BIOETHANOL PRODUCTION FROM CASSAVA BY FERMENTATION PROCESS USING *SACCHAROMYCES CEREVISIAE*

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

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A thesis submitted in fulfilment of the

requirement for the award of the degree of

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DECLARATION

I declare that this thesis entitled

"Bioethanol Production From Cassava By Fermentation Process Using Saccharomyces Cerevisiae" is the result of my own research except as cited in the references. The report has not been accepted for any degree and is not currently submitted in candidature of any other degree.

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Special dedication to:

My Beloved Parents, Mr & Mrs Rajandran and Kamala Devi for their great support and motivation. Their endless care and consultations taught me that even the hardest tasks can be accomplished successfully if full effort has been put in, together with heartfelt gratitude towards the GOD.

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BIOETHANOL PRODUCTION FROM CASSAVA BY FERMENTATION PROCESS USING *SACCHAROMYCES CEREVISIAE*

ABSTRACT

Bioethanol can be produced by fermentation of cassava, which carried out in conical flask by using *Saccharomyces Cerevisiae*. Objective of this experiment is to find out the effect of agitation speed, temperature and substrate concentrations on the bioethanol production, cell growth and the glucose consumption. At the end of this experiment, the best substrate concentrations of cassava, which leads to highest production of bioethanol and the highest cell growth can be identified. In this study substrate concentration varied as 25g (6.25%w/v), 50g (12.5% w/v), 75g (18.75% w/v), 100g (25% w/v) and 150g (32.5%w/v). Follows that, the effect of various fermentation temperature will be analyze, by using temperature varies as 20^{o}C, 30^{O}C, 40^{O}C and 50^{o}C. From this temperature vary the growth of yeast and the bioethanol production will be analyzed. Follows that, third parameter of this experiment is agitation speed of fermentation shake flask. Agitation speed varies from 0 rpm, 150rpm, 200 rpm and 250 rpm. For this part, effect of agitation speed on bioethanol production and cell growth of yeast studied. As for the optimum condition, temperature 30^{o}C maintained, agitation speed fixed at 200 rpm, pH maintained at 4.5 and substrate concentration fixed about 100g (25% w/v). This experiment begins with inoculum preparation step, medium preparation step, following by transferring the inoculum into medium into 1L conical flask, fermentation of cassava in incubator shaker and lastly end with sample analysis. From the experiment, agitation speed 200 rpm, substrate concentration of 150g and temperature 40^{O}C gives highest bioethanol production, highest cell growth and highest glucose consumption.

PENGHASILAN BIOETHANOL DARI UBI KAYU MELALUI PROSES PENAPAIAN DENGAN MENGGUNAKAN *SACCHAROMYCES CEREVISIAE*

ABSTRAK

Bioethanol dapat dihasilkan melalui proses penapaian dalam kelalang kon dengan menggunakan *Saccharomyces Cerevisiae*. Tujuan eksperimen ini adalah bagi mengkaji kesan halaju adukan, suhu dan kepekatan substrat ke atas peghasilan bioethanol,pertumbuhan sel yeast dan penggunaan glukosa.Pada akhir eksperiment ini akan diketahui had laju adukan,suhu dan kepekatan substrat yang paling bagus bagi menghasilkan kepekatan etanol yang paling tinggi. Dalam kajian ini kepekatan substrat 25g (6.25% jisim/isipadu), 50g (12.5% jisim/isipadu), 75g (18.75% jisim/isipadu), 100g (25% jisim/isipadu) and 150g (32.5% jisim/isipadu). Tambahan lagi,kesan suhu yang berlainan juga dikenalpasti.antaranya adalah 20^{o}C, 30^{O}C, 40^{O}C dan 50^{o}C. Pada suhu berlainan ini,pertumbuhan sel *Saccharomyces Cerevisiae* dan kepekatan etanol yang dihasilkan akan dikaji.Selain itu, eksperimen diulangi dengan halaju adukan yang berlainan iaitu pada 0 rpm, 150rpm, 200rpm and 250rpm. Suhu penapaian telah ditetapkan pada 30^{o}C dan pH ditetapkan pada 4.5 dan kepekatan substrat akan ditetapkan pada 100g (25%j/i). Eksperimen bermula dengan persediaan baka penapaian ,persediaan medium penapaian dan seterusnya pemindahan baka yang dikulturkan ke dalam medium penapaian ke dam kelalang kon 1L. Penapaian diakhiri dengan analisis sampel.Daripada kajian ini,diketahui bahawa kepekatam substrat 150g, suhu pada 40^{o}C dan halaju adukan pada 200 rpm akan membawa kepada penghasilan etanol yang tinggi dan pertumbuhan sel *Saccharomyces Cerevisiae* yang tinggi.

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LIST OF ABREVIATIONS / SYMBOLS

^{o}C	Degree Celsius
rpm	Round per minute
g	gram
ml	millilitre
CO_2	Carbon Dioxide
%	Percentage
kg	Kilogram
L	Litre
MJ	Milli Joule
w/v	Weight per volume
hr	hour
Et al	And others
g/l	Gram per litre
nm	nanometre
g^{-1}	Per gram
β	beta
v/v	Volume per volume
R^2	Regression
mg/ml	Milligram per millilitre
RI	Refractive Index

CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

In recent years, researchers has considered necessary to do structural reforms that allow further development to face the needs of the energy sector. One energy source that is little mentioned in national projects and has demonstrated its feasibility in other regions of the world is the production of ethanol. Cassava (*Manihot esculenta*), sometimes also called manioc, is the third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tonnes (Leen et al., 2007).Cassava is highly efficient in producing starch and it is tolerant to extreme stress conditions. Furthermore, it fits nicely within traditional farming systems. Fresh roots contain about 30% starch. Cassava starch is one of the best fermentable substances for the production of ethanol.

Fermentation is the oldest way for humans to produce bioethanol, and this is also the traditional way of making alcoholic beverages (Leen et al., 2007). Bioethanol can be produced from biomass by the hydrolysis process and followed by sugar fermentation processes. Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemi cellulose and lignin. In order to produce sugars from the cassava, the cassava is pre-treated with acids or enzymes to reduce the size of the feedstock and to open up the plant structure (Saoharit et al., 2009). The cellulose and the hemi cellulose portions are broken down (hydrolysed) by enzymes or dilute acids into sucrose sugar that is then fermented into bioethanol. There are three principle methods of extracting sugars from cassava. These are concentrated acid hydrolysis, dilute acid hydrolysis and enzymatic hydrolysis (Akihiko et al., 2008).

Previous studies evaluated the environmental impacts of bio based fuels in various categories, including non-renewable energy consumption, greenhouse gas emissions, acidification, eutrophication, human and ecological health, and photochemical oxidation. Most studies have concluded that the use of bioethanol as liquid fuel could reduce greenhouse gas emissions indicated that life-cycle economic, environment and energy assessment provide an important tool for policy makers to better understand trade-offs among economics, environmental impacts and energy for the most effective use of regional energy resources (Hu et al., 2004).

In another study, presented that cassava-based ethanol has a lower net energy, better carbon dioxide emission and lower external cost of carbon dioxide. However, it has higher production cost than conventional gasoline (CG) does, 0.37 MJ/MJ (49% of

CG), 72.61 g/MJ (83% of CG), 0.87 and 0.14 RMB/MJ (200% of CG) respectively. In Guanxi, China the cassava based bioethanol positive net energy and net renewable energy values of 7.475 MJ/L and 7.881 MJ/L, respectively. A study (Nguyen et al., 2007) on the net energy balance and greenhouse gas (GHG) emissions of ethanol from cassava based on a pilot plant data of the Cassava and Starch Technology Research Unit (CSTRU), Kasetsart University, Thailand and found that the energy balance is positive and net avoided GHG emission is 1.6 kg CO2 eq. per litre of ethanol.

On the other hand, in Thailand based on the pilot plant research on cassava starch by CSTRU, Kasetsart University. It was studied that, an energy efficiency (Yu and Toa, 2009) of cassava-based fuel ethanol in Chinese Guangxi by the Monte Carlo method and showed that the energy balance is a positive net energy and energy input to output ratio of 0.7 MJ/MJ. Several LCA studies indicated that in categories of abiotic depletion, GHG emissions, ozone layer depletion, and photochemical oxidation, bioethanol is better fuel than gasoline whereas gasoline is better in terms of human toxicity, ecotoxicity, acidification and eutrophication (Luo et al., 2009). It concluded that cassava-based (Leng et al., 2008) ethanol is energy efficient as indicated by an energy output to input ratio of 1.28 and a major contribution to energy consumption and sulphur dioxide and CO_2 emissions primarily comes from ethanol conversion phase as a result of the combustion of coal to produce energy (Hu et al., 2004b).

1.2 Problem Statement

1.2.1 High Profitability from the Bioethanol Product in Malaysia and Reduce Importation of Bioethanol (Develop Exportation of Bioethanol)

In Malaysia, cassava tubers are used to produce cassava chips and also to produce bioethanol. Now Malaysian government's plan is to have up to 15 ethanol plants in Perak over the next five years (Azlin et al., 2010). This industry working on identifying high productions raw material to produces bioethanol. From the study, it can be assumed that, the production of bioethanol will have a total capacity of 1.22 billion gallons with total profit of 24.4 billion ringgit that is $4.1 billion (Khatijah and Tan, 2000). Hence to improve their profitability towards their production, cassava can be used as the raw material to produce bioethanol. In 2009, Johor agriculture office stated that Malaysia earned 15.3 billion ringgit from the production of cassava chips (Azlin et al., 2010). Hence, it can be conclude that bioethanol industry contributing about 37.3% higher profit compare to cassava chips industry. Investment into bioethanol industry will increase the annual profitability of Malaysian economics.

In addition, development of bioethanol plants may create opportunity to improve local economy. For instance, developing new bioethanol productions area in Malaysia, it can reduce the dependence of bioethanol importation from foreign countries. Malaysia uses bioethanol in pharmaceutical industry (sterilization purposes), production plants (as a reactant to produce polyesters), and in laboratory (for research and development purposes). Hence, by having own production areas Malaysia can reduce toward foreign imports. Moreover, Malaysia also can export the bioethanol to foreign countries such as

Brazil and United States. Those countries mainly depend on the bioethanol as the fuel for the automobile engines (E85 and E20).

There is a current upsurge of interest in the search for renewable biomass(cassava) for the production of transportation fuels like bioethanol, arising especially from the environmental concerns due to the toxic gas emission from petroleum fuels, squeezing petroleum resources and fossil fuels (Shanavas et at., 2010). Bioethanol is the most important biofuel, accounting for more than 90% of the total biofuel use.

Bioethanol can be used in mixtures with fuels for motor vehicles. It can increase the octane index; reducing it between 10 and 15% the CO. Ethanol can be mixed with unleaded gasoline between 10 to 25% without difficulty (Leticia et al., 2010). Ethanol could therefore replace MTBE (methyl-tert-butyl ether), an oxygenated product used in Mexico since 1989, although it has reduced CO2 emissions it has proved to be a groundwater pollutant and has a carcinogenic effect.

Therefore, this research is focused on the production of bioethanol from cassava without pre-treatment by using enzymes. Hence, hydrolysis process does not take place by addition of enzymes but it occurs simultaneously with fermentation.

1.3 Objectives

The objective of this research is to study the bioethanol production from cassava by (*Saccharomyces Cerevisiae)* without enzyme pre-treatment.

1.4 Research Scope

In order to accomplish the objective, 3 scopes were identified:

a) To study the effect of agitation speed on bioethanol production from cassava (*Manihot*) using yeast (*Saccharomyces Cerevisiae).*

b) To study the effect of temperature on bioethanol production from cassava (*Manihot*) using yeast (*Saccharomyces Cerevisiae)*

c) To study the effect of substrate concentration on bioethanol production from cassava (*Manihot*) using yeast (*Saccharomyces Cerevisiae).*

CHAPTER 2

LITERATURE REVIEW

2.1 Cassava

2.1.1 History of Cassava

Cassava (*Manihot esculenta*), sometimes also called manioc, is the third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tonnes. In Africa, which is the largest centre of cassava production, it is grown on 7.5 million hectare and produces about 60 million tonnes per year. It is a major source of low cost carbohydrates and a staple food for 500 million people in the humid tropics (Leen et al., 2007). The largest cassava market by far is in Nigeria, responsible for 18% of world cassava production. Other important cassava producing countries are Brazil (upcoming), Indonesia, Thailand, Congo and

Mozambique. Approximately 2% of world cassava is traded, mostly in the form of dried chips or pellets (Leen et al., 2007).

2.1.2 Cultivation of Cassava

Major farming activities including land preparing, planting, fertilizing, weeding, and harvesting were covered in this stage (Nguyen et al., 2007). Detailed information on fuel, fertilizers, and herbicides inputs was verified by field survey in the north eastern cultivation area of the country. The total cassava planting area in 2007 was 1.2 million hectare and production yield was 22.9 ton fresh roots per hectare (Pimentel, 1992). When comparing to India which had 0.24 million hectare of cassava planting areas, the production yield was 31.4 ton fresh roots per hectare which was 37% higher than production yield of Thailand (Office of Agricultural Economics, 2008). In traditional agriculture, the most common form of seedbed preparation for cassava planting is on mounts or on unploughed land (Ecoivent, 2006).

On unploughed land, no tillage is done other than required to insert the stem cuttings into the soil. In improved agriculture, the land is first ploughed and then harrowed. Thereafter cassava may be planted on the flat, on ridges or in furrows. Flat plantings of cassava seem to produce higher yields of tuber than ridge or furrow plantings. However, flat planting is unsuitable on heavy clay soils, because the tubers tend to rot. Cassava is propagated vegetatively as clones. Generally, cuttings are taken from the mature parts of the stems, which give a better yield than those taken from the younger portion of the stems (Leen et al., 2007).

Thereafter cassava may be planted on the flat, on ridges or in furrows. Flat plantings of cassava seem to produce higher yields of tuber than ridge or furrow plantings. However, flat planting is unsuitable on heavy clay soils, because the tubers tend to rot. Cassava is propagated vegetatively as clones. Generally, cuttings are taken from the mature parts of the stems, which give a better yield than those taken from the younger portion of the stems. The cuttings should have at least 3 nodes, which serve as origins of shouts and of roots (Leen et al., 2007). Recent releases from agricultural breeding programmes include clones with resistance to many of the major diseases and pests.

Figure 2.1 Cassava plants

Cultivar names are usually based on pigmentation and shape of the leaves, stems and roots. Cultivars may vary in yield, root diameter and length, disease and pest resistance levels, time to harvest, temperature adaptation. Storage root colour is usually

white, but a few clones have yellow-fleshed roots. Each region has its own special clones. Most farmers grow several clones in a field. Cuttings produce root s within a few days and new shoots appear soon afterwards. Early growth is relatively slow, thus weeds must be continuing rolled during the first few months.

Most farmers grow several clones in a field. Cuttings produce root s within a few days and new shoots appear soon afterwards. Early growth is relatively slow, thus weeds must be continuing rolled during the first few months. Although cassava can produce a crop with minimal inputs, optimal yields are recorded from fields with average soil fertility levels (suitable for most food crops) and regular moisture availability. Typically, harvesting can begin eight months after planting. In the tropics, plants can remain unharvested for more than one growing season, allowing the storage roots to enlarge further. However, as the roots age, the central portion becomes woody and inedible (Leen et al., 2007).

2.1.3 Characterization of Cassava

Cassava is a perennial shrub which sometimes reaches the size of a small tree. It stems vary in color from pale to dirty-white to brown marked by numerous nodes formed by scars left by fallen leaves. Pale to dark-green leaves are fan-shape, with 5 to 9 lobes. Roots of cassava plants are few and swallow and some become storage roots. These are clustered around the base of the plant and extend about 60 cm on all sides (Balinghoy, 2009). It is for these roots which contain from 15 to 40 percent starch that

the crop is cultivated. Under favorable conditions, a single root may weigh as much as four kilos.

The number of roots per plant at harvest varies from 2 to 7 each averaging 27.7 to 43.3 cm long and from 4.5 to 7.4 cm in diameter. Cassava is a tropical and sub-tropical plant. It grows in regions with more or less evenly distributed rainfall through out the year. An ambient temperature that ranges from 25°C -30°C. Select an open field with sandy loam or clay loam soil. Be sure that the area is not prone to waterlogging; it must be a well-drained soil (Kamoteng, 2009). Also consider the soil fertility with pH range of 5.5-6.5. Cassava thrives at sea level to 845 meters above sea level. It grows best when planted at the start of the rainy season. Figure 2.2 shows the cassava tubers.

Figure 2.2 Cassava Tuber

2.1.4 Applications of Cassava

Cassava is an important food crop for developing countries, being the main source of energy for between 200 and 300 million people (Gevaudan and Didierm, 1989). In Tanzania, cassava is an important subsistence food crop, although it is still considered by many people outside the production areas as a famine reserve crop when cereals, especially maize, fail. Around 84% of total production in the country is utilized as human food. The remaining fraction is used for livestock feed, starch making and export. This crop is bulky and highly perishable, but is available all year round thus contributing to food security. Its high energy content helps in minimizing incidences of energy malnutrition (Hahn et al., 1970). Cassava also used to produce bioethanol (Akihiko et al., 2008).

Such flours could be blended with cereal flours to improve acceptability of the cassava-based porridges. Exact proportions of these blends have not been fully established. Cassava has also been used in baked products and fried products like doughnuts, buns and *chapati* (a pan-fried unleavened flat round wheat-based product), although not to the extent of the stiff porridges. Another area of utilization of cassava is in the starch industry for food and non-food uses. This product can be obtained from the fresh dried cassava (Hahn et al., 1970). The easiest form of extraction of this cassava starch is from the fresh cassava using graters to grate the cassava into a fine paste.

Yake yake is one paste product obtained after peeling, washing, grating, drying and sieving the cassava to obtain a meal that is moulded and steam-baked. *Agbeli kaklo*

is a second product encountered in literature resembling *yake yake* but instead of steam-baking, the meal is mixed with meat, using the hand it is moulded into small cylinders and palm kernel or coconut oil is used for frying (Doku, 1969**)**. Flour is one of the most important cassava products. Steamed paste and wet paste are common in other places but not Tanzania, and so are toasted and steamed granules (Hweke, 1994**)**.

2.2 *Saccharomyces Cerevisiae*

2.2.1 History of *Saccharomyces Cerevisiae*

Saccharomyces Cerevisiae which in Latin means "sugar fungus" has been utilized by human for thousands of years. It is believed that it was first discovered on the skins of grapes (Polsinelli, 1999). *Saccharomyces Cerevisiae* is budding or brewing yeast, and has been put to use since antiquity to make dough rise and to provide ethanol in alcoholic beverages. The natural history of *Saccharomyces Cerevisiae* has been obscured in part by a long history of domestication. It is the microbial agent responsible for the fermentation of wine, beer and other alcoholic beverages, and the most commonly used microbial leavening agent for bread. Cavalieri has identified *Saccharomyces Cerevisiae* in the residue inside an Egyptian wine jar from c. 3150 B.C (Cavalieri, 2003).

The natural strains of *Saccharomyces Cerevisiae* described in the literature have generally been isolated from vineyard grapes and other fruits (Polsinelli, 1999), fermentation facilities (Mortimer, 1994), insects, oak fluxes (Naumov et al., 1998) or

soil associated with oak and other broad-leafed trees (Sniegowski et al., 2002). Today, a majority of winemakers add commercial yeast to their crushed grapes (wine must), but the historical method of winemaking, natural fermentation, requires *Saccharomyces Cerevisiae* to enter the wine must from the environment (Erlend et al., 2006). The place of origin of yeast strains responsible for natural fermentation has been a matter of debate since the days of Pasteur (Barnett, 2000).

2.2.2 Characterization of Yeast

It exists in single-cell form, or in pseudomyceliac form. *Saccharomyces Cerevisiae* is in the fungi kingdom. The reasons for this classification are because it has a cell wall made of chitin, it has no peptidoglycan in its cell walls, and its lipids are ester linked. It also uses DNA template for protein synthesis and it has larger ribosomes (Marjeta et al., 2001*)*. It is then consider yeast because it is a unicellular organism so it cannot form a fruiting body; like other fungi. Cellular reproduction occurs by budding. The ability of *Saccharomyces Cerevisiae* to ferment specific sugars is a major factor that differentiates it from other yeasts.

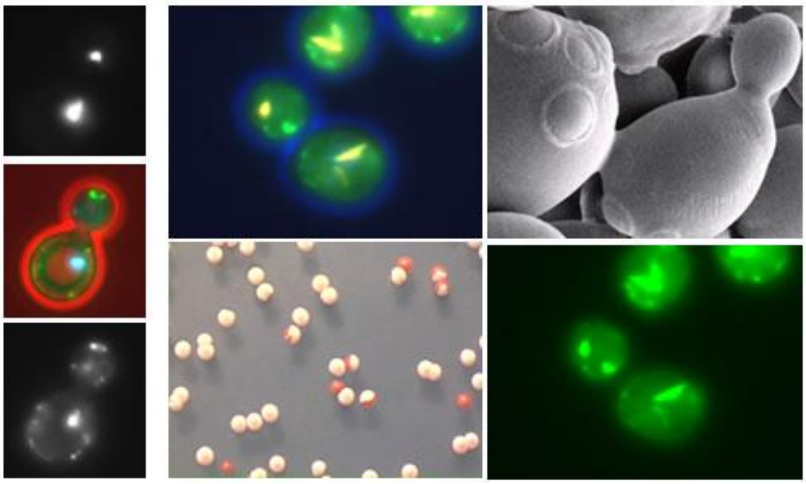


Figure 2.3 Photos of Different types of *Saccharomyces Cerevisiae*

Source: (Madigan et al., 2006)

Saccharomyces Cerevisiae has adapted in several important ways. One is the fact that they are able break down their food through both aerobic respiration and anaerobic fermentation. One of the examples, use of *Saccharomyces Cerevisiae* is in cassava fermentation (Leticia et al., 2010). They can survive in an oxygen deficient environment for a period. Another adaptation they have is their ability to have both sexual and asexual reproduction. And very few organisms can do all four of these processes. This allows this species to live in many different environments.

2.2.3 Cultivation of *Saccharomyces Cerevisiae*

2.2.3.1 Cultivation Using Wickerham's YM Medium

A widely used medium is Wickerham's YM medium, which contains 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose and 2% agar prepared with sea water at a salinity equivalent to the sample site. Bacteria are inhibited by the addition of chloramphenicol (200 mg/l) to the medium prior to autoclaving. An alternative is an antibiotic mixture of penicillin G and streptomycin sulphate (each at 150–500 mg/l), added dry to autoclaved, cooled (45 ◦C) medium. Sediment particles can be placed directly on an agar medium or known quantities of sediment can be placed in a test tube with a given volume of sterile sea water, vortexed and diluted 1: 10 in a sterile sea water series, followed by preparation of standard spread plates from each of the dilution series.

Suspected yeast colonies can be picked and transferred to a microscopic slide for inspection and after confirmation can be transferred from the isolation medium to a growth medium. A selective medium suitable for *Candida* species the chloramphenicol malt agar and chloramphenicol cycloheximide malt agar. Some *Candida* species grow in the presence of cycloheximide, while most other species do not, so it has been used as a differential medium for *Candida* species (Collins and Patricia, 1970). Broad-spectrum antibiotics are both more effective in preventing bacterial growth and less harmful to yeast cells (Mossel, 1962).

2.2.3.2 Cultivation Using Nutrient Agar Medium

The yeast that isolated from dates (fruit of date palm, *Phoenix dactylifera)*, cultured and maintained on the medium containing sucrose 20.0 g/l, agar 20.0 g/l, peptone 5.0 and yeast extract 3.0 at pH 6.0 (Dworschack and Wickerham, 1960). The cultures will be stored at 4°C. Cell suspension was prepared from 2 to 3 days old slant culture of *S.cerevisiae*. Twenty-five ml of seed medium was transferred to each 250 ml Erlenmeyer flask. The medium consisted of (g /l w/v) sucrose 30.0, peptone 5.0 and yeast extract 3.0 at pH 6 unless stated otherwise. The flasks were cotton plugged and autoclaved at 103.5 Pa pressure (121°C) for 15 minutes and cooled at room temperature. One ml of inoculum was transferred to each flask under sterile conditions.

Flasks were then incubated in a rotary incubator shaker at 30°C for 24 hours. Agitation rate was kept at 200 rev/min. Productions of invertase carried out by shake flask technique using 250 ml Erlenmeyer flasks. Same medium composition was used for vegetative inoculum preparation and for fermentation (Ikram and Ali, 2005). One ml of vegetative inoculum was aseptically transferred to each flask, dry cell mass content of vegetative inoculum was 0.45g/l. Flasks were then incubated in a rotary incubator shaker at 30°C for 48 hours. The agitation rate was kept at 200 rev/min. Dry cell mass of yeast was determined by centrifugation of fermented broth incentrifuge at 5000 rev/min using weighed centrifuge tubes. The tubes were oven dried at 105°C for 2 hours in an oven.

2.2.4 Applications of *Saccharomyces Cerevisiae*

A probiotic in terms of its beneficial effects, *Saccharomyces Cerevisiae* has many properties from the most basic to highly advanced. When ingested in a quantity of two tablespoons daily, the commercially prepared product known as "nutritional yeast" provides 52% of the recommended daily amount (RDA) of protein (Marjeta, 2001). Nutritional yeast is also high in fibre, B vitamins and folic acid. Nutritional yeast also has the presence of beta-1,3 glucans, which have been shown to stimulate the body's immune system. While some pharmaceutical drugs are capable of over-stimulating the body's immune system during therapy and are therefore not suitable for people with autoimmune illnesses, beta-glucans appear to assist the immune system without causing over reactivity. Beta-glucans are also apparently capable of lowering LDL cholesterol levels, assisting in the healing of wounds and aid in the prevention of infections. Yeast was used to fermentate biocomponent to produce bioethanol (Akihiko et al., 2008).

2.3 Bioethanol from Cassava

2.3.1 Bioethanol

Bioethanol is a form of renewable energy that can be produced from agricultural feedstock. It can be made from very common crops such as sugar cane, potato, cassava and corn (Thompson, 1979). It is considered an alternative to petroleum and diesel. Its popularity as a fuel for cars it is particularly well established in Brazil. Bioethanol is a high-octane and water-free alcohol produced from the fermentation of sugar or

converted starch. It is a colourless clear liquid with mild characteristic odour that boils at 78°C and freezes at –112°C. It has no basic or acidic properties. It is biodegradable, low in toxicity and causes little environmental pollution if spilt (Thompson, 1979). Bioethanol burns to produce carbon dioxide and water. Bioethanol is a high octane fuel and has replaced lead as an octane container in petrol. There are three main methods of extracting sugars from biomass. These are concentrated acid hydrolysis, dilute acid hydrolysis and enzymatic hydrolysis.

Bioethanol can be produced from biomass by the hydrolysis and sugar fermentation processes. Bioethanol production from starchy materials by conventional fermentation requires saccharification with amylolytic enzymes and subsequent fermentation using the yeast *Saccharomyces Cerevisiae*, because this yeast cannot utilize starchy materials (Akihiko et al., 2008). When it is burned, bioethanol gives off considerably less sulphur and heavy metals, thus contributing to a reduction in acid rain. Emissions of carbon dioxide (CO_2) from bioethanol are also lower than for conventional petrol (Hu et al., 2004). Cassava-based ethanol has a lower net energy, better CO_2 emission and lower external cost of CO_2, but has higher production cost than conventional gasoline (Seksan and Malakul, 2009).

2.3.2 Fermentation and Methods of Fermentation

Fermentation is a metabolic process in which an organism converts a carbohydrate, such as starch or a sugar, into an alcohol or an acid. For example, yeast performs fermentation to obtain energy by converting sugar into alcohol. Bacteria

perform fermentation, converting carbohydrates into lactic acid. There are two types of methods of fermentation which are by using conical flask and bioreactor.

2.3.2.1 Fermentation in Conical Flask

Fermentation also can be carried out in conical flask. It is looks simple and can be carried out a few number of runs. Laboratory scale fermentations are carried out in shaker flasks, and flat bed bottles. Large scale fermentations are carried out in glass or stainless steel tank fermenters. A fermentation vessel should be cheap, not allow contamination of the contents, be non-toxic to the microorganism used for the process, be easy to sterilize, be easy to operate, be robust and reliable, allow visual monitoring of the fermentation process, allow sampling, and be leak proof. These are conical vessels made of glass and are available in different sizes. The typical volume of these flasks is 250 ml (Hartmann, 1997).

There are different types of shaker flasks, such as baffled, unbaffled or Erlenmeyer flask, and flying saucer. Shaker flasks are used for the screening of microorganisms and cultivation of them for inoculation. Shaker beds or shaker tables are used to allow oxygen transfer by their continuous rotary motion. Baffled flasks are used to increase the oxygen transfer. Shaker flasks need to be plugged to prevent contamination with other microorganisms. Cotton-wool, polyurethane foam, glass, and synthetic plugs are commonly used. Fernwald shaker flasks and flat bed Thompson bottles are expensive and are not commonly use (George, 1993).

Figure 2.4 Conical flask

Source: (George, 1993)

2.3.2.2 Fermentation in Bioreactor

A bioreactor on a chip has the potential to highly automate the sample preparation procedures, drastically reduce costs associated with bulky experiments. Moreover, the bioreactor on a chip can be used for experimental study on the dynamical analysis and operation of bioreactors, laboratorial costs can be extraordinarily reduced in several ways, since small quantities of reagents and samples are needed (Oliveira, 2000). We may distinguish three metabolic pathways which are respirative growth on glucose, fermentative growth on glucose and respirative growth on ethanol.

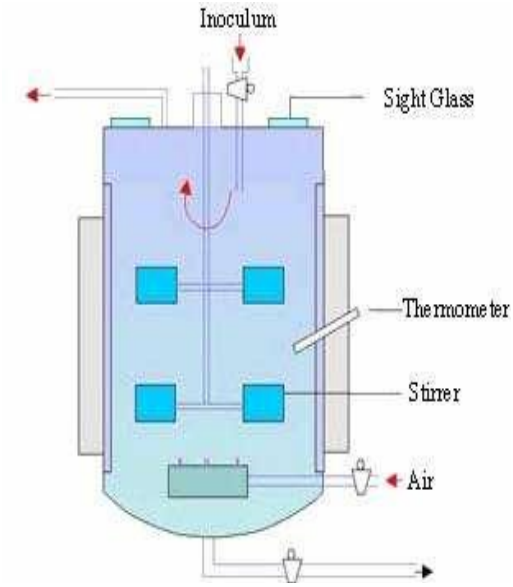


Figure 2.5 Stirred Tank Fermenter

Source: PinkMonkey, Inc.

Respirative pathways occur in presence of oxygen and the fermentative one in its absence (Oliveira, 1996). Biomass, ethanol and pH measurements are carried out by optical spectral analysis, the first two by optical absorption method and pH is determined by fluorescence. The intensity of the transmitted light when measured by the photo detector can give information about the ethanol and biomass concentrations. The biomass detection system (optical absorption) is processed at λAbs 620 nm. The ethanol detection is also based on optical absorption method by using a specific membrane (λAbs 305 nm). When the sample is excited with a wavelength of 403 nm, other photo detector measures the emission light that gives information about pH value (Wolfbeis, 1983).

2.3.3 Parameters in Fermentation Process

2.3.3.1 Effect of Agitation on Bioethanol Production

Agitation is important for adequate mixing, mass transfer and heat transfer. It assists mass transfer between the different phases present in the culture, also maintains homogeneous chemical and physical conditions in the culture by continuous mixing. Agitation creates shear forces, which affect microorganisms, causing morphological changes, variation in their growth and product formation and also damaging the cell structure (Kongkiattikajorn et al., 2007). Agitating the fermentation broth normally satisfy the oxygen demand of a fermentation process. Among other factors having an impact on the operating conditions during fermentation in bioreactors is agitation and mixing. Agitation is important for uniform mixing of the medium components within the fermentor (dispersion of cells and nutrients) as well as mass transfer phenomena (Yuwapin et al., 2008) the effect of agitation on ethanol production is important for the successful progress of the fermentation.

It not only assists mass transfer between the different phases present in the culture, but also maintains homogeneous chemical and physical conditions in the culture by continuous mixing. Agitation creates shear forces, which affect microorganisms in several ways, causing morphological changes, variation in their growth and product formation and also damaging the cell structure (Mittal, 1992). The biomass dry weight increased with the increase of agitation speed from 0 to 200 rpm. Agitation could be beneficial to the growth and performance of the microorganism cells by improving the mass transfer characteristics with respect to substrates, products/byproducts and oxygen.

Thus, agitation results in a better mixing of the fermentation broth, helping to maintain a concentration gradient between the interior and the exterior of the cells (Jirasak, 2008**)**. Such a concentration gradient works in both directions through better diffusion it helps to maintain a satisfactory supply of sugars and other nutrients to the cells, while it facilitates the removal of gases and other byproducts of catabolism from the microenvironment of the cells.

2.3.3.2 Effect of Inoculum Size

In a study it was find out that, size of the inoculum effects the bioethanol fermentation. The fermentation carried out by the process of immobilized cultures of *Saccharomyces Cerevisiae* by varying the inoculum concentration of broth *Saccharomyces Cerevisiae* from 2 to 10 % (v/v). The process with optimized fermentation experiments was carried out for a period of 24 hours at the parameters described in the paper could be used for optimum pH of 6 and optimum temperature of 32.5°C the scaling up of the process to a pilot scale or commercial maximum ethanol concentration at 10% inoculum size for fermenter level thereby making the process more cost the fermentation time was 8.8% (Neelakandan and Usharani, 2009).

In another study, also shows that maximum ethanol yields obtained when using 10% inoculum size were 0.46-0.47 g g-1, with 89.90-92.75% of theoretical ethanol yield (Tatcha et al., 2007). Concerning the interaction between ethanol accumulation and inoculum size, the response surface graph showed that increasing ethanol yields can be obtained using increasing inoculum sizes. As also described in literature review

(D'Amore et al., 1989), the rate and level of ethanol produced increased with the increases in inoculum sizes. In addition, (Vega et al., 1987) proposed a mathematical model, which showed that increasing amounts of inoculum decreased the severity of ethanol inhibition. However, a high level of inoculum leads to rapid fermentation that may not always be favourable to the process depending on the strain and levels of ethanol produced.

2.3.3.3 Effect of Temperature

Temperature is one of the factors that affect the bioethanol production from cassava. Temperature has a marked influence on the production of ethanol (Rivera et al., 2006). Too high temperature kills yeast, and low temperature slows down yeast activity. Thus, to keep a specific range of temperature is required. Normally ethanol fermentation is conducted at temperature range between 30℃ - 35℃ which stated by (Shuler and Kargi, 2002) that the ethanol will be produced at highest concentration. From a study, it was found that ethanol yield increase due the increase temperature from 25℃ to 32.5℃ with the incubation of *Sacharomyces Cervisiae*. Beyond this level concluded that, the ethanol content decreases significantly. The maximum ethanol yield at 32℃ was 8.53% 24 hours of fermentation (Neelakandan and Usharani, 2009).

2.4 Analysis

2.4.1 Analysis of Ethanol Composition Using Gas Chromatography

A method is described for specific quantitative analysis of ethanol in wine by gas chromatography. This method, which uses an internal standard and flame ionization detector, is more accurate and more precise than methods commonly used. Statistical analysis of data from typical winery samples shows that the standard error of estimate for the method is 0.07~/~ (v/v) over the range 7 to 24% (v/v). The estimate of the standard deviation of the chromatography for duplicate injections is 0.02% (v/v). The gas-liquid chromatography method determines ethanol separately from the other wine components that interfere in other methods, and without distillation or chemical reaction. When large numbers of samples are analysed, advantages include short analysis time per sample and potential for extensive automation. Determination of ethanol is perhaps the most important routine analysis in a modern winery. Frequent, fast, and accurate results are needed to control the quality of the wine from grape to bottle, as well as for state and federal government tax and regulatory purposes (Stackler and Christense, 1974).

The alcoholic beverage industry and various regulatory agencies have devoted much effort in recent years to developing a faster, specific, more accurate, and automated method. Gas-liquid chromatography is one of the most modern analytical techniques, dating from 1952. Even more recently, with the utilization of electronic digital data-processing equipment, gas chromatography has become increasingly preferred for accurate quantitative as well as qualitative analyses of many substances. With an appropriately chosen column packing, gas chromatography is inherently

specific, separating volatile compounds on the basis of partitioning properties between a gas phase and a liquid (or a solid) phase. To evaluate the accuracy, precision, and specificity of the gas-liquid chromatography method described here, it was necessary to analyse ethanol standards (Stackler and Christense, 1974).

2.4.2 Analysis of Ethanol Composition by Refractometer Index

The original laboratory refractometer is known as the Abbe, after the scientist Ernst Abbe who invented the instrument in the late 19th century. This manual device measures the index of refraction of a sample sandwiched between two prisms, and is still in use tool today. It surpasses the digital refractometer when measuring solid samples and, with the incorporation of digital displays, has become easier to use but still requires a skilled operator. The portable alternative, offering most of the accuracy and ease-of-use of the digital instrument but in a compact form, is the digital handheld refractometer. These battery-powered devices will, according to model, provide readout of refractive index, Brix, or Oechsle scale quickly and easily in the field (Rex Harrell, 1998).

Portable manual refractometers provide fast and reliable assessment of refractive index and are useful for limited sampling in many process industries. These pocket-sized devices give a direct readout of index of refraction and may have automatic temperature compensation. For more thorough quality control in process industries, a regular automated readout of refractive index may be necessary. In these situations, a process refractometer takes measurements and provides data to the control system. A refractometer with ATC (Auto Thermal Compensation) is unaffected by temperature,

fermentation gases and only takes one to two drops to measure the sugar or alcohols. However, it is affected by the different refractive index of ethanol. As the amount of alcohol increases the refractive index changed algorithmically. Unlike a Hydrometer the reading in a refractometer will never go to zero.

2.4.3 Analysis of Glucose (Reducing Sugar) using DNS method and Nelson-Somogyi Method

Most of the methods for determination of carbohydrase activity are based on the analysis of reducing sugars formed as a result of the enzymatic scission of the glycosidic bond between two carbohydrates or between a carbohydrate and a noncarbohydrate moiety. Different methods for assaying the reducing sugar have been applied in the carbohydrase activity measurements. The Nelson-Somogyi (NS) assay with copper and arsenomolybdate reagents and the 3,5-dinitrosalicylic acids (DNS) assay described by (Miller, 1959) are the most popular methods used by many researchers. Other methods, such as those based on the use of sodium 2,2'-bicinchoninate, *p*-hydroxybenzoic acid hydrazide, or potassium ferricyanide are less frequently used (Alexander et al., 2011).

Although the DNS assay is known to be approximately 10 times less sensitive than the NS assay and it does not provide stoichiometric data with oligosaccharides, giving significantly higher values of RS than the actual number of hemiacetal reducing groups (Robyt and Whelan, 1972), it has been recommended by the IUPAC commission on biotechnology **(**Alexander et al., 2011) for measuring standard cellulase (Ghose, 1987) activities against filter paper and carboxymethylcellulose (CMC). The method for

determination of glucose activity with the DNS reagent, reported by Bailey et al., dominates in laboratories throughout the world. The DNS assay has also been used for measuring activities of other carbohydrases, such as amylases, β-mannanases, pectinases, and xyloglucanase (Aquino et al., 2003).

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter will discuss about the process of bioethanol production via fermentation of cassava using *Saccharomyces Cerevisiae*. Figure 3.1 shown below explains the process flow diagram of production of bioethanol involving cleaning and processing of cassava, grinding and cooking of cassava, preparing acetate buffer solution (pH 4.8), fermentation of cassava and bioethanol composition determination and lastly analysing the result. The further description will be detailed in the following subchapters.

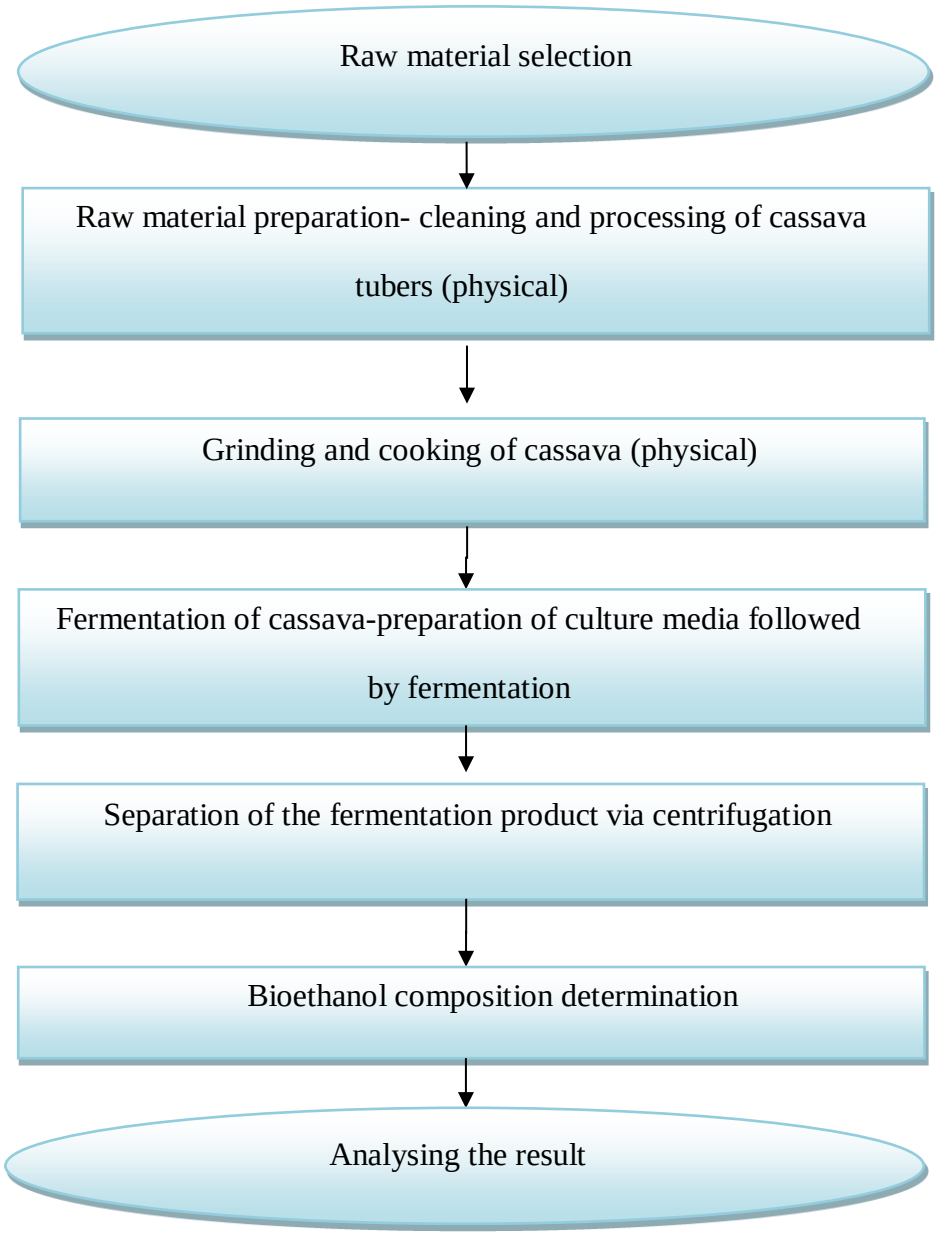


Figure 3.1 Process Flow Diagram for Bioethanol Production from Cassava

3.2 Material and Chemical List

Table 3.1 shows the list of material and chemicals needed for this experiment and the source of those specific materials.

Table 3.1 List of material

Number	Name of Material and Chemicals
1	200g Cassava Tubers
2	10 gram of Dinitro Salicylic Acid (DNS)
3	Sodium sulphate
4	Sodium hydroxide
5	Yeast (*Saccharomyces Cerevisiae*)
6	Distillate water
7	Aluminium foil
9	Ethanol 99%
10	Nutrient agar
11	Nutrient broth
12	Sodium Potassium Tartarate
13	Ethanol (70%)
14	D- glucose

3.3 Apparatus and Equipment

Table 3.2 shows the list of apparatus and equipment needed for this experiment.

Table 3.2 List of apparatus and equipment

Number	Apparatus And Equipment
1	500ml conical flask
2	Laminar Air Flow Cabinet (Model AHC-4A)
3	UV-Visible Single Beam Spectrophotometer (MODEL 1U 1800)
4	Double Stack Shaking Incubater
5	Shaking Water Bath Model (LBS-21)
6	Dry Blender (Model 8010 BU)
7	Thermometer
8	pH meter
9	Refractometer (Mettler Toledo)
10	Inoculating loop
11	Petri dish
12	Anaerobic chamber
13	Bunsen burner

3.4 Cleaning and Processing of Cassava

The cassava tubers will be buying from supermarket in Kuantan. Cassava tubers will be washed to clean up the soil attached on the outer layer of cassava tubers. It is important to make sure the soil present on the outer layer of cassava tubers do not contaminate the inner part when it processed. The outer layer of cassava tubers will be removed using knife (Seksan and Pomthong, 2009). After the outer layer of cassava tuber removed, the inner white colour part only can be seen.

3.5 Grinding and Cooking of Cassava

126g of dried cassava tubers (water content < 5% of the mass) will be prepared using electronic weighing machine (Kosugi, 2008). The cassava tubers will be dried by putting in the oven and the cassava weight measured every 2 hours. This process continuous until the dry cassava weight became constant. Then the cassava tubers put into grinding machine, and ground into a pulp mash（Ping and Mingjun, 2011). The pulp mash will be transferred into conical flask and water was added until reach 20% of weight of cassava by 400 ml of water (20 % w/v).

The conical flask mouth will closed with aluminium foil. Conical flask (250ml) and other apparatus used in this experiment is fully sterile（Nitayavardhana, 2009). The conical flask will be transferred into shaking water bath to cook the slurry cassava solution, and the slurry solution agitated at 90^{o}C for about 4 hours. The agitation speed of water bath was fixed at 150 rpm (Murata, 2008).

3.6 Preparing Acetate Buffer Solution (pH 4.8)

A 1 litre Erlenmeyer flask will fill with distillated water to 500ml. Sodium Acetate anhydrous will be weighed for 16.4 gram in a weighing boat and poured inside the Erlenmeyer flask. The solution stir until all the powder dissolved in water. That contains 988.5ml distillate water. The solution will be stirred until it mixed well. Make sure the pH using pH meter until reaches pH 4.8 (Akihiko and Kondo, 2008).

3.7 Dinitro Salicylic Acid (DNS)

300 g of potassium sodium tartrate tetrahydrate weighed and put into 1 L conical flask. 16 g of sodium hydroxide and 500 ml of water added and dissolved by heating gently. When the solution is clear, 10 g of 3, 5-dinitrosalicylic acid (DNS) added slowly. Then solution cooled to room temperature and made up to 1 L with distilled water. The scott bottle covered with aluminum foil to protect from light. The reagent was protected from light and carbon dioxide.

For the analysis of sample, 3 ml of the liquid sample will mixed with 3ml of DNS solution in the test tube. Then test tube covered with aluminium foil and will be heated up in shaking water bath at temperature 60oC for ten minutes. After that the mixed sample cooled for 20 minutes. Then 1 ml of sodium potassium tetra hydrates to decolorize the sample. After that, sample transferred into cuvette and analysed using uv-vis spectrometer at wavelength 540nm.

3.8 Preparation of Standard Calibration Curve for Glucose

Standard curve for glucose is drawn by using different dilution amount and by DNS method. Amount of glucose and amount of water added for dilution shown in Table 3.3.

Table 3.3 Preparation of stock solution and reading of uv-vis spectrophotometer

Glucose (ml)	Distilled Water (ml)	Glucose (mg/m L)	OD
0	1	0	0
0.1	0.9	0.1	0.15
0.2	0.8	0.2	0.297
0.3	0.7	0.3	0.311
0.4	0.6	0.4	0.423
0.5	0.5	0.5	0.535
0.6	0.4	0.6	0.592
0.7	0.3	0.7	0.683
0.8	0.2	0.8	0.767
0.9	0.1	0.9	0.889
1	0	1	0.983

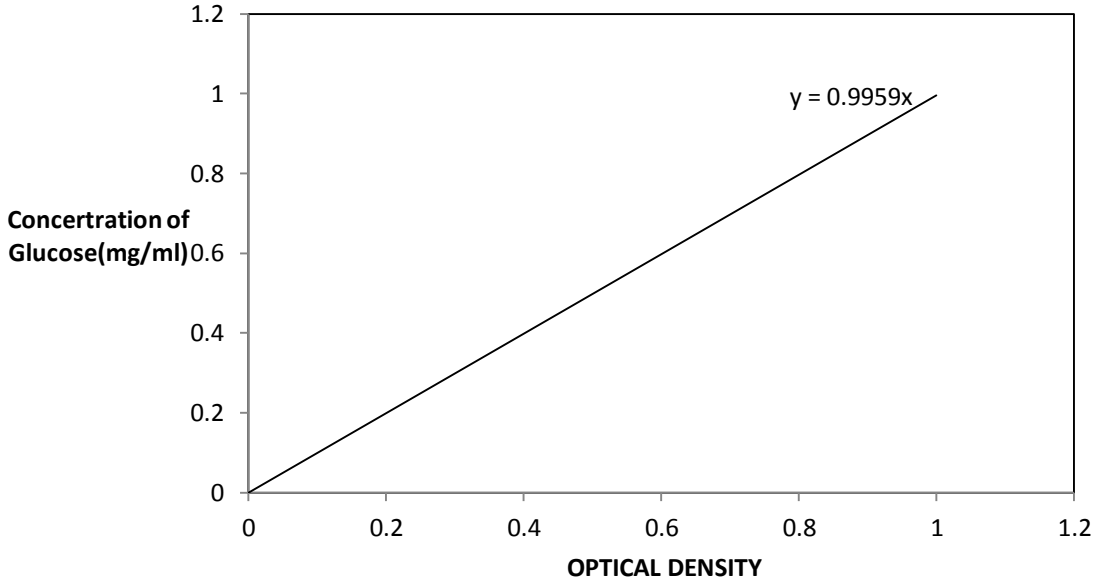


Figure 3.2 Graph of Standard Calibration Curve for Glucose

(Absorbance versus mg/ml)

3.9 Standard Calibration Curve of Ethanol

The standard calibration curve for ethanol has to be done to obtain the composition of ethanol after it is being produced. Analysis of ethanol is done using Metter Table Refractor 30PX and 30 GS, which are portable measuring devices suited for determining the refractive index of liquids. Preparation of (Refractive Index) RI standard calibration curve is used to determine the composition of bioethanol from cassava fermentation and from dilation process in the sample.

A set of mixtures containing ethanol and water will be prepared within a specified range of composition between pure water and pure ethanol. It is advisable to use weight as basis for quantitative analysis. For each mixture, their refractive index readings were obtained using refractrometer (Metter Table Refractor 30PX). The calibration reading of ethanol is shown in Table 3.4. The calibration curve of RI versus composition for the whole range of mixtures was plotted as shown in Figure 3.3.

Table: 3.4 Standard Calibration Reading of Ethanol (Weight and Refractrometer Reading)

Concentration (%v/v)	RI
0	1.3327
10	1.3402
20	1.3427
30	1.3494
40	1.3523
50	1.3598
60	1.3623
70	1.3624
80	1.3625
90	1.3626
100	1.3601

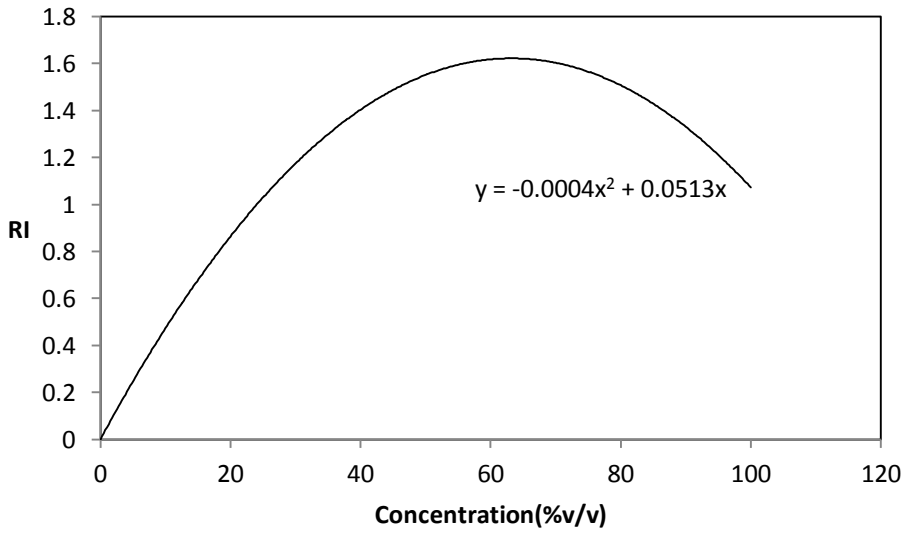


Figure 3.3 Graph of Standard Calibration Curve for Ethanol

3.10 Standard Calibration Optical density and Concentration of Yeast Cell

The optical density and cell concentration are shown in Table 3.5 and the standard calibration curve for cell concentration is shown in Figure 3.4. Yeast cells were cultured in nutrient broth. At time interval, 10 ml sample was for OD analysis and 7 ml from that was filtered using vacuum filter. The filter paper dried in oven temperature 60^{o}C until get a constant dry weight of yeast cells.

Table 3.5 Optical density and Cell Concentration

OD	Cell Concentration (mg/ml)
0	0
0.058	1.285714286
0.571	5.428571429
1.327	9.285714286
1.566	10.42857143
2.066	12.14285714
2.432	13.71428571

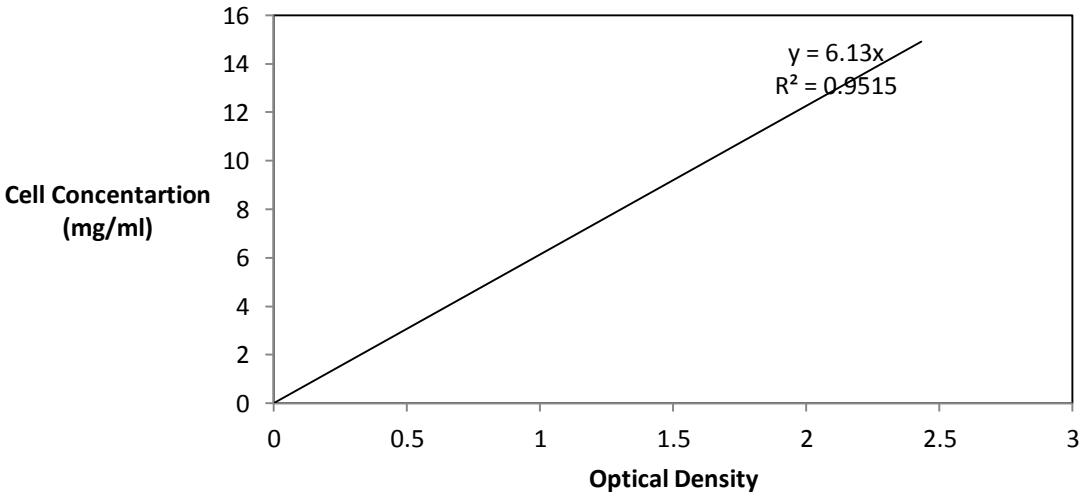


Figure 3.4 Graph of Standard calibration of Cell Concentration (mg/ml)

3.11 Fermentation of Cassava

3.11.1 Preparation of Growth Media and Inoculums

Before fermentation process was carried out, stock cultures were prepared. Growth and dominance of yeast *Saccharomyces Cerevisiae* must be encouraged to ensure complete fermentation. A 0.18g seed culture of *Saccharomyces Cerevisiae* has to be growing in a media of 6g of Saboured Dextrose (SDB) up to a total volume of 300ml of distilled water. SDB powder will weighed in a weighing boat and poured into the Schott bottle. The bottle will hand shacked to mix the solution. The media was then sterilized at 121^{o}C for 15 minutes. After that, the broth solution will be cooled to the room temperature and it will be transferred into three different autoclaved serum bottles. The stock culture of the microorganisms was transferred into to the broth media preparation of seed culture. During the transferring process, the inoculating loop will be flamed with Bunsen burner until it become red hot for sterilization.

The test tube cap that contains the stock culture will be removed and the lip of the test tubes will be flamed with a Bunsen burner. The inoculating loop will be inserted to stock culture test tube and a small portion of closed tight. The inoculating loop that contains the stock culture will be inserted into a serum bottle that contains the broth solution. The inoculating loop will be flamed again after completing the procedure. The same procedure will be repeated for another serum bottle. One of the serum bottles will be as the controller. All the procedures outlined above will be done in a laminar flow cabinet in order to prevent any contamination. The serum bottles then will be incubated in Double stack Shaking Incubator, orbital shaker at 30^{o}C and 180 rpm for 48 hours.

After two days, the bottles will take out from the incubator and keep in the refrigerator at -4^{o}C (Yadav, 2009).

3.12 Fermentation and Separation of Sample Fermentation

The 10% (standard solution, Ping et al., 2011) of inoculated yeast will be transferred into prepared cassava slurry. The mixed solution of inoculum and medium (fermentation broth) was incubated in incubator shaker at temperature 30^{o}C and agitation speed 200 rpm (Arisra et al., 2008). pH of the fermentation solution was maintain using acetate buffer solution. Sample of fermentation were taken at that time interval 2 hours for first 12 hours, then 6 hours once for the following 60 hours and centrifuged at 12 000 rpm for 20 minutes with temperature at 28^{o}C to remove the cells (Akihiko and Kondo, 2008). Samples after centrifugation was analysed for ethanol concentration, glucose concentration and yeast cell concentration. Experiment repeated at 3 different parameters such as temperature (20^{o}C, 30^{o}C, 40^{o}C and 50^{o}C), substrate concentration (25g, 50 g, 75g, 100g and 150g) and agitation speed (0 rpm, 150 rpm, 200 rpm, 250 rpm).

3.13 Analysis Data

Ethanol concentration was measured using refractometer and glucose concentration analysed using DNS method. Cell concentration was analysed by cell washing and reading of optical density using uv-vis spectrometer. Optical density gained from the uv-vis will be compared with standard calibration curve of cell concentration. Data from the analysis was converted into graphs and discussed.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Different Temperature

Saccharomyces Cerevisiae was cultured with cassava slurry in shake flask at 200rpm for 72 hours. To analyse effect of temperature, fermentation was carried out on different temperature, at 20^{o} C, 30^{O} C, 40^{O}C and 50^{O}C. Figure 4.1 shows bioethanol concentration at 4 different temperatures versus time. The cell growth, glucose consumption and bioethanol production were monitored throughout fermentation process. The ethanol concentration, reducing sugar concentration and cell growth are presented in Figure 4.1, 4.2 and 4.3 respectively.

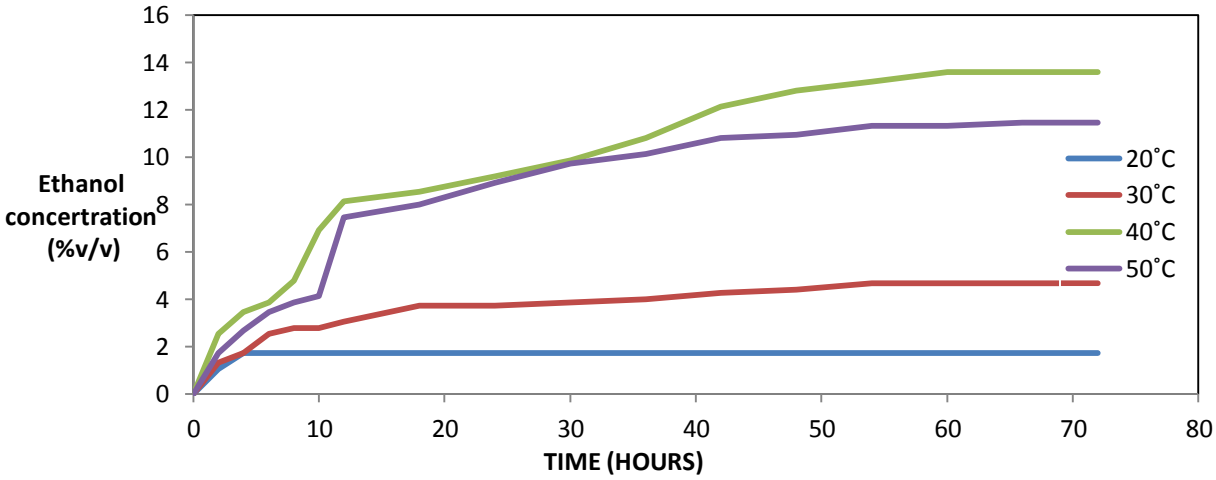


Figure 4.1 Graph of Bioethanol Concentration versus Time

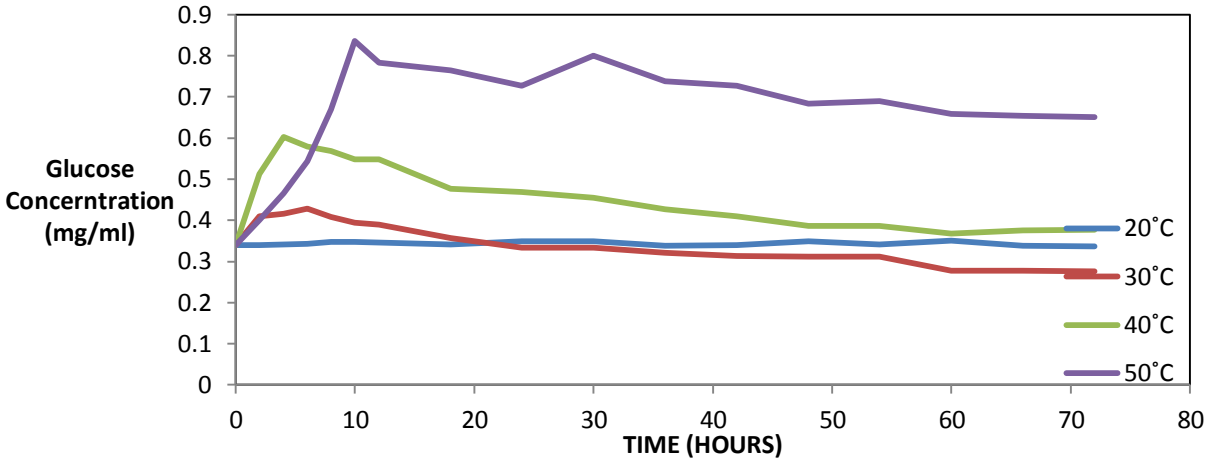


Figure 4.2 Graph of Glucose Concentration (mg/ml) Versus Time (Hours)

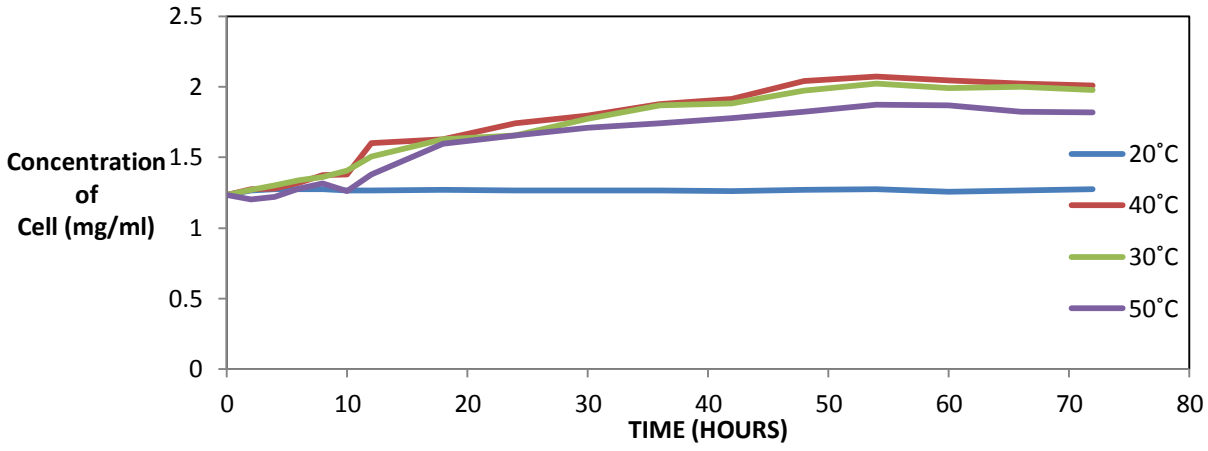


Figure 4.3 Graph of Cell Concentration versus Time (Hours)

From the figures, they clearly show that the maximum concentration ethanol yield from cassava slurry was achieved at 40^{O}C, whereby the highest concentration is 13.6 (%v/v). At 40^{O}C, at 2 hours *Saccharomyces Cerevisiae* produced bioethanol yield of 2.5(%v/v) which is higher compare first reading of other temperatures. It is because at 40^{o}C, the concentration of glucose throughout the experiment was higher compare to the amount of glucose at the other temperatures (Figure 4.2). At high temperature, the starch hydrolysis (conversion of starch into glucose) still continued throughout the experiment which leads to maximum glucose concentration (Manikandan et al., 2006), even though initially the amount of cassava slurry used (100g) same for all experiment.

Hence the availability of substrate (glucose) throughout the experiment helps *Saccharomyces Cerevisiae* to utilize it and to produce maximum concentration of ethanol. Even though the optimum temperature of *Saccharomyces Cerevisiae* is 30^{o}C, but still the maximum ethanol concentration (4.6%v/v) reach at that temperature was lower than the maximum ethanol concentration reached at 40^{O}C.At this temperature, the amount of cassava slurry used was 100g (same as for other temperatures), but this temperature 30^{o}C is not high enough to support the hydrolysis of cassava starch into monomer glucose. Throughout this run, the glucose concentration (Figure 4.2) was lower compare to glucose concentration at temperature 40^{O}C and 50^{O}C. Hence, the substrate (glucose) level was not high to produce maximum ethanol.

Moreover, the maximum ethanol concentration at 20^{O}C is the lowest (1.7 %v/v) compare to the other ethanol concentration at other temperature. *Saccharomyces Cerevisiae* turns into inactive state when placed under lower temperature (20^{O}C) whereby activity of *Saccharomyces Cerevisiae* optimum at temperature 30^{o}C, which a

bit higher than the room temperature. But temperature at 20^{O}C which is very low compare to optimum temperature of yeast, it makes the yeast becomes inactive. Inactivation state of yeast cell causes it does not utilize the substrate (glucose) from the starch, hence the amount ethanol produce is very low (Figure 4.1). But still at the beginning (1^{st} 2 hours) the ethanol concentration at 20^{o}C showing a bit normal compare to the other ethanol concentration at other temperature because at beginning the incubator shaker takes time to change to 20^{O}C from room temperature. In that period of time, yeast utilizes some of substrate (glucose) to produce ethanol before it change in inactive state.

The ethanol production decreased as the incubation temperature increased beyond 40^{o}C. This is because although the highest the glucose concentration was obtained at 50 ^{O}C, as this temperature promote the hydrolysis cassava into glucose. The temperature directly correlated with reaction, the better the hydrolysis the highest glucose concentration was reached but it promotes dysfunctions the yeast cells. Hence at 50^{O}C, the maximum ethanol concentration was 11.46 (%v/v) which is higher than ethanol concentration at 20^{O}C and 30^{O}C.

Temperature at 50^{O}C is high enough to promote the hydrolysis of cassava starch into glucose, hence it leads to high substrate (glucose) concentration (Figure 4.2) compare to other temperature and it helps the yeast to utilize it and produce ethanol. But still the maximum amount of ethanol produce at this temperature lower than ethanol produced at 40^{O}C because at very high temperature *Saccharomyces Cerevisiae* may undergoes slightly denaturation (Manikandan et al., 2006). Higher temperature than the normal, may reduce the activity of yeast by denaturing the structure and leads to lower

production of ethanol at 50 ^{O}C compare to 40^{O}C. Increasing incubation temperature significantly affect bioethanol production.

In order to find out the utilization of substrate by *Saccharomyces Cerevisiae*, concentration of glucose was analyse using DNS method. From the view, it was found for each run concentration of glucose increases at certain level at the beginning, and then reduce until end of the fermentation. The highest glucose concentration was reached at temperature 50^{o}C which is 0.84 mg/ml. At initial, glucose concentration was at 0.40 mg/ml and it reduces throughout the experiment.

The increases in glucose concentration at early stage of fermentation is due to high heat availability at temperature 50^{o} C, which leads to cassava starch hydrolysis at early of the experiment. Heat is required to break the strong cell of cassava which turns into monomer glucose. At time 10 hours, maximum glucose concentration was reached, and after that it reduces for the following 62 hours of fermentation. From the data collected from the research, it was calculated that starts from the time of highest glucose concentration until end of fermentation, about 0.18 mg/ml of glucose was utilize by yeast to produce (7.5% v/v) of ethanol.

The highest reducing sugar (glucose) at temperature 40^{o}C lower compares to glucose concentration at 50^{o}C (Figure 4.2).It is because heat produce at temperature 40^{o}C is not much enough to hydrolysis cassava starch to glucose as converted during temperature at 50^{o}C. It has been proved from data of experiment, the highest glucose concentration and final concentration of glucose at 40^{O}C are 0.79 mg/ml and 0.38 mg/ml respectively, whereby at 50^{O}C highest glucose concentration and final concentration are

0.83mg/ml and 0.65mg/ml, which are higher about 44% than the maximum glucose concentration at 40^{O}C (Lee Man et al., 2010).

The glucose concentration does not deviate much at temperature 20^{o}C. At beginning glucose concentration is 0.326mg/ml and reached a highest level at 0.34 mg/ml at time 24 hours and then become constant throughout the run (Figure 4.2). Temperature 20^{O}C is very low for the yeast to carry out fermentation process, because the optimum temperature of yeast is 30^{O}C, hence yeast converted into inactive form (Manikandan et al., 2006).Due to that glucose in solution do not utilize by *Saccharomyces Cerevisiae* and the fermentation process do not triggered, hence the concentration glucose does not reduce (remains constant) throughout the experiment. Moreover the maximum glucose concentration reached at 20^{O}C is very low compare to glucose concentration at other temperature, it is because at 20^{O}C which is a cooling temperature do not provide enough heat to support the hydrolysis of cassava starch into monomer glucose.

As for the cell concentration, highest growth was reached at temperature 40^{O}C at time 54 hours which is about 2.08mg/ml (Figure 4.3). At 40^{o}C, initially the growth was slow until time 16 hours, it is because at this phase yeast cell adjust themselves to the environment (temperature and agitation speed) hence their growth activity still at beginning stage. Then starts from 16 hours until 54 hours the increase in cell concentration was very rapid and this phase is called as exponential phase, whereby the yeast cell utilizes the glucose rapidly for their growth and produces ethanol. At this exponential phase, the consumption of substrate (glucose) will be optimum. After 54 hours, concentration of cell yeast declines slowly until end of the fermentation. This

phase is called as death phase, whereby the yeast cells reached maturity and the life of yeast reached at the end.

Apart from that, second higher cell concentration reached at temperature 30^{o}C (Figure 4.3).Maximum growth reached at 30^{O}C and time 54 hours which is about 2.03 mg/ml but lower about 2.40% compare to maximum concentration reached at temperature 40^{O}C. Optimum temperature of *Saccharomyces Cerevisiae* is 30^{O}C, but in this case it differs due to highest yeast growth at 40^{o}C which caused by larger of substrate (glucose) availability as shown in Figure 4.2 (Neelekandan and Usharani, 2009). Fermentation at 40^{O}C also promote simultaneously the hydrolysis of cassava starch into monomer glucose, hence it helps in providing extra substrate concentration which leads to higher cell growth.

Furthermore, at 50^{o}C, the cell concentration is lower compare to cell concentration at 40^{O}C and 30^{O}C. At temperature 50^{o}C the maximum cell concentration was reached at time at 54 hours, about 1.90mg/ml (Figure 4.3). But this reading is lower about 6.4% compare to cell concentration at temperature 40^{O}C. This impact most probably results from very high temperature of fermentation, which leads to denaturation of yeast cells. Hence, even glucose concentration high at 50^{o}C, but due to reduction in number of active cells, the growth of cell lesser than cell growth at 40^{o}C.

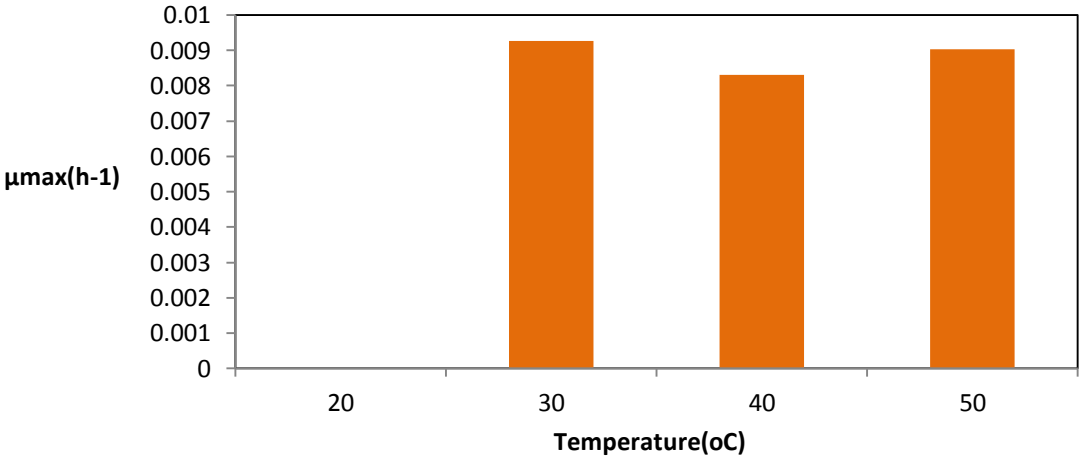
Besides that, at 20^{o}C, the cell concentration does not change much from the initial cell concentration. Moreover, there were absents of lag phase exponential and death phase in the cell concentration graph at temperature 20^{O}C (Figure 4.3). Concentration of cells at this temperature nearly constant throughout experiment.

Temperature 20^{o}C is a very differ condition compare to its optimum temperature (30^{O}C), whereby yeast cells will stay in inactive form.

As conclusion, bioethanol production and cell growth was highest at temperature 40^{O}C. Temperature higher or lower than this temperature gives lower productivity of ethanol and lower growth of yeast cell.

Table 4.1 Temperature Parameter

Weight of cassava slurry(g)	20	30	40	50
Productivity(g/l hr)	0	0.08642	0.226667	0.209876
μnet (h-1)	0.00002	0.006503	0.006344	0.00595
μmax (h-1)	0.00001	0.00927	0.008308	0.009034



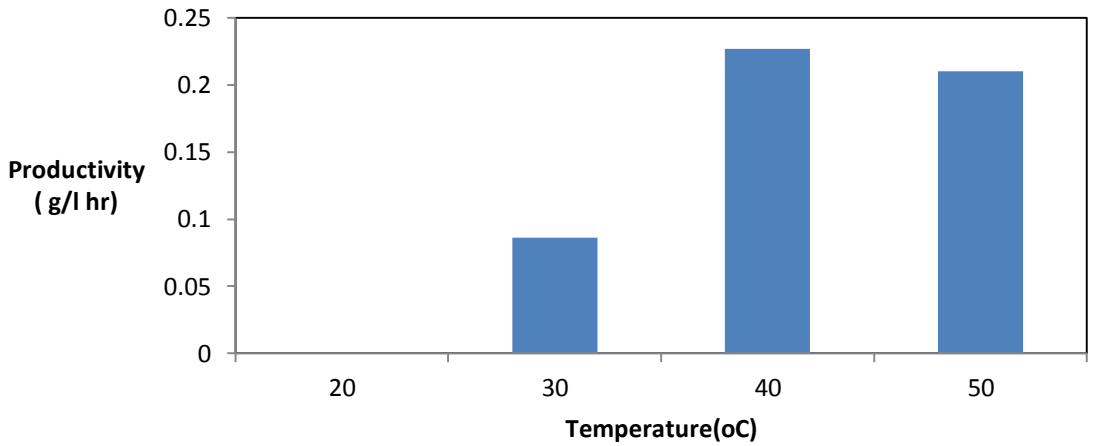


Figure 4.4 Bar graph of Productivity and Maximum Growth versus Temperature

Figure 4.4 shows the ethanol productivity, net growth of yeast cell and maximum growth of yeast cell. Ethanol productivity increases as the temperature increase from 20^{o}C to 40^{o}C but decrease at temperature 50^{o}C. It is because the optimum temperature of *Saccharomyces Cerevisiae* is (30-40) ^{o} C; hence any temperature that deviates in a wide range from the optimum temperature will lead to lower ethanol productivity.

Ethanol productivity nearly 0 (g/ l hour) at temperature 20^{o}C (which is a very low temperature than optimum temperature of yeast) because at that temperature the yeast conformation become freeze and become inactive state. Then, at temperature 50^{o}C, ethanol productivity lower than at 40^{o}C, because a very high temperature than optimum will causes conformation change of yeast cell, whereby there will less number of active yeast cell that utilizes glucose, hence the ethanol productivity lesser than the highest (Rahmat et al., 2012).

The net growth of yeast cells has the same trend of graph as the ethanol productivity. Table 4.1 and figure 4.4 shows that the reading of $\mu_{max\ and}$ μ_{net} is at

temperature 40^{o}C is higher compare to other temperatures (Ijogbeme et al., 2011). At this a bit higher temperature, more amount of fermentable sugar available by hydrolysis process, hence yeast cell gained adequate amount of substrate for its growth. Hence, when substrate does not become a limiting reactant here, hence the growth of yeast cell reached at maximum.

Reading of $\mu_{max\ and}$ $\mu_{net\ at}$ temperature 20^{o}C is the almost zero. It is because at lowest temperature there is no hydrolysis process to produce fermentable sugar. At the same time, a very low temperature causes the yeast cell become inactive and do not participate in utilization of glucose. Hence, the there is almost no growth of yeast cells at this temperature.

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4.2 Different Substrate Concentration

Fermentation of cassava was repeated at various substrate concentrations which are at 25g (6.25%w/v), 50g (12.5%w/v), 75g (18.75%w/v), 100g (25%w/v) and 150g (37.5%w/v) of cassava slurry in order to find its effect on ethanol concentration, reducing sugar concentration and cell growth. Results from the experiment were analysed as follows. The ethanol concentration, reducing sugar concentration and cell growth are presented in Figure 4.5, 4.6 and 4.7 respectively.

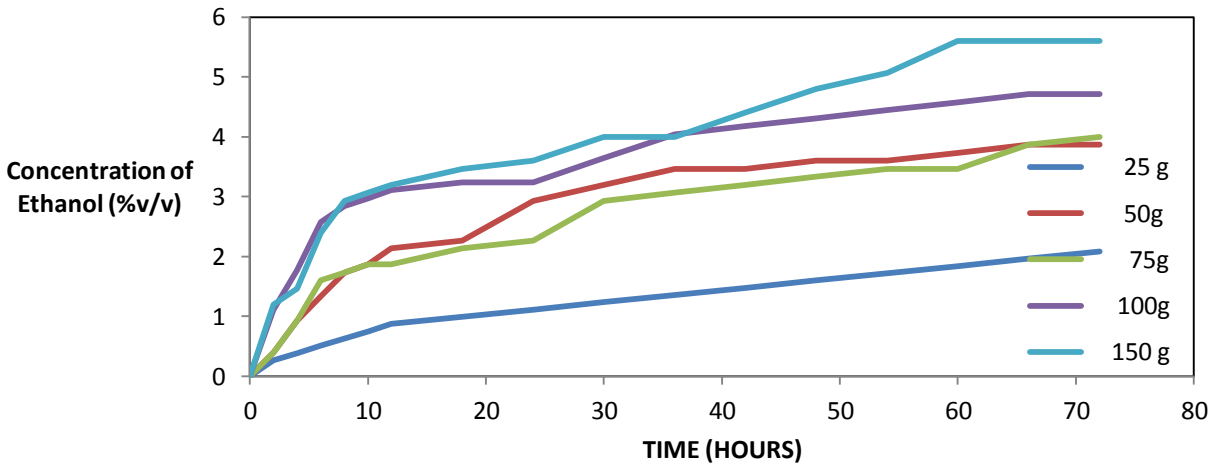


Figure 4.5 Graph of Ethanol (%v/ v) Concentration versus Time (Hours)

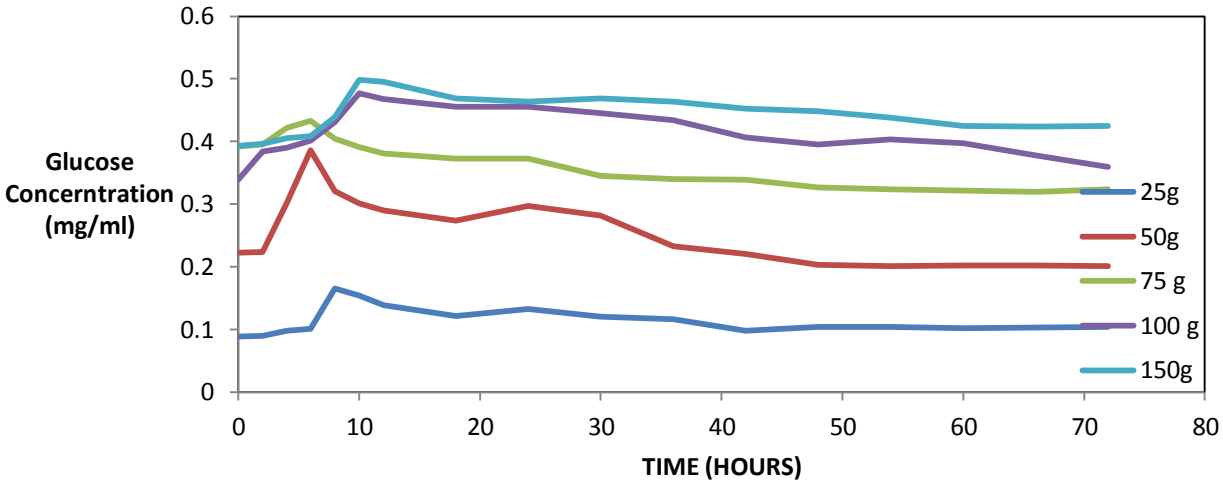


Figure 4.6 Graph of Glucose Concentration versus Time

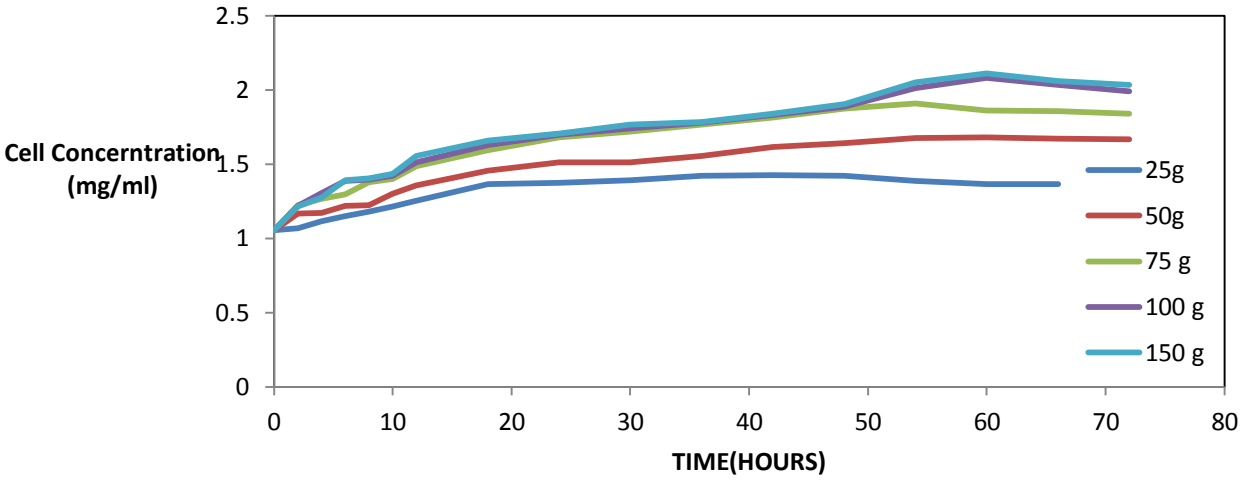


Figure 4.7 Graphs of Cell Concentrations versus Time (Hours)

From the figures it can be concluded that the highest ethanol reached at cassava concentration 150g (37.5%w/v) which is about (5.6%v/v) (Figure 4.5). This is because at highest cassava slurry concentration, it contains more amount of glucose (Figure 4.6) compare to other cassava concentration that can be utilized by the yeast cells to produce ethanol. Hence maximum availability of substrate trough out the experiment leads to highest utilization of glucose by yeast cells and further leads to highest production of ethanol (Kademi and Barati, 1996).

Follows that, cassava concentration at 100g (25%w/v) also produces almost highest amount ethanol but still lower about 15% compare to substrate level at 150g. Substrate (glucose) present in 100g lower compare to substrate present in 150g of cassava (Figure 4.6), which reduces the substrate availability at 100g and leads to lower production of ethanol. The same situation happens at substrate concentration at 75g, 50 g and 25g whereby the maximum ethanol production reduces as the substrate concentration reduces. The highest concentration reached at cassava weight 75g, 50g, and 25g are (4%v/v), 3.8(%v/v), 2.08(%v/v) respectively. These ethanol reading is less about 28 %, 32% and 62.8 % respectively compare to ethanol reading at 150g (Figure 4.5).

The results obtained from this study showed that the percentage yields of bio-ethanol were dependent upon the substrate concentration until all the enzyme was saturated with the substrates at the active sites at maximum yield. This relationship suggested that at very low substrate concentrations, most of the active sites of the enzyme were unoccupied. Increasing the substrate concentration allowed more active sites to be occupied, thus, resulting in increased yield. On the other hand, at higher

concentrations of substrate molecules, most of the active sites of the enzyme were occupied and the observed yield depended only on the concentration at which the bound substrates were converted to products. Consequently, further increase in the substrate concentration produced little or no appreciable effect (Abara et al., 2006).

Based on figure 4.6, at all 5 substrate concentrations studies the amount of detected glucose increases initially and decreases throughout the experiment. It is because the supply of heat at temperature 30^{o}C causes conversion insoluble starch component by hydrolysis process into soluble and fermentable sugar (glucose). Hence, this leads an increase in glucose concentration at beginning of each run. By the way, the initial and maximum glucose concentration differs at all the 5 substrate concentration. It is because the alteration in percentage of cassava slurry as the fermentation medium cause the differences mentioned above (Ping, 2011).

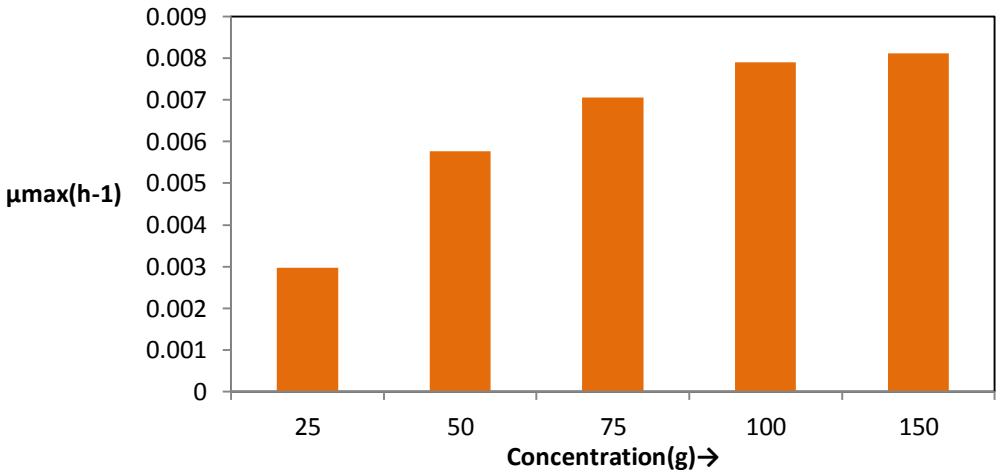
For an example, at substrate concentration 6.25 (%v/w) the maximum glucose concentration reached is lower about 67% compare to substrate concentration at 37.5 (%w/v) (Figure 4.6). This cause by the alteration in weight of cassava slurry used to be mixed in water as the fermentation medium. As the weight of cassava used increases, the amount of starch present in the fermentation medium also increases, which later will be converted in to fermentable sugar (Kademi and Barati, 1996). Hence, as the starch amount increases, the amount of glucose present in the solution too increases which leads to difference in highest glucose concentration at various substrate concentration level.

As for overview of Figure 4.7, all the lines on the graph increases to a certain time then decreases at the end of fermentation. Patterns of yeast growth are nearly same, which consist of a lag phase stationary phase, and death phase. But difference occurs at the maximum yeast growth and at the net yeast growth as shown in Table 4.2. From the Table 4.2 and the Graph 4.8, it can be find that the μ_{max} and μ_{net} increases as the substrate concentration increases.

The increase in μ_{max} and μ_{net} is caused by increasing substrate concentration which provides substrate for the growth of yeast cells. When more amount of substrate available, the yeast undergoes maximum rate of utilization for its growth hence increase in mass of yeast cells. The growth of yeast cells at the beginning is very slow for all substrate concentration level. It is because yeast cell slows up its utilization at beginning, because it takes time to adapt itself to the new environment. At the end, the yeast cells reached its maturity and mortality stage whereby substrate utilization become slows and the mass of yeast cell start decreases after the inflection point.

Table 4.2 Parameters at different substrate Concentrations

Weight of cassava slurry(g)	25 g	50g	75g	100g	150 g
Productivity(g/ l hr)	0.028956	0.053704	0.055556	0.082296	0.093333
$\mu_{net}(h_{-1})$	0.003479	0.005101	0.005812	0.00699	0.007368
$\mu_{max}(h_{-1})$	0.002976	0.00576	0.00706	0.007897	0.008106



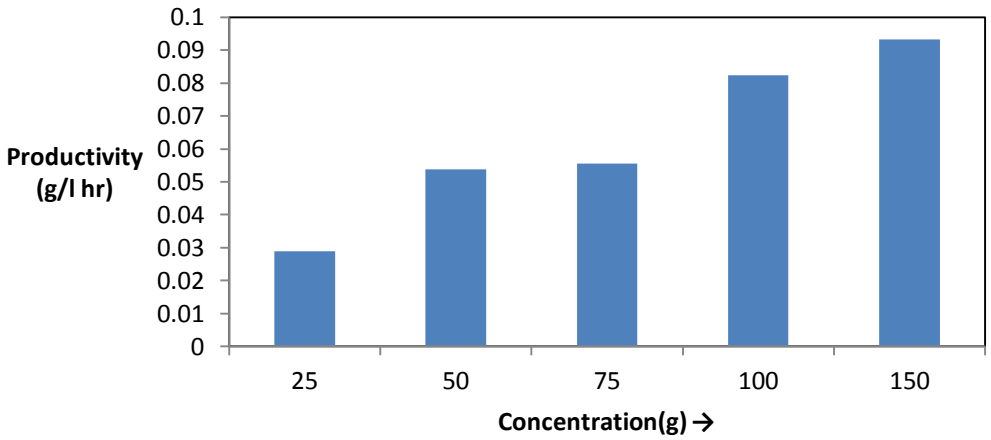


Figure 4.8 Bar graph of ethanol productivity and maximum growth rate versus increasing substrate concentration

Table 4.2 and Figure 4.8 show the data of ethanol productivity, maximum growth of yeast cell and net growth of yeast cells. Ethanol productivity by the *Saccharomyces Cerevisiae* increases with the increase of the concentration substrate concentration. Ethanol productivity was highest at 150g (37.5%w/v) of cassava slurry. It is because at higher concentration of cassava slurry, more starch polymers are available to be

converted into reducing sugars (glucose) which provide highest substrate supply for yeast. Hence, this continues substrate supply causes in maximum ethanol productivity at cassava weight at 150g.

Moreover at substrate concentration (25%w/v) the ethanol productivity reached as second highest which is about 0.08 (g/l hour). This productivity is lower about 11% compare to the highest productivity. This deviation may due to less availability of starch component in 100g compare to 150g, where by the conversion of starch into utilizable glucose is less. But still the difference in ethanol productivity between substrate concentration of 150g and 100g does not much, compare to large difference between productivity at substrate concentration at 75g and 150g which is about 44% lower compare to the highest productivity (Kademi and Barati, 1996).

This explains by the limiting factor concept, whereby at 150g, there is adequate amount of glucose present in solution but still the amount of yeast that utilizes the glucose is limited trough out the experiment. Hence a very large supply of substrate to limited growing yeast does not give big changes to the ethanol productivity (Abara et al., 2006). As for the overall view, by increasing substrate concentration, productivity of ethanol also increases at a certain level, whereby yeast becomes as limiting reactant.

4.3 Agitation Speed

Fermentation of cassava slurry was carried out at different agitation speed (0 rpm, 150 rpm, 200 rpm, and 250 rpm) in order to find out the effect of agitation speed on

ethanol concentration, reducing sugar concentration and cell growth. Temperature kept at 30^{o}C and other parameter kept at optimum. Data from the experiment was converted into graph. The ethanol concentration, reducing sugar concentration and cell growth are presented in Figure 4.9, 4.10 and 4.11 respectively.

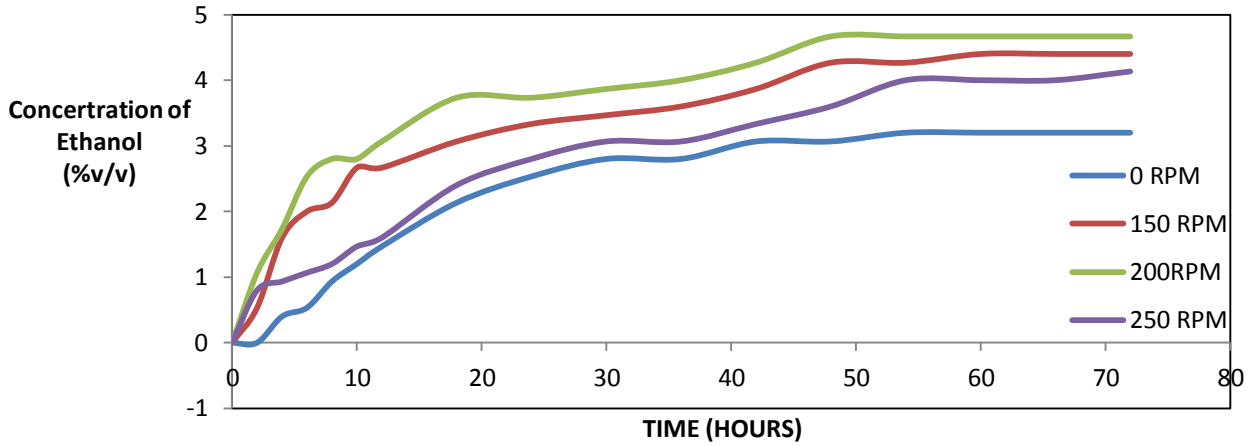


Figure 4.9 Graph of Ethanol Concentration versus Time

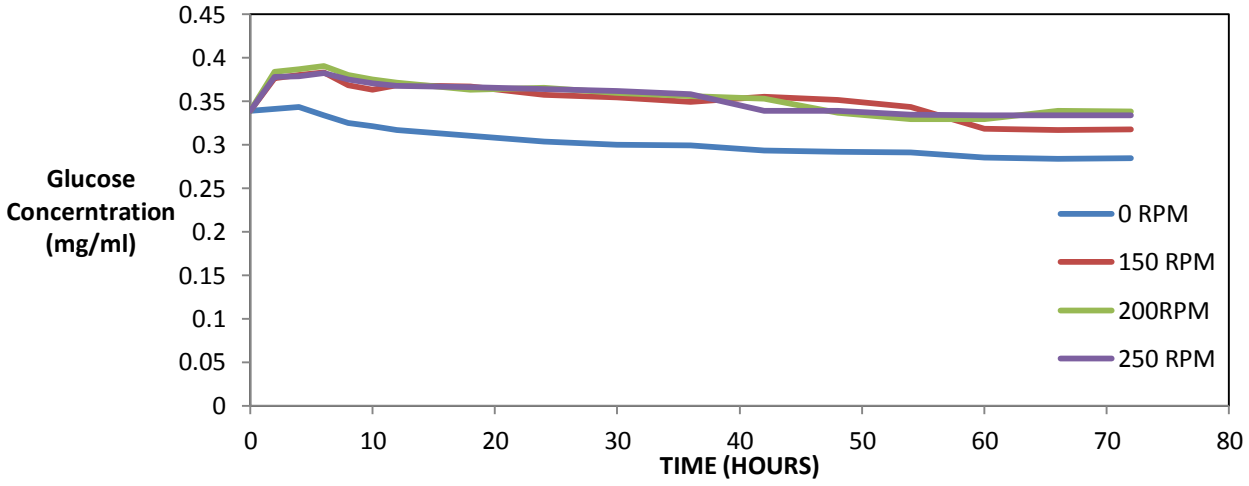


Figure 4.10 Graph of Glucose Concentration versus Time

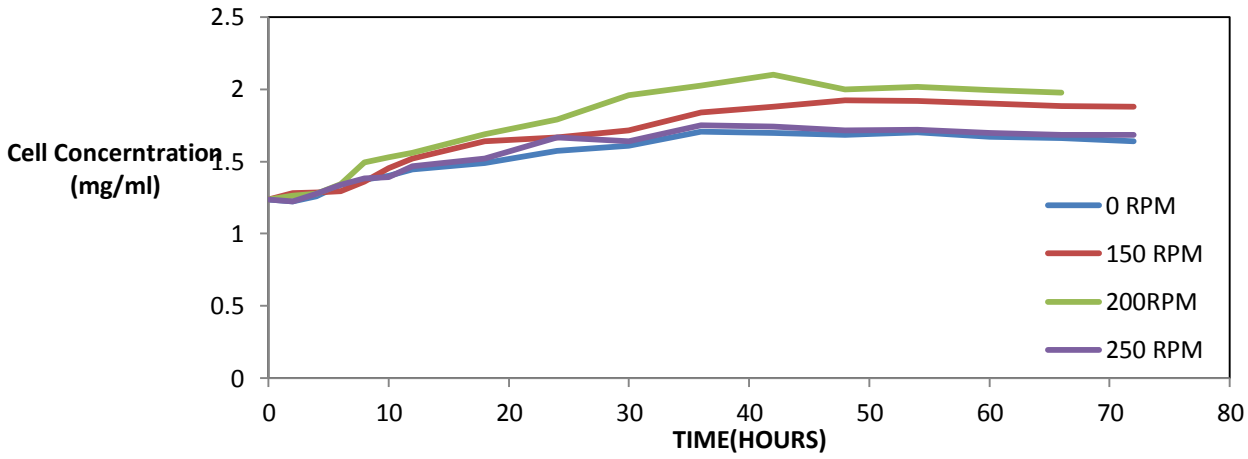


Figure 4.11 Graph of Cell Concentration versus Time (Hours)

Table 4.3 Parameter at Various Agitation Speeds

Agitation Speeds (rpm)	0	150	200	250
Productivity (g/l hr)	0.120542	0.140267	0.146842	0.120785
μ_{net} (h_{-1})	0.004168	0.005492	0.006109	0.004555
μ_{max} (h^{-1})	0.004413	0.006303	0.006258	0.004841

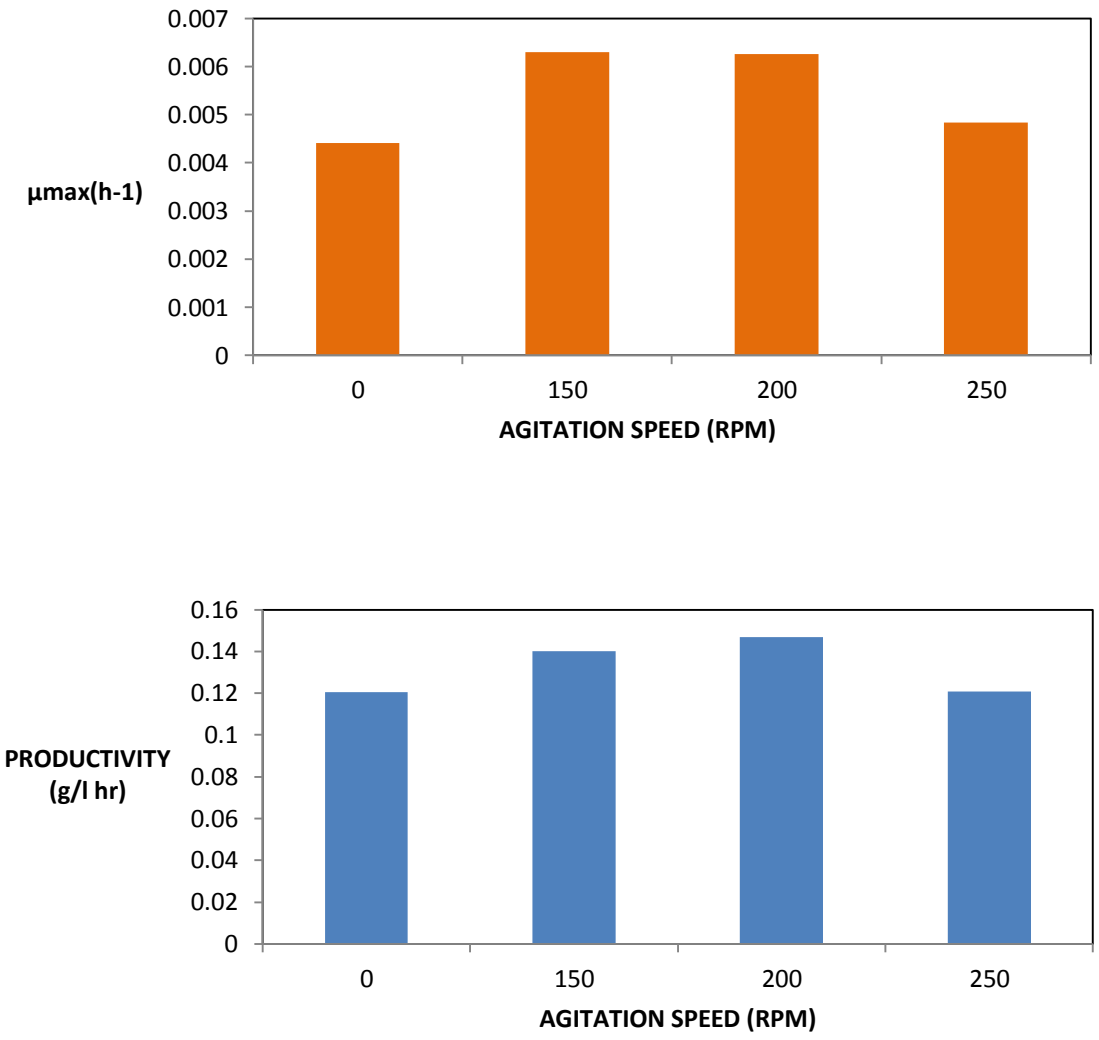


Figure 4.12 Graphs of Ethanol Productivity and Maximum Cell Growth versus Increasing Agitation Speed

Highest bioethanol (Figure 4.9) concentration was reached at agitation speed of 200 rpm which is about 4.63(%v/v). This reading is cause by the increase of dissolved oxygen concentration, generated by the increase of agitation speed, resulted in a quick start of growth (shortened lag time) which leads to higher production of ethanol. The availability of oxygen is very important for aerobic fermentation of *Saccharomyces*

Cerevisiae. Hence the better way to increase the dissolve oxygen in the fermentation medium is by increasing the agitation speed of shaking incubator. At 200 rpm, the availability of oxygen is adequate for the yeast; hence there was a largest production of ethanol (Arisra et al., 2008).

Furthermore, maximum ethanol (Figure 4.9) production at 250 rpm is also high which about 3.99 (%v/v). But still the maximum ethanol production reached at 250 rpm lower compare to 200 rpm which differ about 9%. Agitation speed 250 rpm is a bit higher speed and reduces the ethanol production. At higher agitation speed there is a lot of dissolved oxygen in fermentation media, but very higher shaking causes the formation of complex yeast-substrate could not bind together for long time. Hence, the complex of yeast-substrate will break immediately into yeast and substrate and leads to less production of ethanol. So, the yeast-substrate complexes which withstand at higher shaking end with formation of product.

At 0 rpm (Figure 4.9), there is no agitation and the conical flask was incubated at static state. Highest ethanol concentration gained at this state is about 3.19 (%v/v) which is lower compare to other three agitation speeds. This reading differ about 31% compare to ethanol production at 200 rpm. At 0 rpm, there is no shaking of fermentation media which helps in mixing of the air in the solution. When there is no presents of agitation there will be less oxygen dissolved in the mixture. Hence, low dissolved oxygen concentration in the fermentation solution causes fermentation process less efficient, whereby utilization of glucose by *Saccharomyces Cerevisiae* become less due to lack of dissolved oxygen (Liu et al., 2009).

Figure 4.10 shows the reading of reducing sugar (glucose) present at different agitation speeds. At overall, glucose concentration was increases initially and starts decline trough the fermentation time. At 250rpm the glucose concentration was the highest compare to other agitation speed. It is because when there is higher shaking, it helps the in dissolving of the reducing sugar which hydrolyses from cassava starch. When reducing sugar that dissolved in the solution increases, it provides more substrate availability for the yeast (Arisra et al., 2008).

Follows that, at 0 rpm (Figure 4.10) whereby there conical flask maintain in static position in incubator shaker, the highest glucose concentration is the lowest compare to other agitation speed. When there are no presents of shear or shaking, reducing sugar which hydrolysis from cassava starch would not dissolved fully in the fermentation solution. Furthermore, there will be formation of layers of liquid and cassava slurry. Hence, the cassava slurry do not dissolved well in the solution which leads to low glucose concentration in the fermentation solution.

Based on figure 4.11, the highest cell growth was reached at agitation speed of 200 rpm with maximum cell concentration about 2.1 (mg /ml). Furthermore, from Table 4.3 and Figure 4.12 it can be seen that, μ_{max} and μ_{net} at 200 rpm is the highest compare to other agitation speeds. At 200 rpm, oxygen from the air dissolved fully in the fermentation media which provide continuous oxygen supply for yeast cells. At the same time, reducing sugars from the cassava starch dissolved fully into liquid solution at this agitation speeds provides continuous substrate supply for yeast. These both continuous supplies of oxygen and substrate induce growth of yeast cells at agitation speed 200 rpm (Liu et al., 2009).

At 0 rpm, the maximum cell concentration was about 1.7 (mg/ml) which is the lowest reading compare to other agitation speeds (Figure 4.11). Moreover, μ_{max} and μ_{net} at 0 rpm also the lowest which are 0.004168h-1 and 0.004413 h-1 respectively (Table 4.3). Because, at 0 rpm conical flask incubated at static position. So, there is no effect of shaking and shear on to the fermentation media. Hence, when there is no effect of shaking, the mixing glucose and yeast was not encouraged well. Furthermore, amount of dissolved oxygen into the fermentation solution is not much to induce glucose utilization by yeast. Besides that, at 0 rpm there is no mixing process of glucose and yeast cells. Hence, the distribution of yeast cells in the fermentation not much efficient at 0 rpm. All the factors mentioned above results in lower cell growth at 0 rpm (Arisra et al., 2008).

At 250 rpm, highest cell growth (Figure 4.11) reached is about 1.75 (mg/ml) which is lower than cell growth at 200 rpm and 150 rpm. Hence, μ_{max} and μ_{net} at 250 rpm also less than the μ_{max} and μ_{net} gained at agitation speed 200 and 150 rpm. Even though, higher agitation speed encourage in amount of dissolved oxygen and dissolved reducing sugar, but higher shaking and shear impact cause reduction in time formation of yeast-substrate complex formation. Time for the yeast-substrate complex binding becomes less and causes incomplete reaction between yeast and substrates. Hence, those effects causes in low yeast cell growth.

The biomass dry weight increased with the increase of agitation speed from 0 to 200 rpm. Agitation could be beneficial to the growth and performance of the microorganism cells by improving the mass transfer characteristics with respect to substrates, products/by products and oxygen. Thus, agitation results in a better mixing of the fermentation broth, helping to maintain a concentration gradient between the interior

and the exterior of the cells. Such a concentration gradient works in both directions; through better diffusion it helps to maintain a satisfactory supply of sugars and other nutrients to the cells, while it facilitates the removal of gases and other by-products of catabolism from the microenvironment of the cells. Agitation also favours oxygen supply to the cells that is important for high biomass concentration (Farman et al., 2011).

CHAPTER 5

CONCLUSION & RECOMMENDATION

5.1 Conclusion

It can be concluded that bioethanol can be produced by fermentation process of cassava using *Saccharomyces Cerevisiae*. Productivity of ethanol is depended on the substrate concentration, temperature and agitation speed. From this experiment, it can be concluded that agitation speed 200 rpm, substrate concentration of 150g (37.5 % w/v) and temperature 40^{O}C will gives highest bioethanol production, highest cell growth and highest glucose consumption. At this temperature, agitation speed and substrate concentration, there are presents of adequate amount of reducing sugars. Follow that,

effective mixing of fermentation solution and dissolved oxygen at 200 rpm induces growth of yeast cells.

Temperature 40^{o}C helps in continuous hydrolysis of cassava starch into glucose which provides adequate substrate throughout the experiment and induces utilization by yeast which results in highest bioethanol production and highest cell growth. Besides that, substrate concentration at (32.5 %w/v) is containing large amount of cassava starch which provide maximum substrate supply for the yeast cells. Any changes for the optimum parameter will lead to reduction in bioethanol production and reduction in cell growth. Hence, for the optimum production bioethanol fermentation should be carried out at agitation speed 200 rpm, substrate concentration of 150g (37.5 % w/v) and temperature 40^{O}C.

If compare to the study which had done by (Letha & Raj., 2004) ethanol production from sugarcane bagasse without using enzyme pre-treatment, this study proved that bioethanol can be produce from cassava tubers by the same method. Even though the selected raw material is differ from that study, but still the end product is the bioethanol.

5.2 Recommendation

As for the recommendation there are few criteria need to take consideration. First the source of raw material (cassava tubers) should be the same all the time. It is because moisture content and starch content inside the cassava tubers differs according to the

sources. Hence when the source of cassava tubers differs each time of the experiment, in will affect the amount glucose present for bioethanol production. Secondly, the maturity of the cassava tubers must take in consideration. Total starch content in cassava tubers differs according to its maturity .For example, the percentage of moisture content and starch content reduces as increases its maturity. Hence, during the whole experiment it is important to use same maturity of cassava tubers to get an accurate result. Follows that, during cooking of the cassava in shaking water bath, it is important to make sure that there is no split of any other chemical from surroundings. Because while sharing the shaking water bath with others there is possibility chemicals from surroundings touches the cassava tubers. Any effective chemicals that contaminate cassava tubers will results in failure of the experiment.

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APPENDICES

[1] Appendix A

Table A Temperature RI Value versus Time (Hours)

Time	Initial Concentration	20 ˚ C	30˚C	40˚C	50˚ C
0	4.26667	0	0	0	0
2	4.26667	1.066663	1.33333	2.53333	1.73333
4	4.26667	1.73333	1.73333	3.466663	2.666663
6	4.26667	1.73333	2.53333	3.866663	3.466663
8	4.26667	1.73333	2.799997	4.799997	3.866663
10	4.26667	1.73333	2.799997	6.93333	4.13333
12	4.26667	1.73333	3.066663	8.13333	7.466663
18	4.26667	1.73333	3.73333	8.53333	7.999997
24	4.26667	1.73333	3.73333	9.199997	8.93333
30	4.26667	1.73333	3.866663	9.866663	9.73333
36	4.26667	1.73333	3.999997	10.8	10.13333
42	4.26667	1.73333	4.266663	12.13333	10.8
48	4.26667	1.73333	4.399997	12.8	10.93333
54	4.26667	1.73333	4.666663	13.2	11.33333
60	4.26667	1.73333	4.666663	13.6	11.33333
66	4.26667	1.73333	4.666663	13.6	11.46666
72	4.26667	1.73333	4.666663	13.6	11.46666
Standard Deviation		0.440884	1.348976	4.401737	3.964153

[2] Appendix B

Table B Agitation Speed RI Value versus Time (Hours)

Time	Initial Concentration	0 RPM	150 RPM	200RPM	250 RPM
0	4.26667	0	0	0	0
2	4.26666	6.67E-06	0.53334	1.066673	0.800007
4	4.26667	0.399997	1.599997	1.73333	0.93333
6	4.26667	0.53333	1.999997	2.53333	1.066663
8	4.26667	0.93333	2.13333	2.799997	1.199997
10	4.26667	1.199997	2.666663	2.799997	1.466663
12	4.26667	1.466663	2.666663	3.066663	1.599997
18	4.26667	2.13333	3.066663	3.73333	2.399997
24	4.26667	2.53333	3.33333	3.73333	2.799997
30	4.26667	2.799997	3.466663	3.866663	3.066663
36	4.26667	2.799997	3.599997	3.999997	3.066663
42	4.26667	3.066663	3.866663	4.266663	3.33333
48	4.26667	3.066663	4.266663	4.666663	3.599997
54	4.26667	3.199997	4.266663	4.666663	3.999997
60	4.26667	3.199997	4.399997	4.666663	3.999997
66	4.26667	3.199997	4.399997	4.666663	3.999997
72	4.26667	3.199997	4.399997	4.666663	4.13333
Mean		1.98	2.98	3.34	2.44
Standard Deviation		1.23	1.35	1.38	1.35

[3] Appendix C

Table C Substrate Concentration

RI Value versus Time (Hours)

25 g	50g	75g	100g	150 g
0	0	0	0	0
0.266667	0.399997	0.4	1.110633	1.2
0.387877	0.93333	0.933333	1.7773	1.466667
0.509087	1.33333	1.6	2.5773	2.4
0.630297	1.73333	1.733333	2.843967	2.933334
0.751507	1.866663	1.866667	2.9773	3.066667
0.872717	2.13333	1.866667	3.110633	3.2
0.993927	2.266663	2.133333	3.243967	3.466667
1.115137	2.93333	2.266667	3.243967	3.6
1.236347	3.199997	2.933333	3.643967	4
1.357557	3.466663	3.066667	4.043967	4
1.478767	3.466663	3.2	4.1773	4.4
1.599977	3.599997	3.333333	4.310633	4.8
1.721187	3.599997	3.466667	4.443967	5.066667
1.842397	3.73333	3.466667	4.5773	5.6
1.963607	3.866663	3.866667	4.710633	5.6
2.084817	3.866663	4	4.710633	5.6
0.62731	1.271713	1.195143	1.32854	1.622715

[4] Appendix D

Table D Temperature OD of Glucose Value versus Time (Hours)

TIME	20 ˚ C	30˚C	40˚C	50˚ C
0	0.33942	0.33942	0.33942	0.33942
2	0.33942	0.409873	0.512934	0.401134
4	0.341209	0.415444	0.602374	0.46553
6	0.342998	0.42886	0.579119	0.544238
8	0.34747	0.408289	0.568386	0.669454
10	0.34747	0.393978	0.547815	0.835812
12	0.346575	0.389506	0.547815	0.783579
18	0.342103	0.357308	0.476263	0.76426
24	0.348364	0.333159	0.468214	0.726695
30	0.349258	0.333159	0.455692	0.80093
36	0.337631	0.320638	0.426177	0.737428
42	0.340314	0.312588	0.409183	0.72759
48	0.349258	0.311694	0.386823	0.683764
54	0.341209	0.311694	0.385929	0.68913
60	0.351047	0.277706	0.367146	0.658721
66	0.338526	0.277706	0.375196	0.653354
72	0.336737	0.275918	0.376985	0.651566
Standard Deviation	0.00467	0.05165	0.085364	0.140111

[5] Appendix E

Table E Agitation Speed Glucose OD versus Time (Hours)

TIME	0 RPM	150 RPM	200RPM	250 RPM
0	0.33942	0.33942	0.33942	0.33942
2	0.341209	0.376985	0.38414	0.377879
4	0.343892	0.380562	0.386823	0.378774
6	0.334054	0.383246	0.390401	0.382351
8	0.32511	0.368935	0.380562	0.375196
10	0.321532	0.363569	0.375196	0.370724
12	0.31706	0.368935	0.371618	0.368041
18	0.310799	0.367146	0.363569	0.366252
24	0.303644	0.357308	0.365358	0.364463
30	0.300066	0.354625	0.359991	0.36178
36	0.299172	0.349258	0.356414	0.358202
42	0.293806	0.355519	0.352836	0.33942
48	0.292017	0.351942	0.336737	0.33942
54	0.291122	0.343892	0.329582	0.334948
60	0.285756	0.318849	0.329582	0.334054
66	0.283967	0.31706	0.33942	0.334054
72	0.284862	0.317954	0.338526	0.334054
Standard Deviation	0.021027	0.020997	0.020612	0.018281

[6] Appendix F

Table F Substrate Concentration

OD Glucose versus Time (Hours)

TIME	25g	50g	75 g	100 g	150g
0	0.088888	0.222154	0.39209	0.33932	0.392984
2	0.089782	0.223942	0.395667	0.38404	0.396562
4	0.097832	0.30265	0.421605	0.390301	0.405506
6	0.100515	0.385829	0.433232	0.401034	0.408189
8	0.164912	0.320538	0.404611	0.431443	0.439493
10	0.154179	0.300861	0.391195	0.477058	0.498523
12	0.138974	0.290128	0.380462	0.468114	0.49584
18	0.121086	0.274029	0.372413	0.455592	0.469008
24	0.132714	0.297283	0.372413	0.455592	0.463642
30	0.120192	0.282078	0.344686	0.445754	0.469008
36	0.116614	0.232886	0.340214	0.434126	0.463642
42	0.097832	0.220365	0.33932	0.4064	0.452909
48	0.104093	0.203371	0.326798	0.394773	0.448437
54	0.104093	0.200688	0.324115	0.403717	0.438598
60	0.10141	0.202477	0.321432	0.397456	0.425182
66	0.103198	0.201582	0.319643	0.377779	0.424288
72	0.104093	0.200688	0.324115	0.359891	0.425182
Standard Deviation	0.022032	0.054641	0.037569	0.038986	0.032102

[7] Appendix G

Table G Temperature Yeast OD versus Time (Hours)

TIME	20 ˚ C	40˚C	30˚C	50˚ C
0	1.235	1.235	1.235	1.235
2	1.265	1.275	1.27	1.2
4	1.275	1.28	1.3	1.22
6	1.275	1.32	1.34	1.28
8	1.275	1.375	1.36	1.315
10	1.265	1.38	1.405	1.26
12	1.265	1.6	1.505	1.38
18	1.27	1.63	1.63	1.595
24	1.265	1.74	1.655	1.655
30	1.265	1.795	1.775	1.71
36	1.265	1.88	1.87	1.74
42	1.26	1.915	1.885	1.78
48	1.27	2.04	1.975	1.825
54	1.275	2.075	2.025	1.875
60	1.255	2.045	1.99	1.87
66	1.265	2.025	2	1.825
72	1.275	2.01	1.98	1.82
Standard Deviation	0.00988	0.316444	0.297067	0.265557

[8] Appendix H

Table H Agitation Speed Yeast OD versus Time (Hours)

TIME	0RPM	150 RPM	200RPM	250 RPM
0	1.235	1.235	1.235	1.235
2	1.23	1.28	1.265	1.225
4	1.26	1.285	1.275	1.275
6	1.34	1.295	1.345	1.34
8	1.375	1.36	1.495	1.385
10	1.4	1.455	1.53	1.39
12	1.445	1.52	1.56	1.465
18	1.49	1.64	1.69	1.52
24	1.575	1.6665	1.79	1.665
30	1.61	1.715	1.96	1.64
36	1.705	1.84	2.025	1.75
42	1.695	1.88	2.1	1.74
48	1.685	1.925	2	1.715
54	1.7	1.92	2.015	1.72
60	1.67	1.9	1.995	1.695
66	1.66	1.885	1.975	1.685
72	1.64	1.88	1.94	1.685
Standard Deviation	0.176504	0.263647	0.311087	0.19263

[9] Appendix I

Table I Substrate Concentration Yeast OD versus Time (Hours)

Time	25g	50g	75 g	100 g	150 g
0	1.055	1.055	1.055	1.055	1.055
2	1.07	1.165	1.225	1.22	1.215
4	1.115	1.17	1.265	1.305	1.275
6	1.15	1.22	1.295	1.385	1.39
8	1.18	1.225	1.38	1.395	1.405
10	1.215	1.3	1.4	1.42	1.435
12	1.255	1.355	1.485	1.51	1.555
18	1.365	1.455	1.595	1.63	1.66
24	1.375	1.51	1.68	1.695	1.705
30	1.39	1.51	1.72	1.74	1.765
36	1.42	1.555	1.765	1.78	1.785
42	1.425	1.615	1.815	1.83	1.84
48	1.42	1.64	1.875	1.885	1.905
54	1.385	1.675	1.91	2.01	2.05
60	1.365	1.68	1.86	2.08	2.11
66	1.365	1.67	1.855	2.035	2.06
72	1.365	1.665	1.84	1.99	2.035
Standard Deviation	0.130758	0.213368	0.273729	0.312115	0.324821

[10] Appendix J

Table J Standard Curve of Ethanol (g/g)

Weight (g/g)	RI	Y bar	(Y-Y BAR)
0	1.3327	1.353364	0.000427
10	1.3402	1.353364	0.000173
20	1.3427	1.353364	0.000114
30	1.3494	1.353364	1.57E-05
40	1.3523	1.353364	1.13E-06
50	1.3598	1.353364	4.14E-05
60	1.3623	1.353364	7.99E-05
70	1.3624	1.353364	8.16E-05
80	1.3625	1.353364	8.35E-05
90	1.3626	1.353364	8.53E-05
100	1.3601	1.353364	4.54E-05
	14.887	1.353364	0.001148
Mean	1.353363636		0.010714
Standard Deviation	0.010714128		Deviation %
			0.032732
			3.273244

[11] Appendix K

Table K Optical Density and Cell Dry Weight (g)

OD				WEIGTH(g)	y bar	(y-y_)^2
		mg/ml	mg/ml			
0	10000	0	0	0	0.005229	2.73424E-05
0.058	10000	0.000129	1.285714	0.0009	0.005229	2.60144E-05
0.571	10000	0.000543	5.428571	0.0038	0.005229	2.19599E-05
1.327	10000	0.000929	9.285714	0.0065	0.005229	1.84937E-05
1.566	10000	0.001043	10.42857	0.0073	0.005229	1.75238E-05
2.066	10000	0.001214	12.14286	0.0085	0.005229	1.61179E-05
2.432	10000	0.001371	13.71429	0.0096	0.005229	1.48809E-05
SUM		0.005229		0.0366		0.000114991
MEAN				0.0061	0	
					Standard Deviation	
					0.004377796	0.003737965
					% deviate	28.23285694
						0.850435558

[12] Appendix L

Table L Standard Calibration Curve of Glucose

Stock Solution 1000microgram					
Glucose (ml)	**Distilled Water (ml)**	**Glucose (mg/m L)**	**OD**	**y_**	**(y-y_)^2**
0	1	0	0		
0.1	0.9	0.1	0.15	0.563	0.170569
0.2	0.8	0.2	0.297	0.563	0.070756
0.3	0.7	0.3	0.311	0.563	0.063504
0.4	0.6	0.4	0.423	0.563	0.0196
0.5	0.5	0.5	0.535	0.563	0.000784
0.6	0.4	0.6	0.592	0.563	0.000841
0.7	0.3	0.7	0.683	0.563	0.0144
0.8	0.2	0.8	0.767	0.563	0.041616
0.9	0.1	0.9	0.889	0.563	0.106276
1	0	1	0.983	0.563	0.1764
		Mean	0.563		0.664746
		Standard Deviation	0.271773		
				Standard Deviation	0.271773
					0.30553

[13] Appendix M

Figure A GANTT CHART PSM I

PROJECT TITLE (PSM I) : PRODUCTION OF BIOETHANOL FROM CASSAVA BY FERMENTATION PROCESS																
Project Task	Mon-yr-week	Feb 2012				March 2012				April 2012				May 2012		
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Identify project (problem to investigate & scope of the study)																
Plan work schedule																
Review related literature																
Determine methodology																
Write proposal and abstract (summary of proposal)																
Present and defend proposal in oral presentation																
Submit written research proposal & abstract																

Figure B GANTT CHART PSM II

PROJECT TITLE (PSM II) : PRODUCTION OF BIOETHANOL FROM CASSAVA BY FERMENTATION PROCESS																									
PROJECT TASK	MON-YR-WEEK	SEPT 2012				OCT 2012				OCT 2012				NOV 2012				DEC 2012				JAN 2013			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Collect and analyse data																									
Interpret result																									
Evaluate result																									
Draw conclusion and suggest recommendation																									
Revise and edit of introduction, literature review and methodology (from proposal)																									
Write first draft of result and discussion, and recommendation																									
Revise and edit abstract (from proposal)																									
Compile entire final report and revise abstract																									
Present in oral presentation																									
Submit written final report and abstract																									

[14] Appendix N

PICTURES

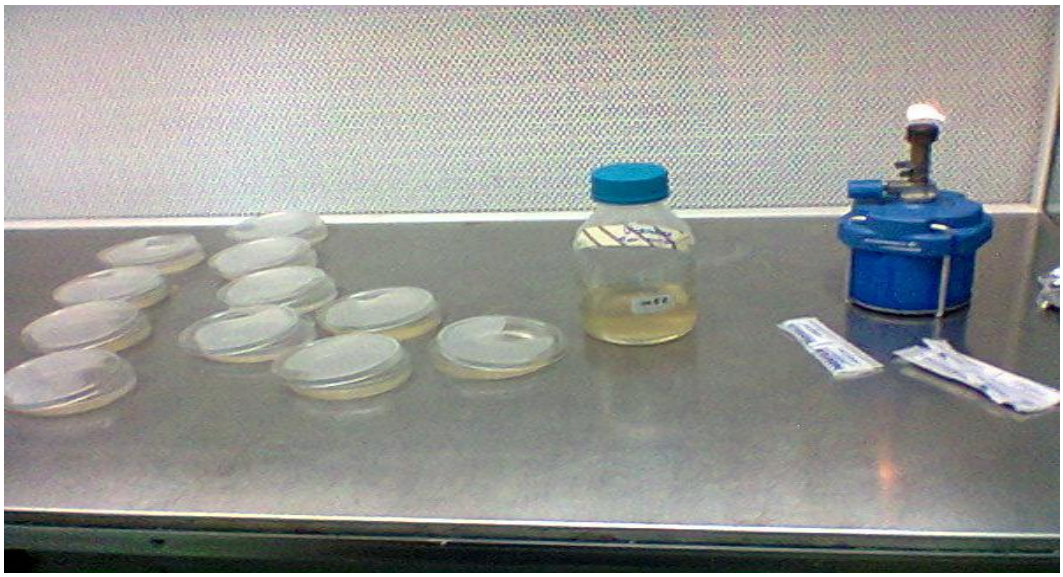

Figure C Preparation of Agar Slant

Figure D Prepared Nutrient Agar after Sterilization

Figure E Preparation of Acetate Buffer Solution

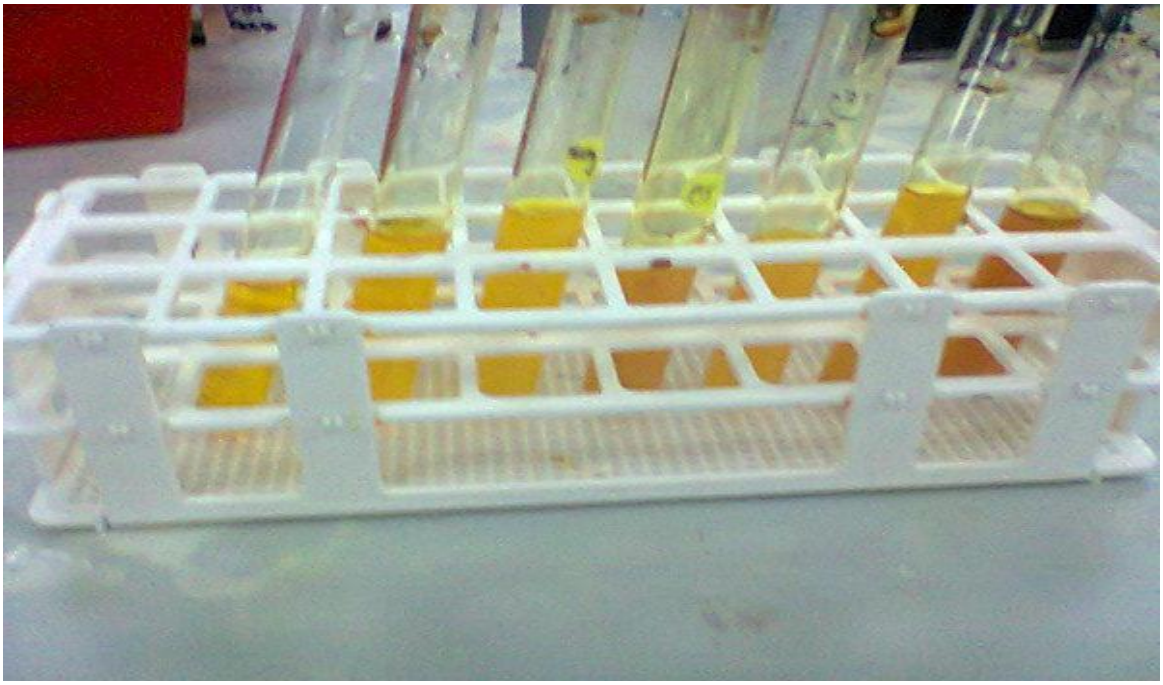

Figure F Sample analysed Using DNS Method

Figure G Cassava Slurry after Sterilization

Figure H Filtration of Yeast Cells Using Vacuum Pump