Investigation of Various Local Fungi for Biomass Digestion

by

Khairul Amir Bin Sukarman

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Universiti Teknologi PETRONAS

Bandar Seri Iskandar

31750 Tronoh

Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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A project dissertation submitted to the

Chemical Engineering Programme

Universiti Teknologi PETRONAS

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Approved by,

_____

(Dr Shuhaimi Mahadzir)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

MAY 2013

CERTIFICATION OF ORIGINALITY

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

_____

KHAIRUL AMIR BIN SUKARMAN

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

ABSTRACT

Consumption of ethanol produced from biomass carries wide opportunity to reduce the use of gasoline and cut CO_2 emissions. According to The National Development and Reform Commission, the biomass availability in Malaysia and its potential energy generated are 50,919 dry kton/year and 13,343 kton/year for 2011. Therefore, the fermentation of waste banana branch cellulose with various local fungi include *Schizophyllum commune*, *Pycnoporus sanguineus*, *Micropus xanthopus*, *Perenial Ganoderma lipsiense*, *Microporus affinis*, *Microporus xanthopus* and *Mycena sp* has been investigated for their ability to produce bioethanol. The production patterns of fermentation were studied during the growth of the organisms for a period of interval of two day until day 10. The cell wall breaking banana branch cellulose has been done using the technique of ultrasound bath. The resulted bioethanol generation has been referred by ethanol standard calibration of refractive index. Among seven types of the collected fungi, fermentation yield resulted maximum 11% volume ethanol which represented by *Perenial Ganoderma lipsiense* fungi at day 2. Very low levels of bioethanol production were detected by the Microporus affinis fungi. The 11% yield percent of ethanol produced by the experiment show the better ability of alternative fungi for bioethanol industry compared to conventional fungi, yeast which also being included as a standard of fungi fermentation.

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CHAPTER 1

INTRODUCTION

1.1 PROJECT BACKGROUND

In future energy consumption, non-renewable fossil energy sources like oil and coal use nowadays will become depleted. People tend to find biomass as an alternative energy which is more clean and renewable. Biomass including organic matter wood, crops, seaweed, and animal wastes carries potential to become primary energy source in industries such as power generation of electric utilities, transportation, and residentials. One of the significant products of biomass is biofuel, primarily bioethanol which mostly used as additives to petroleum gasoline.

Ethanol also known as ethyl alcohol, C_2H_5OH can be produced synthetically from fossil fuel or by microbial conversion through fermentation process. The chemical equation of fermentation process can be described by the two steps below:

Steps 1:

$$\text{Carbohydrate} \longrightarrow \text{simple sugar + glucose}$$

Step 2:

$$C_6H_{12}O_6\ (\text{glucose}) \longrightarrow C_2H_5OH\ (\text{ethanol}) + CO_2\ (\text{carbon dioxide}) + H_2O\ (\text{water})$$

Step 1 described the conversion carbohydrate into simple sugar and glucose through the process of pretreatment of biomass by breaking the structure of the source. The glucose then will react with microorganism such as yeast to produce ethanol, carbon dioxide and water as shown in step 2.

Sugars, starches and cellulose are the common sources use in the fermentation process nowadays and a lot of research has been done to improve this technology. Fermentation process converts sugar into ethanol while complex carbohydrate and cellulose need to be hydrolyzed to become simple sugar first with the help of conventional fungi *Saccharomyces cerevisiae* also known as yeast.

Yeast is useful for the production of bread and enzymes, pigments, antioxidants, and for various other medical applications that benefit mankind. The huge mainstream of bioethanol produced internationally is made by using *Saccharomyces cerevisiae* or yeast. Some research has been done to improve the performance of the yeast such as transforming and cloning for fermentation of bioethanol at high temperature. The expensive cost, time constraint of experiment and difficulties of re-engineered yeast are common problem faced by researcher to improve bioethanol fermentation process.

Therefore, this research will be focus on experimental set up on the ability of various local fungi towards bioethanol fermentation process including conventional fungi, yeast as a standard sample. Seven samples of different local fungi has been collected, which are *Schizophyllum commune, Pycnoporus sanguineus, Micropus xanthopus, Perenial Ganoderma lipsiense, Microporus affinis, Microporus affinis and Mycena sp.* Specifically, the research being done on the cellulose biomass type which is banana tree branch to solve the issue of human food based feedstock. Banana tree branch is an agriculture waste and offer huge source of biomass in tropical climate.

1.2 PROBLEM STATEMENT

Yeasts or *Saccharomyces cerevisiae* is the common and conventional microorganisms currently used for huge scale industrial ethanol production. Nowadays, there is a lot of technology and research has been develop to improve the bioethanol fermentation including re-engineered yeast and improves the yeast strains. This is because yeast has some weaknesses such as low productivity, low cell density, longer fermentation and prolonged downtimes. Moreover, yeast gene need to be re-engineered to prevent the fermentation inhibitor, acetic acid which is normally released by all the plant.

However, high cost and time constraints of conducting experiments and the difficulties of producing re-engineered yeast become problems in bioethanol fermentation.

One option is to investigate suitable fungi that are specific for biomass cellulose. In this study, banana branch which is non-food feedstock is selected. Several collected sample of different local fungi will lead to a cheaper solution for fermentation improvement process. With the technique of ultrasound bath which break the cellulose structure, the reaction between local fungi and banana tree branch will be more favorable. Thus, this research looks into the selection of suitable fungi for the conversion biomass cellulose into ethanol by fermentation process.

1.3 OBJECTIVES

The objectives in this project are:

- To identify the various local fungi for biomass digestion
- To evaluate the capability of local fungi in the fermentation of cellulosic biomass.

1.4 SCOPE OF STUDY

In order to achieve the objective of the research project, some scope needs to be done successfully. Firstly, the study on details of fermentation process as a part of literature review should be done continue with the collection of 7 local fungi and banana tree branch. The difference local fungi need to identify according their type. Then, the ethanol standard sample need to be complete as a refractive index references. Lastly, the experimental study on fermentation of banana branch and collected local fungi need to be set up to gain the result of ethanol produced which to be analyze.

1.5 FEASIBILITY OF THE PROJECT WITHIN SCOPE AND TIME FRAME

The process of pretreatment of banana branch will be conducted by Ultrasonic Bath and the preparation of fungus enzyme will be done by heating oven. The calibration of standard ethanol sample also should be done first before start the experiment as the standard reference. The experiment will be done on a few stages. The first stage is

research and some literature review of bioethanol, fungi and cellulose from banana branch. Then, the second stage continues with experimental design and conducting the experiment. This project will be carried out in the given timeframe.

CHAPTER 2

LITERATURE REVIEW

This chapter discusses previous work and critical analysis towards the problems, methodologies and result used.

2.1 BIOETHANOL FERMENTATION

Energy demand and climate change require large scale substitution of petroleum based fuels. The decreasing of petroleum-based fuels and environmental problems has triggered the development of inexpensive production of bioethanol fermentation (Yadav, 2011). The history of first fermentation occurred by accident of formation of "crude" alcoholic beverage by unattended grain or fruit along the river Tigris in the empire of Mesopotamia, currently called Iraq six to seven thousand years ago (Bouthyette,2008**).** Since the discovery of fermentation process, lot of research and improvements has been done on fermentation technology. By using yeast and bacteria or combination of both, the fermentation process successfully convert carbohydrate to alcohol and carbon dioxide or organic acid especially on food technology industry (Chisti, 1999). Fermentation occurs by process of the activity of micro-organisms such as yeast and bacteria under anaerobic condition. Fermentation process can be classified into several types including ethyl alcoholic fermentation, lactic acid fermentation, acetic acid fermentation and butyric acid fermentation (Srivastava, 2008).

The fermentation of ethanol can be produced from sugar, starch or lignocellulosic biomass where sugar and starch-based plant residues are presently major at the industrial level and an economically favorable. Its production typically involves a

hydrolysis-fermentation route, which has three main steps: pretreatment to get fermentable sugars, fermentation to produce bioethanol, and a separation process to obtain highly concentrated bioethanol. According to (Nikolic´, 2010), source of biomass can be described on the figure 2.1 below:

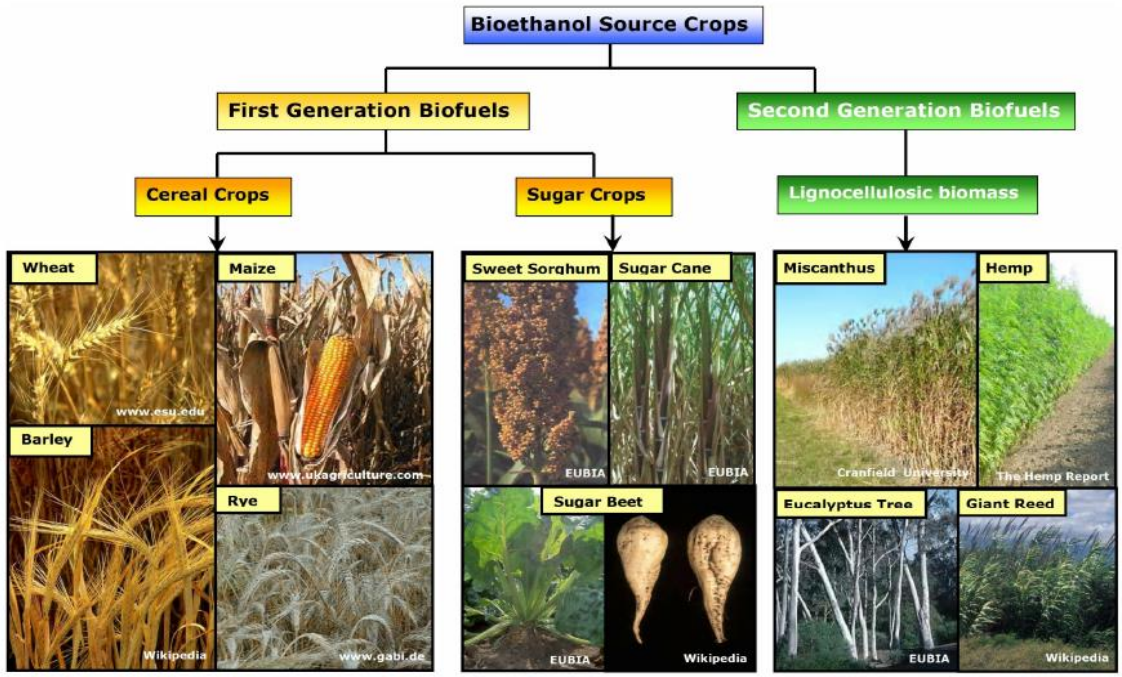


Figure 2.1: Bioethanol source crop

A number of chemicals are produced in the ethanol industry. The second generation bioethanol industry, serves a wide range of uses in the pharmaceuticals, cosmetics, beverages and medical sectors as well as for industrial uses. The second generation bioethanol industry comprise of lignocellulosic biomass such as miscanthus, hemp, Eucalyptus tree and Giant Reed while first generation mostly are crops from cereal and sugar. First generation of biomass can be easily extracted the glucose compared to second generation of biomass. The market potential for bioethanol is therefore not just limited to transport fuel or energy production but has potential to supply the existing chemicals industry.

2.2 FERMENTATION FROM CELLULOSIC BIOMASS

According to (Manea, 2010), sugars which extracted from molasses, fruits, sugarcane, and sugar beets will be straight away converted into ethanol. Corn, cassava, potatoes, and root crops are sources of starch which should go through process of hydrolysis to convert into sugars by the action of enzymes. From figure 2.2, the most abundant source of biomass, cellulose located at the most inner part of the plant structure after hemicellulose and lignin (Menon, 2012). The extraction of simple sugar from the cellulosic biomass needs technique of breaking the cellulose such as ultrasonic bath.

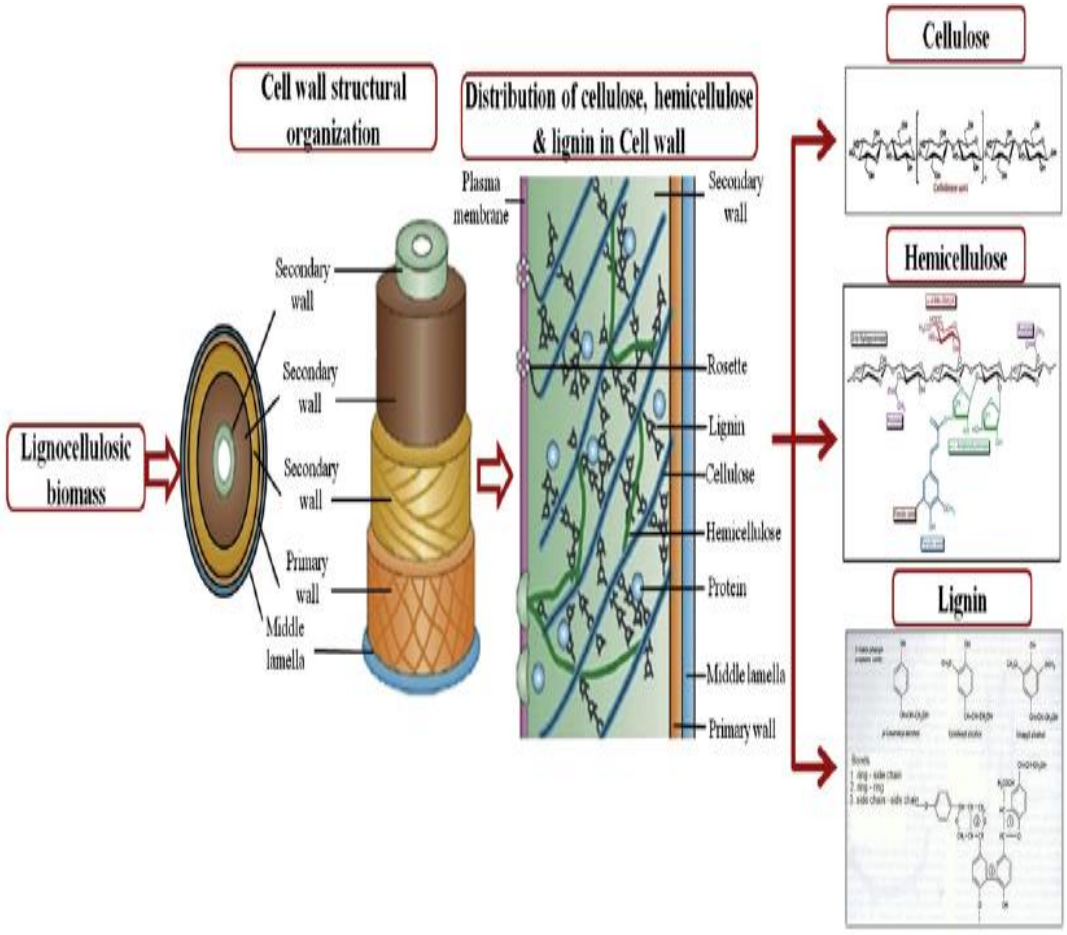


Figure 2.2: Diagrammatic illustration of lignocellulose.

Then, cellulose from wood, agricultural residues, waste sulfite liquor from pulp, and paper mills should be converted into sugars by the favor of mineral acids (Lin &

Tanaka, 2005). Compare to starch and sugar cellulose materials represent the most abundant global source of biomass and have been largely unutilized. According to the. (El-Halwagi M., 2011) stated that lignocellulosic materials, which consist mainly of cellulose, hemicellulose and lignin, are among the most promising renewable feedstock's for the production of energy and chemicals. Bioethanol is a major biofuel that can be produced from lignocellulosic materials. From the Table 2.1, most of raw material abundant in the world made up of structure of cellulose as well as in hardwood, softwood and agricultural residue (Garrote et al, 1999).

Table 2.1: Cellulose, hemicellulose and lignin content of representative lignocellulose.

Raw material	Cellulose	Hemicellulose	Lignin
Hardwoods			
White birch	41	36.2	18.9
Poplar aspen	50.8–53.3	26.2–28.7	15.5–16.3
Red maple	44.1	29.2	24
Hybrid poplar	41.7	20.2	29.3
Eucalyptus viminalis	41.7	14.1	31
Softwoods			
Pinus banksiana	41.6	25.6	28.6
Pinus pinaster	42.9	17.6	30.2
fir	43.9	26.5	28.4
Agricultural residues			
Corn cobs	33.7–41.2	31.9–36	6.1–15.9
Sugar cane bagasse	40–41.3	27–37.5	10–20
Wheat straw	32.9–50	24–35.5	8.9–17.3
Rice straw	36.2–47	19–24.5	9.9–24
Corn stalks	35–39.6	16.8–35	7–18.4
Barley straw	33.8–37.5	21.9–24.7	13.8–15.5
Soya stalks	34.5	24.8	19.8
Cotton stalks	38.4–42.6	20.9–34.4	21.45

Source: Garrote et al. (1999).

Lot of investigation has been done for developing economically pretreatments for generating simple sugar from cellulose and hemicellulose (Yang & Wyman, 2008). Pretreatment or either chemical, biological or mechanical, is required to breakdown cellulose, hemicellulose and lignin to allow the reaction between fungi and extracted sugar. The operative pretreatment can be concluded as characteristic of avoiding size reduction, preserving hemicellulose fractions, limiting formation of inhibitors due to degradation products, minimizing energy input, and being low cost. According to National Renewable Energy Laboratory (NREL) lignin is the hardest part of lignocellulose. Lignin residue normally used as a fuel for the ethanol production plant boilers in the cellulosic ethanol production

2.3 FERMENTATION BY LOCAL FUNGI

Ethanol fermentation is a biological process in which microorganism convert organic materials to simpler compounds, such as sugars (Lin Y. & Tanaka S., 2005). In the context of fermentation, two parts for microorganisms are being applied for the conversion of fermentable substrates into ethanol, and the other is to produce the enzyme to catalyze chemical reactions that hydrolyze the complicate substrates into simpler compounds. The common type of fungi used for fermentation process are *Saccharomyces Cerevisiae*, *Saccharomyces*, and *Aspergillus*, *Ellipsogdeus* (Bouthyette P.S, 2008). The most preferable type of fungi for fermentation process is *Saccharomyces cerevisiae* which resulted concentration of 18% of the fermentation broth (Lin & Tanaka, 2005). Different fungi can produce different chemicals during fermentation such as *Rhizopus stolonifer* familiarly produced dye and carotene and *Penicillium chrysogenum* used in fermentation to produce penicilin. Meanwhile, *Aspergillus niger* produces citric acid or vitamin C that is added to soft drinks. The ability of various fungi also has been shown by Hossin et al, (2010) in which the fermentation of bioethanol from pretreated and untreated wheat straw with fungi *Fusarium oxysporum* in Continuous Stir Batch Reactor (CSBR) was found to be an effective biofuel production process.

2.4 ULTRASOUND BATH TECHNIQUE

Effective pretreatment is an important step in the fermentation process to break the lignocellulose from the lignin hemicellulose structure so as to make it accessible for a following reaction step. The total content of hydrolysable biopolymers in a typical lignocellulosic biomass such as wood is approximately cellulose: 50%; hemicellulose: 25% and lignin: 20% by mass (Bobleter, 1994). Pretreatments can be done through physical methods, for example size reduction or chemical process (Mosier, 2005). The example of pretreatment technique is acid hydrolysis, steam explosion, ammonia fiber expansion, organ solve and sulfite pretreatment. Organ solve pretreatment can be describes as process of using organic solvent or aqueous solution to remove lignin from the plant structure. Meanwhile, sulfite pretreatment is a process of a short chemical treatment of lignocellulose with sulfite follow by mechanical size reduction.

In order to improve the bioconversion of lignocellulosic materials in an environmentally friendly manner, ultrasound is the best technique by expansion of the lignocellulose cells due to the effect of making pore through the ultrasonic waves (Vinatoru, 2009).

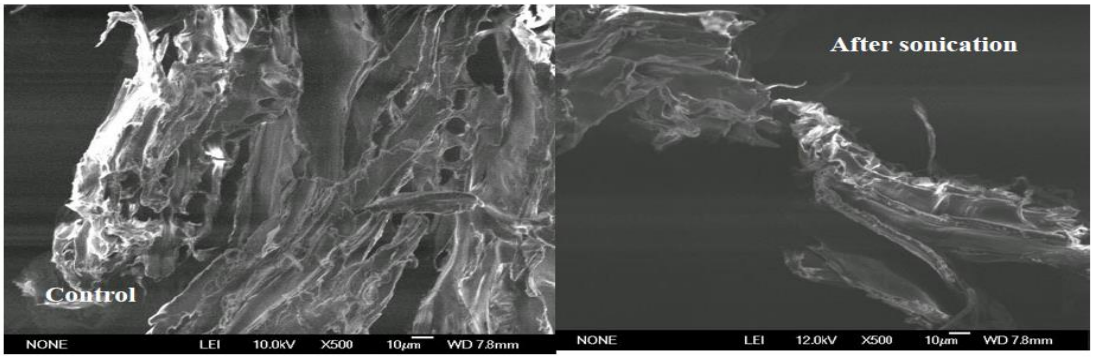


Figure 2.3: The wood microstructure before and after sonication

Paul Langevin was the first person who uses sound waves in water to avoid hitting of iceberg by the ship (Bremner, 1990). Sound frequency above 18 kHz is considered to be ultrasound (US) (Kwiatkowska et al, 2009). Large amount of research has gone to analyzed ultrasound at both high and low power. Theory of ultrasound wave can be explained as mechanical vibration by intense ultrasonic wave. Ultrasonic wave is able to penetrate blind hole, crack, and cell wall of cellulosic material. The purpose of ultrasound bath is to generate a pretreated substrate that is more easily hydrolyzed via increasing enzyme accessible surface area sonication have been reported to decrease cellulase requirements by 1/3 to 1/2 and to increase ethanol production from mixed waste office paper by approximately 20% (Wood,1997). Sakakibara, et al. (1996) have reported that sucrose hydrolysis by invertase is also accelerated by ultrasound irradiation. The application of ultrasound pretreatment may significantly increase the conversion of starch materials to glucose as well as overall ethanol yield (Khanal, 2007) which represent on figure 2.4 below.

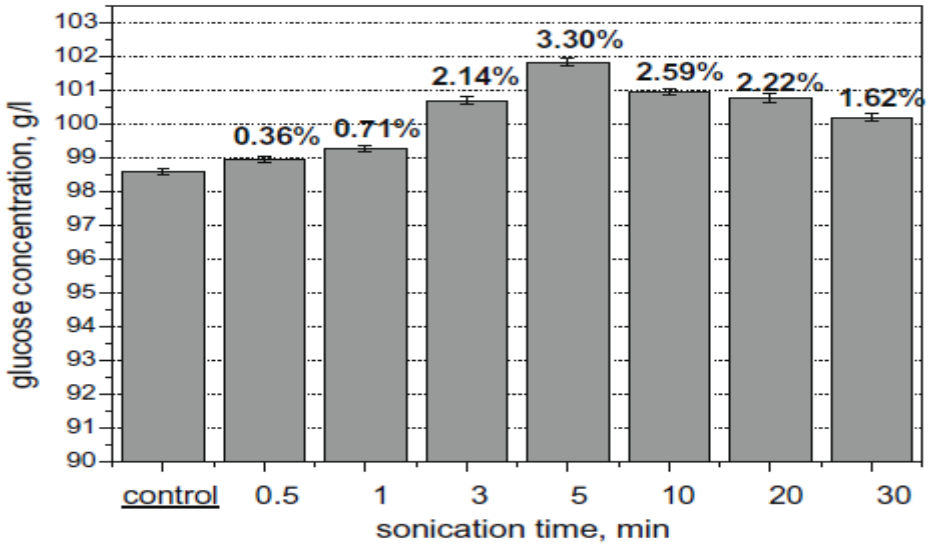


Figure 2.4: Glucose concentration of corn vs. sonication time

Direct exposure of the lignocellulosic substrate to the 20 kHz high power ultrasound is in the main benefit of both pretreatment and ongoing hydrolysis (Vinotoru, 1999). Ultrasound operates by the bubbling of water within the lignocellulosic material to expand and contract. The wave also causes compression and extension of the particle, resulting in a destructive effect. Ultrasound produces high frequency oscillations and causes mutual friction inside the medium, thus generating heat, resulting in a large local temperature differences. In this sonication, the sonic horn or transducer imparts energy to a bath of fluid surrounding the vessel containing the material being processed (Nikolić et al., 2011).

2.5 ECONOMIC POTENTIAL OF BANANA BRANCH AS SOURCE OF BIOMASS

With the inevitable depletion of the world's energy supply, there has been an increasing worldwide interest in alternative sources of energy (Aristidou and Penttila 2000; Jeffries and Jin 2000; John 2004; Kerr 1998; Wheals et al. 1999; Zaldivar et al. 2001). Consider the cleanest liquid fuel alternative to fossil fuel, growing attention has been dedicated to the conversion of biomass into fuel ethanol for long years ago that can contribute to sustainable development (Van den Broek 2000; Monique et al. 2003).According to Centre for Monitoring Indian Economy (ICME) banana is one the

most extensively consumed fruits in the world and represents 40% of world trade in fruits. Any country with a banana agronomic-based economy can use current technology for fuel ethanol fermentation. This is possible because, during the last two decades, technology for ethanol production from nonfood-plant waste has been developed to the point at which large-scale production will be a reality in the next few years. Extensive research has been carried out in this field for decades (Yu & Zhang, 2004), and the first demonstration plant using lignocellulose feedstock has been in operation in Canada since April 2004 (Tampier et al., 2004).

Malaysia has been recognized as the country in Asia that developed agriculture as one of its focus industries of economic aspect. With the significant amount of agricultural activities, lignocellulose by banana branch as a waste agriculture product become one of the source of non-food biomass for ethanol production. The estimated availability of the biomass and its potential energy generated in Malaysia are 50,919 dry ktonne / year and 13343 ktonne / year (Tye et al, 2011). There is 29,270 hectare of banana planted in 2012 producing 294,530 metric ton of fresh banana. According to (Anem, 2012), total value of banana production in 2012 estimated about RM 294.5 million concentrated area of planting located in Johor, Pahang, Selangor, Perak and Sabah in Malaysia. High biomass feedstock of banana waste material such as banana branch will cause of high potential of production of bioethanol. Furthermore, banana plant also grows well due to suitable climate of Malaysia.

Table 2.2: Potential power generation of banana component

Banana component	Method	Potential power generation (MW)
Pseudostem and leaf	Direct combustion	80.52
Banana peels	Anaerobic digestion	269.13
Reject banana	Anaerobic digestion	600
	Total	949.65

Table 2.2 described how banana waste product can generate potential power that is relevant to be developing in Malaysia. Therefore, the market for bioethanol industry is larger than biodiesel since most automobile in Malaysia run in gasoline.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION.

This project is done based on literature review and the general view of bioethanol, the production of bioethanol, source of biomass and enzyme. This project also has been given a work station at Reaction Engineering Laboratory. There are 8 sample of different fungus including yeast as a standard reference. Pretreatment of cellulose from banana branch has been specifying by ultrasound bath and the preparation of fungus enzyme done by oven heating process.

3.2 TOOLS AND EQUIPMENT.

There are tools and equipment need to be used during the entire experiment. All these tools and equipment are available at Universiti Teknologi PETRONAS laboratory. Below is list of the tools and equipment used in the project are listed in table 3.1 below:

Table 3.1 : Equipment and apparatus for experimental study

Equipment	Description	Apparatus	Description
1) Electronic weight balance	To measure the weight of the banana branch and fungi samples.	1) Plastic container	To keep the process of batch fermentation of the mixture of banana branch and fungi.
2) Grinder	To reduce the size of the banana branch and fungi samples.	3) Beaker	To keep the banana branch during the ultrasonic bath process.

3) Centrifuge	To separate the fermentation samples to solid and liquid.	4) Centrifuge tube	To keep the fermentation samples at the centrifuge machine.
4) Refractometer	To measure the refractive index of the ethanol produce by the samples.	5) Spatula	To scooping out the measured yeast and fungi.
5) Oven	To remove the water content of the banana branch and fungi samples.	6) Pipette	To measure the amount of standard ethanol for calibration.
6) Ultrasound Bath	To break the cellulose structure of the banana branch.	7) Aluminum foil	To cover the beaker during ultrasound bath process

3.3 CHEMICAL AND MATERIALS

The chemical and material also has to be preparing according to experimental step and procedure. The chemical and material require for this experiment are listed on the table 3.2 below:

Table 3.2: Chemical and material for experimental setup.

Chemical and Materials	Description
1) Ethanol 95% V/v	To calibrate the standard concentration of ethanol
2) Distilled water	To be mix with ethanol for calibration of standard ethanol sample.
3) *Saccharomyces Cerevisiae* (yeast)	To be as a standard or references fermentation fungi.
4) 7 samples of different unknown type of fungi	To be as fermentation fungi.
5) Banana branch	To be as source of biomass.

3.4 CALIBRATION OF ETHANOL CONCENTRATION STANDARD SAMPLE

Preparation of standard ethanol sample are the vital part need to be done before proceed with the experiment. The standard samples are measured by their refractive index according to the ethanol concentration.

Procedure:

The calibration of ethanol concentration standard sample begins with measurement of 1 ml of 95% concentration of EtOH by measuring cylinder. Then, the experimental step continues with measurement of 9ml of distilled water by the other measuring cylinder. Next, mix the 1 ml EtOH with 9 ml of distilled water into the test tube. After mixing step, the mixture sample will be test at refractometer to get the reading of the refractive index. Lastly, repeat the experiment by using different volume of ethanol and distilled water as shown in the table 3.1 below.

Table 3.3: Ratio on ethanol and distill water for calibration**.**

Volume of Ethanol (ml)	Volume of Distilled Water (ml)	Purity (%) of Ethanol
0	10	0
2	8	19
4	6	38
6	4	57
8	2	76
10	0	95

3.5 EXPERIMENT AND PROCEDURES

Pretreatment, Fungus enzyme preparation and fermentation technique are included in this experimental procedure on the figure 3.2. The experimental begin with the pretreatment or reducing size of banana branch or either preparation of fungi enzyme. Both fungi and banana branch will be mix together for the fermentation process.

Figure below show the flow sheet procedure for pretreatment, preparation of enzyme and fermentation process.

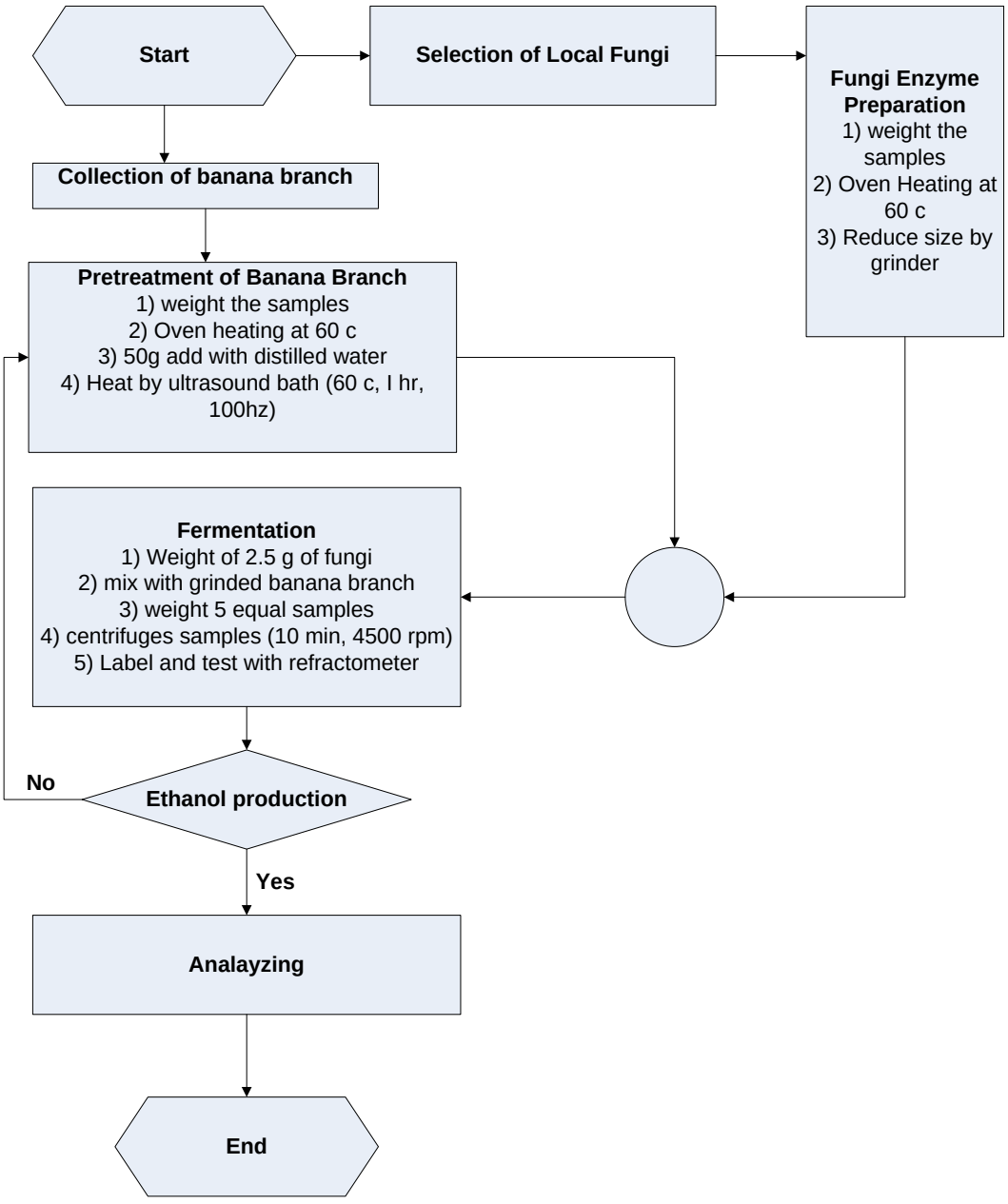


Figure 3.1: Procedure of the experiment

3.5.1 Pretreatment Technique

Procedure:

The technique that has been chosen for pretreatment which breaking the lignocellulose of the banana branch is ultrasound bath technique. First, the pretreatment of banana branch start with the measurement of weight of the sample of banana branch then put the banana branch samples in oven at temperature 60 ^{o}c for three hour for drying process. Then, sample is grinded to smaller size by using grinder and prepares the 50 g eight samples and added 100 ml of distilled water for each sample. After that, the beaker of the mixture is wrapped by the aluminum foil. Lastly, The 8 samples are heated in ultrasound bath at 40-50°c for 1 hour with frequency 100 Hz. This temperature is suitable with vibration from ultrasound wave at this frequency and power.

3.5.2 Preparation of fungi

Procedure:

In order to prepare the fungi pretreatment, 7 samples of local fungi and yeast need to weight first. Then, put the fungi and yeast samples in oven at temperature at 60°c for 8 hour for drying. Lastly, the fungi samples are grinded to smaller size by using grinder.

3.5.3 Fermentation

Procedure:

Fermentation stage begins with the measurement of 2.5g of each of the 7 samples of fungi and 2.5 g of *Saccharomyces cerevisiae.* Next, mix the samples with the grinded banana branches and stir the mixtures completely. Divide the mixtures into 5 equal weights and put in closed plastic container. Then, label each of the containers with date with the interval of day 2, day 4, day 6, and day 10. After that, centrifuge the samples for 10 minutes and speed of 4500 rpm for interval of 2 day and test it with refractometer. Record the data and repeat the step for each type of fungi.

3.6 PROJECT ACTIVITIES

The project activities are conducted according with the objectives that been stated in previous chapter.

Based on the first objective of the investigation of this project which to identify the various local fungi for biomass digestion, the project activity is:

a) **Collection or identification of various local fungi and banana branch.**

Banana branch and various local fungi have been collected around UTP. The fungi have been kept in each closed container. The fungi also has been labeling for sampling and identification purpose. The fungi sample is later being recognized and identified by referring to fungi physical and chemical properties.

Based on the second objective of the investigation of this project which is to evaluate the capability of local fungi in the fermentation of cellulosic biomass, the project activity are:

a) **To study the concept of ethanol fermentation.**

In order to achieve the first objective, the author needs to do some background study on the concept of fermentation. Background study helps the author to have better understanding on the subject thus the author can plan the whole process of the project through a Gantt chart. During preliminary research, it is found out that most study was done by experiment. This is whereby the analysis of ethanol produce and the result was referring to standard ethanol sample graph.

The first step is to study the general concept of fermentation. Following that, the author also read and study the journals of previous works related to biomass digestion by various fungi study. During this phase, the author outlined the methodologies used by other researchers and tries to find the best method. However, not much analysis was done using experimental on fermentation of cellulose with fungi as secreted enzyme. Most of the study has been focused on the conventional method which by

yeast. Using all these references, the author developed the methodologies for this experimental study.

b) Develop the experimental studies.

After preliminary research is done, there are some equipment and tools need to be prepared. The author needs to identify the biomass degradation method such as ultrasound bath. Then, the author will have to ensure the type of various local fungi and also its general characteristics.

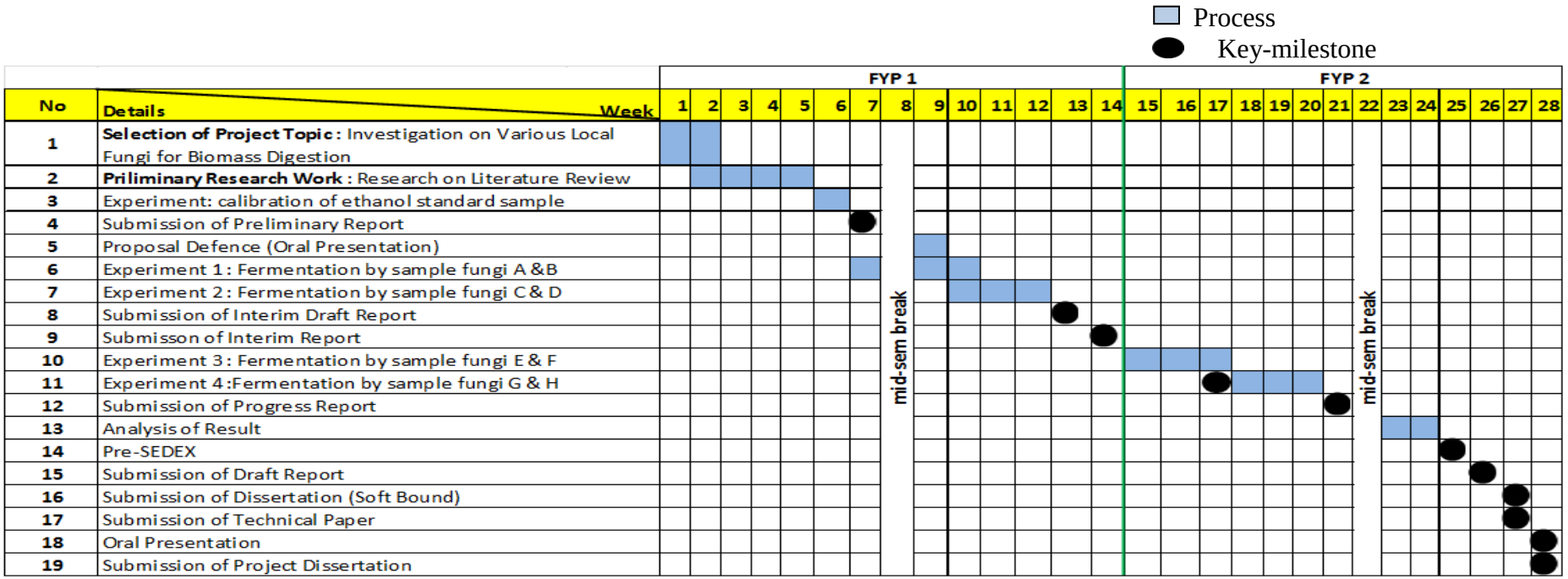
Before go for further procedure of experiment, the author has to do the calibration of ethanol standard as a reference point for percent of ethanol produced for every sample of fungi later. Then the experiment will undergo procedure of biomass pretreatment, preparation of fungi enzyme and fermentation process.

c) Analysis of ethanol production

Based on the refractive index of the 8 samples which has been measure by refractometer, the standard calibration of ethanol sample graph will set as reference point for the percentage of ethanol produce. The data will be recorded and will tabulate in the graph and table form for analysis, discussion and recommendation.

3.6 GANTT CHART

In order for the project going within the time and key-milestone, Gant Chart of project has been developed. The Gant chart has been designed for FYP1 and FYP2 further plan.



No	Details \ 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 :** Investigation on Various Local Fungi for Biomass Digestion																												
2	**Priliminary Research Work** : Research on Literature Review																												
3	Experiment: calibration of ethanol standard sample																												
4	Submission of Preliminary Report																												
5	Proposal Defence (Oral Presentation)																												
6	Experiment 1 : Fermentation by sample fungi A &B																												
7	Experiment 2 : Fermentation by sample fungi C & D																												
8	Submission of Interim Draft Report																												
9	Submisson of Interim Report																												
10	Experiment 3 : Fermentation by sample fungi E & F																												
11	Experiment 4 :Fermentation by sample fungi G & H																												
12	Submission of Progress Report																												
13	Analysis of Result																												
14	Pre-SEDEX																												
15	Submission of Draft Report																												
16	Submission of Dissertation (Soft Bound)																												
17	Submission of Technical Paper																												
18	Oral Presentation																												
19	Submission of Project Dissertation																												

Figure 3.2: Gantt chart of activity

CHAPTER 4

RESULTS AND DISCUSSION

This section explains the result obtained from experiments. There from the experimental set up, the result will be analyzed and discuss in this part. Therefore, the results and discussion might be divided into:

I. Calibration of standard ethanol sample.
II. Identification of various local fungi.
III. Fermentation by fungi *Saccharomyces cerevisiae* or *yeast* (standard sample).
IV. Fermentation by fungi *Schizophyllum commune.*
V. Fermentation by fungi *Pycnoporus sanguineus.*
VI. Fermentation by fungi *Micropus xanthopus* .
VII. Fermentation by fungi *Perenial ganoderma lipsiense.*
VIII. Fermentation by fungi *Microporus affinis.*
IX. Fermentation by fungi *Stereum hirsutum.*
X. Fermentation by fungi *Mycena sp.*

Each type of fungi has two graphs for value of refractive index and ethanol concentration.

4.1) RESULTS AND DISCUSSION

4.1.1 Calibration of ethanol concentration

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

Table 4.1: Refractive Index of Standard Ethanol Concentration

Volume of Ethanol (ml)	Volume of Distilled Water (ml)	Purity (%) of Ethanol	Refractive Index
0	10	0	1.33260
2	8	19	1.33921
4	6	38	1.34455
6	4	57	1.34930
8	2	76	1.35668
10	0	95	1.35932

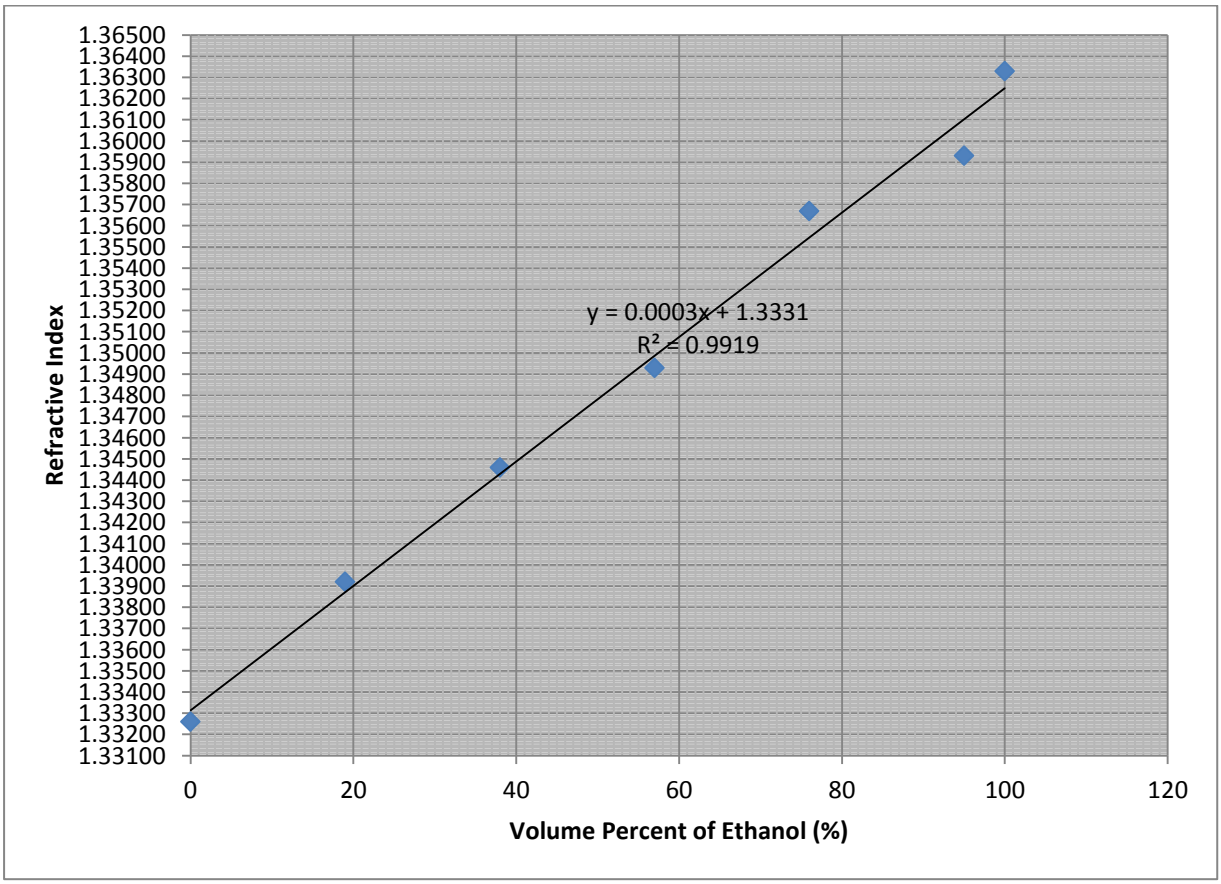


Figure 4.1: Graph of refractive index of ethanol.

As shown on figure 4.2, the refractive index is linear to ethanol concentration with equation of:

$$Y = 0.003x + 1.3333$$

The significant of standard ethanol calibration is to indicate the present of ethanol component by calculating ethanol concentration (%). The graph 4.1 above resulted of

correlation coefficient, R^2 = 0.9919. The value R^2 is a fraction between 0.0 and 1.0, and has no units. When r2 equals 1.0, all points lie exactly on a straight line with no scatter. Knowing volume percent of ethanol, *x* then the value of refractive index, y can be predicted. Therefore, the calibration of standard ethanol concentration is acceptable since it has R^2 = 0.9919.

4.1.2 Identification of various local fungi

This section will discuss the details about the 8 local collected fungi based on this research. The identification of each fungus was based on the internet website http://malaysianfungi.blogspot.com.

a) Schizophyllum commune

Figure 4.2*: Schizophyllum commune*

Schizophyllum commune is world's widely dispersed fungi included under species of mushroom in the genus *Schizophyllum*. In Mexico, it is commonly used as well as elsewhere at the tropic. By producing reproductive spore on the surface split when the mushroom dries out, *Schizophyllum commune* can be found from autumn to spring on dead wood, in coniferous and deciduous place. Structure of *Schizophyllum commune* made of a cap which is in shell-shaped with the attachment of the tissue at the end which also known as stem. With the properties of tough, felty and hairy, old *Schizophyllum commune* will wavy and lobed with a rigid margin. The color of *Schizophyllum commune* is greyish white and maximum 4 cm in diameter and mostly capable by movement. Known as the gills, *Schizophyllum commune* becomes in rolled when wet and have very narrow a longitudinal split edge.

b) Pycnoporus sanguineus (Red Fungus)

Figure 4.3: *Pycnoporus sanguineus*

Pycnoporus sanguineus or syn. P. coccineus is a species fungus under white rot saprobes fungus. *Pycnoporus sanguineus* commonly growing on dead hardwoods was found on Guana Island but occurs throughout the tropics. *Pycnoporus sanguineus* can be found in the form of a thin dry conk with a lateral attachment to its substrate. Moreover with the properties of bright orange on all surfaces with center zonation it is inedible and possibly toxic.

c) Micropus xanthopus

Figure 4.4: *Micropus xanthopus*

Micropus xanthopus made up of funnel-shaped caps around 1 and 3 millimetres thick that are concentrically zoned in various shades of brown and which are connected by a yellow-footed stem. Under the type of polypore genus, this species is found on rotting wood and is common from the Australasian, Asian and African tropics except the American tropics.

d) Perenial Ganoderma lipsiense

Figure 4.5: *Perenial Ganoderma lipsiense*

Perenial Ganoderma lipsiense are fungi under the species of Ganoderma family. Ganoderma are generated from Greek word with the combination of *ganos* means brightness and *derma* means skin. *Perenial Ganoderma lipsiense* grow on wood and scientifically have 80 species especially from tropically region. Also known as shelf mushroom or bracket fungi, it highly potential in the medicine field especially in traditional Asian medicine. They are lignicolous and leathery either with or without a stem instead of grow in a fan-like or hoof-like structures on the trunks of living or dead trees. They have double-walled, truncate spores with yellow to brown decked inner layers.

e) Microporus affinis

Figure 4.6: *Microporus affinis*

Microporus affinis always can be found on fallen branches in rainforests. This type of fungi can be identified by their fan-shaped bracket with velvety, ridged cap with concentric zones of brown, red, yellow and black. On the underside of the cap are little white pores. The most common characteristic of having *Microporus affinis* is a concave shape like a saucer.

f) Stereum hirsutum

Figure 4.7: *Stereum hirsutum*

Stereum hirsutum is a hardwood-loving shell fungus that develops fairly substantial, middle-sized cap structures. The ecology of these fungi always grows on dead wood of hardwoods, especially oaks laterally attached, without a stem. The fruiting body is around 5-3 cm and has structures of fan shaped, semicircular and apprised with hair. Most of this species are widely distributed at North America.

g) Mycena sp.

Figure 4.8: *Mycena sp.*

Mycena sp. is a big genus of small saprotrophic mushrooms that are randomly more than a few centimeters in width. The white spores, tiny conical or bell-shaped cap, and thin fragile stem are the common characteristic of *Mycena sp.* few species has brighter colour but most of them are gray and brown. Other than have translucent cap,it has also incurved margin. This type of fungi has been collected by the researcher at the stem of banana plant.

h) *Saccharomyces cerevisiae* / yeast

Figure 4.9: Saccharomyces cerevisiae / yeast

Saccharomyces cerevisiae or known yeast is a conventional fungi used in the industrial fermentation. This fungus is set to be standard sample for fermentation process to be compared to performance of other fungi.

4.1.3 Fermentation by fungus *Saccharomyces cerevisiae* or *yeast* (standard fungi sample).

Table 4.2: Refractive index and ethanol volume percent for yeast

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33401	2.50		2	1.33367	1.00		2	1.33354	0.90
	4	1.33378	1.50		4	1.33355	0.40		4	1.33376	1.50
	6	1.33389	2.00		6	1.33388	2.00		6	1.33382	1.70
	8	1.33389	2.00		8	1.33394	2.20		8	1.33370	1.40
	10	1.33414	3.00		10	1.33392	2.00		10	1.33362	1.00

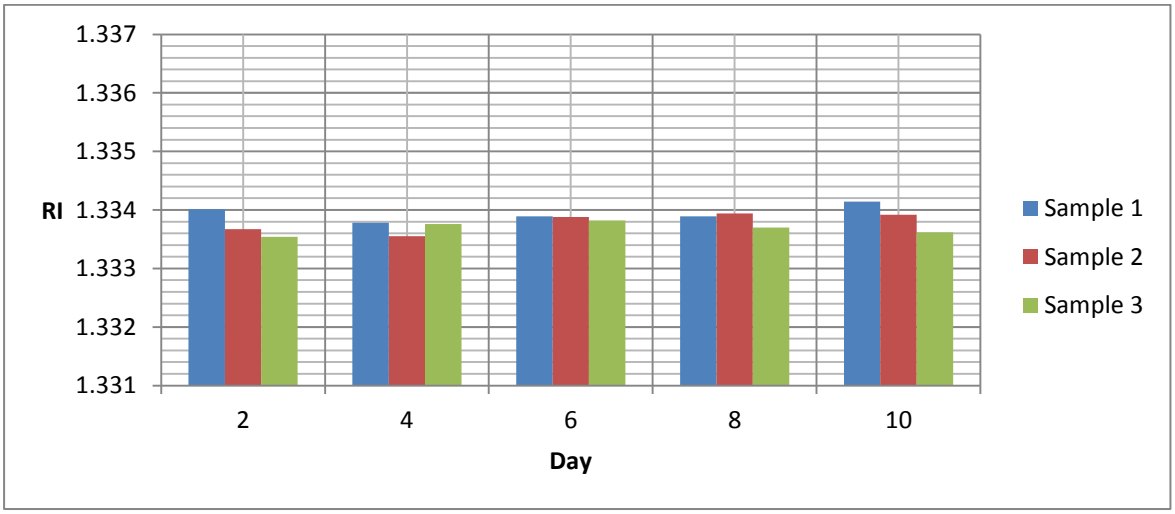


Figure 4.10: RI vs. Time graph for fungi yeast

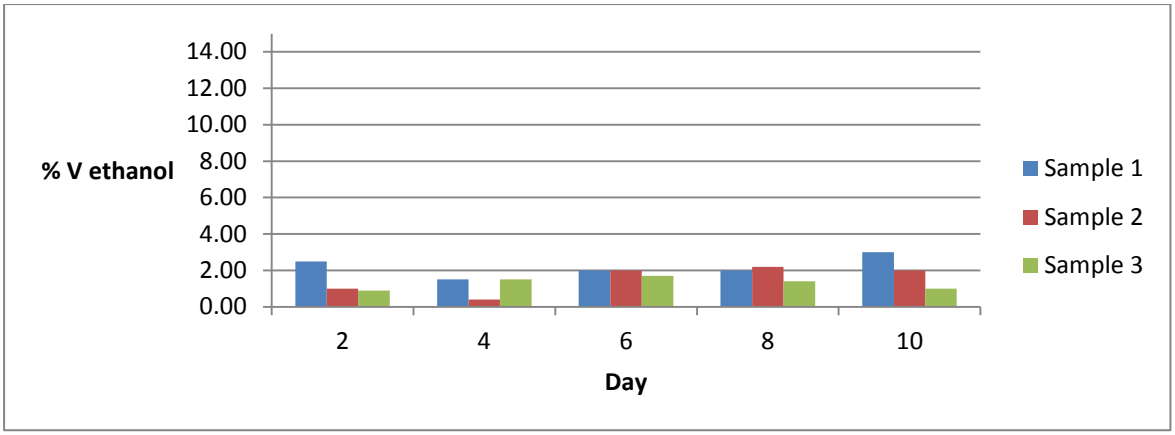


Figure 4.11: Ethanol Concentration vs. Time by yeast

Saccharomyces cerevisiae or yeast is the conventional fungi used in the world wide fermentation. Based on this research, yeast was used as a standard sample for comparison of ethanol production. The maximum ethanol production by yeast has been produced on day 10 at the maximum of 3% volume of ethanol.

4.1.4 Fermentation by fungus *Schizophyllum commune.*

Table 4.3: Refractive index and ethanol volume percent by fungi *Schizophyllum commune.*

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33326	0.00		2	1.3333	0.00		2	1.33332	0.00
	4	1.33322	0.00		4	1.33337	0.00		4	1.33331	0.00
	6	1.33329	0.00		6	1.33334	0.00		6	1.33334	0.00
	8	1.33351	0.70		8	1.33332	0.00		8	1.33398	2.00
	10	1.33342	0.50		10	1.33344	0.50		10	1.33354	0.70

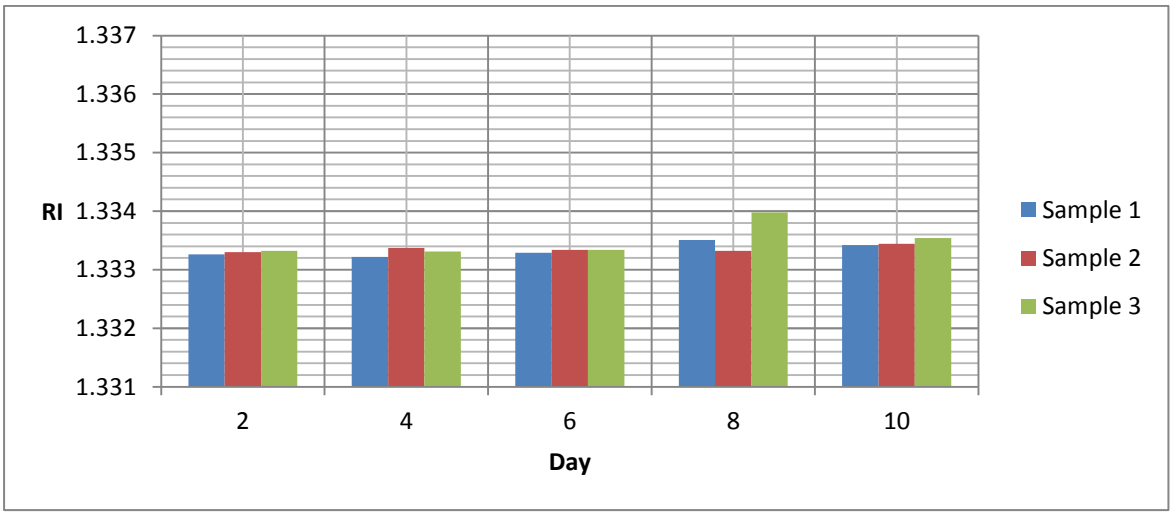


Figure 4.12: RI vs. Time graph for fungi *Schizophyllum commune.*

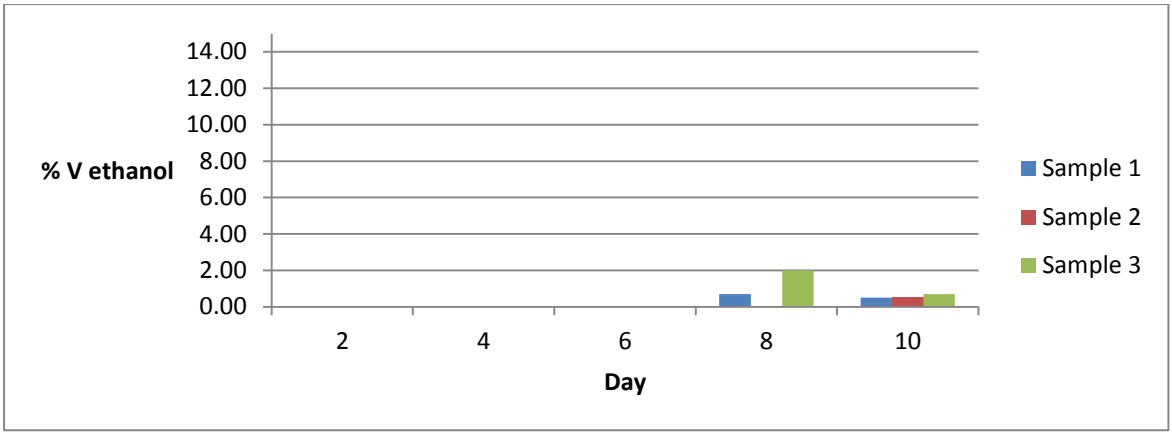


Figure 4.13: Ethanol Concentration vs. Time by *Schizophyllum commune.*

Production of ethanol by *Schizophyllum commune* resulted late production of ethanol where it represent zero from initial day 2 until day 6. However, small rise on production of ethanol can be seen during day 8 at 2% volume ethanol but decrease to 0.7% volume ethanol at day 10. The zero production of ethanol at the beginning of the day because slow reaction by active microorganism of *Schizophyllum commune* with cellulose of banana branch. Then, the decreasing of the ethanol production at the day 10 is because of the production of other chemical such as lignin which inhibits and deactivates the microorganism activity toward fermentation process.

4.1.3 Fermentation by fungus *Pycnoporus sanguineus.*

Table 4.4: Refractive index and ethanol percent by fungi *Pycnoporus sanguineus.*

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33386	2.00		2	1.33383	1.50		2	1.33388	1.80
	4	1.33357	1.00		4	1.33348	0.50		4	1.33346	0.70
	6	1.3332	0.00		6	1.33328	0.00		6	1.33336	0.20
	8	1.33321	0.00		8	1.33315	0.00		8	1.33312	0.00
	10	1.33319	0.00		10	1.3331	0.00		10	1.33307	0.00

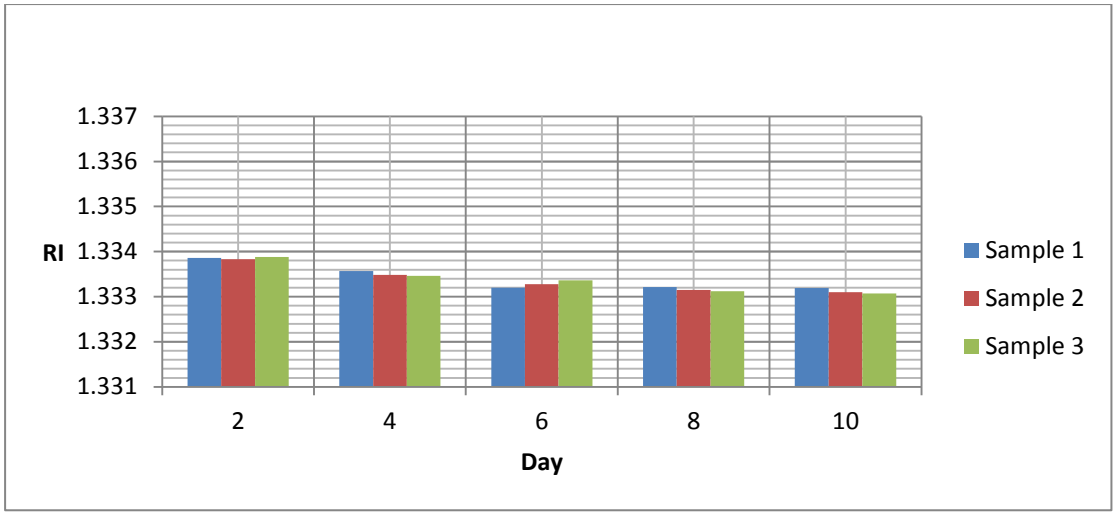


Figure 4.14: RI vs. Time graph for fungi *Pycnoporus sanguineus.*

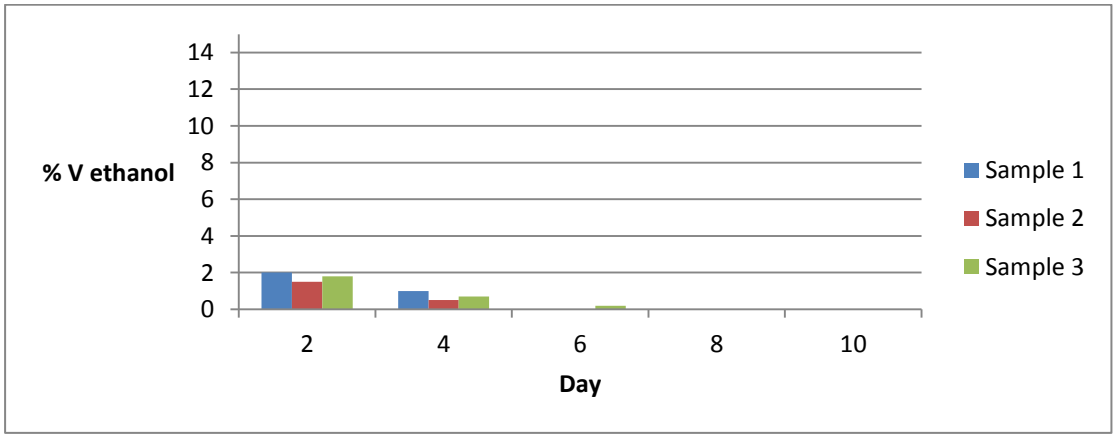


Figure 4.15: Ethanol Concentration vs. Time by *Pycnoporus sanguineus.*

Ethanol production by fermentation of banana branch with *Pycnoporus sanguineus* achieve highest production at day two with the 2 % ethanol volume but suddenly decreases for the next day. At day eight and 10, the ethanol production starts depleted to 0% volume ethanol. The highest production of ethanol at the beginning of the day indicates that the *Pycnoporus sanguineus* microorganism reacts faster with the sugar in the cellulose structure inside the banana branch to ethanol production. The lignin that dissolve during the pretreatment technique actually inhibit the production ethanol by slowly deactivate the fungi microorganism. The presence of weak acid along the 10 day and ethanol also kill the microorganism by attack the cell membrane of the microorganisms. Therefore, the enzyme leak out of the cell and slow the rate of reaction of the fermentation process.

4.1.4 Fermentation by fungus *Micropus xanthopus.*

Table 4.5: Refractive index and ethanol percent for fungi *Micropus xanthopus.*

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33324	0.00		2	1.33329	0.00		2	1.33327	0.00
	4	1.33334	0.00		4	1.33336	0.00		4	1.33328	0.00
	6	1.33338	0.00		6	1.33337	0.00		6	1.33327	0.00
	8	1.33339	0.00		8	1.33343	0.50		8	1.33342	0.30
	10	1.33338	0.00		10	1.33339	0.00		10	1.33363	1.00

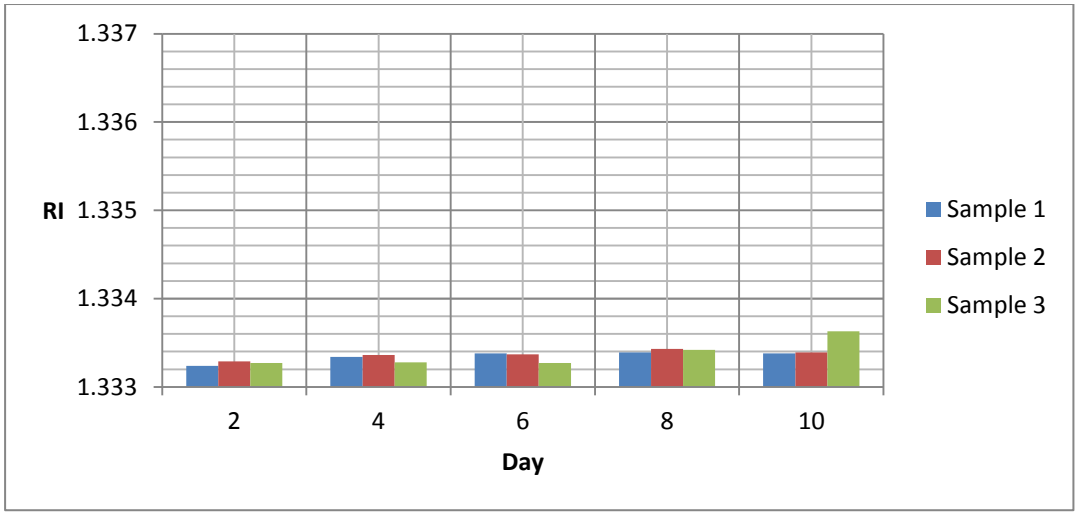


Figure 4.16: RI vs. Time graph for fungi *Micropus xanthopus.*

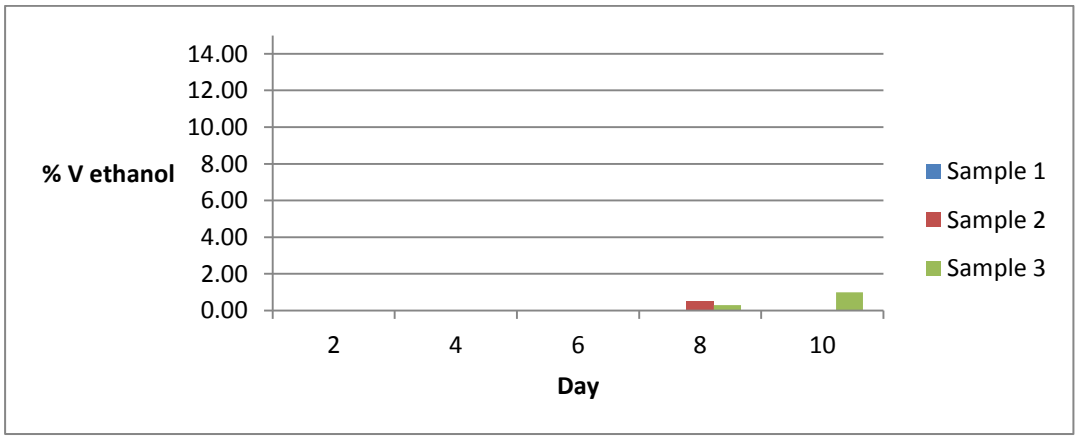


Figure 4.17: Ethanol Concentration vs. Time by *Micropus xanthopus*

Micropus xanthopus fungi resulted low performance on fermentation process where it takes long time fermentation where the production of ethanol start at day 8 with small amount of 0.5% volume ethanol and 1% volume ethanol at day 10. Since the experiment are not extracted the active microorganism from the fungi,

there are some possibility of disturbance of the ethanol production. Same as previous fungi, the *Micropus xanthopus* microorganism has slow reaction with the cellulose. There also fast presence of other chemical such as weak acid at the beginning of the day which kill most of the microorganism cell and left only small amount of healthy microorganism cell to react with the cellulose at day 8 and 10. Therefore, at the end of the day, very small amount of volume percent of ethanol is detected.

4.1.5 Fermentation by fungus *Perenial ganoderma lipsiense.*

Table 4.6: Refractive index and ethanol percent for fungi *Perenial ganoderma lipsiense.*

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33565	6.00		2	1.33616	9.00		2	1.33666	11.00
	4	1.33456	4.00		4	1.33465	1.50		4	1.33573	8.00
	6	1.33359	1.00		6	1.33359	1.00		6	1.33551	7.50
	8	1.33353	0.90		8	1.33363	1.50		8	1.33456	4.50
	10	1.33350	0.90		10	1.33352	0.90		10	1.33382	2.00

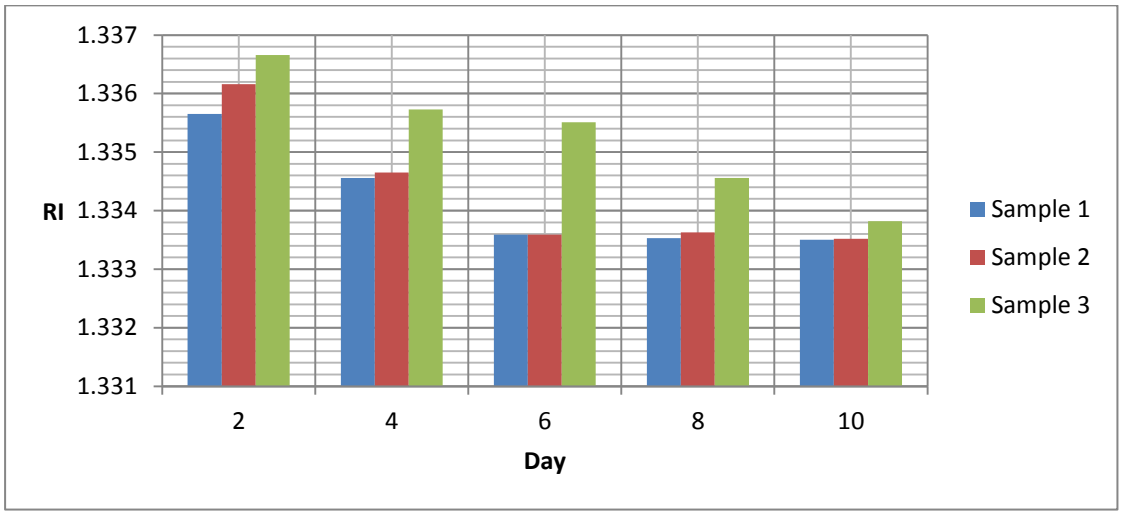


Figure 4.18: RI vs. Time graph for fungi *Perenial ganoderma lipsiense.*

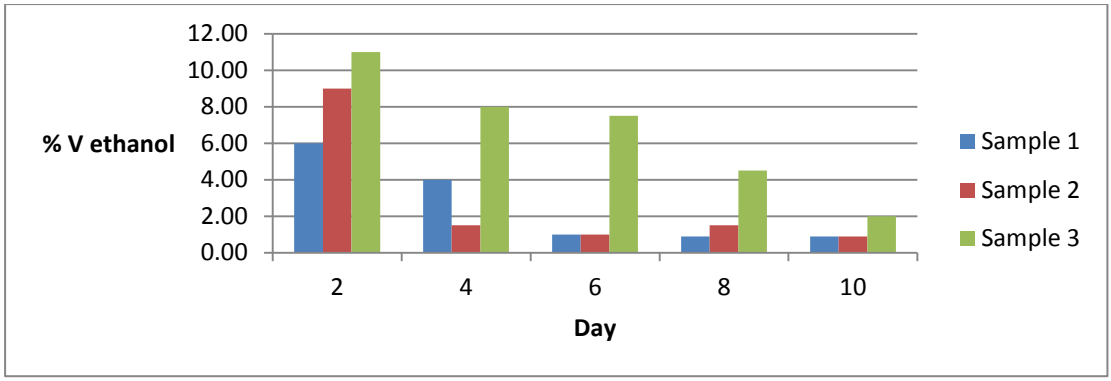


Figure 4.19.: Ethanol Concentration vs. Time by *Perenial ganoderma lipsiense.*

Perenial ganoderma lipsiense show the highest yield of fermentation at day two with 11% volume production of ethanol. The decreasing of ethanol production from day 4 to day 10 indicates the lowest production at day 10 which is around 2 % volume ethanol. From the graph above, *Perenial ganoderma lipsiense* microorganisms react with cellulose faster at the beginning of the fermentation process. The presence of weak acid is slowly appearing day by day where weak acid slowly attack and kill the cell of the microorganism. The highest productions of ethanol by *Perenial ganoderma lipsiense* is favor by the slow production of acidic acid and create a lower acidic condition. As the pH increase, the ethanol production is decrease.

4.1.6 Fermentation by fungus *Microporus affinis.*

Table 4.7: Refractive index and ethanol percent for fungi *Microporus affinis.*

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33322	0.00		2	1.33322	0.00		2	1.33321	0.00
	4	1.33315	0.00		4	1.33321	0.00		4	1.33333	0.00
	6	1.33321	0.00		6	1.33366	1.00		6	1.33358	0.70
	8	1.33352	0.80		8	1.33365	1.00		8	1.33362	1.00
	10	1.33355	0.90		10	1.33382	1.80		10	1.33362	1.00

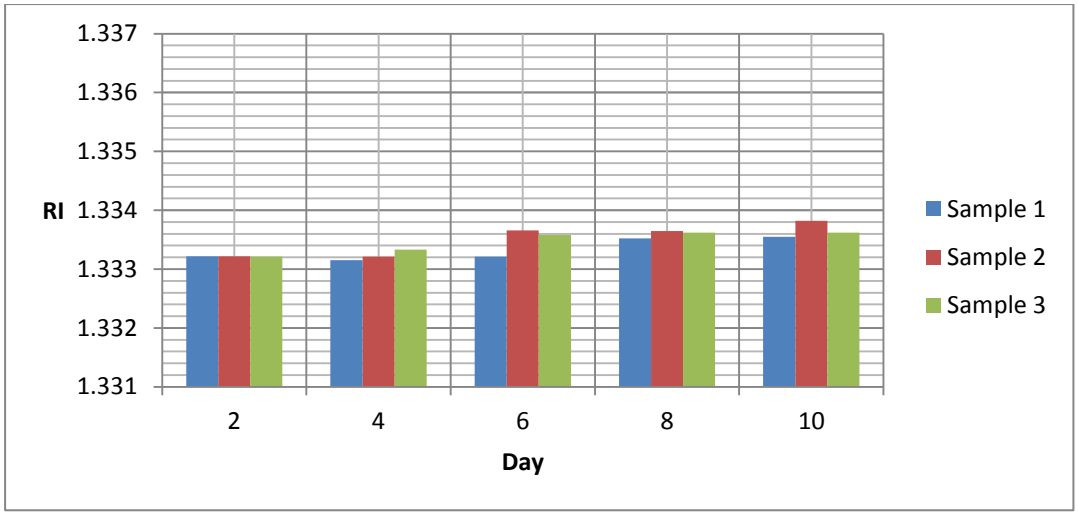


Figure 4.20: RI vs. Time graph for fungi *Microporus affinis.*

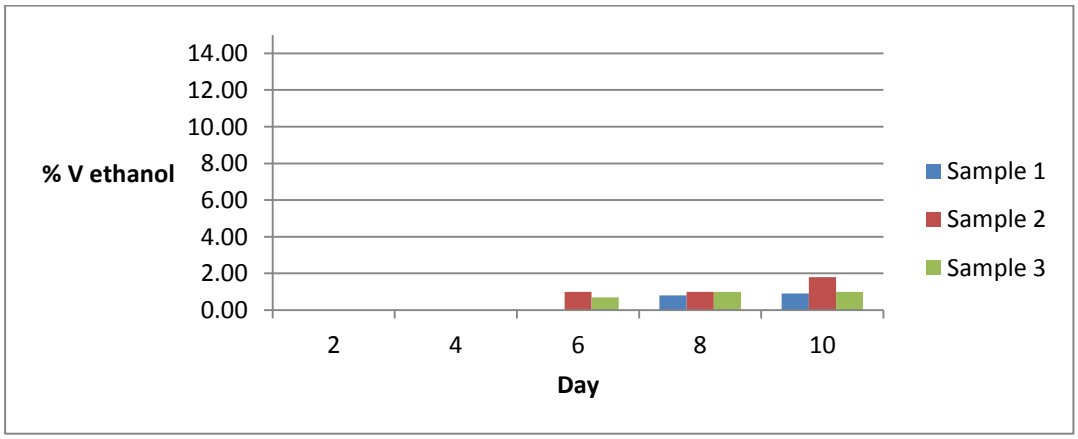


Figure 4.21.: Ethanol Concentration vs. Time by *Microporus affinis.*

The highest production of ethanol by *Microporus affinis* experimentally achieve at day 10 with 1.8%. Same as other fungi, *Microporus affinis* slowly favor the ethanol production slowly with the no production of ethanol at the initial day of 2 and 4. Since the experiment are not extracted the active microorganism from the fungi, there are some possibility of disturbance of the ethanol production. Same as *Micropus xanthopus* fungi, the *Micropus affinis* microorganism has slow reaction with the cellulose. At the beginning of the day, the *Micropus affinis* microorganism still not in good condition to release fermentation enzyme, cellulase to react with cellulose. The microorganism starts to react at day 6 and increase at day 8 and 10 and require long fermentation time to reach maximum production time.

4.1.7 Fermentation by fungus *Stereum hirsutum.*

Table 4.8: Refractive index and ethanol percent for fungi *Stereum hirsutum.*

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	0.00	0.00		2	0.00	0.00		2	0.00	0.00
	4	0.00	0.00		4	0.00	0.00		4	0.00	0.00
	6	1.33342	0.40		6	1.33341	0.30		6	1.33339	0.20
	8	1.33389	2.00		8	1.33343	0.40		8	1.33362	1.00
	10	1.33363	1.00		10	1.33353	0.80		10	1.33363	2.00

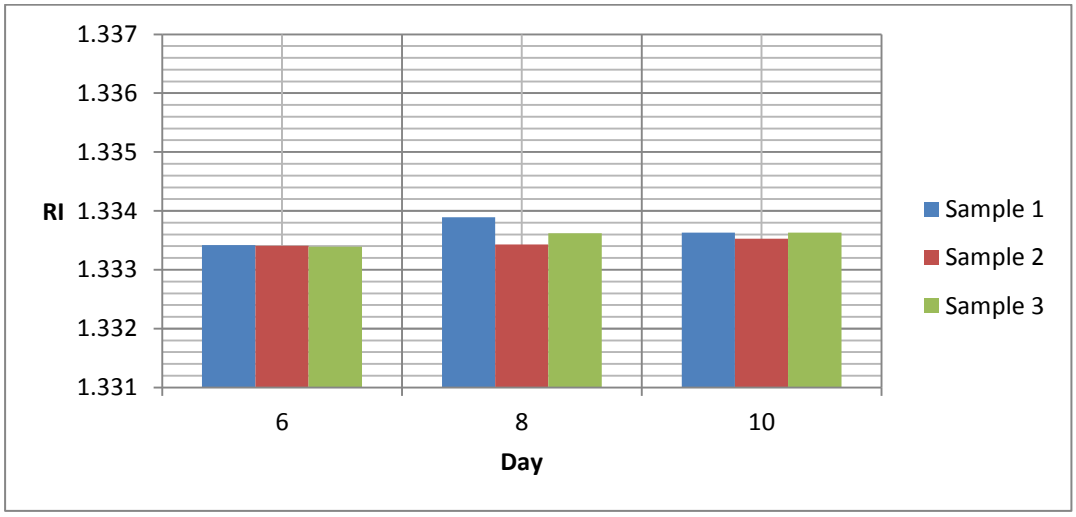


Figure 4.22: RI vs. Time graph for fungi *Stereum hirsutum.*

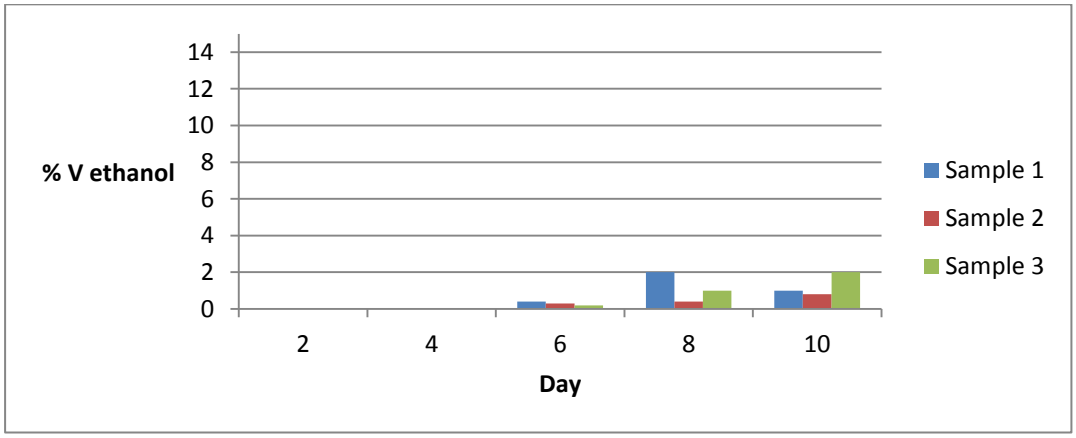


Figure 4.23.: Ethanol Concentration vs. Time by *Stereum hirsutum.*

Stereum hirsutum fungi also show long fermentation time where the production of ethanol starts at day 6. The highest production represent by day 10 with the value of 2% volume of ethanol. Same as other fungi, *Stereum hirsutum* slowly favor the ethanol production slowly with the no production of ethanol at the initial day of 2

and 4. Same as *Micropus xanthopus* fungi, the *Stereum hirsutum.* Microorganism has slow reaction with the cellulose. At the beginning of the day, the *Micropus affinis* microorganism still not in good condition to release fermentation enzyme, cellulase to react with cellulose. The microorganism starts to react at day 6 and increase at day 8 and 10 and require long fermentation time to reach maximum production time.

4.1.8 Fermentation by fungi *Mycena sp.*

Table 4.9: Refractive index and ethanol percent by fungi *Mycena sp.*

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33343	0.50		2	1.33331	0.00		2	1.33341	0.50
	4	1.33461	4.50		4	1.33411	3.00		4	1.33434	3.50
	6	1.33348	0.60		6	1.33447	4.00		6	1.33323	0.00
	8	1.33394	2.00		8	1.33332	0.00		8	1.33323	0.00
	10	1.33358	0.70		10	1.33332	0.00		10	1.33337	0.00

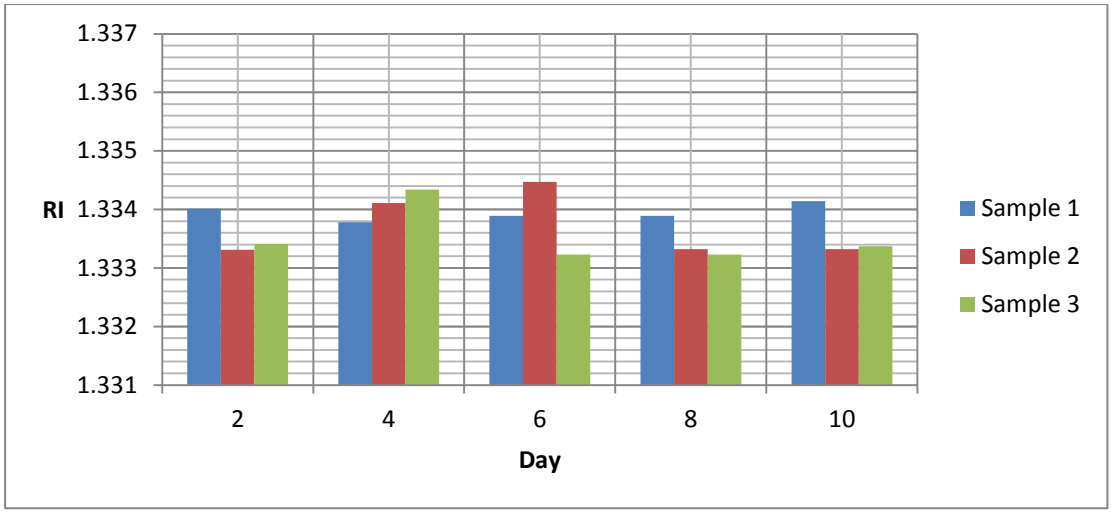


Figure 4.24: RI vs. Time graph for fungi *Mycena sp.*

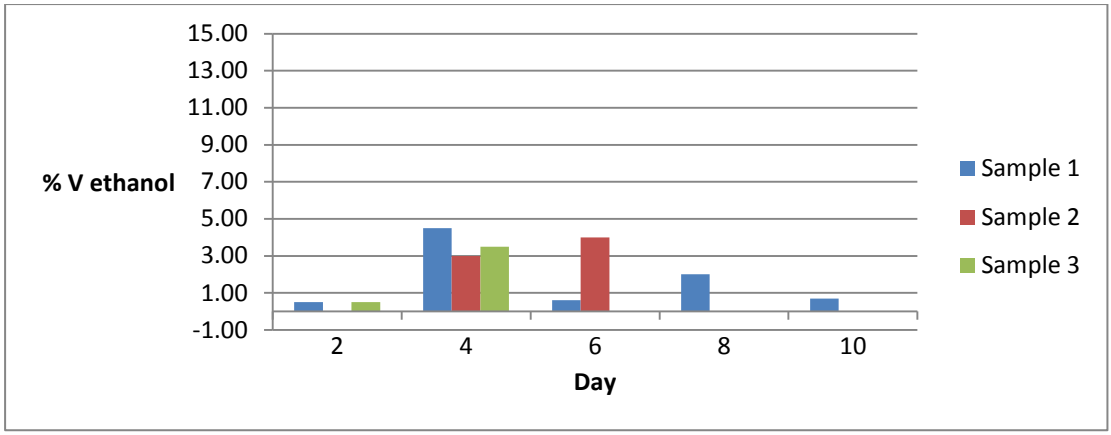


Figure 4.25.: Ethanol Concentration vs. Time by *Mycena sp.*

Mycena sp. fungi are one of the collected fungi grow at banana stem. The highest production of ethanol by *Mycena sp* can be show at day 4 with the percentage of 4.5% volume ethanol. Small production of ethanol is detected at day 2, where the small amount of microorganism reacts with cellulose but continue react until day 4. By the time, as the microorganism digest the glucose, weak acid such as acetic acid and formic acid is formed during rest of the day as by product. The weak acid which is able to diffuse to the microorganism membrane and increase the pH inside the cell. Therefore, in order to maintain the pH of the cell, the microorganism needs to expand Adenosine triphosphate (ATP) and suddenly have less ability for cell reproduction for ethanol production.

4.2.2) Summary of performance of local fungi.

Based on the fermentation sample of 8 types of fungi, the highest ethanol production is 11% represent by fungi *Perenial ganoderma lipsiense* at day 2. The standard sample of fungi, yeast or *Saccharomyces cerevisiae* resulted of 3% at day 10 being used as a reference or benchmark for the ability of the other 7 type of fungi toward the biomass digestion for fermentation process. Among the 7 type of fungi, there are several fungi has the higher ability for fermentation fungi such as *Perenial ganoderma lipsiense* where 11% at day 2 and *Mycena sp* where 4.5% ethanol produced at day 4 while the other fungi are below 3% production of ethanol. *Perenial ganoderma lipsiense* are one of 80 type of Ganoderma family and extensive used in the Asia region. *Mycena sp* are type of fungus where it grows at the banana tree stem.

However, the yield of ethanol by fermentation of local fungi is still in lower level where it below 15%. Since this research not extracted the pure ethanol during centrifugal of sample, there were present of the other chemical other than ethanol. Therefore, the refractive index reading is influenced by the present of the other chemical and resulted lower reading of refractive index.

Other than that, the low production of fermentation ethanol also reasonably because of the long period of ultrasound technique which is 60 minute. Longer time for ultrasonic sound during pretreatment resulted increasing concentration of glucose which affected the enzyme inhibition. During the ultrasound technique, the degradation of banana branch will reach maximum, but the longer time will lead to the inhabitation of enzyme produced during fermentation process. This research experimental procedure also using raw banana branch lignin, hemicellulose and cellulose are not being separated. Therefore, lignin dissolved under the pretreatment step can deceptively redeposit onto cellulose, creating a barrier to effective cellulose hydrolysis and reducing sugar yield. The detrimental effect of dissolve lignin potentially reduces the yield of ethanol.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Based on the 7 sample of fungus and conventional yeast that has been investigated through this research, the unconventional fungi indicate positive results and prospect to become industrial fermentation fungi since the yield of ethanol of the *Mycena sp* (4.5%) *and Perenial ganoderma lipsiense* (11%) are higher than conventional yeast in term of ethanol production. Therefore, the production of bioethanol can be improved by replacing the conventional yeast experimentally or industrial purpose with a lot of further research and development. The banana branch also shows positively as one of the non-food biomass source for local energy industry. Therefore, this investigation meets the objective of this research by successfully identify 7 local fungi for biomass digestion and evaluate the capability of local fungi in the fermentation of cellulosic biomass.

5.2 RECOMMENDATION

From this study, fungi *Perenial ganoderma lipsiense* are recommended for next potential to replace yeast since it has higher ability as source of enzyme to produce fermentation bioethanol. Then, the longer fermentation study also needs to be studied since some fungi took longer fermentation time to reach optimum production of ethanol. Lastly, the shorter ultrasound technique applied to the banana branch and the author also would like to recommend for the study of other method of determination of ethanol content such as specific gravity method, GC-MS method and HPLC method for accurate calibration and measurement.

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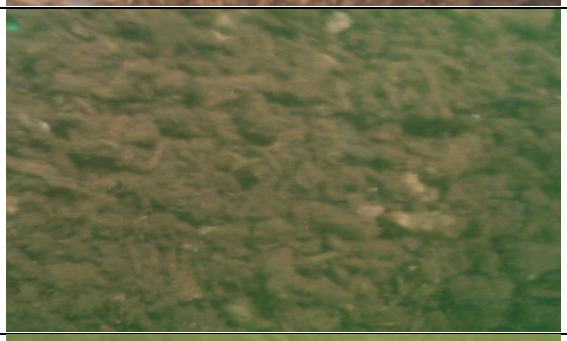
APPENDICES

i) Grinded and Dried Fungi

A		*Spilt-Gill (Schizophyllum commune)*
B		*Pycnoporus sanguineus - Red Fungus*
C		*Micropus xanthopus*
D		*Perenial Ganoderma lipsiense*
E		*Microporus affinis*

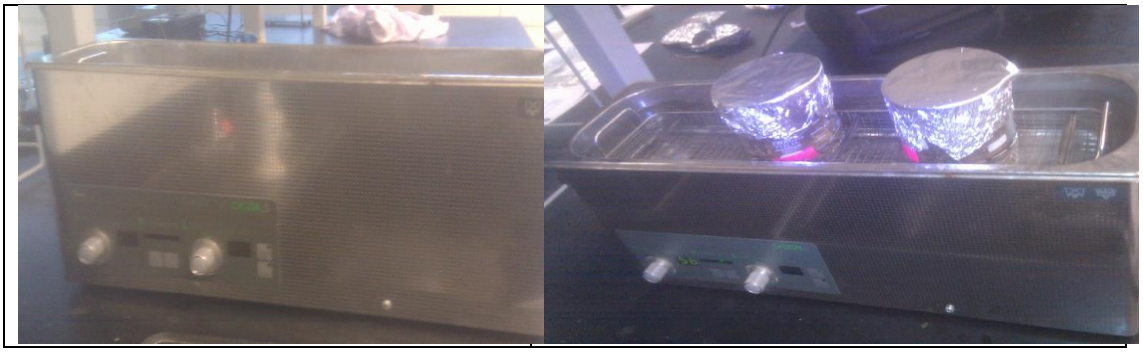
F		*Stereum hirsutum*
G		*Mycena sp.*
H		*Saccharomyces cerevisiae*

ii) Grinded and Dried Banana Branch

iii) Equipment

1) Ultrasonic bath.



2) Oven

3) Centrifuge

4) Refractometer

INVESTIGATION OF VARIOUS LOCAL FUNGI FOR BIOMASS DIGESTION

Khairul Amir Bin Sukarman
Chemical Engineering Department
Universiti Teknologi PETRONAS
Tronoh, Malaysia
kas5490@gmail.com

Shuhaimi Bin Mahadzir
Chemical Engineering Department
Universiti Teknologi PETRONAS
Tronoh, Perak
shuham@petronas.com.my

***Abstract*— Consumption of ethanol produced from biomass carries wide opportunity to reduce the use of gasoline and cut CO_2 emissions. Therefore, the fermentation of waste banana branch cellulose with various local fungi include *Schizophyllum commune, Pycnoporus sanguineus, Micropus xanthopus, Perenial Ganoderma lipsiense, Microporus affinis, Microporus xanthopus and Mycena* sp has been investigated for their ability to produce bioethanol. The production patterns of fermentation were studied during the growth of the organisms for a period of interval of two day until day 10. The cell wall breaking banana branch cellulose has been done using the technique of ultrasound bath. The resulted bioethanol generation has been referred by ethanol standard calibration of refractive index. Among seven types of the collected fungi, fermentation yield resulted maximum 11% volume ethanol which represented by *Perenial Ganoderma lipsiense* fungi at day 2. Very low levels of bioethanol production were detected by the *Microporus affinis* fungi. The 11% yield percent of ethanol produced by the experiment show the better ability of alternative fungi for bioethanol industry compared to conventional fungi, yeast which also being included as a standard of fungi fermentation.**

Keywords-component; fermentation, bioethanol, cellulose, ultrasound bath, fungi.

I. INTRODUCTION

In future energy consumption, non-renewable fossil energy sources like oil and coal use nowadays will become depleted. People tend to find biomass as an alternative energy which is more clean and renewable. Biomass including organic matter wood, crops, seaweed, and animal wastes carries potential to become primary energy source in industries such as power generation of electric utilities, transportation, and residential. One of the significant products of biomass is biofuel, primarily bioethanol which mostly used as additives to petroleum gasoline.

Sugars, starches and cellulose are the common sources use in the fermentation process nowadays and a lot of research has been done to improve this technology. Fermentation process converts sugar into ethanol while complex carbohydrate and cellulose need to be hydrolyzed to become simple sugar first with the help of conventional fungi *Saccharomyces cerevisiae* also known as yeast.

Energy demand and climate change require large scale substitution of petroleum based fuels. The decreasing of petroleum-based fuels and environmental problems has triggered the development of inexpensive production of bioethanol fermentation [1]. The fermentation of ethanol can be produced from sugar, starch or lignocellulose biomass where sugar and starch-based plant residues are presently major at the industrial level and an economically favorable. Its production typically involves a hydrolysis-fermentation route, which has three main steps: pretreatment to get fermentable sugars, fermentation to produce bioethanol, and a separation process to obtain highly concentrated bioethanol [2]. Source of biomass can be described on the figure 1 below [3] where the major composition of raw material of biomass consists of cellulose.

Table 1: Composition of cellulose, hemicellulose and lignin of raw material

Raw material	Cellulose	Hemicellulose	Lignin
Hardwoods			
White birch	41	36.2	18.9
Poplar aspen	50.8–53.3	26.2–28.7	15.5–16.3
Red maple	44.1	29.2	24
Hybrid poplar	41.7	20.2	29.3
Eucalyptus viminalis	41.7	14.1	31
Softwoods			
Pinus banksiana	41.6	25.6	28.6
Pinus pinaster	42.9	17.6	30.2
fir	43.9	26.5	28.4
Agricultural residues			
Corn cobs	33.7–41.2	31.9–36	6.1–15.9
Sugar cane bagasse	40–41.3	27–37.5	10–20
Wheat straw	32.9–50	24–35.5	8.9–17.3
Rice straw	36.2–47	19–24.5	9.9–24
Corn stalks	35–39.6	16.8–35	7–18.4
Barley straw	33.8–37.5	21.9–24.7	13.8–15.5
Soya stalks	34.5	24.8	19.8
Cotton stalks	38.4–42.6	20.9–34.4	21.45

Source: Garrote et al. (1999).

Sugars which extracted from molasses, fruits, sugarcane, and sugar beets will be straight away converted into ethanol [4]. Corn, cassava, potatoes, and root crops are sources of starch which should go through process of hydrolysis to

convert into sugars by the action of enzymes. The most abundant source of biomass, cellulose located at the most inner part of the plant structure after hemicellulose and lignin [5].The extraction of simple sugar from the cellulosic biomass needs technique of breaking the cellulose such as ultrasonic bath.

Pretreatments can be done through physical methods, for example size reduction or chemical process [6]. The example of pretreatment technique is acid hydrolysis, steam explosion, ammonia fiber expansion, organ solve and sulfite pretreatment. In order to improve the bioconversion of lignocellulosic materials in an environmentally friendly manner, ultrasound is the best technique by expansion of the lignocellulose cells due to the effect of making pore through the ultrasonic waves [7].

Any country with a banana agronomic-based economy can use current technology for fuel ethanol fermentation [8]. High biomass feedstock of banana waste material such as banana branch will cause of high potential of production of bioethanol. Furthermore, banana plant also grows well due to suitable climate of Malaysia.

II. PROBLEM DEFINITION

Yeasts or *Saccharomyces cerevisiae* is the common and conventional microorganisms currently used for huge scale industrial ethanol production. Nowadays, there is a lot of technology and research has been develop to improve the bioethanol fermentation including re-engineered yeast and improves the yeast strains. This is because yeast has some weaknesses such as low productivity, low cell density, longer fermentation and prolonged downtimes. Moreover, yeast gene need to be re-engineered to prevent the fermentation inhibitor, acetic acid which is normally released by all the plant.

However, high cost and time constraints of conducting experiments and the difficulties of producing re-engineered yeast become problems in bioethanol fermentation.

One option is to investigate suitable fungi that are specific for biomass cellulose. In this study, banana branch which is non-food feedstock is selected. Several collected sample of different local fungi will lead to a cheaper solution for fermentation improvement process. With the technique of ultrasound bath which break the cellulose structure, the reaction between local fungi and banana tree branch will be more favorable. Thus, this research looks into the selection of suitable fungi for the conversion biomass cellulose into ethanol by fermentation process.

III. METHODOLOGY

There are 8 sample of different fungus including yeast as a standard reference. Pretreatment of cellulose from banana branch has been specifying by ultrasound bath and the preparation of fungus enzyme done by oven heating process.

A) Calibration of ethanol concentration standard sample

Preparation of standard ethanol sample are the vital part need to be done before proceed with the experiment. The standard samples are measured by their refractive index according to the ethanol concentration.

Procedure:

The calibration of ethanol concentration standard sample begins with measurement of 1 ml of 95% concentration of EtOH by measuring cylinder. Then, the experimental step continues with measurement of 9ml of distilled water by the other measuring cylinder. Next, mix the 1 ml EtOH with 9 ml of distilled water into the test tube. After mixing step, the mixture sample will be test at refractometer to get the reading of the refractive index. Lastly, repeat the experiment by using different volume of ethanol and distilled water as shown in the table 2 below.

Table 2: Ratio on ethanol and distill water for calibration.

Volume of Ethanol (ml)	Volume of Distilled Water (ml)	Purity (%) of Ethanol
0	10	0
2	8	19
4	6	38
6	4	57
8	2	76
10	0	95

B) Experiment and procedures

The experimental begin with the pretreatment or reducing size of banana branch or either preparation of fungi enzyme. Both fungi and banana branch will be mix together for the fermentation process. Figure 2 below show the flow sheet procedure for pretreatment, preparation of enzyme and fermentation process.

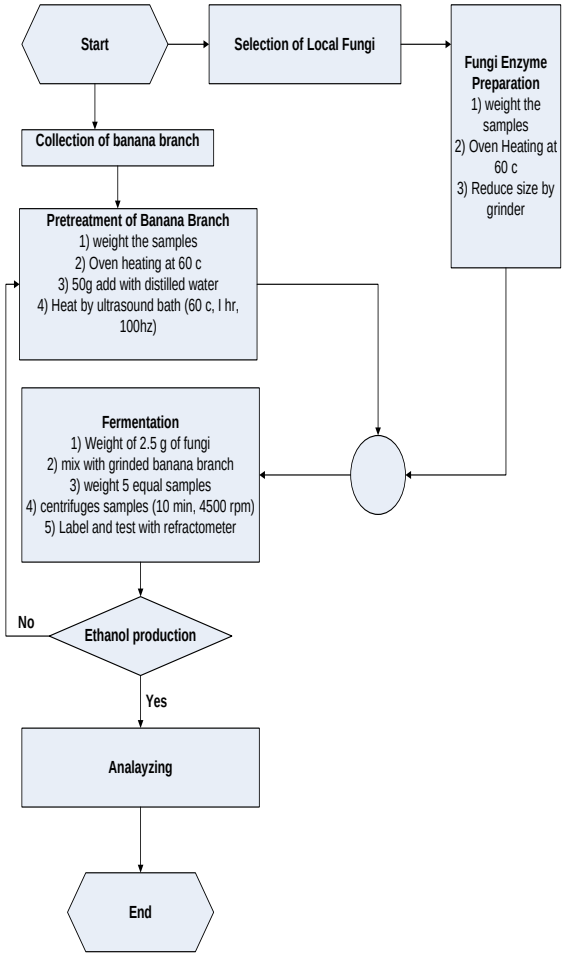


Figure 1: Experimental procedure

i) Pretreatment Technique

Procedure:

The technique that has been chosen for pretreatment which breaking the lignocellulose of the banana branch is ultrasound bath technique. First, the pretreatment of banana branch start with the measurement of weight of the sample of banana branch then put the banana branch samples in oven at temperature 60 oc for three hour for drying process. Then, sample is grinded to smaller size by using grinder and prepares the 50 g eight samples and added 100 ml of distilled water for each sample. After that, the beaker of the mixture is wrapped by the aluminum foil. Lastly, The 8 samples are heated in ultrasound bath at 40-50°c for 1 hour with frequency 100 Hz. This temperature is suitable with vibration from ultrasound wave at this frequency and power.

ii) Preparation of fungi

Procedure:

In order to prepare the fungi pretreatment, 7 samples of local fungi and yeast need to weight first. Then, put the fungi and yeast samples in oven at temperature at 60°c for 8 hour for drying. Lastly, the fungi samples are grinded to smaller size by using grinder.

iii) Fermentation

Procedure:

Fermentation stage begins with the measurement of 2.5g of each of the 7 samples of fungi and 2.5 g of *Saccharomyces cerevisiae*. Next, mix the samples with the grinded banana branches and stir the mixtures completely. Divide the mixtures into 5 equal weights and put in closed plastic container. Then, label each of the containers with date with the interval of day 2, day 4, day 6, and day 10. After that, centrifuge the samples for 10 minutes and speed of 4500 rpm for interval of 2 day and test it with refractometer. Record the data and repeat the step for each type of fungi.

IV. RESULT AND DISCUSSION

The results and discussion might be divided into:

I. Calibration of standard ethanol sample.
II. Identification of various local fungi.
III. Fermentation by fungi *Saccharomyces cerevisiae* or yeast (standard sample).
IV. Fermentation by fungi *Schizophyllum commune.*
V. Fermentation by fungi *Pycnoporus sanguineus.*
VI. Fermentation by fungi *Micropus xanthopus* .
VII. Fermentation by fungi *Perenial ganoderma lipsiense.*
VIII. Fermentation by fungi *Microporus affinis*.
IX. Fermentation by fungi *Stereum hirsutum*.
X. Fermentation by fungi *Mycena sp.*

Each type of fungi has two graphs for value of refractive index and ethanol concentration.

i) Calibration of ethanol concentration

Table 3 shows the refractive index for different ethanol concentrations. The sample used is in ratio of volume ethanol to distilled water (v/V).

Table 3: Refractive Index of Standard Ethanol Concentration

Volume of Ethanol (ml)	Volume of Distilled Water (ml)	Purity (%) of Ethanol	Refractive Index
0	10	0	1.33260
2	8	19	1.33921
4	6	38	1.34455
6	4	57	1.34930
8	2	76	1.35668
10	0	95	1.35932

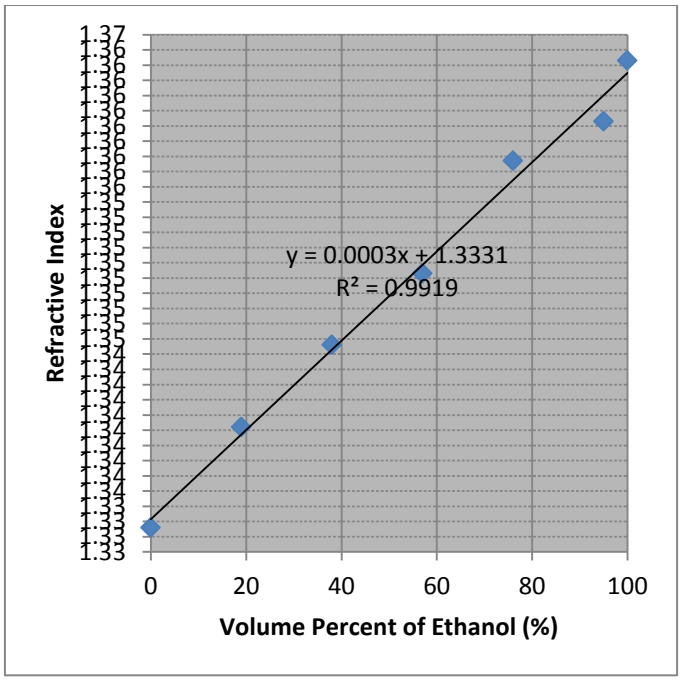


Figure 2: Graph of standard ethanol concentration

As shown on figure 2, the refractive index is linear to ethanol concentration with equation of:

$$Y = 0.003x + 1.3333$$

The significant of standard ethanol calibration is to indicate the present of ethanol component by calculating ethanol concentration (%). The figure 2 above resulted of correlation coefficient, R2 = 0.9919. The value R^2 is a fraction between 0.0 and 1.0, and has no units. When r2 equals 1.0, all points lie exactly on a straight line with no scatter. Knowing volume percent of ethanol, x then the value of refractive index, y can be predicted. Therefore, the calibration of standard ethanol concentration is acceptable since it has R^2 = 0.9919.

ii) Identification of various local fungi

This section will discuss the details about the 8 local collected fungi based on this research.

a) Schizophyllum commune


Figure 3: Schizophyllum commune

Schizophyllum commune is world's widely dispersed fungi included under species of mushroom in the genus *Schizophyllum*. In Mexico, it is commonly used as well as elsewhere at the tropic. By producing reproductive spore on the surface split when the mushroom dries out, *Schizophyllum commune* can be found from autumn to spring on dead wood, in coniferous and deciduous place. Structure of *Schizophyllum commune* made of a cap which is in shell-shaped with the attachment of the tissue at the end which also known as stem. With the properties of tough, felty and hairy, old *Schizophyllum commune* will wavy and lobed with a rigid margin. The color of *Schizophyllum commune* is greyish white and maximum 4 cm in diameter and mostly capable by movement. Known as the gills, *Schizophyllum commune* becomes in rolled when wet and have very narrow a longitudinal split edge.

b) Pycnoporus sanguineus (Red Fungus)


Figure 4: Pycnoporus sanguineus

Pycnoporus sanguineus or syn. *P. coccineus* is a species fungus under white rot saprobes fungus. *Pycnoporus sanguineus* commonly growing on dead hardwoods was found on Guana Island but occurs throughout the tropics. *Pycnoporus sanguineus* can be found in the form of a thin dry conk with a lateral attachment to its substrate. Moreover with the properties of bright orange on all surfaces with center zonation it is inedible and possibly toxic.

c) Micropus xanthopus


Figure 5: Micropus xanthopus

Micropus xanthopus made up of funnel-shaped caps around 1 and 3 millimetres thick that are concentrically zoned in various shades of brown and which are connected by a yellow-footed stem. Under the type of polypore genus, this species is found on rotting wood and is common from the Australasian, Asian and African tropics except the American tropics.

d) Perenial Ganoderma lipsiense

Figure 6: Perenial Ganoderma lipsiense

Perenial Ganoderma lipsiense are fungi under the species of *Ganoderma* family. *Ganoderma* are generated from Greek word with the combination of ganos means brightness and derma means skin. *Perenial Ganoderma lipsiense* grow on wood and scientifically have 80 species especially from tropically region. Also known as shelf mushroom or bracket fungi, it highly potential in the medicine field especially in traditional Asian medicine. They are lignicolous and leathery either with or without a stem instead of grow in a fan-like or hoof-like structures on the trunks of living or dead trees. They have double-walled, truncate spores with yellow to brown decked inner layers.

e) Microporus affinis

Figure 7: Microporus affinis

Microporus affinis always can be found on fallen branches in rainforests. This type of fungi can be identified by their fan-shaped bracket with velvety, ridged cap with concentric zones of brown, red, yellow and black. On the underside of the cap are little white pores. The most common characteristic of having *Microporus affinis* is a concave shape like a saucer.

f) Stereum hirsutum

Figure 8: Stereum hirsutum

Stereum hirsutum is a hardwood-loving shell fungus that develops fairly substantial, middle-sized cap structures. The ecology of these fungi always grows on dead wood of hardwoods, especially oaks laterally attached, without a stem. The fruiting body is around 5-3 cm and has structures of fan shaped, semicircular and apprised with hair. Most of this species are widely distributed at North America.

g) Mycena sp.

Figure 9: Mycena sp.

Mycena sp. is a big genus of small saprotrophic mushrooms that are randomly more than a few centimeters in width. The white spores, tiny conical or bell-shaped cap, and thin fragile stem are the common characteristic of *Mycena sp*. few species has brighter colour but most of them are gray and brown. Other than have translucent cap,it has also incurved margin. This type of fungi has been collected by the researcher at the stem of banana plant.

h) Saccharomyces cerevisiae / yeast

Figure 10: Saccharomyces cerevisiae / yeast

Saccharomyces cerevisiae or known yeast is a conventional fungi used in the industrial fermentation. This fungus is set to be standard sample for fermentation process to be compared to performance of other fungi.

iii) Performance of local fungi

a) Fermentation by fungus Saccharomyces cerevisiae or yeast (standard fungi sample).

Table 4 : RI and % ethanol by yeast

Sample 1				Sample 2				Sample 3			
	Day	RI	% EtOH		Day	RI	% EtOH		Day	RI	% EtOH
	2	1.33401	2.50		2	1.33367	1.00		2	1.33354	0.90
	4	1.33378	1.50		4	1.33355	0.40		4	1.33376	1.50
	6	1.33389	2.00		6	1.33388	2.00		6	1.33382	1.70
	8	1.33389	2.00		8	1.33394	2.20		8	1.33370	1.40
	10	1.33414	3.00		10	1.33392	2.00		10	1.33362	1.00

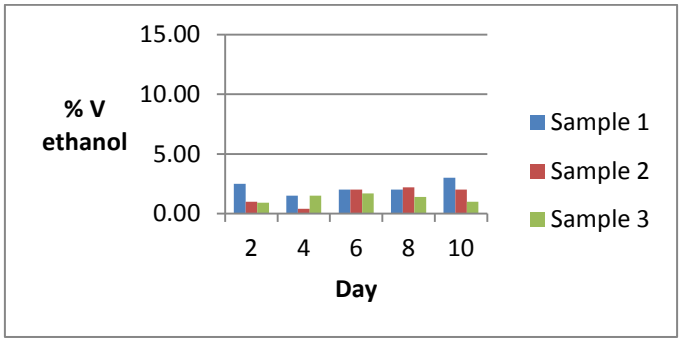


Figure 11: Ethanol Concentration vs. Time by yeast

Saccharomyces cerevisiae or yeast is the conventional fungi used in the world wide fermentation. Based on this research, yeast was used as a standard sample for comparison of ethanol production. The maximum ethanol production by yeast has been produced on day 10 at the maximum of 3% volume of ethanol

b) Fermentation by fungus Schizophyllum commune.

Table 5 : RI and % ethanol by Schizophyllum commune.

Sample 1				Sample 2				Sample 3			
	Day	RI	% EtOH		Day	RI	% EtOH		Day	RI	% EtOH
	2	1.33326	0.00		2	1.3333	0.00		2	1.33332	0.00
	4	1.33322	0.00		4	1.33337	0.00		4	1.33331	0.00
	6	1.33329	0.00		6	1.33334	0.00		6	1.33334	0.00
	8	1.33351	0.70		8	1.33332	0.00		8	1.33398	2.00
	10	1.33342	0.50		10	1.33344	0.50		10	1.33354	0.70

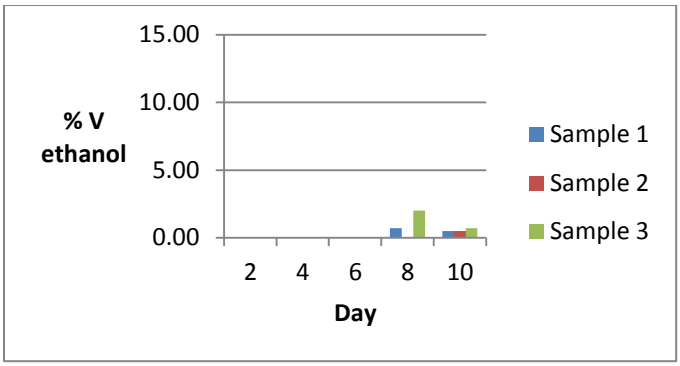


Table 12: Ethanol Concentration vs. Time by Schizophyllum commune.

Production of ethanol by *Schizophyllum commune* resulted late production of ethanol where it represent zero from initial day 2 until day 6. However, small rise on production of ethanol can be seen during day 8 at 2% volume ethanol but decrease to 0.7% volume ethanol at day 10. The zero production of ethanol at the beginning of the day because slow reaction by active microorganism of *Schizophyllum commune* with cellulose of banana branch. Then, the decreasing of the ethanol production at the day 10 is because of the production of other chemical such as lignin which inhibits and deactivates the microorganism activity toward fermentation process.

c) Fermentation by fungus Pycnoporus sanguineus.

Table 6 : RI and % ethanol by Pycnoporus sanguineus.

Sample 1				Sample 2				Sample 3			
	Day	RI	% EtOH		Day	RI	% EtOH		Day	RI	% EtOH
	2	1.33386	2.00		2	1.33383	1.50		2	1.33388	1.80
	4	1.33357	1.00		4	1.33348	0.50		4	1.33346	0.70
	6	1.3332	0.00		6	1.33328	0.00		6	1.33336	0.20
	8	1.33321	0.00		8	1.33315	0.00		8	1.33312	0.00
	10	1.33319	0.00		10	1.3331	0.00		10	1.33307	0.00

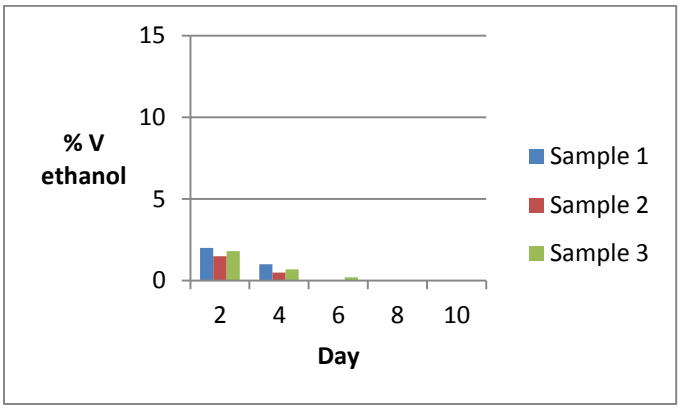


Table 13: Ethanol Concentration vs. Time by Pycnoporus sanguineus.

Ethanol production by fermentation of banana branch with *Pycnoporus sanguineus* achieve highest production at day two with the 2 % ethanol volume but suddenly decreases for the next day. At day eight and 10, the ethanol production starts depleted to 0% volume ethanol. The highest production of ethanol at the beginning of the day indicates that the *Pycnoporus sanguineus* microorganism reacts faster with the sugar in the cellulose structure inside the banana branch to ethanol production. The lignin that dissolve during the pretreatment technique actually inhibit the production ethanol by slowly deactivate the fungi microorganism. The presence of weak acid along the 10 day and ethanol also kill the microorganism by attack the cell membrane of the microorganisms. Therefore, the enzyme leak out of the cell and slow the rate of reaction of the fermentation process.

d) Fermentation by fungus Micropus xanthopus.

Table 7: RI and % ethanol by Micropus xanthopus.

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33324	0.00		2	1.33329	0.00		2	1.33327	0.00
	4	1.33334	0.00		4	1.33336	0.00		4	1.33328	0.00
	6	1.33338	0.00		6	1.33337	0.00		6	1.33327	0.00
	8	1.33339	0.00		8	1.33343	0.50		8	1.33342	0.30
	10	1.33338	0.00		10	1.33339	0.00		10	1.33363	1.00

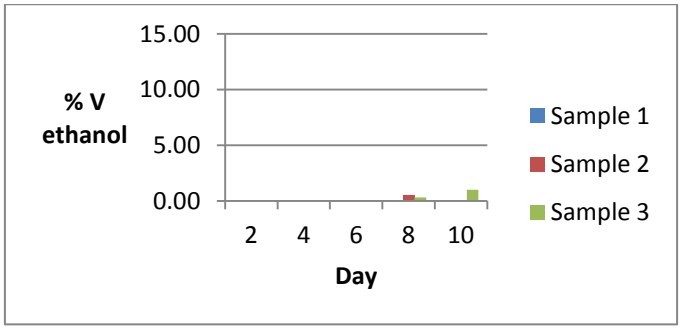


Figure 14: Ethanol Concentration vs. Time by Micropus xanthopus.

Micropus xanthopus fungi resulted low performance on fermentation process where it takes long time fermentation where the production of ethanol start at day 8 with small amount of 0.5% volume ethanol and 1% volume ethanol at day 10. Since the experiment are not extracted the active microorganism from the fungi, there are some possibility of disturbance of the ethanol production. Same as previous fungi, the *Micropus xanthopus* microorganism has slow reaction with the cellulose. There also fast presence of other chemical such as weak acid at the beginning of the day which kill most of the microorganism cell and left only small amount of healthy microorganism cell to react with the cellulose at day 8 and 10. Therefore, at the end of the day, very small amount of volume percent of ethanol is detected.

e) Fermentation by fungus Perenial ganoderma lipsiense.

Table 8: RI and % ethanol by Perenial ganoderma lipsiense

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33565	6.00		2	1.33616	9.00		2	1.33666	11.00
	4	1.33456	4.00		4	1.33465	1.50		4	1.33573	8.00
	6	1.33359	1.00		6	1.33359	1.00		6	1.33551	7.50
	8	1.33353	0.90		8	1.33363	1.50		8	1.33456	4.50
	10	1.33350	0.90		10	1.33352	0.90		10	1.33382	2.00

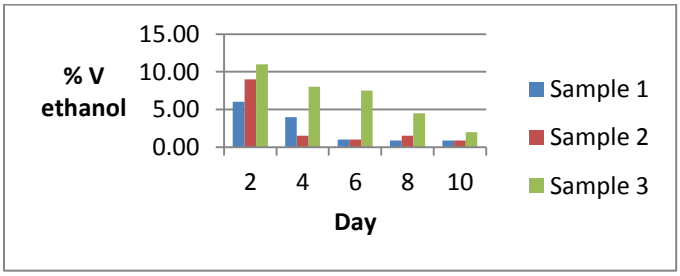


Figure 15: Ethanol Concentration vs. Time by Perenial ganoderma lipsiense

Perenial ganoderma lipsiense show the highest yield of fermentation at day two with 11% volume production of ethanol. The decreasing of ethanol production from day 4 to day 10 indicates the lowest production at day 10 which is around 2 % volume ethanol. From the graph above, *Perenial ganoderma lipsiense* microorganisms react with cellulose faster at the beginning of the fermentation process. The presence of weak acid is slowly appearing day by day where weak acid slowly attack and kill the cell of the microorganism. The highest productions of ethanol by *Perenial ganoderma lipsiense* is favor by the slow production of acidic acid and create a lower acidic condition. As the pH increase, the ethanol production is decrease.

f) Fermentation by fungus Microporus affinis.

Table 9: RI and % ethanol by Microporus affinis.

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33322	0.00		2	1.33322	0.00		2	1.33321	0.00
	4	1.33315	0.00		4	1.33321	0.00		4	1.33333	0.00
	6	1.33321	0.00		6	1.33366	1.00		6	1.33358	0.70
	8	1.33352	0.80		8	1.33365	1.00		8	1.33362	1.00
	10	1.33355	0.90		10	1.33382	1.80		10	1.33362	1.00

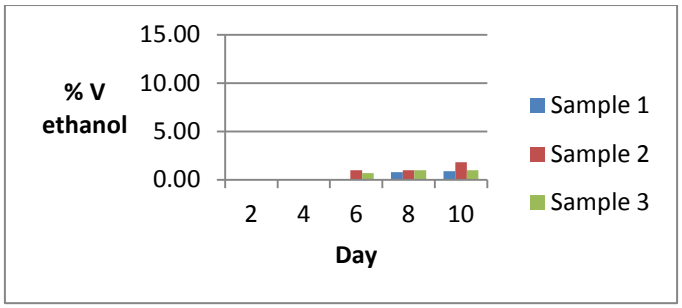


Figure 16: Ethanol Concentration vs. Time by Microporus affinis.

The highest production of ethanol by *Microporus affinis* experimentally achieve at day 10 with 1.8%. Same as other fungi, *Microporus affinis* slowly favor the ethanol production slowly with the no production of ethanol at the initial day of 2 and 4. Since the experiment are not extracted the active microorganism from the fungi, there are some possibility of disturbance of the ethanol production. Same as *Micropus xanthopus* fungi, the *Micropus affinis* microorganism has slow reaction with the cellulose. At the beginning of the day, the *Micropus affinis* microorganism still not in good condition to release fermentation enzyme, cellulase to react with cellulose. The microorganism starts to react at day 6 and increase at day 8 and 10 and require long fermentation time to reach maximum production time.

g) Fermentation by fungus Stereum hirsutum.

Table 10: RI and % ethanol by Stereum hirsutum.

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	0.00	0.00		2	0.00	0.00		2	0.00	0.00
	4	0.00	0.00		4	0.00	0.00		4	0.00	0.00
	6	1.33342	0.40		6	1.33341	0.30		6	1.33339	0.20
	8	1.33389	2.00		8	1.33343	0.40		8	1.33362	1.00
	10	1.33363	1.00		10	1.33353	0.80		10	1.33363	2.00

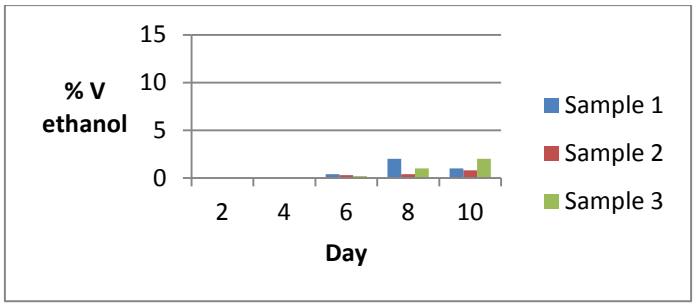


Figure 17: Ethanol Concentration vs. Time by Stereum hirsutum

Stereum hirsutum fungi also show long fermentation time where the production of ethanol starts at day 6. The highest production represent by day 10 with the value of 2% volume of ethanol. Same as other fungi, *Stereum hirsutum* slowly favor the ethanol production slowly with the no production of ethanol at the initial day of 2 and 4. Same as *Micropus xanthopus* fungi, the *Stereum hirsutum*. microorganism has slow reaction with the cellulose. At the beginning of the day, the *Micropus affinis* microorganism still not in good condition to release fermentation enzyme, cellulase to react with cellulose. The microorganism starts to react at day 6 and increase at day 8 and 10 and require long fermentation time to reach maximum production time.

h) Fermentation by fungi Mycena sp.

Table 11: RI and % ethanol by Mycena sp.

Sample 1	Day	RI	% EtOH	Sample 2	Day	RI	% EtOH	Sample 3	Day	RI	% EtOH
	2	1.33343	0.50		2	1.33331	0.00		2	1.33341	0.50
	4	1.33461	4.50		4	1.33411	3.00		4	1.33434	3.50
	6	1.33348	0.60		6	1.33447	4.00		6	1.33323	0.00
	8	1.33394	2.00		8	1.33332	0.00		8	1.33323	0.00
	10	1.33358	0.70		10	1.33332	0.00		10	1.33337	0.00

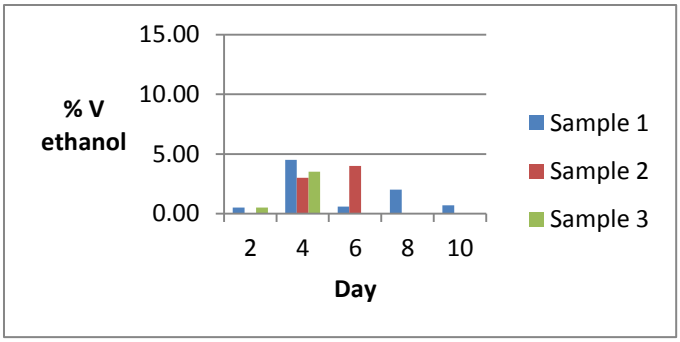


Figure 18: Ethanol Concentration vs. Time by Mycena sp.

Mycena sp. fungi are one of the collected fungi grow at banana stem. The highest production of ethanol by *Mycena sp* can be show at day 4 with the percentage of 4.5% volume ethanol. Small production of ethanol is detected at day 2, where the small amount of microorganism reacts with cellulose but continue react until day 4. By the time, as the microorganism digest the glucose, weak acid such as acetic acid and formic acid is formed during rest of the day as by product. The weak acid which is able to diffuse to the microorganism membrane and increase the pH inside the cell. Therefore, in order to maintain the pH of the cell, the microorganism needs to expand Adenosine triphosphate (ATP) and suddenly have less ability for cell reproduction for ethanol production.

V. CONCLUSION

Based on the 7 sample of fungus and conventional yeast that has been investigated through this research, the

unconventional fungi indicate positive results and prospect to become industrial fermentation fungi since the yield of ethanol of the *Mycena sp* (4.5% by volume) and *Perenial ganoderma lipsiense* (11% by volume) are higher than conventional yeast in term of ethanol production. Therefore, the production of bioethanol can be improved by replacing the conventional yeast experimentally or industrial purpose with a lot of further research and development. The banana branch also shows positively as one of the non-food biomass source for local energy industry. Therefore, this investigation meets the objective of this research by successfully identify 7 local fungi for biomass digestion and evaluate the capability of local fungi in the fermentation of cellulosic biomass.

From this study, fungi *Perenial ganoderma lipsiense* are recommended for next potential to replace yeast since it has higher ability as source of enzyme to produce fermentation bioethanol. Then, the longer fermentation study also needs to be studied since some fungi took longer fermentation time to reach optimum production of ethanol. Lastly, the shorter ultrasound technique applied to the banana branch and the author also would like to recommend for the study of other method of determination of ethanol content such as specific gravity method, GC-MS method and HPLC method for accurate calibration and measurement.

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