nausea, headache, dizziness, unconsciousness and coma. Causes respiratory tract irritation. May cause narcotic effects in high concentration. Vapors may cause dizziness or suffocation.
Chronic Exposure: May cause reproductive and fetal effects. Laboratory experiments have resulted in mutagenic effects. Animal studies have reported the development of tumors. Prolonged exposure may cause liver, kidney, and heart damage.

Section 4 - First Aid Measures

Eye Contact: Get medical aid. Gently lift eyelids and flush continuously with water.
Skin Contact: Get medical aid. Wash clothing before reuse. Flush skin with plenty of soap and water.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.
Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid. Do NOT use mouth-to-mouth resuscitation.
Notes to Physician: Treat symptomatically and supportively. Persons with skin or eye disorders or liver, kidney, chronic respiratory diseases, or central and peripheral nervous sytem diseases may be at increased risk from exposure to this substance.
Antidote: None reported.

Section 5 - Fire Fighting Measures

General Information: Containers can build up pressure if exposed to heat and/or fire. As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Vapors may form an explosive mixture with air. Vapors can travel to a source of ignition and flash back. Will burn if involved in a fire. Flammable Liquid. Can release vapors that form explosive mixtures at temperatures above the flashpoint. Use water spray to keep fire-exposed containers cool. Containers may explode in the heat of a fire.
Fire Extinguishing Media: For small fires, use dry chemical, carbon dioxide, water spray or alcohol-resistant foam. For large fires, use water spray, fog, or alcohol-resistant foam. Use water spray to cool fire-exposed containers. Water may be ineffective. Do NOT use straight streams of water.
Autoignition Temperature: 363°C (685.40°F)
Flash Point: 16.6°C (61.88°F)
Explosion Limits, lower: 3.3 vol%
Explosion Limits, upper: 19.0 vol%
NFPA Rating: (estimated) Health: 2; Flammability: 3; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Remove all sources of ignition. Use a spark-proof tool. Provide ventilation. A vapor suppressing foam may be used to reduce vapors.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Use only in a well-ventilated area. Ground and bond containers when transferring material. Use spark-proof tools and explosion proof equipment. Avoid contact with eyes, skin, and clothing. Empty containers retain product residue (liquid and/or vapor) and can be dangerous. Keep container tightly closed. Avoid contact with heat, sparks and flame. Avoid ingestion and inhalation. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose empty containers to heat, sparks or open flames.
Storage: Keep away from heat, sparks, and flame. Keep away from sources of ignition. Store in a tightly closed container. Keep from contact with oxidizing materials. Store in a cool, dry, well-ventilated area away from incompatible substances. Flammables-area. Do not store near perchlorates, peroxides, chromic acid or nitric acid.

Section 8 - Exposure Controls, Personal Protection

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs	OSHA - Vacated Pels
Ethanol	1000 ppm	1000 ppm TWA 1900 mg/m3 TWA 3300 ppm IDLH	1000 ppm TWA 1900 mg/m3 TWA	1000 ppm TWA 1900 mg/m3 TWA

Engineering Controls: Use explosion-proof ventilation equipment. Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant a respirator's use.

Section 9 - Physical and Chemical Properties

Physical State: Clear liquid
Appearance: Colorless
Odor: Mild, pleasant
pH: Not available
Vapor Pressure: 59.3 mm Hg @ 20° C
Vapor Density: 1.59
Evaporation Rate: Not available
Viscosity: 1.200 cP @ 20° C

Boiling Point: 78° C
Freezing/Melting Point: -114.1° C
Decomposition Temperature: Not available
Solubility: Miscible
Specific Gravity/Density: 0.790 @ 20° C
Molecular Formula: C2H5OH
Molecular Weight: 46.0414

Section 10 - Stability and Reactivity

Chemical Stability: Stable under normal temperatures and pressures.
Conditions to Avoid: Incompatible materials, ignition sources, excess heat, oxidizers.
Incompatibilities with Other Materials: Strong oxidizing agents, acids, alkali metals, ammonia, hydrazine, peroxides, sodium, acid anhydrides, calcium hypochlorite, chromyl chloride, nitrosyl perchlorate, bromine pentafluoride, perchloric acid, silver nitrate, mercuric nitrate, potassium-tert-butoxide, magnesium perchlorate, acid chlorides, platinum, uranium hexafluoride, silver oxide, iodine heptafluoride, acetyl bromide, disulfuryl difluoride, tetrachlorosilane plus water, acetyl chloride, permanganic acid, ruthenium (VIII) oxide, uranyl perchlorate, potassium dioxide.
Hazardous Decomposition Products: Carbon monoxide, irritating and toxic fumes and gases, carbon dioxide.
Hazardous Polymerization: Will not occur.

Section 11 - Toxilogical Information

Carcinogenicity: ACGIH: A4 - Not Classifiable as a Human Carcinogen
Epidemiology: Ethanol has been shown to produce fetotoxicity in the embryo or fetus of laboratory animals. Prenatal exposure to ethanol is associated with a distinct pattern of congenital malformations that have collectively been termed the "fetal alcohol syndrome".
Teratogenicity: Oral, Human - woman: TDLo = 41 gm/kg (female 41 week(s) after conception) Effects on Newborn - Apgar score (human only) and Effects on Newborn - other neonatal measures or effects and Effects on Newborn - drug dependence.
Reproductive Effects: Intrauterine, Human - woman: TDLo = 200 mg/kg (female 5 day(s) pre-mating) Fertility - female fertility index (e.g. # females pregnant per # sperm positive females; # females pregnant per # females mated).
Neurotoxicity: No information available.
Mutagenicity: DNA Inhibition: Human, Lymphocyte = 220 mmol/L.; Cytogenetic Analysis: Human, Lymphocyte = 1160 gm/L.; Cytogenetic Analysis: Human, Fibroblast = 12000 ppm.; Cytogenetic Analysis: Human, Leukocyte = 1 ppb/72H (Continuous).; Sister Chromatid Exchange: Human, Lymphocyte = 500 ppm/72H (Continuous).

Section 11 - Toxilogical Information (continued)

Other Studies: Standard Draize Test(Skin, rabbit) = 20 mg/24H (Moderate) Standard Draize Test: Administration into the eye (rabbit) = 500 mg (Severe).

Section 12 - Ecological Information

Environmental Toxicity: Fish: Rainbow trout: LC50 = 12900-15300 mg/L; 96 Hr; Flow-through @ 24-24.9°C Rainbow trout: LC50 = 11200 mg/L; 24 Hr; Fingerling (Unspecified) na: Phytobacterium phosphoreum: EC50 = 34900 mg/L; 5-30 min; Microtox test 250 ppm/6hr/goldfish/lethal/fresh water.
Environmental: Ethanol: In water, will volatilize and probably degrade.
Physical: No information available.
Other: Not expected to bioconcentrate in fish.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name	Ethanol	Ethanol
Hazard Class	3	3 (8.1)
UN Number	UN1170	UN1986
Packing Group	II	II
Other		FP 18C

Section 15 - Regulatory Information

US Federal
TSCA: CAS# 64-17-5 is listed on the TSCA inventory.
Health & Safety Reporting List: None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules: None of the chemicals in this product are under a Chemical Test Rule.
Section 12b: None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule: None of the chemicals in this material have a SNUR under TSCA.
SARA:
CERCLA Hazardous Substances and corresponding RQs: None of the chemicals in this material have an RQ.
SARA Section 302 Extremely Hazardous Substances: None of the chemicals in this product have a TPQ.
SARA Codes: CAS # 64-17-5: acute, chronic, flammable.
Section 313: No chemicals are reportable under Section 313.
Clean Air Act: This material does not contain any hazardous air pollutants. This material does not contain any Class 1 Ozone depletors. This material does not contain any Class 2 Ozone depletors.
Clean Water Act: None of the chemicals in this product are listed as Hazardous Substances under the CWA. None of the chemicals in this product are listed as Priority Pollutants under the CWA. None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA: None of the chemicals in this product are considered highly hazardous by OSHA.
STATE: Ethanol can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.
This product contains Ethanol, a chemical known to the state of California to cause birth defects or other reproductive harm.
California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations
European Labeling in Accordance with EC Directives
Hazard Symbols: F
Risk Phrases:
R 11 Highly flammable.
Safety Phrases:
S 7 Keep container tightly closed.
S 9 Keep container in a well-ventilated place.
S 16 Keep away from sources of ignition - No smoking.
S 33 Take precautionary measures against static discharges.

Section 15 - Regulatory Information (continued)

WGK (Water Danger/Protection): CAS# 64-17-5: 0
Canada - DSL/NDSL: CAS# 64-17-5 is listed on Canada's DSL List.
Canada - WHMIS: This product has a WHMIS classification of B2, D2A.
Canadian Ingredient Disclosure List: CAS# 64-17-5 is listed on Canada's Ingredient Disclosure List.
Exposure Limits: CAS# 64-17-5: OEL-AUSTRALIA:TWA 1000 ppm (1900 mg/m3); OEL-BELGIUM:TWA 1000 ppm (1880 mg/m3); OEL-CZECHOSLOVAKIA:TWA 1000 mg/m3;STEL 5000 mg/m3; OEL-DENMARK:TWA 1000 ppm (1900 mg/m3); OEL-FINLAND:TWA 1000 ppm (1900 mg/m3);STEL 1250 ppm (2400 mg/m3); OEL-FRANCE:TWA 1000 ppm (1900 mg/m3);STEL 5000 pp; OEL-GERMANY:TWA 1000 ppm (1900 mg/m3); OEL-HUNGARY:TWA 1000 mg/m3;STEL 3000 mg/m3; OEL-THE NETHERLANDS:TWA 1000 ppm (1900 mg/m3); OEL-THE PHILIPPINES:TWA 1000 ppm (1900 mg/m3); OEL-POLAND:TWA 1000 mg/m3; OEL-RUSSIA:STEL 1000 mg/m3; OEL-SWEDEN:TWA 1000 ppm (1900 mg/m3); OEL-SWITZERLAND:TWA 1000 ppm (1900 mg/m3); OEL-THAILAND:TWA 1000 ppm (1900 mg/m3); OEL-TURKEY:TWA 1000 ppm (1900 mg/m3); OEL-UNITED KINGDOM:TWA 1000 ppm (1900 mg/m3) JANS OEL IN BULGARIA, COLOMBIA, JORDAN, KOREA check ACGIH TLV OEL IN NEW ZEALAND, SINGAPORE, VIETNAM check ACGI TLV

Section 16 - Additional Information

MSDS Creation Date: 09/23/2004

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall VEE GEE Scientific be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if VEE GEE Scientific has been advised of the possibility of such damages.