The Effectivity of Dry Yeast, *Saccharomyces cerevisiae,* in Ethanol Production

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Researchers:
Rhona Mae B. Mendoza
Dennis L. Dumaplin
John Alain Leal
IV-GRAVITON

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ABSTRACT

Active dry yeast, *Saccharomyces cerevisiae*, was used for the alcoholic fermentation of 11% pure D-Glucose solution in an incubator at 35^0C – 42^0C for 7 days. Carbon dioxide production was observable during the process by the bubbles formed.

Determination of ethanol by the distillation method was done on four determinations. The alcohol concentrations of the fermented liquids were assessed to have a mean of 13.1±1.6% (v/v) and a specific gravity of 0.97965±0.0042.

Determination for the presence of ethanol was done on each distillate of four determinations.

APPROVAL SHEET

This research paper entitled "The Effectivity of Dry Yeast, *Saccharomyces cerevisiae*, in Ethanol Production" submitted by Rhona Mae B. Mendoza, Dennis L. Dumaplin, and John Alain Leal, in partial fulfillment of the requirements in Science Research 2, has been examined and is recommended for acceptance and approval.

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Date **Prof. Josette T. Biyo,**
Science Research 2 Consultant

This research paper is accepted and approved in partial fulfillment of the requirements in Science Research 2.

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Date **Prof. Rebecca Yandog,**
School Director, PSHS-WVC

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CHAPTER 1
INTRODUCTION

A. BACKGROUND AND RATIONALE

The use of microorganisms in making bread, cheese, and wine is as old as civilization itself. Long before any microorganisms were identified, milk was being made into cheese and fermented beverages, and bread was being leavened by microbes. In modern food production, specific organisms are purposely used to make a variety of foods.

People first started putting microbes to work long before microorganisms were first observed. Processes discovered by accident thousands of years ago are still used today to produce bread, beer, wine, cheeses, yogurt, and many oriental foods. Sophisticated techniques are providing new and exciting ways of tapping the technological potential of microorganisms (Mckane, 1996).

Some of the more advanced technologies used today would have astonished many early microbiologists. So far, however, the applied fields of microbiology are still dominated by traditional applications of microbial activities --- industrial fermentation and production of alcoholic beverages, foods, antibiotics and vaccines. One of the goals of the new biotechnology is to improve the efficiency of the microorganisms performing these established processes or to develop new strains of organisms that can perform desirable tasks not accomplished by their naturally occurring microbial counterparts. Another objective is to develop strains of

organisms that will readily provide resources that either are unavailable or are difficult to obtain from conventional sources (Mckane, 1996).

Nowadays, there is a great interest in the genetic improvement of yeast and in the production of thermotolerant and high alcohol-yielding yeast by chemical mutagenis and hybridization. Yeast has been used in leavening breads and pastries, and in the production of alcoholic beverages such as wine and beer. The only limiting activities of the yeast are that it could be destroyed easily at a high temperature, and that it ceases to function once the alcohol concentrations has reached to 15% or more. At this level, the alcohol could be very toxic to the yeast and thus disrupts the yeast's ability to produce more alcohol and carbon dioxide. This is the reason why most wines must have to undergo distillations to make the alcohol concentrations higher.

Previous researches had given full attention in the genetic manipulation of yeast for improved alcoholic fermentation. One of the goals of biotechnology is to develop new strains of yeast that are thermotolerant and that could still work at higher alcohol concentrations. Before all of these, it is necessary first to know the performance of yeast in its natural state. So this study focuses on the metabolic activity of dry yeast in the production of ethyl alcohol (ethanol) from D-Glucose, determining the ethanol, produced from a particular sugar concentration after a particular fermentation period.

B. STATEMENT OF THE PROBLEM

This study is basically designed to answer this question: Is the dry yeast effective enough to produce more than 15% (v/v), (m/v), and (w/w) ethanol?

C. OBJECTIVES OF THE STUDY

This study was conducted to assess the metabolic activity of dry yeast in the production of ethyl alcohol as a possible basis or as a conventional source for further researches concerning on the improvement of the yeast's performance. Specifically, the study aimed to determine:

a.) The alcohol concentrations of the fermented liquid, either by weight or by volume.

b.) The Specific gravities of the alcohol solutions by calculation and by comparing it to appropriate tables.

D. HYPOTHESIS OF THE STUDY

Based on the objectives, the alcohol concentration of a solution fermented from a particular sugar concentration would not exceed 15% (v/v), (m/v) and (w/w), as a fact.

E. SIGNIFICANCE OF THE STUDY

This assessment of the metabolic activity of the dry yeast could be one step for further researches dealing on the improvement of yeast in certain microbial activities like industrial fermentation and production of alcoholic beverages and

foods. It could be a conventional source for the manipulation of microorganisms to increase their practical benefit in microbial food production.

The quality of wines based on their proof spirits could be credited on the yeast's performance during the fermentation. Thus, this descriptive study is necessary to determine how concentrated ethanol is would be produced from a particular sugar (D-Glucose) concentration using dry yeast instead.

F. SCOPE AND LIMITATIONS

The study is limited only to the determination of alcohol concentrations by weight, by volume or by specific gravity. It is also limited only to the confines of the Science Research laboratory, with the apparatuses used ranging from cork stoppers to water-baths, ovens and autoclaves.

The study includes only the use of one organism: the active dry yeast (*Saccharomyces cerevisiae*), which is commonly used in home baking. The study was wholly conducted in the laboratory in PSHS-WVC within a four week period, with the distillation of the fermented liquids constituting about 70% of the time.

F. DEFINITION OF TERMS

Distillation – the chemical procedure of evaporating a liquid from one container and recondensing it into another container (Barnes and Nobles Encyclopedia, 1993).

Ethyl alcohol – also known as ethanol or grain alcohol, is a colorless liquid. It boils at 78.5^0C and has a pleasant odor and burning sensations. It is miscible with water in all proportions and also soluble in other polar solvents. It is prepared from ethylene by catalytic hydration and by enzymatic fermentation of carbohydrates (Solomon et al., 1988).

Fermentation – a chemical reaction in which an organic compound is broken through the action of an enzyme. This process is typically carried out using bacteria or yeast to metabolize carbohydrates in the absence of oxygen. The two most common end products of fermentation are ethanol and lactic acid, but the exact end product depends on the type of bacteria and the nature of the material used (Barnes and Nobles Encyclopedia, 1993).

Glucose – also called dextrose; by far the most common of the six carbon sugars. It is the primary product of plant photosynthesis. Starch and cellulose are both condensation polymers of glucose. Maltose, lactose, and sucrose contain at least one glucose residue, and glucose may be obtained from them by acid or enzymatic hydrolysis (Barnes and Nobles Encyclopedia, 1993).

Proof Spirit – is the measurement of the amount of ethanol (the active constituent) in alcoholic drinks, commonly expressed using either volumetric measures or proof strength. Proof spirit is a mixture containing a standard amount of ethanol (Barnes and Nobles Encyclopedia, 1993).

Specific Gravity – density measured to some standard, typically water at 20^0C; symbol d, expressed as a pure number (Barnes and Nobles Encyclopedia, 1993).

Yeast - a fungus that can occur as single cells; typically reproduces by budding or by fission; used in fermentation processes in the brewing and baking industries (Barnes and Nobles Encyclopedia, 1993).

CHAPTER 2
REVIEW OF RELATED LITERATURE

A. ETHANOL

Ethanol (ethyl alcohol or grain alcohol), C_2H_5OH, is a lipid, colorless liquid, with a burning taste and characteristic, agreeable odor. Because of its low freezing point, it has been used as a fluid in thermometers for temperatures below -40^0C (-40^0F), the freezing point of mercury, and for other special low temperature purposes, such as for antifreeze in automobile radiators (Funk and Wagnall's New Encyclopedia, 1986).

Ethanol is normally concentrated by distillation of dilute solutions, but the concentrations cannot proceed beyond 97.2% by volume. Commercial ethanol contains 95% by volume of ethanol and 5% of water and produce absolute ethanol. Ethanol melts at -114^0C (-173.4^0), boils at 78.5^0C (173.3^0F), and has a sp. gr. of 0.789 at 20^0C (Funk and Wagnall's New Encyclopedia, 1986).

A.1 ETHANOL PRODUCTION

Ethanol has been made since ancient times by fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Starch from potatoes, corn, or other cereals can be the raw material. The yeast enzyme, zymase, changes the simple sugars into ethanol and carbon dioxide. The fermentation reaction, represented by the simple equation

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

is actually very complex because impure cultures of yeast produce varying amounts of other substances, including fusel oil, glycerin, and various organic acids. The fermented liquid, containing from 7 to 12% ethanol, is concentrated to 95% by a series of distillations. In the production of beverages such as whiskey and brandy, some of the impurities, which supply the flavor, are of great value. Much ethanol not intended for drinking is now made synthetically, either from acetaldehyde made from acetylene, or ethylene made from petroleum. A small amount is made from wood pulp (Funk and Wagnall's New Encyclopedia, 1986).

Ethanol can be oxidized to form acetaldehyde and acetic acid. It can be dehydrated to form others. Butadiene, used in making synthetic rubber, maybe made from ethanol, as chloroform and many other organic chemicals. Ethanol is miscible (mixable) in all proportions with water and with most organic solvents. It is an excellent solvent for many substances and is used in making such products as perfumes. Lacquer, celluloid, and explosives (Funk and Wagnall's New Encyclopedia, 1986).

A.2 SIGNIFICANCE

Alcohol, the end product of fermentation by yeast cells can be burned and can even be used as an automobile fuel; obviously, it contains a great deal of energy that the yeast cells are unable to extract using anaerobic methods (Solomon et al., 1993).

The present government plan to use alcohol as fuel additive has changed research directions toward improving fermentation efficiency. There is considerable interest in the genetic improvement of yeast for alcohol fermentation (Halos et al., 1987).

A.3 DETERMINATION OF ETHANOL

Ethanol is determined by distilling a measured volume of sample and, after making up the distillate to the same volume, the alcohol content is assessed from its gravity by reference to appropriate tables. For high levels of alcohol, dilution of the original sample may be necessary and use made of modified tables.

Proof spirit (100%) has a specific gravity of 0.91702 at 20^0C and contains 49.276% ethanol by weight and 57.155% ethanol by volume.

The test is not specific for ethanol and the presence of other water-soluble, volatile compounds such as methanol may cause erroneous results unless a check is made of the refractive index of the distillate against its specific gravity (James, 1995).

B. FERMENTATION

Fermentation is a chemical change in organic substances produced by the action of enzymes. This general definition includes virtually all chemical reactions of physiological importance, and scientists today often restrict the term to the specific

colorless, one-celled, and are mostly round or oval-shaped. They are usually solitary, but sometimes, small clusters are formed when growth is rapid, and daughter cells begin to bud before separating from parent. Wild yeast is found abundantly in the air; they are able to survive and retain their vitality without warmth, moisture, or food, by passing into a resting stage. They are destroyed, however, by being exposed to moist heat at temperature of 212^0F. Because of their powers of fermentation, yeast was used to raise bread, to make bread, to make wine, and to brow beer for thousands of years before their true nature was understood. It is now known that moist foods containing small amounts of sugar are exposed to the air at a suitable temperature, yeast multiply rapidly and secrete a ferment, or enzyme, which changes the sugar into carbon dioxide and alcohol (Collier's Encyclopedia. 1995).

There are various kinds of yeast that are used commercially; brewer's yeast, which is the top-yeast taken from vats in which beer and ale have fermented; compressed yeast, which is cultivated by sowing selected varieties of wild yeast in a warm mash of dextrinized starch. The yeast cells rise to the top of the mash, where they are skimmed off, washed, and purified, then pressed to removed part of the water, and finally cut into cakes. Dried yeast, commonly used in home baking, is compressed yeast dehydrated at low temperatures (Collier's Encyclopedia, 1995).

Genetic manipulation of yeast for improved alcohol production could be undertaken using several techniques: induced mutation by ultraviolet irradiation,

protoplast fusion technique, and spontaneous mutation by adaptation to higher sugar and alcohol concentration and high temperature (Joson et al., 1988).

C. YEAST

The importance of the economically useful yeast can be attributed to two main factors: (1) fermentation – the transformation of simple sugars and other organic chemicals to other, more desirable chemicals; and (2) respiratory (oxidative) metabolism – the great capacity of some yeast for a protein synthesis during growth in richly aerated media containing a wide variety of carbonaceous and nitrogenous nutrients. Thus, yeast serve in many ways: (1) As living cells, they are bio-catalysts in the production of bread, wine beer, distilled beverages, among other important food products. (2) As dried, non-fermentive whole cells or hydrolyzed cell matter, yeast contribute nutrition and flavor to human diets and animal rations. (3) As producers of vitamins and other bio-chemicals, yeast are a rich source of enzymes, coenzymes, nucleic acid, nucleotides, sterols, and metabolic intermediates. (4) As a versatile bio-chemical tool, yeast aid research students in nutrition, enzymology, and molecular biology (Scientific Encyclopedia, 1995).

C.1 PROPERTIES OF YEAST

Gross chemical composition of compressed baker's yeast is approximately 70% moisture. The dry matter is made up of 55% protein (N x 6.25), 6% ash, 1.5% fat, and the remainder mostly polysaccharides, including about 15% glycogen and 8% trehalose (Scientific Encyclopedia, 1995).

Food-yeast, molasses-grown, is dried to about 5% moisture and has the same chemical composition as baker's yeast. In terms of micrograms per gram of yeast, the vitamin content is: 165 thiamine; 100 riboflavin; 590 niacin; 20 pyridoxine; 13 folacin; 100 pantothenic acid; 0.6 biotin; 160 para-aminobenzoic acid; 2170 choline; and 3000 inositol. Yeast crude protein contains 80% amino acids; 12% nucleic acids; and 8% ammonia. The latter components lower the true protein content to 40% of the dry cell weight (Scientific Encyclopedia. 1995).

Yeast protein is easily digested (87%) and provides amino acids essential to human nutrition. Most commercial yeast shows the following pattern of amino acids, among others, as percent of protein: 8.2% lysine; 5.5% valine; 7.9% leucine; 2.5% methionine; 4.5 % phenylalanine; 1.2% tryptophan; 1.6% cystine; 4% histidine; 5% tyrosine; and 5% arganine. The usual therapeutic dose of dried yeast is 40 grams per day, which supplies significant daily needs of thiamine, riboflavin, niacin, pyridoxine, and general protein (Scientific Encyclopedia, 1995).

The ash content of food yeast ranges from 6 to 8% (dry basis), consisting mainly of calcium, phosphorus, and potassium. Contained in quantities of less than 15 are magnesium, sulfur, and sodium. At the microgram level are included iron, copper, lead, manganese, and iodine (Scientific Encyclopedia, 1995).

Triglycerides, lecithin, and ergosterol are the main constituents of yeast lipid (fat). Oleic and palmitic acids predominate in yeast fat. Ergosterol, the precursor of calciferol (vitamin D_2) varies from 1 to 3% of yeast dry matter (Scientific Encyclopedia. 1995).

C.2 METABOLIC ACTIVITY

This is generally associated with the familiar alcoholic fermentation in which theoritically 100 parts of glucose are converted to 51.1 parts of ethyl alcohol (ethanol), 48.9 parts carbon dioxide (CO_2), and heat. In addition, however, the anaerobic reaction also yields minor byproducts in small amounts – mainly glycerol, succinic acid, higher alcohols (fusel oil), acetic acid, acetaldehyde, and lactic acid. Fusel oil is a mixture of alcohols, including *n* – propyl, *n* – butyl, isobutyl, amyl, and isoamyl alcohols (Scientific Encyclopedia, 1995).

Respiratory activity of oxidative dissimilation is characteristic of many species of yeast. During aerobic growth, sugar is oxidized to carbon dioxide and water, with release of large amounts of energy (about 680 kcal when complete oxidation occurs). Aerobiosis produces a variety of byproducts, some in unusually high concentration, such as acetic acid, succinic acid, zymonic acid, polyhydric alcohols (glycerol, erythritol, etc), extracellular lipids, carotenoid pigments in shades of red and yellow, black pigment (melanin), and capsular polysaccharides (Phosphomannan) (Scientific Encyclopedia, 1995).

D. MICROBES AND FOOD PRODUCTION

Microorganisms continue to be used in the food industry primarily in three ways.

1.) Specific metabolic activities, usually fermentation reactions, generate organic compounds that accumulate and transform some marginally edible substances into foods with more desirable characteristics. These altered properties usually help preserve foods, and they often enhance their flavor, texture, or digestibility. Dairy products, breads, soy sauce, pickled vegetables, alcoholic beverages, and vinegar are just few of the edibles produced by microbial fermentation.

2.) Microbial cells, cultured in large quantities, and used as protein supplements in feed for livestock. Such microbe-generated proteins have also been suggested as alternative food resources for humans.

3.) Certain microbes produce metabolic byproducts that have nutritional or flavor-enhancing properties when added to foods and feeds. Enzymes isolated from microorganisms are also instrumental in food production (Mckane, 1996).

D.1 NONDAIRY FOODS

Vegetables, particularly cucumbers, cabbages, and olives, can be preserved by the fermenting activities of the lactic acid bacteria and yeast that naturally reside on their surfaces. The growth of these microbes is selectively encouraged by placing the vegetables in salt solutions called brines. Lactic acid production usually continues until no fermentable carbohydrates remain. The salt, low pH, and absence

of carbohydrates so effectively prevent the growth of spoilage organisms that pickles, sauerkraut, and olives can usually be kept indefinitely (Mckane, 1996).

So sauce originated in China centuries ago. Its production requires two microbial transformations. Cooked soybeans and crushed wheat are first incubated with the mold *Aspergillus oryzae*, which converts much of the starch to fermentable sugars. The mash is then fermented for 6 to 12 months with a mixture of lactic acid bacteria and yeast. The remaining liquid is soy sauce; the remaining solids are used as livestock feed (Mckane, 1996).

Bread production by fermentation is another ancient process. Leavened breads are made by adding baker's yeast, *Saccharomyces cerevisiae*, to dough. The yeast metabolizes sugar in the dough aerobically. The oxygen minimizes alcohol production and maximizes carbon dioxide. Bubbles of carbon dioxide gas become trapped in the dough, which rises – literally inflates – and acquire a light texture. The small amount of alcohol that is produced evaporates during baking, so you can eat bread without fear of inebriation (Mckane, 1996).

Sourdough and various other breads acquire there characteristic flavors from additional microbial processes. In sourdough production, two additional organisms produce the acid byproducts responsible for the bread's tanginess. One organism, *Saccharomyces exiguus*, leavens the dough, while the bacterium *Lactobacterium sanfrancisco* generates the lactic acid and acetic acids that impart that elusive taste

once believed achievable only if the bread were baked in San Francisco (Mckane, 1996).

D.2 ALCOHOLIC BEVERAGES

The term *fermentation*, from the Latin word for boiling, was originally applied to the production of alcoholic beverages because the generation of carbon dioxide gas gives the fermenting liquid a frothy appearance. Most alcoholic beverages are manufactured by the metabolic activity of yeasts in the genus *Saccharomyces*. Wines are prepared by the direct action of *S. cerevisiae* or *S ellipsoideus* on sugars in fruit juices. Beers, on the other hand, are made from the starch in barley or other grain extracts. Starch is a nonfermentable substrate, so beer production requires that the starch be hydrolyzed to fermentable sugars, a process that begins with malting. During malting the barley germinates and produces starch-digesting enzymes. In next step called mashing, the crushed malted grain is mixed with water, at which time the enzymes digest starch to form the fermentable sugars glucose and maltose. These sugars are dissolved in the aqueous extract of the malted barley, called wort, which is then boiled with hops, a flower with bacteriostatic properties that imparts a characteristic bitter flavor to the brew while preventing unwanted bacterial growth. The boiling also denatures the starch-digesting enzymes, halting the enzymatic release of sugars. Brewers prepare mash in concentration that provide only enough sugar to produce between 3 to 6 percent ethanol (drinking alcohol). After boiling, the hops is removed and yeasts are added to ferment the sugars to alcohol and flavor-enhancing compounds. Because the undigested starch in

the wort cannot be fermented by brewer's yeasts, most beers have a high carbohydrate content, containing about 4 percent unfermented starch (each gram of which adds about 4 calories to the beverage). Yeasts that digest this starch have been mated with brewing yeasts to yield hybrid organisms that produce beers containing less than 15 percent undigested carbohydrate. These yeasts are used to manufacture some brands of low-calorie ('light') beer. Other light beers depend on predigestion of starch in the wort by adding enzymes instead of whole organisms (Mckane, 1996).

E. RELATED STUDIES

Bench and pilot-scale studies were conducted by del Rosario (1987) on continuous-flow ethanol fermentation and tubular fermenter containing carageenan-immobilized yeast, using as substrate sugarcane molasses, sugarcane juice or sweet sorghum juice.

Elegado et al. (1990) experimented on simultaneous saccharification and fermentation (SSF) of raw cassava starch powder by a mix culture of *Rhizopus sp.* and *S. cerevisiae* in a gas-circulating bio-reactor has an optimum substrate concentration of the product was 88.4 g/L after 3 days fermentation.

The effect of temperature on the purification of hydrous ethanol by preferential adsorption of water in an ethanol-water vapor mixture in starchy materials was also investigated to explore some possible savings in energy.

Pham et al. (1990) studied the dehydration process for absolute ethanol using cornstarch as packing and using interaction effects of process parameters such as temperature feed concentration. The starch material used was corn. It was dried in an oven at 80^0C for 24 hours. It was then ground using the Wiley mill then passed through a sieve with a screen size of 0.05 mm.

S. Yu and M. Maywan (1986) of the University of Toronto faculty recommended the use of *Candid shetatae,* a bacteria, in ethanol production by use of its superior hexose and pentose formation

The design and operation used for the system was previously described by Marasigan et al. (1990). Ethanol concentration was determined by gas chromatography using Shimadzu GC-&A. It was statistically analyzed by means of stepwise multiple regression.

CHAPTER 3
METHODOLOGY

A. CHEMICALS AND EQUIPMENT

A.1 Preparation of Glucose Solutions

200 ml pure D-Glucose
1 hot plate
stirring rod
spatula
600 ml beaker
4 filtering flasks
wire gauze
distilled water

A.2 Rehydration of Active Dry Yeast

600 ml beaker
28 g active dry yeast
distilled water
stirring rod
wire gauze
top-loading balance
1 hot plate

A.3 Alcoholic Fermentation

Incubator/water-bath
Rubber tubing
4 large cork stoppers
distilled water
100 ml beaker
4 filtering flasks

A.4 Filtration

4 funnels
4 iron rings
600 ml beaker
filter papers
1 iron stand

A.5 Distillation Set-up

Water-bath/Bunsen burner
Hot plate
1 conical flask
1 adapter
1 thermometer
3 iron clamps
3 small cork stoppers
wire gauze
1 distilling flask
1 condenser
rubber tubing
3 iron stands
aluminum foil
distilled water

A.6 Chemical Reaction Tests

1 test tube rack
10 test tubes

3 droppers
graduated cylinders
7 ml 95% v/v
Absolute ethanol
7 ml Hydrochloric acid
distilled water
7 ml Potassium
dichromate
7 ml Sulfuric acid

B. METHODS AND PROCEDURES

B.1 Sterilization of Glassware

All laboratory glassware, particularly the ones used for the alcoholic fermentation were subjected to 170^0C in the autoclave for one hour to avoid unwanted bacterial contamination during the processes. Before all glassware was put in the autoclave, they were first cleaned with detergent, air-dried, then wrapped with paper.

B.2 Preparation of Glucose Solutions

Fifty milliliter of pure D-Glucose was placed into four separate beakers, each representing a determination. Hot distilled water was poured into each beaker until the volume reach 400 ml to make 12% (v/v) D-Glucose solution. The solutions were continuously stirred until no visible traces of D-Glucose left. The solutions were transferred to each four previously sterilized filtering flasks.

B.3 Rehydration of Active Dry Yeast

Seven grams of active dry yeast were placed in a 50 ml pre-heated distilled water (about 42^0C) in a beaker and was allowed to rehydrate for 10 to 20 minutes. It was then stirred gently until all the yeasts are dissolved. This was done four times for replication.

B.4 Alcoholic Fermentation

Into each filtering flasks, which already contained 400 ml of 12% (v/v) pure D-Glucose, the rehydrated active dry yeast was poured. Each flask was occassionally shaken to mix well the glucose solution and the yeast solution. Each fermentation medium (flask) now contained 450 ml of 11% (v/v) pure D-Glucose solution. The flasks were stoppered properly to avoid entrance of oxygen during the process. Rubber tubing was connected from the sidearm of each flask to a reservoir of distilled water in a 1000 ml beaker. Then the four filtering flasks, including the beaker, were put inside an incubator set at a temperature range of $35^0C - 40^0C$ for 7 days of controlled temperature.

B.5 Filtration

After 7 days of alcoholic fermentation, the fermented liquid was filtered using a filter paper to separate the supernatant of free yeast cells from the liquid part. The filter funnel was put atop an iron ring attached to an iron stand.

B.6 Determination of Ethyl Alcohol by the Distillation Method

Simple distillation set-up (see the plates section) was used to separate the volatile ethanol from the original sample. Only 50 ml of the fermented liquid from each flasks was transferred to the distilling flask, each was allowed to be distilled for 2 days. The set-up, using Bunsen burner and was later replaced by a water-bath for temperature control, was switched on in the following settings:

Water-bath settings: $77^0C - 95^0C$
Solution temperature range: $74^0C - 90^0C$

Duration: continually 2 days

Water was allowed to enter the inlet rubber tube to cool off the condenser during the process and was allowed to go out through the outlet rubber tube. Specific gravities of each distillate obtained were determined according to the formula:

$$\textbf{Sp. gr.} = (X_2 - X_1) / (X_3 - X_1)$$

Where: X_1 = weight (g) of Specific gravity bottle

X_2 = weight (g) of Specific gravity bottle + sample

X_3 = weight (g) of Specific gravity bottle + water

Percent (v/v), (m/v), and (w/w) Ethanol were calculated by getting the volume and weight of the distillant and the volume and weight of the distillate obtained as well.

B.7 Determination for the Presence of Ethanol by Reaction Tests

About 3 ml of each distillate was pipetted to separate test tubes. Two samples sere taken for each distillate and into the first sample, about 5 drops of hydrochloric acid were added, and into the second sample, also 5 drops of Potassium dichromate and sulfuric acid were added. This was done four times since there were four determinations. The absolute ethanol was used as standard.

CHAPTER 4
RESULTS AND DISCUSSIONS

Preparation of the 12% (v/v) pure D-Glucose solution was the first step. Pure D-Glucose is very sticky and almost non-viscous. It was made to dissolve in solution by adding hot water until no visible trace of it is left. Rehydration of active dry yeast was done by sticking to the fact that yeast must be optimally rehydrated in water at a temperature not higher than 45^0C. A temperature higher than that would kill the yeast and it would not produce enzymes necessary for the conversion of glucose into ethyl alcohol and carbon dioxide. The inoculation of the yeast solution to the flasks made it to become 11% (v/v) pure D-Glucose solution in the fermentation media.

The four determinations were subjected to 7 days of alcoholic fermentation. As the process progresses, the production of carbon dioxide was evident by the bubbles forming at the end of each rubber tube, which was immersed in water. The fermentation media were incubated at a temperature range of 35^0C – 42^0C, which at this range, the enzymes could work in optimum performance.

Ethanol was determined by distilling a measured volume of the fermented liquid and the alcohol content was assessed from its gravity by reference to appropriate tables. Table 1.0 shows the alcohol concentrations (v/v and m/v) and the specific gravities of four determinations.

The determination of alcohol by the distillation method was not specific for ethanol and the presence of other water-soluble, volatile compounds such as methanol may cause erroneous results unless a check is made of the refractive index of the distillate against its specific gravity (James, 1995).

Table 1.0 Alcohol concentrations and specific gravities of four determinations.

Determination	**%v/v**	**%m/v**	**%w/w**	**Specific gravity**
D_1	12.0	9.50	10.87	0.9854
D_2	14.0	11.12	13.21	0.9823
D_3	15.4	11.94	14.16	0.9771
D_4	11.0	8.90	10.01	0.9737
Mean ± S.D.	**13.1 ± 1.6**	**10.37 ± 1.17**	**12.1 ± 1.62**	**0.9796 ± 0.0042**

The chemical reaction tests for ethanol were done to test if the distillate obtained after distillation is really absolute ethanol, since other organic byproducts in small amounts such as glycerol, lactic acid, acetic acid, and higher alcohols (fusel oil) could be produced by the yeast as well. Table 2.0 shows the qualitative observations of the chemical reactions of the distillate to hydrochloric acid, and potassium dichromate and sulfuric acid.

The distillation process took a very long time. Condensed vapors of ethanol took just about 1 drop for every two- minute interval. It requires a constant temperature water bath for the whole distillation period. Using a Bunsen burner

instead could waste time, since the temperature could barely be controlled at a range of $78^0C - 84^0C$, with the boiling point as 78^0C. Distillation temperatures must have to fall under these circumstances to minimize the condensation of water alone that could make the distillate less concentrated.

Table 2.0 Qualitative observations of the Chemical Reaction Tests.

Reactant	**$K_2Cr_2O_4 + H_2SO_4$**	**HCl**
Absolute ethanol	Green to light blue; precipitate formed	Colorless; slow reaction; no precipitate
Distillate 1	Light blue; no precipitate	Colorless; no precipitate
Distillate 2	Light green to light blue; no precipitate formed	Colorless; no precipitate
Distillate 3	Light blue; no observable precipitate	Colorless; no precipitate
Distillate 4	Light blue; hazy	Colorless; no precipitate

A similar experiment (trial) was conducted using *Musa compressa* bananas as the only substrate for the alcoholic fermentation. The proportion used for the concentrations of the medium was 2 grams of banana: 1 ml of distilled water (see the plates section for pictures) with 15 grams of dry yeast to start the fermentation. The mixtures that were to be fermented were not put under critical conditions. Instead, they were put under room temperature conditions. The experiment yielded satisfactory results. Table 3.0 shows the concentrations of ethanol solutions collected after the distillation.

Table 3.0 Alcohol concentrations of two determinations with means±s.d.

Determination	% v/v	% w/w	% m/v
M_1	6.8	6.27	5.49
M_2	7.7	7.1	6.17
Mean±s.d.	**7.25±0.45**	**6.68±0.415**	**5.83±0.34**

Figure 1. % v/v Alcohol concentrations of four determinations.

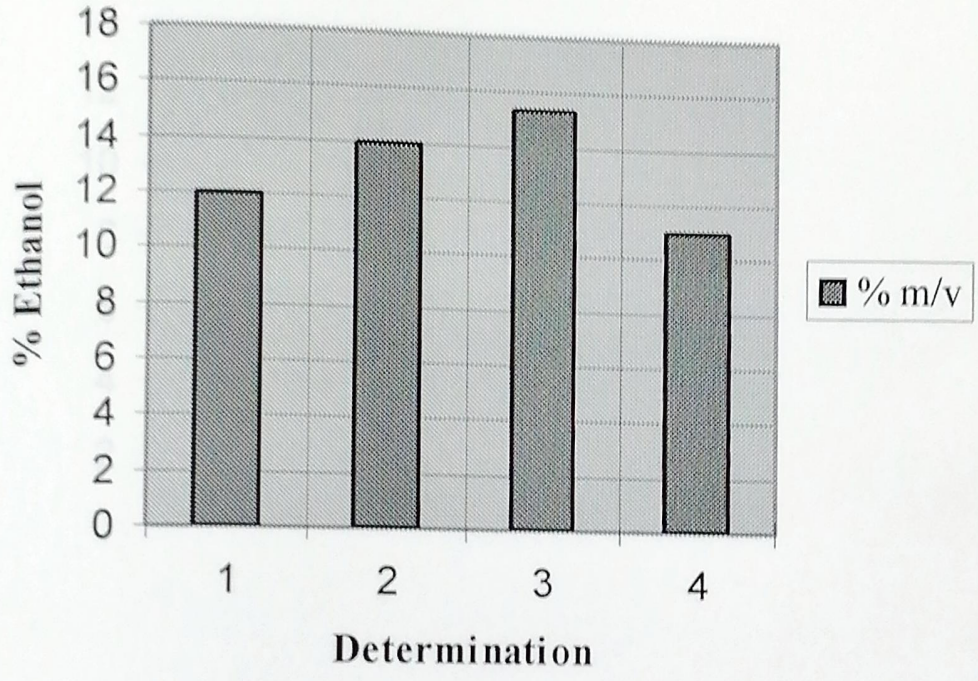


Figure 2. % m/v Alcohol concentrations of four determinations.

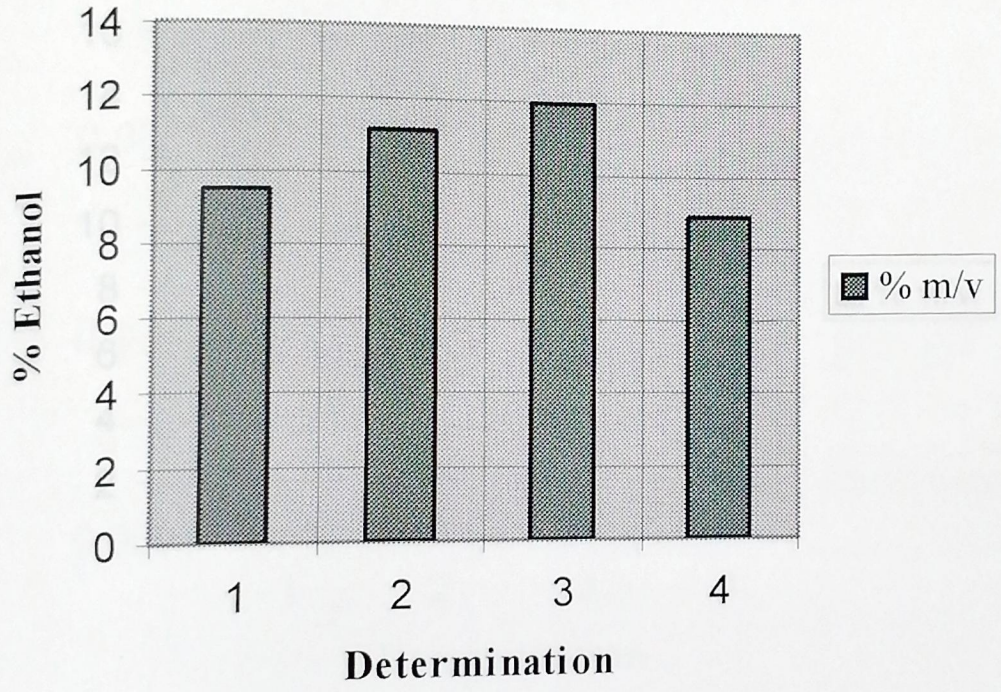


Figure 3. % w/w Alcohol concentrations of four determinations.

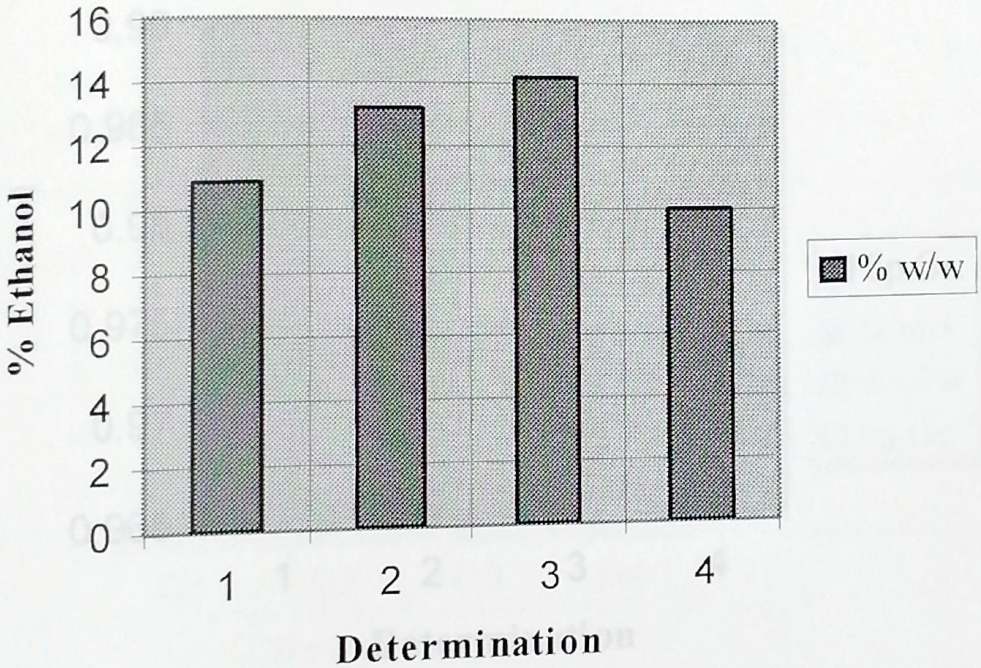


Figure 4. Specific Gravities of four determinations.

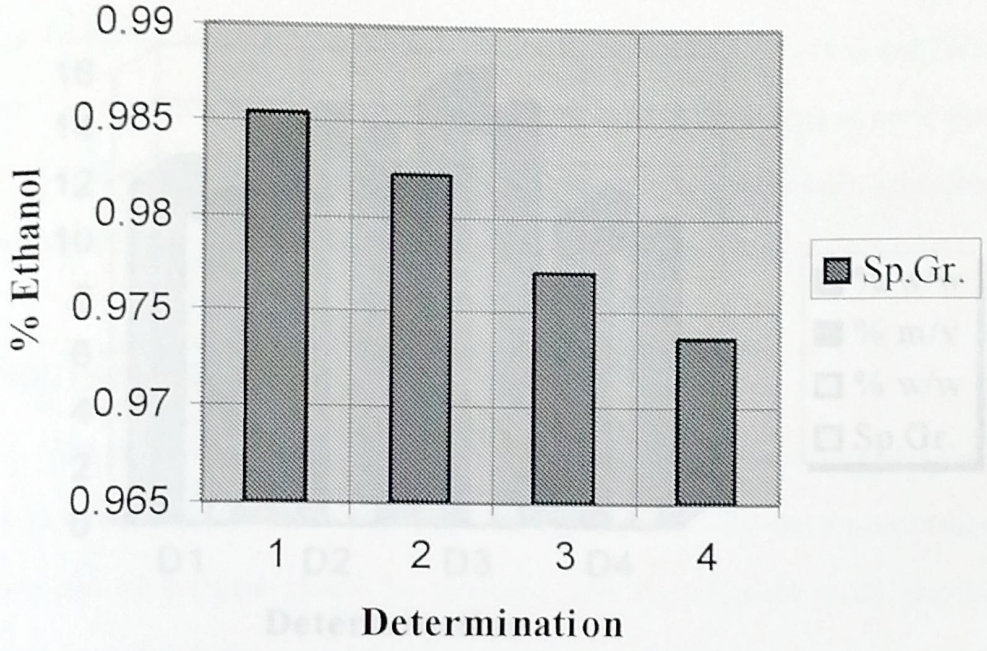
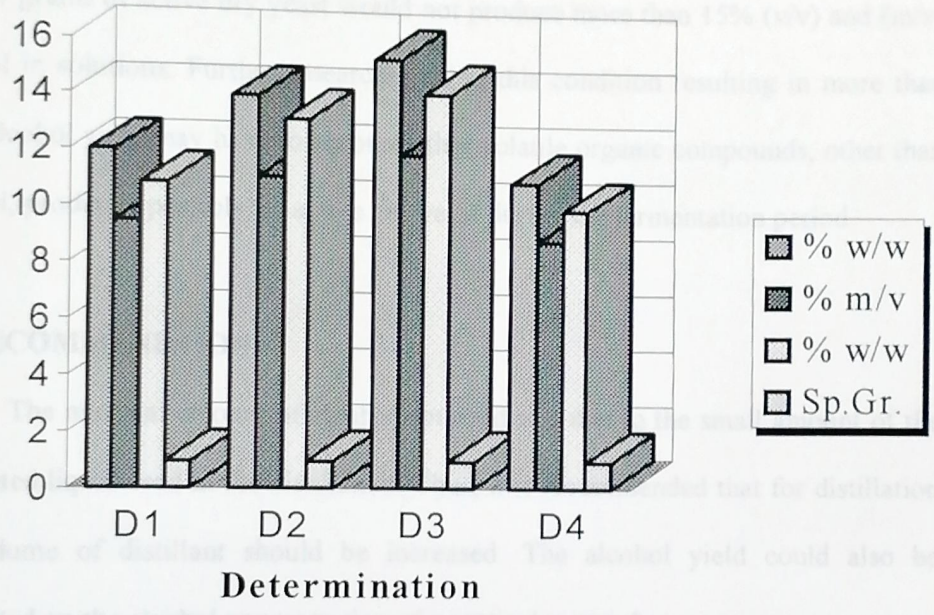


Figure 5. Various alcohol concentrations and Specific gravities(Sp.Gr.) of four determinations.



CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

A. CONCLUSIONS

The alcoholic fermentation of 450 ml of 11% (v/v) pure D-Glucose solution using 7 grams of active dry yeast would not produce more than 15% (v/v) and (m/v) alcohol in solutions. Further researches using this condition resulting in more than 15% alcohol yield may have to account other volatile organic compounds, other than ethanol, produced possibly by active dry yeast during the fermentation period.

B. RECOMMENDTIONS

The minimal amount of alcohol obtained was due to the small amount of the fermented liquid used in the distillation. Thus, it is recommended that for distillation the volume of distillant should be increased. The alcohol yield could also be accounted on the alcohol concentration of a particular sample.

Further, it is recommended that more replicates or determinations should be used to account for the precision and accuracy of the results. As many replicate as possible should be performed in order to minimize effects of random errors.

To gather more information about the metabolic activity of yeast before advancing to other related researches, it is necessary to evaluate the ability of the yeast to consume sugar during the fermentation. Sugar analysis should be done

before and after fermentation. In this case, volumetric determination of sugars by copper reduction (Lane and Eynon Method) or the Benedict's Test for carbohydrates are recommended to be performed.

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APPENDIX A
TABLE

Appendix A.1 Relationship between the specific gravity and the proportion of ethanol in alcohol solutions at 20^0C.

Specific gravity	Proof spirit	% Ethanol		Specific gravity	Proof spirit	% Ethanol	
		m/v	v/v			m/v	v/v
1.0000	0.00	0.00	0.00	0.9800	27.27	12.65	15.68
0.9995	0.58	0.26	0.33	0.9795	28.04	13.02	16.13
0.9990	1.16	0.53	0.67	0.9790	28.87	13.40	16.59
0.9985	1.74	0.80	1.01	0.9785	29.68	13.78	17.06
0.9980	2.33	1.06	1.34	0.9780	30.49	14.17	17.53
0.9975	2.92	1.33	1.68	0.9775	31.30	14.55	17.99
0.9970	3.52	1.61	2.02	0.9770	32.11	14.93	18.46
0.9965	4.12	1.88	2.37	0.9765	32.92	15.32	18.92
0.9960	4.73	2.16	2.72	0.9760	33.73	15.70	19.38
0.9955	5.34	2.44	3.07	0.9755	34.54	16.09	19.85
0.9950	5.96	2.72	3.43	0.9750	35.36	16.47	20.31
0.9945	6.58	3.01	3.75	0.9745	36.17	16.86	20.78
0.9940	7.21	3.30	4.15	0.9740	36.97	17.24	21.24
0.9935	7.84	3.59	4.51	0.9735	37.78	17.63	21.70
0.9930	8.47	3.88	4.88	0.9730	38.59	18.01	22.16
0.9925	9.12	4.18	5.25	0.9725	39.39	18.39	22.62
0.9920	9.77	4.48	5.62	0.9720	40.19	18.77	23.06
0.9915	10.43	4.78	6.00	0.9715	40.98	19.15	23.53
0.9910	11.09	5.09	6.36	0.9710	41.77	19.53	23.98
0.9905	11.76	5.40	6.77	0.9705	42.55	19.90	24.43
0.9900	12.44	5.71	7.16	0.9700	43.34	20.28	24.48
0.9895	13.12	6.03	7.55	0.9695	44.12	20.66	25.33
0.9890	13.80	6.35	7.94	0.9690	44.90	21.03	25.77
0.9885	14.50	6.67	8.34	0.9685	45.67	21.40	26.21
0.9880	15.20	7.00	8.75	0.9680	46.44	21.77	26.65
0.9875	15.92	7.33	9.16	0.9675	47.19	22.13	27.06
0.9870	16.63	7.67	9.57	0.9670	47.94	22.49	27.51
0.9865	17.36	8.00	9.96	0.9665	48.69	22.85	27.93
0.9860	18.09	8.34	10.40	0.9660	49.43	23.21	28.36
0.9855	18.82	8.69	10.83	0.9655	50.16	23.57	28.78
0.9850	19.56	9.03	11.25	0.9650	50.89	23.92	29.19
0.9845	20.31	9.38	11.68	0.9645	51.61	24.27	29.60
0.9840	21.06	9.73	12.11	0.9640	52.32	24.61	30.01
0.9835	21.82	10.09	12.55	0.9635	53.02	24.95	30.41
0.9830	22.58	10.44	12.58	0.9630	53.72	25.29	30.80
0.9825	23.34	10.80	13.42	0.9625	54.41	25.63	31.20
0.9820	24.12	11.17	13.87	0.9620	55.06	25.96	31.58
0.9815	24.90	11.53	14.32	0.9615	55.75	26.29	31.97
0.9810	26.69	11.91	14.87	0.9610	56.42	26.61	43.34
0.9805	26.48	12.28	15.83	0.9605	57.06	26.93	32.72
0.9800	27.27	12.65	15.68	0.9600	57.73	27.25	33.09

APPENDIX B
RAW DATA

Appendix B.1 Raw data of the four determinations for calculations of %(v/v).

Determination	V_d (ml)	V_2 (ml)
1	6.0	50.0
2	7.0	50.0
3	7.7	50.0
4	5.5	50.0

FORMULA : %(v/v) Ethanol = (V_d / V_s) x 100

Where: V_d = volume(ml) of distillate

V_s = volume(ml) of solution

Appendix B.2 Raw data of four determinations for calculations of %(m/v).

Determination	M_d (g)	V_s (ml)
1	4.75	50.0
2	5.56	50.0
3	5.97	50.0
4	4.45	50.0

FORMULA: %(m/v) Ethanol = (M_d / V_s) x 100

Where: M_d = Mass(g) of distillate

V_s = Volume(ml) of solution

Appendix B.3 Raw data of four determinations for calculations of %(w/w).

Determination	M_d (g)	M_s (g)
1	4.75	43.59
2	5.56	42.10
3	5.97	42.17
4	4.45	44.45

FORMULA: %(w/w) Ethanol = (M_d / M_s) x 100

Where: M_d = Mass(g) of distillate

Ms = Mass(g) of solution

APPENDIX B.4 Raw data of four determinations for calculations of Specific gravity.

Determination	X_1 (g)	X_2 (g)	X_3 (g)
1	154.00	158.75	158.82
2	154.00	159.56	159.66
3	154.00	159.97	160.11
4	154.00	158.45	158.57

FORMULA: Specific gravity (Sp. gr.) $= \frac{X_2 - X_1}{X_3 - X_1}$

Where: X_1 = Weight (g) of Specific gravity empty

X_2 = Weight (g) of Specific gravity bottle + sample

X_3 = Weight (g) of Specific gravity bottle + distilled water

APPENDIX C
CALCULATIONS

Appendix C.1 Percent (v/v) of four determinations.

$$\% (v/v)_1 = (V_d / V_s) \times 100$$

$$= (6.0 \text{ ml} / 50.0 \text{ ml}) \times 100$$

$$= \mathbf{12.0\ \%}$$

$$\% (v/v)_2 = (V_d / V_s) \times 100$$

$$= (7.0 \text{ ml} / 50 .0 \text{ ml}) \times 100$$

$$= \mathbf{14.0\ \%}$$

$$\% (v/v)_3 = (V_d / V_s) \times 100$$

$$= (7.7 \text{ ml} / 50.0 \text{ ml}) \times 100$$

$$= \mathbf{15.4\ \%}$$

$$\% (v/v)_4 = (V_d / V_s) \times 100$$

$$= (5.5 \text{ ml} / 50.0 \text{ ml}) \times 100$$

$$= \mathbf{11.0\ \%}$$

Appendix C.2 Percent (m/v) of four determinations.

$$\% (m/v)_1 = (M_d / V_s) \times 100$$

$$= (4.75 \text{ g} / 50.0 \text{ ml}) \times 100$$

$$= \mathbf{9.5\ \%}$$

$$\% (m/v)_2 = (M_d / V_s) \times 100$$

$$= (5.56 \text{ g} / 50.0 \text{ ml}) \times 100$$

$$= \mathbf{11.12\ \%}$$

$$\% (m/v)_3 = (M_d / V_s) \times 100$$

$$= (5.97 \text{ g} / 50.0 \text{ ml}) \times 100$$

$$= \mathbf{11.94\ \%}$$

$$\% (m/v)_4 = (M_d / V_s) \times 100$$

$$= (4.45 \text{ g} / 50.0 \text{ ml}) \times 100$$

$$= \mathbf{8.90\ \%}$$

Appendix C.4 Specific gravities (Sp. gr.) of four determinations.

$$\text{Sp. gr.}_1 = (X_2 - X_1) / (X_3 - X_1)$$

$$= (158.75\ \text{g} - 154.0\ \text{g}) / (158.82\ \text{g} - 154.0\ \text{g})$$

$$= \mathbf{0.9854}$$

$$\text{Sp. gr.}_2 = (X_2 - X_1) / (X_3 - X_1)$$

$$= (159.56\ \text{g} - 154.0\ \text{g}) / (159.66\ \text{g} - 154.0\ \text{g})$$

$$= \mathbf{0.9823}$$

$$\text{Sp. gr.}_3 = (X_2 - X_1) / (X_3 - X_1)$$

$$= (159.97\ \text{g} - 154.0\ \text{g}) / (160.11\ \text{g} - 154.0\ \text{g})$$

$$= \mathbf{0.9771}$$

$$\text{Sp. gr.}_4 = (X_2 - X_1) / (X_3 - X_1)$$

$$= (158.45\ \text{g} - 154.0\ \text{g}) / (158.57\ \text{g} - 154.0\ \text{g})$$

$$= \mathbf{0.9737}$$

APPENDIX D
PLATES

Plate 1.0 The materials used for the descriptive study.

Plate 2.0 Preparation of the glucose solutions and rehydration of active dry yeast.

Plate 3.0 The Fermentation set-up at 35^0C – 42^0C for 7 days.

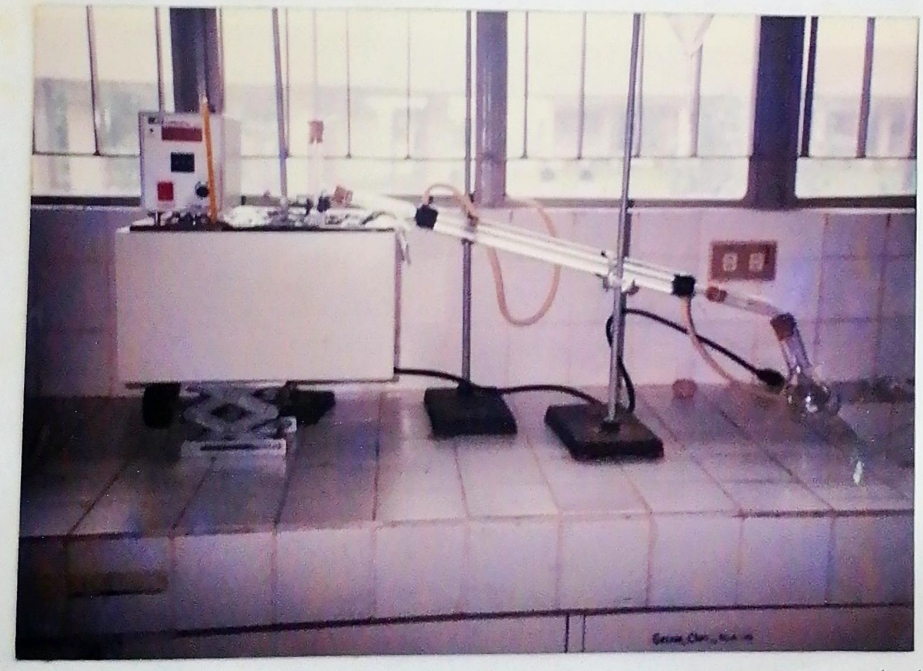

Plate 4.0 The distillation set-up using the constant temperature water-bath for temperature control.

Plate 5.0 The test tubes containing the distillates and the chemicals.

Plate 6.0 Replicates 1 and 2 subjected to the determination for the presence of ethanol by chemical reaction tests.

Plate 7.0 Bubbles formed as a result of carbon dioxide production from the flasks.

Plate 8.0 Organism and substrate preparation in the trial experiment.

Plate 9.0 Solutions of the two determinations used in the trial experiment.