KINETICS OF BIOETHANOL PRODUCTION USING SAWDUST AS FEEDSTOCK

BY

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DELARATION

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CERTIFICATION

This is to certify that this Thesis entitled Kinetics of Bioethanol Production Using Sawdust as Feedstock, has been approved as meeting the requirement for the award of Masters of Engineering Degree in Chemical Engineering Department, Federal University of Technology, Minna.

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DEDICATION

This research project is dedicated to the Almighty God, the God of all things.

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ABSTRACT

The production of bioethanol using sawdust as a feedstock has been undertaken. First, the cellulose from the sawdust was hydrolysed with sulphuric acid at varying temperatures and acid concentrations. The rate of hydrolysis as measured by the rate of glucose formation increased with increase in temperature and the optimum concentration of the acid was determined to be 6M at 80°C. The hydrolysis process was found to follow zero order kinetics based on integral method of analysis with activation energy of 39.652 KJ/mol. The hydrolysate was fermented by two microorganisms, namely *Saccharomyces cerevisiae* and *Zymomonas mobilis* to bioethanol. The kinetic fermentation parameters obtained with *Saccharomyces cerevisiae* for the yeast maximum specific growth rate (μ_m), maximum biomass concentration (X_m), the yield coefficient of ethanol on biomass ($Y_{p/x}$), lag time (Δt), the yield coefficient of biomass on substrate ($Y_{x/s}$) and maintenance coefficient (m) were found to be $0.0395h^{-1}$, 5.5182g/l, 3.3949g/g, 4.8214hrs, 0.1996g/g and $0.1325hr^{-1}$ respectively whilst the corresponding values on using *Zymomonas mobilis* were $0.0385h^{-1}$, 5.4718g/l, 3.6439g/g, 4.6350hrs, 0.1992g/g and $0.1328hr^{-1}$ respectively. The percentage yield of ethanol obtained using *Zymomonas mobilis* was 82.2% which is higher than 64.8% when *Saccharomyces cerevisiae* was used.

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LIST OF SYMBOLS

Symbol	Description	Unit
X	Biomass concentration	g/l
X_m	Maximum biomass concentration	g/l
X_o	Initial biomass concentration	g/l
m	Maintenance coefficient	g/g
t	Time	h
P	Produced ethanol concentration	g/l
S	Fermentable sugar concentration	g/l
S_o	Initial fermentable sugar concentration	g/l
$Y_{p/x}$	Yield coefficient of ethanol on biomass	g/g
$Y_{x/s}$	Yield coefficient of biomass on sugar	g/g
μ	Specific growth rate	h^{-1}
μ_m	Maximum specific growth rate	h^{-1}
C_B	Concentration of cellulose	g/l
E	Activation energy	KJ/mol
R	Universal gas constant	J/mol.k
T	Temperature	^oC
A	Exponential factor	min^{-1}
K	Rate constant	min^{-1}
X_c	Amount of monosaccharide (glucose) produced	g/l

CHAPTER ONE

1.0 INTRODUCTION

The burning of fossil fuel at the current rate is creating an environmental crisis globally. Use of fossil fuel generates carbon dioxide, methane and a significant quantity of nitrous oxide. Most of these harmful gases are formed due to incomplete combustion of fossil fuel but ethanol contains 35% oxygen that may result in a more complete combustion of fuel and thus reduces tailpipe emissions (Chandel *et al.,* 2007). It is worth while to know that the problems associated with petroleum and fossil fuel sources are that they are limited in supply and cannot be renewed hence depletion is eminent. It is therefore evident that there is the need to search for alternative fuel sources which can be renewed with time and ethanol has been found as one of such fuel sources (Akpan *et al.,* 2008).

However, the importance of bioethanol as a clean and sustainable renewable fuel has increased in recent years because of the anticipated shortage of fossil fuels and increased environmental pollution (Sun and Cheng, 2002). Bioethanol is the most widely used alternative transport fuel in the world (Cardona and Sanchez, 2007). Ethanol produced from various lignocellulosic materials such as wood, agricultural and forest residues has the potential to be a valuable substitute for or complement to gasoline (Galbe *et al.,* 2002). Recent studies have shown that researchers in this field have successfully converted many cellulosic materials such as sawdust, solid animal wastes, crop residues etc (Aderemi *et al.*, 2008; and Sun and Cheng, 2002) to more valuable products such as fermentable sugars.

Unfortunately, the current method of producing bioethanol is expensive because of the high cost of raw materials (sugar cane and cereals). Therefore, attention has now turned towards lignocellulosic biomass, especially residues from agricultural and forestry operations to provide a

cheaper source of carbohydrate for fermentation (Pan *et al.*, 2002). Lignocellulosic biomass is the most abundant renewable organic material on earth with an annual supply of approximately 200 billion metric tons. Bioconversion of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation and product separation/ distillation.

As part of the works carried out on production of bioethanol, Megawati *et al.,* (2010) modelled the kinetics of dilute- acid hydrolysis of rice husk for ethanol production using pseudo-homogeneous approach. It was observed that high ethanol yield results from high acid concentration. Similarly, ethanol concentration increased with increase in catalyst concentration. Akpan *et al.*, (2005) investigated the acid hydrolysis of groundnut shell using *S. cerevisiae*. Results obtained from the study shows that glucose yield during acid hydrolysis of cellulose increases with increase in temperature.

Wang *et al.,* (*2004*) studied the fermentation kinetics of different sugars with apple wine yeast, *Saccharomyces cerevisiae*. The results obtained indicated that the fermentation kinetic model could predict optimum fermentation performance using different sugar as the substrate with various initial sugar concentrations. Various kinetic models have been proposed for freely suspended cells in either batch or continuous operation (Lin and Tanaka, 2006).

Unstructured models usually gives the most fundamental observations concerning microbial metabolic processes and can be considered a good approximation when the cell composition is time dependent or when the substrate concentration is high compared to the saturation constant (Sonnleitner *et al.*, 1997).

A developed mathematical kinetic model capable of predicting the cell, substrate and ethanol concentrations during the continuous anaerobic fermentation is necessary. However, it cannot be expected that any kinetic model will be directly applicable to a real process situation.

Therefore, mathematical modelling should start with the simplest type, but it must be reiterated, modified and extended until it eventually leads to an adequate process kinetic model.

Gulnur *et al.*, (1998) investigated the mathematical description concerned with the basic metabolic processes of *S. cerevisiae* in immobilized form. Glucose utilization, ethanol production and growth pattern of yeast cells immobilized in calcium alginate gel beads were determined in a stirred batch system using four different initial substrate concentrations.

It is obvious that fermentation of simple sugar follows first order kinetics and the interpretation of the fermentation kinetics is often based on the simple linear model of Monod's equations but some bio-reactions that occur during the fermentation process are not described by linear model because several factors such as lag time, amount of micro-organisms (yeast) present and amount of fermentable sugar available affect the production rate of ethanol (Wang *et al.*, 2004). They went further to report different suitable mathematical models for the bio-reactions that occur during the fermentation process of apple wine. Several other factors such as temperature, concentration, starter media culture volume e.t.c also affect the production rate of ethanol by fermentation, and a suitable mathematical description of the fermentation process has been developed. This helps in interpreting fermentation measurements with a view to early detection of poor fermentation performance, the ability to predict future fermentation behavior and application to design and advanced control of fermentation and optimization (Boulton, 1996).

Akpan *et al.*, (2008) studied the kinetic model for ethanol production from organic food waste by *Saccharomyces cerevisiae*. It was found out that the modified Monod's equations as described by Wang *et al.*, (2004) are suitable for predicting fermentation processes.

Few works have been published on the production of bioethanol using sawdust as feedstock. Hence, the importance of this study

1.1 Aim and objectives

The aim of this work is to study the kinetics of ethanol production from sawdust, through acid hydrolysis and fermentation using two microorganisms: *Saccharomyces cerevisiae* and *Zymomonas mobilis*. In order to realize the above stated aim, the following objectives have been set:

- To determine the kinetic parameters of cellulose hydrolysis using tetraoxosulphate (VI) acid.
- To determine the fermentation kinetic parameters of the hydrolysate, which includes: Yeast Maximum Specific Growth rate (μ_m), maximum biomass concentration (X_m), lag time (Δt), yeast coefficient ($Y_{x/s}$), maintenance coefficient (m), and the yield coefficient ($Y_{p/x}$). Also some thermodynamics parameters such as the activation energy and the exponential factor will be determined.
- To compare the yield from the two different microorganisms used: *Saccharomyces cerevisiae* and *Zymomonas mobilis.*

1.2 Scope of study

The area of coverage of this work is based on the four major unit operations involved in the bioconversion of lignocellulosic materials into ethanol namely: isolation of the cellulose from the lignocellulosic material, acid hydrolysis of the cellulose, fermentation of the hydrolysate and distillation of the bio-ethanol produced.

Also, the effect of different acid concentrations on the hydrolysis, effect of different temperatures on the hydrolysis process at a constant acid concentration will be studied. Comparative study of the yield of ethanol obtained from *Saccharomyces cerevisia* and *Zymomonas mobilis* on the fermentation process will be investigated. Finally, a modified Monod equation will then be use to obtain the fermentation kinetic parameters.

1.3 Significance/Justification

It is clear that biomass based energy can play an important role in reducing green house gas emissions, since ethanol produced from this process only uses energy from renewable sources. Hence no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source (Kheshgi *et al.*, 2000). The ability to produce ethanol from agricultural waste materials will make it more competitive to gasoline.

Furthermore, ethanol from lignocelluloses such as wood sawdust may also open new employment opportunities in rural areas, and thus make a positive socio-economic impact (Wyman *et al.,* 2005; Chandel *et al.*, 2007).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Ethanol Production

Ethanol is an important product in the fuel market. Its market grew from less than a billion liters in 1975 to more than 39 billion liters in 2006 and is expected to reach 100 billion in 2015 (Licht, 2006).

Approximately 80% of the ethanol produced in the world is still obtained from fermentations, the remainder comes largely by synthesis from the petroleum product, ethylene. The alcohol produced in the U.S. is primarily used in alcoholic beverages, but this is not always the case elsewhere in the world. Brazil has embarked on a major programme to produce ethanol for fuel and thereby diminish petroleum imports. As of 1984, approximately 7.9 million tons of ethanol was produced by fermentation in Brazil, with sucrose from sugarcane as the carbon source. The U.S. is also substantially increasing its fuel alcohol production, originally because of the rapid increase in petroleum costs during the 1970s and the subsequent need for developing alternative energy sources. In spite of extensive research on fuel ethanol production from biomass, until 1995 not a single plant capable of converting cellulosic feedstock to ethanol, via biological processing on the industrial scale, has been put into operation anywhere in the world, although some pilot scale plants have been commissioned (Szczodrak and Fiedurek, 1996).

During World War II, when wartime conditions changed economic conditions and priorities several ethanol-from-cellulose (EFC) plants were built and operated in various countries to provide an alternative fuel source. These countries include Germany, Russia, China, Korea, Switzerland and the U.S. among others. Since the end of the war, competition from synthetically produced ethanol has forced many of these plants to close (Lin and Tanaka, 2006).

Since April 2004, the first demonstration plant using lignocellulosic feedstocks in Canada has been in operation. The target volume of 100 million litres of ethanol, anticipated by 2006, is met or exceeded by 2007. There is also progress on pretreatment of softwood residues and pentose fermentation (Lin and Tanaka, 2006).

Currently, some countries in locations with higher ethanol and fuel prices are producing ethanol from cellulosic feedstocks. It is only recently that cost-effective technologies for producing EFC in the U.S. have started to emerge. In Canada, Iogen Corporation built a small commercial-scale cellulose-ethanol plant using proprietary enzymatic hydrolysis technology. In 1997 they partnered with Petro-Canada to produce cellulose-ethanol beginning with 4 million liters per year ethanol demonstration facility, located at Iogen's headquarters in Ottawa, using corn stover and switch grass (Lin and Tanaka, 2006).

In summer 2005, a Swedish plant in Örnsköldsvik started to produce ethanol from sawdust. The production is still in a start up phase, but the optimism is high. In a not so distant future, Sweden could become self-sufficient of ethanol from wood and wood residues, which would be a much more sustainable way of supplying ethanol to the Swedish market (Lin and Tanaka, 2006).

Nowadays, in the field of sugar and starch utilization, the large-scale application of modern bioenergy conversion technologies has already occurred in a number of countries, both in the industrialised and developing world. In the U.S., the Minnesota Pollution Control Agency (MPCA) has scheduled a public information meeting in early 2005 to discuss the proposed Heron Lake BioEnergy ethanol project. The proposed plant would cover 37 acres at a site about one mile northeast of the City of Heron Lake in Jackson County. It would process 21.7 million bushels of corn annually to produce 55 million gallons of ethanol and 193,300 tons of distillers

dried grains (Sullivan, 2005). Another example is that of Brazil, a country that has committed itself to the development of its modern bioenergy potential. Its sugar cane based ethanol industry annually produces around 15 billion liters from about 350 distilleries, and satisfies over 33% of the country's gasoline needs .For the Global ethanol market, Brazil has more than 300 plants, producing 15 billion liters per year and supplying 3 million cars with pure ethanol. In the U.S., there are more than 80 plants producing 10 billion liters per year which it intends to increase to 19 billion liters by 2010. China could create 3 billion liters of ethanol per year. India's annual production of ethanol is 2.7 billion liters, Eastern Europe's 2.5 billion liters. Western Europe's production ability is 2 billion liters and in Canada, 0.24 billion liters could be achieved and possibly expanded to 1.4 billion liters (Klein, 2005).

2.1.1 Biomass Resources

Biomass is seen as an interesting energy source for several reasons. The main reason is that bioenergy can contribute to sustainable development (Monique *et al.*, 2003). There are various forms of biomass resources in the world, which can be grouped into four categories.

1. Wood residues are by far the largest current source of biomass for energy production. It comes from the wood product industry which includes paper mills, sawmills and furniture manufacturing.
2. Municipal solid waste is the next largest.
3. Agriculture residues.
4. Dedicated energy crops.

Among these biomass resources including short-rotation woody crops and herbaceous crops, primarily tall grasses, dedicated energy crops seem to be the largest, most promising, future resource of biomass. This is because of the ability to obtain numerous harvests from a

single planting, which significantly reduces average annual costs for establishing and managing energy crops, particularly in comparison to conventional crops (Monique *et al.*, 2003).

2.1.2 Raw Materials for Ethanol Production

Fermentation processes from any material that contains sugar could derive ethanol. The varied raw materials used in the manufacture of ethanol via fermentation are conveniently classified into three main types of raw materials:

a) Sugars (from sugar cane, sugar beets, molasses and fruits) can be converted to ethanol directly.

b) Starches (from corn, cassava, potatoes and root crops) must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds.

c) Cellulose (from wood, agricultural residues, waste sulfite liquor from pulp and paper mills) must likewise be converted into sugars, generally by the action of mineral acids. Once simple sugars are formed, enzymes from microorganisms can readily ferment them to ethanol.

2.1.2.1 Ethanol Production from Molasses (Sugar)

The most widely used sugar for ethanol fermentation is molasses which contains about 50 wt% of sugar and about 50 wt% of organic and inorganic compounds, including water. It is thick, dark-colored syrup produced during refinement of sugar. Since molasses contains microorganisms which can disturb the fermentation, the molasses is taken first to the sterilizer and then to the fermentor. Then it is diluted with water to the mass fraction of 10±18% to reduce its viscosity in the pipeline. In addition, a very high concentration of sugar can give too much ethanol and results in a prolonged fermentation time and an incomplete sugar conversion. After

the pH of the mash is adjusted to about 4-5 with mineral acid, it is inoculated with yeast or bacteria and the fermentation is carried out non-aseptically at 20-32°C for about 1-3 days.

The products obtained are ethanol and carbon dioxide as shown in equation (2.1)

$$C_6H_{12}0_6 \xrightarrow{yeast} 2CH_3CH_20H + 2CO_2 \quad (2.1)$$

2.1.2.2 Ethanol Production from Starch

Most agricultural biomass containing starch can be used as a potential substrate for the ethanol fermentation by microbial processes. These substrates include corn (maize), wheat, oats, rice, potato and cassava. On a dry basis, corn, wheat, sorghums (milo) and other grains contain around 60 to 75% (wt/wt) of starch, hydrolysable to hexose with a significant weight increase (stoichiometrically, the starch to hexose ratio is 9:10), and these offer a good resource in many fermentation processes. Fermentation of starch is somewhat more complex than fermentation of sugars because starch must first be converted to sugar and then to ethanol. Starch is first hydrolysed by adding α-amylase to avoid gelatinization, and then cooked at high temperature (140–180°C). Next, the liquefied starch is hydrolysed to glucose with glucoamylase. The resulting dextrose is fermented to ethanol with the aid of microorganisms producing CO_2 as a co-product. During the process currently employed for industrial-scale ethanol fermentation from starchy materials, high-temperature cooking (140–180°C) is very effective for fermentation of starchy materials because it raises starch saccharification efficiency and achieves high levels of ethanol production under complete sterilization of harmful microorganisms (Jackman, 1987).

However, production costs are high due to the high energy consumption in the cooking process and the addition of large amounts of amylolytic enzymes. So processes to reduce the high production costs are required. To resolve these difficulties, non-cooking and low

temperature cooking fermentation systems have been developed (Matsumoto *et al.*, 1985). Industrial ethanol production has been reported using various starchy materials such as corn, wheat, starch and potatoes, cassava root (Lindeman and Rocchiccioli, 1979), corn stover (Kadam and McMillan, 2003) and starch (Maisch *et al.*, 1979). Among many starchy materials, cassava starch is an inexpensive fermentable source. It is a tropical root crop produced in more than 80 countries (Sasson, 1990). About 20% of the cassava starch was incorporated into animal feed. A similar amount was converted into starch for industrial use and another portion used for human food in some developing countries. The rest was lost since cassava is perishable after harvest. Harnessing the lost portion in addition to gains from new high-yielding varieties with outputs of 100 tones per hectare could provide the fermentation industry with an abundance of raw material. Fresh cassava has very high starch content, up to 30%. The content of sucrose is about 4%. Dried cassava has 80% fermentable substrate (Anthony *et al.*, 1996).

2.1.2.3 Ethanol Production from Cellulose

Among the three main types of raw materials, cellulose materials represent the most abundant global source of biomass and have been largely unutilized. The global production of plant biomass, of which over 90% is lignocellulose, amounts to about 200×109 ton per year, where about 8 to 20×109 ton of the primary biomass remains potentially accessible. However, the effective utilization of the lignocellulosic feedstock is not always practical because of its seasonal availability, scattered stations, and the high costs of transportation and storage of such large amounts of organic material (Polman, 1994). Recently, the enzymatic hydrolysis of biomass cellulose is considered to be the most promising technology available (Yu and Zhang, 2004). However, despite the work done, the industrial scale-up of this process appears to still be hindered by technological issues or by the lack of a biomass refinery approach in which ethanol

is one of several products. In fact, because raw material cost comprises more than 20% of the production cost (Kaylen *et al.*, 2000), the optimization of the cellulose conversion should be accomplished by correct management and utilization of all process streams. A consequence of this situation is that even limited government intervention is still crucial to maintaining ongoing research.

Furthermore, lignocellulose is a more complex substrate than starch. It is composed of a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin. The carbohydrate polymers are tightly bound to lignin mainly by hydrogen bonds but also by some covalent bonds. The biological process for converting the lignocellulose to fuel ethanol requires: delignification to liberate cellulose and hemicelluloses from their complex with lignin, depolymerization of the carbohydrate polymers to produce free sugars, and fermentation of mixed hexose and pentose sugars to produce ethanol. Among the key processes described above, the delignification of lignocellulosic raw materials is the rate-limiting and most difficult task to be solved. Another problem is that the aqueous acid used to hydrolyze the cellulose in wood to glucose and other simple sugars destroys much of the sugars in the process. Extensive research has been carried out in this field for decades (Yu and Zhang, 2004), and the first demonstration plant using lignocellulosic feedstocks has been in operation in Canada since April 2004 (Lin and Tanaka, 2006). It is expected that the cost of lignocellulosic ethanol can undercut that of starch-based ethanol because low-value agricultural residues can be used.

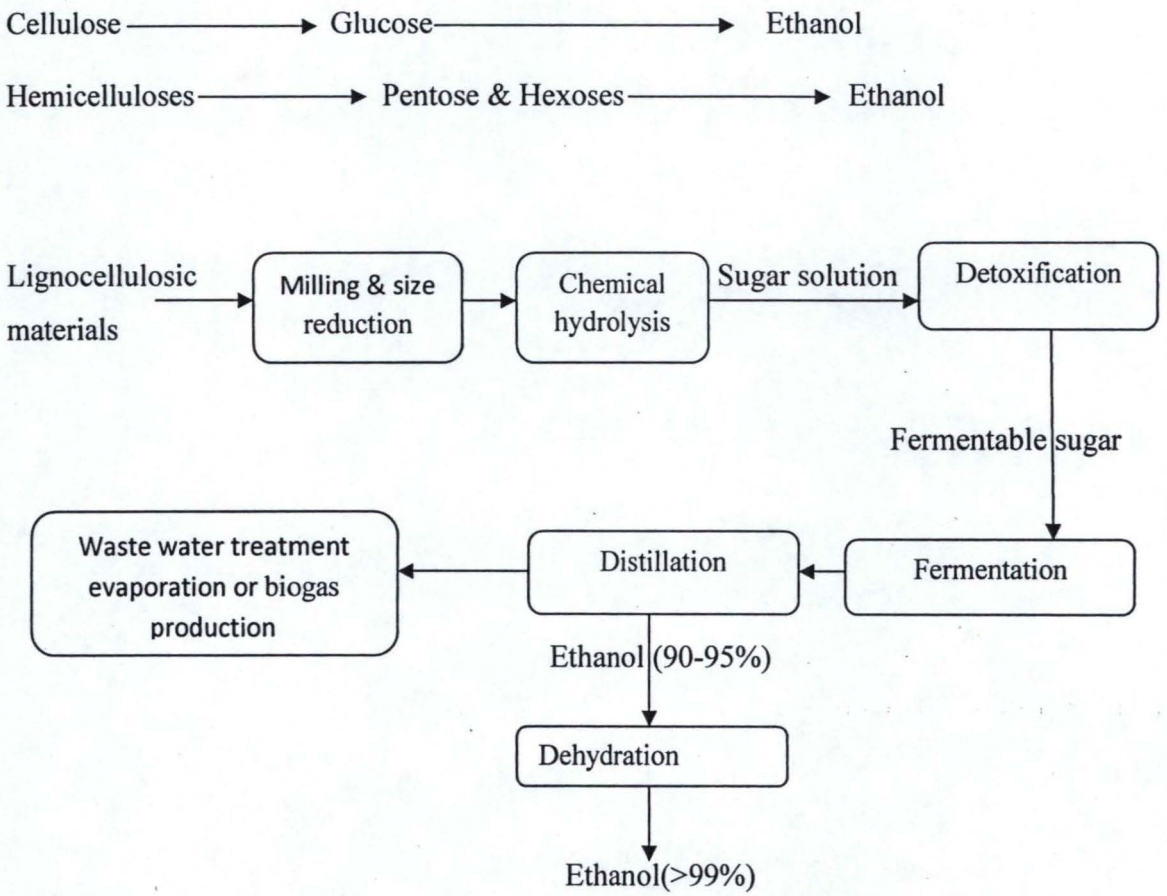

Fig. 2.1 Overall process scheme for ethanol production from lignocellulosic materials

When cellulose was used as the raw material, the cellulase responsible for enzymatic hydrolysis of pretreated cellulosic biomass is strongly inhibited by hydrolysis products: glucose and short cellulose chains. One way to overcome cellulase inhibition is to ferment the glucose to ethanol as soon as it appears in solution. Simultaneous saccharification and fermentation (SSF) combines enzymatic hydrolysis with ethanol fermentation to keep the concentration of glucose low. The accumulation of ethanol in the fermentor does not inhibit cellulase as much as high concentrations of glucose, so SSF is a good strategy for increasing the overall rate of cellulose to ethanol conversion. In comparison to the process where these two stages are sequential, the SSF method enables attainment of higher (up to 40%) yields of ethanol by removing end-product

inhibition, as well as by eliminating the need for separate reactors for saccharification and fermentation (Hari *et al.*, 2001). Other advantages of this approach are a shorter fermentation time and a reduced risk of contamination with external microflora, due to the high temperature of the process, the presence of ethanol in the reaction medium and the anaerobic conditions (Emert and Katzen, 1980). In spite of the obvious advantages presented by the SSF, it has some drawbacks. These lie mainly in different temperature optima for hydrolysis (45-50°C) and fermentation (28-35°C) (Ballesteros *et al.*, 2004; Jeffries and Jin, 2000)

2.1.3 Ethanol Production Technologies

Bioconversion of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation and product separation/ distillation.

2.1.3.1 Pretreatment

Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields (Sun and Cheng, 2002; Moiser *et al.*, 2005). Pretreatment affects the structure of biomass by solubilizing hemicellulose, reducing crystallinity and increase the available surface area and pore volume of the substrate. Pretreatment has been considered as one of the most expensive processing step in biomass to fermentable sugar conversion with cost as high as 30 cents/gallon ethanol produced (Moiser *et al.*, 2005). To asses the cost and performance of pretreatment methods, technoeconomic analysis have been made recently (Chandel *et al.*, 2007). There is huge scope in lowering the cost of pretreatment process through extensive R&D approaches. Pretreatment of cellulosic biomass in cost effective manner is a major challenge of cellulose to ethanol technology research and development.

Native lignocellulosic biomass is extremely recalcitrant to enzymatic digestion. Therefore, a number of thermochemical pretreatment methods have been developed to improve digestibility (Wyman *et al.*, 2005). Recent studies have clearly proved that there is a direct correlation between the removal of lignin and hemi-cellulose on cellulose digestibility (Kim and Holtzapple, 2006). Thermochemical processing options appear more promising than biological options for the conversion of lignin fraction of cellulosic biomass, which can have a detrimental effect on enzyme hydrolysis. It can also serve as a source of process energy and potential co-products that have important benefits in a life cycle context (Sheehan *et al.*, 2003). Pretreatment can be carried out in different ways such as mechanical combination (Cadoche and Lopez, 1989), steam explosion (Gregg and Saddler, 1996), ammonia fiber explosion (Kim *et al.*, 2003), acid or alkaline pretreatment (Damaso *et al.*, 2004) and biological treatment (Keller *et al.*, 2003).

2.1.3.2 Hydrolysis

After pretreatment there are two types of processes to hydrolyze the feed stocks into monomeric sugar constituents required for fermentation into ethanol. The hydrolysis methods most commonly used are acid (dilute and concentrated) and enzymatic. To improve the enzymatic hydrolytic efficiency, the lignin-hemicellulose net work has to be loosened for the better amenability of cellulases to residual carbohydrate fraction for sugar recovery. Dilute acid treatment is employed for the degradation of hemicellulose leaving lignin and cellulose network in the substrate. Other treatments are alkaline hydrolysis or microbial pretreatment with white-rot fungi (*Phaenerochate chrysosporium*, *Cyathus stercoreus*, *Cythus bulleri* and *Pycnoporous cinnabarinus* etc.) preferably act upon lignin leaving cellulose and hemicellulose network in the residual portion. However during both treatment processes, a considerable amount of

carbohydrates are also degraded, hence the carbohydrate recovery is not satisfactory for ethanol production.

2.1.3.2.1 Acid hydrolysis

There are two types of acid hydrolysis process commonly used - dilute and concentrated acid hydrolysis. The dilute acid process is conducted under high temperature and pressure and has reaction time in the range of seconds or minutes. It is a method that can be used either as a pre-treatment preceding enzymatic hydrolysis, or as the actual method of hydrolyzing lignocelluloses to the sugars (Qureshi and Manderson, 1995). The concentrated acid process uses relatively mild temperatures, but at high concentration of sulfuric acid and a minimum pressure involved, which only creates by pumping the materials from vessel to vessel. Reaction times are typically much longer than for dilute acid process.

i. Dilute acid hydrolysis

In dilute acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulosic fraction. Dilute sulfuric acid is mixed with biomass to hydrolyse hemicellulose to xylose and other sugars. Dilute acid is interacted with the biomass and the slurry is held at temperature ranging from 120 - 220°C for a short period of time. Thus hemicellulosic fraction of plant cell wall is depolymerised and will lead to the enhancement of cellulose digestibility in the residual solids (Sun and Cheng, 2002). Dilute acid hydrolysis has some limitations. If higher temperatures (or longer residence time) are applied, the hemicelluosic derived monosaccharides will degrade and give rise to fermentation inhibitors like furan compounds, weak carboxylic acids and phenolic compounds (Klinke *et al.*, 2004). These fermentation inhibitors are known to affect the ethanol production performance of fermenting microorganisms (Chandel *et al.*, 2007). In order to remove the inhibitors and increase the

pollution is the main advantage of utilizing biomass conversion into ethanol. Ethanol contains 35% oxygen that helps complete combustion of fuel and thus reduces particulate emission that pose health hazard to living beings. A report by Chandel *et al.*, (2007) on the ethanol blended diesel (E10 and E30) combustion at different loads shows that the addition of ethanol to diesel fuel simultaneously decreases cetane number, high heating value, aromatics fractions and kinematic viscosity of ethanol blended diesel fuels and changes distillation temperatures. These factors lead to the complete burning of ethanol and less emissions. With its ability to reduce ozone precursors by 20 - 30%, bioethanol can play a significant role in reducing the harmful gasses in metro cities worldwide. Ethanol blended diesel (E-15) causes the 41% reduction in particulate matter and 5% NO_x emission.

One of the disadvantage in using ethanol as fuel is that aldehyde predominantly acetaldehydes emissions are higher than those of gasoline. However acetaldehydes emissions generate less adverse health effects in comparison to formaldehydes emitted from gasoline engines (Chandel *et al.*, 2007).

2.2 Wood Sawdust

Wood is an essential material for man. It is a material source for energy and constructional works. Sawdust is composed of fine particles of wood. This material is produced from cutting with a saw, hence its name. Wood sawdust which is a byproduct of wood processing pollutes the environment even though these debris are materials suited for biodegradation (Williams, 2001). Wastes and their disposal have become enough substances of environmental concern worldwide especially when these wastes are biodegradable to useful goods and services. Three major wastes management routes have been identified (Williams, 2001) namely, sewage disposal, compositing

and landfill and bioremediation. Amongst these, the sewage disposal provides opportunity for any possible recovery of useful product after biodegradation.

Wood sawdust or wood is composed essentially of moisture, cellulose, resin and wax, water-solubles and lignin at varying percentages. Wood sawdust, as a lignocellulosic material can undergo enzymatic degradation to produce protein, glucose, and subsequently ethanol, without loosing any other component that is nonbiodegradable. Cultivation of enzymes for degradation of lignocellulosic materials has been reported (Shide *et al.,* 2004). The negative concerns other people have regarding wastes differ from scientific views; wastes are no longer scraps instead they are unused resources.

Table 2.3 Composition of sawdust (%, *w* dry) (Yuan-chuan *et al.*, 2004)

Component	Moisture	Ash	Extractives	Hemi-cellulose	Cellulose	Lignin
Content(%, *w*)	14.5	1.4	5.2	14.1	48.5	30.8

2.2.1 Uses of wood sawdust

Wood sawdust has a variety of practical uses. It is employed in several sectors including construction works, pulp and paper production, manure in the agricultural sector, and fuel in the energy sector. It serves as a mulching material, or as an alternative to clay cat litter and for the manufacture of particleboard. Until the advent of refrigeration, it was often used in icehouses to keep ice frozen during the summer. Historically, it has been treated as a by-product of manufacturing industries and can easily be understood to be more of a hazard, especially in terms of its flammability. It has also been used in artistic displays, and as scatter. It is also sometimes used in bars in order to soak up spills, allowing the spill to be easily swept out the door. Perhaps the most interesting application of sawdust is in pykrete, a slow-melting, much stronger ice

composed of sawdust and frozen water. It is used to make Cutler's resin (http://wikipedia-sawdust/the free encyclopedia, 2010).

2.3 Kinetic models

A developed mathematical model capable of predicting the cell, substrate and ethanol concentrations during fermentation is necessary. However, it cannot be expected that any kinetic model will be directly applicable to a real process situation. Therefore, mathematical modeling should start with the simplest type, but it must be reiterated, modified and extended until it eventually leads to an adequate process kinetic model. Kinetic models describing the behavior of microbiological systems can be a highly appreciated tool and can reduce tests to eliminate extreme possibilities (Lin and Tanaka, 2006).

2.3.1 Kinetics of Acid hydrolysis

Both cellulose and hemi-cellulose are polysaccharides that can be acidically hydrolyzed into monosaccharide (glucose, xylose). In hydrolysis of sawdust, monosaccharide was the desired product that is readily degraded consecutively into undesired products (Yuan-chuan *et al.*, 2004).

A variety of kinetic models for acidic hydrolysis of hemi-cellulose and cellulose have been reported (Lavarak *et al.*, 2002; Yuan-chuan *et al.*, 2004). The commonly accepted kinetic behavior of hydrolysis model is consecutive irreversible reactions from cellulose (hemi-cellulose) to monosaccharides and then to decomposed products (Lavarak *et al.*, 2002).

Hydrolysis of lignocellulosic biomass can be approached as sequential reaction of lignocelluloses hydrolysis. Schematically, the process can be represented as

$$B + H_2O \xrightarrow{K_{hyd}} D \qquad (2.2)$$

Where B = polysaccharide (cellulose and hemi-cellulose)

D = monosaccharide (sugar)

Basically, solid-liquid reactions are usually modeled by heterogeneous or pseudo-homogeneous approach. A pseudo-homogeneous model was applied in this case. For a pseudo-homogeneous model, the mass balance of B in a batch reactor can be written as

$$\frac{dC_B}{dt} = -K_{hyd} C_{H_2O} C_B \quad (2.3)$$

During the reaction, the amount of H_2O is assumed to be constant, because it is in excess compared to the lignocelluloses, so equation (2.3) can be simplified as

$$\frac{dC_B}{dt} = -K_{hyd} C_B \quad (2.4)$$

The reaction rate constants are influenced by the temperatures. These rate constants are then correlated by the Arrhenius equation as follows

$$K_{hyd} = A_{hyd} exp\left(\frac{-E_{hyd}}{RT}\right) \quad (2.5)$$

The activation energy and the exponential constant will be estimated by plotting the rate constants K_{hyd} (min^{-1}) against the inverse of the temperatures in degree Kelvin (^{O}K) as described by the Arrhenius equation:

$$lnK_{hyd} = lnA_{hyd} - \left(\frac{E_{hyd}}{RT}\right) \quad (2.6)$$

The yield of monosaccharide (glucose) can thus be expressed as follows:

$$Yield = \frac{Total\ amount\ of\ monosaccharide(glucose)in\ hydrolysate}{Total\ amount\ of\ cellulose\ and\ hemicellulose\ in\ sawdust\ sample} \times 100\% \quad (2.7)$$

2.3.2 Fermentation Kinetics

Microbial fermentation involves the use of micro-organisms as a biocatalyst in the fermentation process. This process is autocatalytic as the cells replicate themselves when the substrate is being decomposed and the new cells participate in the continuing process (Ekumankama *et al.*, 2001). In such a process, a selected strain of micro-organism is inoculcated into the medium containing the substrate. They feed on the cells by the aid of intracellular enzymes and secrete their byproduct into the system to help in the breakdown of substrate into assimilable nutrients. Generally, all micro-organisms (yeast) perform their activities more effectively between the pH of 3M to 6M (Daniel *et al.*, 1978) while Casida *et al.*, (1964) reported that the optimum pH for *Saccharomyces cerevisiae* (yeast) to metabolise its substrate (sugar) during fermentation is between 4.5M to 5M.

Kinetic data are needed to develop basic understanding of fermentation processes, for improvement of batch process performance and to predict a future fermentation and its application in the designing of reactors for continuous fermentation process. A better understanding of the fermentation kinetics is becoming increasingly important, although the advantages and economics of continuous fermentation have been widely reported (UnderKofler and Hickey, 2000) though it was discovered that product yields and substrate conversion were the only criteria of performance in early commercial fermentations (Ekumankama *et al.*, 2001). With introduction of improved technologies, attention was directed towards the use of time factor 'productivity', the average rate of product formation has became popular as a basis for comparison (Ekumankama *et al.*, 2001).

The rate of growth of micro-organism is given as shown in equation (2.8) (Ekumankama *et al.*, 2001)

$$r_c = \frac{dc}{dt} = \mu c \tag{2.8}$$

where c is the concentration of the organism (cell concentration g/l), t is time (hr) and μ the specific growth rate (h^{-1}).

The specific growth rate μ is related to the substrate concentration S in accordance with Monod's equation (Ekumankama *et al.*, 2001).

$$\mu = \frac{\mu_m S}{K_S + S} \tag{2.9}$$

where μ_m is the yeast maximum specific growth rate, K_s is the saturation constant and S is the concentration of growth-limiting substrate.

Then combining (2.8) and (2.9)

$$\frac{dc}{dt} = \frac{\mu_m C S}{K_s + S} \tag{2.10}$$

Separating variable and integrating

$$\ln c = \frac{\mu_m S}{K_s + S} t \tag{2.11}$$

Differentiating both sides with respect to t gives

$$\frac{d \ln c}{dt} = \frac{\mu_m S}{K_s + S} \tag{2.12}$$

Taking a reciprocal of both side gives

$$\left(\frac{d \ln c}{dt}\right)^{-1} = \frac{K_s}{\mu_m S} + \frac{1}{\mu_m} \tag{2.13}$$

A plot of $\left[\frac{d\ ln\ c}{dt}\right]^{-1}$ against $\frac{1}{S}$ gives a slop of $\frac{k_s}{\mu_m}$ and an intercept of $\frac{1}{\mu_m}$ at the ordinate.

The rate of product formation, r_p when a substrate is stoichiometrically converted to a single product, P is related to the cell growth rate as shown in equation (2.14) (Ekumankama *et al.*, 2001).

$$r_p = \frac{dp}{dt} = \propto \frac{dc}{dt} = \propto r_c \tag{2.14}$$

where $\propto$ is a stoichiometic constant. Equation (2.14) is a growth associate model which has been in use to describe the kinetics of product formation of alcohol fermentation (Ekumankama *et al.*, 2001). Thus the product yield coefficient Y_p is shown in equation (2.15)

$$Y_p = \frac{p}{s} = \frac{dp}{ds} = \frac{dp/dt}{ds/dt} \tag{2.15}$$

Equations (2.13), (2.14) and (2.15) have been in used to interpret the fermentation kinetics based on simple linear process. Several factors affect the production rate of ethanol by fermentation and a suitable mathematical description of the fermentation process has been developed in resent years, for better understanding of fermentation kinetics (Wang *et al.*, 2004). This helps in interpreting fermentation measurements with view to early detection of poor fermentation performance, the ability to predict future fermentation behavior and application to design and control of fermentation and optimization (Boulton, 1996).

Among the numerous models developed (Wang *et al.*, 2004), the majority of the models are biochemically knowledge-based models, which consist of a set of mathematical equations describing the phenomena occurring during wine fermentation. The main advantage of this type of model is that they account for biological phenomena. The model parameters with some biological significance can be obtained, but their structures may be strongly nonlinear and

complex. In the development of non-linear modeling techniques, there has been an increase in the use of sigmoidal shaped growth models in the predictive microbiology field to predict fermentation process. The details of non-linear modeling have been described by Wang *et al.*, (2004).

In general, the kinetics model for the fermentation is subdivided into a growth model, a substrate model, and a product model. There are three different equations which have been derived to describe the kinetic behavior of the concentration of yeast cells, the sugars (glucose, fructose or sucrose, respectively), and ethanol (Wang *et al.,* 2004).

There are many models describing the growth kinetics of microorganisms but the Monod's model structures were chosen. The Monod type models does not fit processes of fermentation very well in many cases, although there are many modified types (Wang *et al.*, 2004). Recently the logistic model, as a sigmoidal shaped model, has been a most popular one due to its "goodness of fit" and has been widely used in describing the growth of microorganism (www.usda.manlib.cornel.edu, May 2009). Usually, the logistic model was used to show the self-regression made by the increase of cell concentration common in batch-fermentation.

For cell (yeast) concentration, X, the logistic model was derived as follows, (Wang *et al.*, 2004).

$$\frac{dX}{dt} = \mu_m X\left(1 - \frac{X}{X_m}\right) \tag{2.16}$$

where, μ_m is the yeast maximum specific growth rate with respect to the fermentation conditions, as the form of the Monod relationship. With the following boundary conditions: $t = 0$, $X = X_0$, $S = S_0$ and $P = 0$

By integration of equation (2.16), the kinetic model can be formulated. The yeast (cell) production rate yields the following equation (the logistic equation)

$$X = \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} \tag{2.17}$$

Equation (2.17) shows the relationship between biomass and the fermentation time, which is used to fit the experimental data of yeast concentration. There are two parameters, μ_m and X_m, in this equation, and they are estimated from the experimental data by the mathematical software-MATLAB 7.0. However, a delay of ethanol production was found compared with the cell growth and little quantity of ethanol were always produced during the yeast lag growth phase (Wang *et al.*, 2004). Therefore, a parameter of the lag time, Δt, was introduced to describe the delay of ethanol production to cell growth, and the equation of ethanol production rate was modified as equation (2.18) Wang *et al.*, (2004).

$$\frac{dp}{dt} = Y_{p/x} \frac{dX}{d(t - \Delta t)} \tag{2.18}$$

This equation can be integrated using two estimated parameters from equation (2.17), μ_m and X_m, and the model is described by the equation (2.19). After the experimental data of ethanol production was fitted, two parameters in equation (2.19), the yield coefficient $Y_{p/x}$ and Δt, were estimated.

$$P = Y_{p/x} \left[\frac{X_0 X_m e^{\mu_m (t-\Delta t)}}{X_m - X_0 + X_0 e^{\mu (t-\Delta t)}} - \frac{X_0 X_m e^{-\mu_m \Delta t}}{X_m - X_0 + X_0 e^{-\mu_m \Delta t}} \right] \tag{2.19}$$

For the alcoholic fermentation process, the equation describing the substrate consumption rate takes into account two aspects, the sugar consumption in the formation of biomass and the

maintenance of biomass (www.usda.manlib.cornel.edu, May 2009). The consumption rate of sugar was described by Wang *et al.,* (2004) as shown below

$$-\frac{dS}{dt} = \frac{1}{Y_{x/s}}.\frac{dX}{dt} + \mathrm{m}.X \tag{2.20}$$

$Y_{x/s}$ is the product yield coefficient, m is the maintenance factor, combining equations (2.16), (2.18), (2.20) and the estimated parameters, the integration gives sugar consumption equation as shown in equation (2.21) (Wang *et al.,* 2004). The related parameters were estimated ($Y_{x/s}$ and m).

$$S = S_0 - \frac{1}{Y_{x/s}}\left[\frac{X_o X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} - X_0\right] - \left[\frac{X_m \mathrm{m}}{\mu_m} In \frac{X_m - X_0 + X_0 e^{\mu_m t}}{X_m}\right] \tag{2.21}$$

The initial values of X_0 and S_0 were fixed by the experimental conditions. The other parameters, such as μ_m, X_m, Δt, M and some yield coefficients can be estimated by the Newton nonlinear regression method of Polymath, Matlab, Mathcad and any other mathematical software package system using batch experimental data (Wang *et al.,* 2004). In the non-linear program, iterative search techniques, including Newton nonlinear regression method, Newton-Gauss, Marguardt, Gradient and Dud technique, are used to minimize the residual sum of squares. All the techniques gave similar results; however the Newton nonlinear regression method tends to give slight better fits as evidenced by examination of the residual sum of squares and F-value.

Literature has revealed that there area possibilities of producing ethanol from agricultural materials (biomass) which will serve as a substitute for gasoline. Meanwhile, the study of the kinetic parameters of the fermentation gives better understanding of the fermentation process.

hydrolysate fermentability, several chemicals and biological methods have been used. These methods include over liming (Martinez *et al.*, 2000), charcoal adsorption (Chandel *et al.*, 2007), ion exchange (Nilvebrant, 2001), detoxification with lactase (Martin *et al.*, 2002; Chandel *et al.*, 2007), and biological detoxification (Lopez *et al.*, 2004). The detoxification of acid hydrolysates has been shown to improve their fermentability; however, the cost is often higher than the benefits achieved (Palmqvist and Hahn- Hagerdal, 2000). Dilute acid hydrolysis is carried out in two stages- First-stage and two-stage.

a) First-stage dilute acid hydrolysis

The lignocellulosic material is first contacted with dilute sulfuric acid (0.75%) and heated to approximately 50°C followed by transferring to the first stage acid impregnator where the temperature is raised to 190°C. Approximately, 80% of the hemicellulose and 29% of cellulose are hydrolyzed in the first reactor. The hydrolysate is further incubated at a lower temperature for a residence time of 2 h to hydrolyze most of the oligosaccharides into monosaccharides followed by the separation of solid and liquid fractions. The solid material again washed with plentiful of water to maximize sugar recovery. The separated solid material is sent to second stage acid hydrolysis reactor (Chandel *et al.*, 2007).

b) Two-stage dilute acid hydrolysis

In two-stage dilute acid hydrolysis process, first, biomass is treated with dilute acid at relatively mild conditions during which the hemicelluose fraction is hydrolyzed and the second stage is normally carried out at higher temperature for depolymerisation of cellulose into glucose. The liquid phase, containing the monomeric sugars is removed between the treatments, thereby avoiding degradation of monosaccharides formed. It is very important to avoid monosaccharide degradation products for improving the ethanol yield. Sanchez *et al.*, (2004)

carried out the two-stage dilute acid hydrolysis using Bolivian straw material, *Paja brava*. In first stage, *P. brava* material was pretreated with steam followed by dilute sulfuric acid (0.5 or 1.0% by wt) hydrolysis at temperatures between 170 and 230°C for a residence time between 3 and 10 min. The highest yield of hemicellulose derived sugars were found at a temperature of 190°C, and a reaction time of 5 – 10 min, whereas in second stage hydrolysis considerably higher temperature (230 °C) was found for hydrolysis of remaining fraction of cellulose.

The two-stage dilute-acid process is usually preferred to one-stage dilute-acid hydrolysis because:

a) The separate stages for hydrolysis of the hemicelluloses and cellulose should result in higher sugar yield. Furthermore, a product with high hexose sugar content can be obtained in the second-stage hydrolysis, which can easily be fermented to ethanol. Mixtures of pentose and hexose are usually problematic for fermentation, because of the difficulty in fermentation of pentoses.
b) The energy consumption should be minimized, since liquid is removed before the second-stage hydrolysis.
c) The resulting sugar solution should be more concentrated.
d) Less sugar degradation from the hydrolyzed materials in the first stage leads to a higher overall yield of sugars.
e) Fewer fermentation-inhibiting components are formed during the two-stage hydrolysis.

Generally, the maximum yield of pentoses and hexoses recovered from hemicelluloses in the first stage of the hydrolysis is high (i.e. 80-95% of the total sugars available), while the yield of cellulose hydrolysis to glucose is usually low (e.g. 40-60%). However, a low yield of glucose may not be considered as a serious problem because of the low price of lignocellulosic materials,

as well as the possibility of drying and burning the residual cellulose and lignin in an energy complex to produce electricity and heat (Sanchez *et al.*, 2004).

ii. Concentrated acid hydrolysis

This method uses concentrated sulfuric acid followed by a dilution with water to dissolve conversion of cellulose to glucose and hemicellulose to xylose with a little degradation. The concentrated acid process uses 70% sulfuric acid at 40 - 50°C for 2 to 4 h in a reactor. The low temperatures and pressure will lead to minimize the sugar degradation. The hydrolyzed material is then washed to recover the sugars.

In the next step, the cellulosic fraction has to be deploymerized. The solid residue from first stage is de-watered and soaked in 30 - 40% sulfuric acid for 50 min. at 100°C for further cellulose hydrolysis. The resulting slurry mixture is pressed to obtain second acid–sugar stream (approximately 18% sugar and 30% acid). Both the sugar steams from two hydrolysis steps are combined and may be used for subsequent ethanol production. Chandel *et al.*, (2007) reported on the concentrated acid hydrolysis of mixed wood chips and found that maximum sugar recovery (78 - 82% of theoretical yields) was achieved at sulfuric acid concentration (26%) for 2 h of residence time.

The primary advantage of the concentrated acid process is the potential for high sugar recovery efficiency, about 90% of both hemicellulose and cellulose fraction gets depolymerized into their monomeric fractions. The acid and sugar syrup are separated via ion exchange and then acid is reconcentrated through multiple effect evaporators. The remaining lignin rich solids are collected and optionally palletized for fuel generation (Chandel *et al.*, 2007).

Table 2.1 Comparison between Concentrated acid and Dilute acid Hydrolysis (Taherzadeh and Karimi, 2007)

Hydrolysis method	Advantages	Disadvantages
Concentrated-acid process	-Operated at low temperature -High sugar yield	-High acid consumption -Equipment corrosion -High energy consumption for acid recovery -Long reaction time (e.g. 2-6 h)
Dilute-acid process	-Low acid consumption -Short residence time	-Operated at high temperature -Low sugar yield -Equipment corrosion -Formation of undesirable by-products

2.1.3.2.2 Enzymatic hydrolysis

The acid, alkaline or fungal pretreated lignocellulosics can be saccharified enzymatically to get fermentable sugars (Itoh *et al.*, 2003). Bacteria and fungi are the good sources of cellulases, hemicellulases that could be used for the hydrolysis of pretreated lignocellulosics. The enzymatic cocktails are usually mixtures of several hydrolytic enzymes comprising of cellulases, xylanases, hemicellulases and mannanases. In the last decade, new cellulases and hemicellulases from bacterial and fungal sources have continued to be isolated and regular efforts have been made for the improved production of enzymetic titers (Aro *et al.*, 2005). However, the cellulases were produced at a concentration too low to be useful. There is a group of microorganisms (*Clostridium*, *Cellulomonas*, *Tricho-derma*, *Penicillium*, *Neurospora*, *Fusarium*, *Aspergillus* etc.) showing a high cellulolytic and hemicellulolytic activity, which are also highly capable of fermenting monosaccharides. Genetic engineering is used to produce super strains, which are capable of hydrolyzing cellulose and xylan along with fermentation of glucose and xylose to

ethanol (Lin and Tanaka, 2006). The utilization of cellulose by microorganisms involves a substantial set of fundamental phenomena beyond those associated with enzymatic hydrolysis of cellulose (Lynd *et al.*, 2002).

2.1.3.2.3 Factors that Influence Hydrolysis of Lignocellulosic Materials

Lignocellulosic material and hydrolysis processes are very complicated. Factors influencing the yield of the lignocelluloses to the monomeric sugars and the by-products are:

(a) Properties of the Substrate

The properties of the substrate can affect the hydrolysis. These properties are: neutralizing capacity, proportion of easily hydrolysable hemicelluloses and cellulose, amount and rate of hydrolysis of the difficult-to-hydrolyze materials, the length of the macromolecules, degree of polymerization of cellulose, configuration of the cellulose chain, and association of cellulose with other protective polymeric structures within the plant cell wall such as lignin, pectin, hemicelluloses, proteins, mineral elements, etc. Particle size is also one of the factors (Taherzadeh *et al.*, 2007).

(b) The Acidity of the System

Another parameter affecting the hydrolysis is the acidity system. The acidity is dependent on the type and concentration of the acid used, amount of acid (e.g. acetic acid) released from the biomass during hydrolysis, liquid to solid ratio, the neutralizing capacity of the lgnocellulose, and movement of the solution during heating. When dilute-acid hydrolysis is applied in a continuous process such as a screw-fed co-current reactor, it requires a relatively short residence time. Therefore, the penetration of acid catalyst into the biomass, as well as dispersion in the reactor, can significantly affect the overall reaction, and consequently the reactor performance. Diffusivity of sulfuric acid is dependent on the nature of the lignocellulosic materials. It has been

shown that the diffusivity of sulfuric acid is significantly higher in agricultural residues than in hardwood (Kim and Lee, 2003).

(c) Rate of Decomposition of Hydrolysis Products during Hydrolysis

The rate of decomposition of the products during the hydrolysis process depends on temperature, reaction time, and the concentration of sugars. Under hydrolysis conditions that produce a solution containing in excess of 10 percent glucose, reversion phenomena are suggested to be very important. The reversion phenomena result in much of the glucose being present not as free glucose but as dimmers, oligomers, and anhydrosugars which are unavailable to the microorganisms used in fermentation (Harris *et al.*, 1984). It was recently reported that metals ions can also catalyze glucose decomposition during the acid hydrolysis of lignocelluloses materials. Thus, the material used in the construction of the hydrolysis reactor should also be carefully selected (Taherzadeh and Karimi, 2007).

2.1.3.3 Fermentation of Hydrolysates

Fermentation of the lignocellulosic hydrolysates is more difficult than the well-established processes of ethanol production from sugar-cane juice or grains. Hydrolysates contain a broader range of inhibitory compounds, whose composition and concentration depend on the type of lignocellulosic materials and on the chemistry and nature of the pretreatment and hydrolysis processes. Secondly, the hydrolysates of hemicelluloses contain not only hexoses but also pentoses, where xylose is the dominant sugar in the hydrolysates from hardwood hemicelluloses (Nigam, 2001). Therefore, the fermenting microorganism should be able to produce ethanol from the hydrolysates with a high yield and productivity, withstand potential inhibitors, and produce ethanol from pentoses, as well as being safe for humans. Baker's yeast (*saccharomyces cerevisiae*) is the most commercially used microorganism for ethanol production, but it cannot

ferment xylose (Taherzadeh and Karimi, 2007; Jeffries and Jin, 2006). A large number of yeasts, bacteria, and filamentous fungi are reported to produce ethanol as the main fermentation product, and have been reviewed (Jeffries and Jin, 2006; Lin and Tanaka 2006).

Bioconversion of various biomass sources into ethanol by different microorganisms has been summarized in Table 2.2. The sugar syrup obtained after cellulosic hydrolysis is used for ethanol fermentation. The ability to ferment pentoses along with hexoses is not widespread among microorganisms; *S. cereviseae* is capable of converting only hexose sugars to ethanol. The most promising yeasts that have the ability to use both C5 and C6 sugars are *Pichia stipitis*, *Candida shehatae* and *Pachysolan tannophilus*. However, ethanol production from sugars derived from starch and sucrose has been commercially dominated by the yeast *S. cereviseae* (Lin and Tanaka, 2006). Thermotolerant yeast could be more suitable for ethanol production at industrial level. In high temperature process energy savings can be achieved through a reduction in cooling costs. Considering this approach, Sree *et al.*, (2000) developed solid state fermentation system for ethanol production from sweet sorghum and potato employing a thermotolerant *S. cereviseae* strain (VS3).

Researches are now focusing on developing recombinant yeast, which can greatly improve the ethanol production yield by metabolizing all forms of sugars, and reduce the cost of operation. In this contention the researchers have made efforts by following two approaches. The first approach has been to genetically modify the yeast and other natural ethanologens additional pentose metabolic pathways. The second approach is to improve ethanol yields by genetic engineering in microorganisms that have the ability to ferment both hexoses and pentoses (Jeffries and Jin, 2000; Dien *et al.*, 2003). Jeffries and Jin, (2006) compiled the recent developments that took place towards the genetic engineering of yeast metabolism and

concluded that strain selection through mutagenesis, adaptive evolution using quantitative metabolism models may help to further improve their ethanol production rates with increased productivities. Chandel *et al.*, (2007) showed the recent developments in comparative genomics and bioinformatics to elucidate the high ethanol production mechanism from *Saccharomyces* sp.

Though new technologies have greatly improved bioethanol production yet there are still a lot of problems that have to be solved. The major problems include maintaining a stable performance of genetically engineered yeast in commercial scale fermentation operation (Taherzadah and Karimi, 2007), developing more efficient pre-treatment technologies for lignocellulosic biomass, and integrating optimal component into economic ethanol production system. Sridhar and co-workers (2002) made an effort to improve the thermo tolerance of yeast isolates by treating them with UV radiation.

Fermentation can be performed as a batch, fed batch or continuous process. The choice of most suitable process will depend upon the kinetic properties of microorganisms and type of lignocellulosic hydrolysate in addition to process economic aspects.

i. Batch fermentation

Traditionally, ethanol has been produced batch wise. At present, nearly, all of the fermentation ethanol industry uses the batch mode. In batch fermentation, the microorganism works in high substrate concentration initially and a high product concentration finally. The batch process is a multi-vessel process, allows flexible operation and easy control over the process. Generally batch fermentation is characterized by low productivity with an intensive labour (Shama, 1988). For batch fermentation, elaborate preparatory procedures are needed and because of the discontinuous start up and shut down operations, high labour costs are incurred.

This inherent disadvantage and the low productivity offered by the batch process has led many commercial operators to consider other fermentation methods.

ii. Fed batch fermentation

In fed batch fermentation, the microorganism works at low substrate concentration with an increasing ethanol concentration during the course of fermentation process. Fed batch cultures often provide better yield and productivities than batch cultures for the production of microbial metabolites. For practical reasons, therefore, some continuous operations have been replaced by fed batch process. Keeping the low feed rate of substrate solution containing high concentration of fermentation inhibitors such as furfural, hydroxymethyl furfural and phenolics, the inhibitory effect of these compounds to yeast can be reduced. Complete fermentation of an acid hydrolysate of spruce, which was strongly inhibiting in batch fermentation, has been achieved without any detoxification treatment (Taherzadeh, 1999). The productivity in fed batch fermentation is limited by the feed rate which, in turn, is limited by the cell mass concentration. The specific ethanol productivity has also been reported to decrease with increasing cell mass concentration (Palmqvist *et al.*, 2000). Ideally, the cell density should be kept at a level providing maximum ethanol productivity and yield.

iii. Continuous fermentation

Continuous fermentation can be performed in different kind of bioreactors – stirred tank reactors (single or series) or plug flow reactors. Continuous fermentation often gives a higher productivity than batch fermentation, but at low dilution rates which offers the highest productivities. Alexender *et al.*, (1989) studied the effect of shift in temperature and aeration in steady state continuous culture of *C. shehatae* to determine the effects of ethanol on xylose metabolism. The accumulation of ethanol exerted a delayed inhibitory effect on the specific rate

of substrate utilization. Continuous operation offers ease of control and is less labor intensive than batch operation. However contamination is more serious in this operation. Since the process must be interrupted, all the equipments must be cleaned, and the operation started again with the growth of new inoculum. The continuous process eliminates much of the unproductive time associated with cleaning, recharging, adjustment of media and sterilization. A high cell density of microbes in the continuous fermenter is locked in the exponential phase, which allows high productivity and overall short processing of 4 - 6 h as compared to the conventional batch fermentation (24 - 60 h). This results in substantial savings in labour and minimizes investment costs by achieving a given production level with a much smaller plant. It should be noted that during the course of the fermentation process, immobilized cells are preferred to use.

2.1.3.3.1 Immobilized cells

A limitation to continuous fermentation is the difficulty of maintaining high cell concentration in the fermenter. The use of immobilized cells circumvents this difficulty. Immobilization by adhesion to a surface (electrostatic or covalent), entrapment in polymeric matrices or retention by membranes has been successful for ethanol production from hexoses. The applications of immobilized cells have made a significant advance in fuel ethanol production technology. Immobilized cells offer rapid fermentation rates with high productivity – that is, large fermenter volumes of mash put through per day, without risk of cell washout. In continuous fermentation, the direct immobilization of intact cells helps to retain cells during transfer of broth into collecting vessel. Moreover, the loss of intracellular enzyme activity can be kept to a minimum level by avoiding the removal of cells from downstream products (Najafpour, 1990). Immobilization of microbial cells for fermentation has been developed to eliminate inhibition caused by high concentration of substrate and product and also to enhance ethanol productivity

and yield. Abbi *et al.*, (1996) observed that the rate of sugar consumption by immobilized cells of *C. shehatae* NCL-3501 was slightly lower than that of free cells, thus leading to higher ethanol production. When microorganisms are attached to solid supports, fluid viscosity is lower which contributes to better mixing and mass transfer in the system. The work on ethanol production in an immobilized cell reactor (ICR) showed that ethanol production using *Z. mobilis* was doubled. Yamada *et al.*, (2002) successfully used recombinant *Z. mobilis* with high sugar concentration (12-15%) and further observed the significant role of increased biomass concentration in bioreactor performance for the improved ethanol production. A repeated batch fermentation system was used to produce ethanol using an immobilized osmotolerant *S. cereviseae*, in which ethanol concentration as high as 93 g/l was recorded at 200 g/l glucose concentration (Sree *et al.*, 2000). Also Nigam, (2000) reported that the ethanol production rate as high as 42.8 g/l/h was achieved from the fermentation of pineapple canary derived sugars by *S. cereviseae* ATCC 24553.

2.1.3.4 Product Separation/Distillation

The fermentation broth (mash or beer) is a mixture of ethanol, water, cell mass, fuel oil, and other components available in the fermentation media such as residual sugars, nonfermentable sugars, and hydrolysis by-products. The concentration of ethanol produced from dilute-acid hydrolysate is typically low (e.g. 10-35 g/l), since the total concentration of sugar is usually low (e.g. 20-80 g/l). Furthermore, it is difficult to work with highly concentrated hydrolysates, since the concentration of inhibitors will increase by concentrating the hydrolysates. On the other hand, if one could get rid this problem, then the ethanol concentration in mash would be generally up to 10%. The cells can tolerate this level of ethanol concentration at 30°C, but their tolerance decreases with increasing temperature (Hamelinck *et al.*, 2005).

Ethanol can be purified by distillation to a concentration just below its azeotropic point, i.e 95%, which will be called "hydrated ethanol". However, the solid particles and fermenting microorganisms should be separated before distillation, by e.g. centrifuges or decanters. On the other hand, if flocculant yeast is used, then the natural capacity of these microorganisms for aggregation and flocculation can eliminate the need for separation of biomass and reduce the investment costs. Hydrated ethanol can be employed in high-ethanol-content fuel (e.g. E95). However, for mixing of the ethanol with gasoline, the ethanol should contain no more than 1% of water (anhydrous ethanol). Molecular-sieve technology is the common method for production of anhydrous ethanol (Hamelinck *et al.*, 2005).

2.1.3.4.1 Residual Solids and Wastewater Treatment

The main solid residual from the process is lignin. Its amount and quantity depend on the feedstock used and the process applied. The lignin and remaining solid materials can be burned to produce steam for the process (hydrolysis, distillation, and evaporation), electricity, and perhaps central heat (Hamelinck *et al.*, 2005). This is possible due to the high energy value of the lignin that is released during its combustion (Cardona and Sanchez, 2007). However, it can also be processed through gasification and Fischer-Tropsch process to produce synthesis gas and hydrocarbon fuel additives. Lignin can replace phenol in the widely used phenol formaldehyde resins, although the production costs and market value of these products are prohibitive.

Table 2.2 Various raw materials for ethanol production and process conditions (Chandel *et al.*, 2007)

Raw Material	Pretreatment and Saccharification	Fermentation conditions	Microorganism	Reference
Sugarcane baggase	Dilute acid hydrolysis	Batch	*C. shehatae* NCIM3501	Chandel et al. 2006b
Wheat straw	Dilute acid, Enzymatic hydrolysis	SSF, SHF	*E. coli FBR5*	Saha et al. 2005
Rice straw	Auto hydrolysis	Batch	*C. shehatae* NCIM3501	Abbi et al. 1996
Sorghum straw	Steam explosion, enzymatic	SSF	*Kluyveromyces marxianus* CECT10875	Ballesteros et al. 2004
Corn stover	Steam, enzymatic	Fed-batch	*S. cerevisiae* TMB3400	Ohgren et al. 2006
Barley husk	Steam, enzymatic	SSF	*S. cerevisiae*	Palmarola et al, 2005
Sun flower stalk	Steam, enzymatic	Batch	*S. cerevisiae var ellipsoideus*	Sharma et al. 2002
Sugarcane leaves	Alkaline H202	SSF	*S. cerevisiae* NRRL-Y-132	Krishna et al. 2001
Wheat bran	Dilute acid, Enzymatic hydrolysis	Batch	*S. cerevisiae*	Palmarola et al.2005
Groundnut shell	Acid hydrolysis	Batch	*S. cerevisiae*	Akpan et al. 2005
Alfalfa fibres	Liquid hot water	SSF, SHF	*C. shehatae* FPL-702	Sreenath et al. 2001
Aspen	Acid hydrolysis	Continuous, Immobilized cell	*P. stipitis* R	Parekh et al. 1987
Saw dust	Acid hydrolysis	Batch, Continuous upflow reactors	*Clostridium thermosaccharolyticum* ATCC31925	Liu et al. 1988
Pine	Acid hydrolysis	Continuous stirred tank reactor, Immobilized cells	*P. stipitis* NRRL-1724	Qureshi et al. 1991
Poplar	Steam explosion, Enzymatic	SSF, SHF	*S. cerevisiae*	Cantarella et al. 2004
Birch	Acid hydrolysate	Batch	*S. cerevisiae*	Johanssen et al. 2001
Spruce	Dilute acid hydrolysis	Fed batch	*S. cerevisiae*	Taherzadeh,1999
Willow	Steam	Batch	*E. coli* K011	Olsson et al. 1995
Paja brava	Dilute acid hydrolysis, Two stage	Batch	*C. shehatae, P. stipitis, Pachylen tannophilus*	Sanchez et al. 2004
Eicchornia crassipes	Dilute acid hydrolysis	Batch	*P. stipitis*	Nigam, 2002
Saccharum spontaneum	Dilute acid hydrolysis, Enzymatic	Batch,Fed batch	*P. stipitis* NCIM 3498	Gupta, 2006
Cassava starch	Starch liquifaction	Batch, Continuous co-immobilized cells	*S. diastaticus zymomonas mobilis*	Amutha anc Gunashekhran, 2001
Apple pomace		SSF	*S. cerevisiae* ATCC 24702	Ngadi and Correial, 1992 Nigam, 2000
Pineapple canary waste	Juice Extraction	Continuous, Immobilized	*S. cerevisiae* ARCC 24553	

Banana pulp waste	Juice Extraction	cells Continuous, Cell recycles	*S. uvarum* NCIM *culture* 3528	Joshi et al. 2001
Finger millet (Eleusine corcana flour)		High gravity fermentation	*S. cerevisiae*	Reddy and Reddy, 2006
Municipal solid waste (MSW)	Acid pretreatment	Batch	*S. cerevisiae*	Mtui and Nakamur, 2005
News print	Acid hydrolysate	Batch	*E. coli* B(pLO1297)	Lawford and Roussea 1993
Industerial waste		SSF	*K. marxianus, S.cerevisiae*	Kadar et al. 2004

2.1.4 Economics of ethanol Production

The estimated cost of producing ethanol from wood varies widely between different investigations, with production costs in the range US$ 0.32–1.0/l ethanol (Galbe *et al.*, 2002). The lower costs are usually obtained for rather large plant capacities – above 600,000 metric tons of dry raw material per year. Most cost analyses are performed for hardwoods although some are for softwoods (Galbe *et al.*, 2002; Kadam *et al.*, 2000). The cost of raw material is a major contributor (about 25–40%) to the total production cost, although it varies between different studies due to differences in the local price of raw material (US$ 22–61/metric ton dry matter). Most cost estimations are based on lab-scale and, to some extent, pilot-scale data for individual process steps and should be treated with caution and not used to obtain an absolute production cost. The cost estimations are useful, though, for identification of bottlenecks and to compare the relative costs of different process strategies and the effect of changes in process configurations, e.g. plant capacity, ethanol yield, energy efficiency, etc. As shown in the production cost for the base case without recirculation of any process streams was SEK 4.30/l ethanol. This was based on an income corresponding to about SEK 1.0/l for solid fuel as by-product. A breakdown of the production cost into various process parts showed that the raw material constituted about 30%,

distillation and evaporation about 30% and SSF about 25%. Half the cost for the SSF was due to cost of the cellulase enzymes, which is highly variable. The high cost for the distillation and evaporation steps is due to the low concentration of suspended solids in the SSF (5% dry matter used in the base case), which results in an ethanol concentration in the distillation of around 2 wt%. An interesting possibility that may reduce the production cost is to integrate ethanol production with a combined heat and power plant or with a pulp and paper mill. In 1994, the environmental consulting company ÅF-IPK AB performed a feasibility study on integration of an ethanol plant with a pulp mill, a combined power and heating plant and a sawmill with production of solid fuels (Ångpanneföreningen-IPK, 1994). The result showed that the integration could reduce the ethanol production cost by up to 20%. A similar study (Kadam *et al.*, 2000) was recently performed for co-production of ethanol and electricity from softwood, based on the conditions in California. Their study also showed that co-production is more favourable than stand-alone production of ethanol.

To be competitive, and find economic acceptance, the cost for bioconversion of biomass to liquid fuel must be lower than the current gasoline prices (Chandel *et al.*, 2007). It seems however; now much more attainable because of increasing efforts of researchers working towards improvisation in the efficiency of biomass conversion technologies. However, there is still huge scope to bring down the cost of biomass-to-ethanol conversion. The cost of feedstock and cellulolytic enzymes are two important parameters for low cost ethanol production. Biomass feedstock cost represents around 40% of the ethanol production cost (Hamelinck *et al.*, 2005). An important factor for reducing the cost of bioethanol production is to use larger industrial facilities rather than smaller ones. Chandel *et al.*, (2007) also suggested the integrated approach (Process engineering, fermentation, enzyme and metabolic engineering) could improve the

ethanol production economics. By increasing the plant size, the investment per unit output of product falls off, a ten-fold increase in size reducing the unit cost to less than one-half and thereby reducing unit capital cost charges and conversion cost reducing profitability. To further improve the economy of ethanol production, energy integration of the ethanol production, to already existing plants such as pulp and paper plants is necessary. O'Boyle *et al.*, (1991) reported that the cost of producing ethanol from pine with a diluted acid hydrolysate process was estimated to be 3.22 SEK L-1 in a stand alone plant in comparison to 2.54 SEK L-1 with an integrated plant. It was projected from the study that the cost of bioethanol can be reduced from US$ 1.22 per liter to about US $ 0.31 per liter on the basis of continuous improvement in pretreatment of biomass, enzyme application and fermentation.

2.1.5 Ethanol and Environment

Ethanol represents closed carbon dioxide cycle because after burning of ethanol, the released carbon dioxide is recycled back into plant material because plants use CO_2 to synthesize cellulose during photosynthesis cycle (Chandel *et al.*, 2007). Ethanol production process only uses energy from renewable energy sources; no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source. In addition, the toxicity of the exhaust emissions from ethanol is lower than that of petroleum sources. Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect (Foody, 1988).

As energy demand increases the global supply of fossil fuels cause harm to human health and contributes to the green house gas (GHG) emission. In a study by Chandel *et al.*, (2007), it was observed that alarmed to the security of oil supply and the negative impact of the fossil fuel on the environment, particularly on GHG emissions could be disastrous. The reduction of GHG

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials and Reagents

Table 3.1 Materials/Reagents used during experiment

Materials/Reagents	Grade/Manufacturer
Sulphuric acid	BDH Chemical Ltd Poole England- General purpose reagent
Dinitrosalicylic reagent	DNS reagent- BDH Chemical Ltd Poole England
Potassium iodide	Laboratory BURGOYNE Reagent
Distilled water	Biochemistry Lab, FUT, Minna, Niger State
Sodium hydroxide	BDH Chemical Ltd Poole England
Diethyl ether	SIGMA-ALDRICH USA

3.1.1 Sawdust

The sawdust sample used was collected from Maitunbi sawmill, Minna, Niger State.

3.1.2 Equipment

Table 3.2 Equipment used during experiment

Equipment	Specification	Manufacturer
Water bath	B$T	A SEARLE Company England
pH meter	CRISON-MicropH 2000	Spain
Spectrophotometer	JENWAY 6305	England
Digital Incubator	E8SEX	Baird and Tat lock London
Alcohol Distillation Apparatus		England
Glass vessel	10litres	Germany
Magnetic heating stirrer	78HW-1	B.Bran Scientific and Instrument Company England
Digital weighing balance	Brainwieght B300	OHAUS SCALE CORP.USA
Autoclave	YX-2803, volume 18L	England
Oven	ISOTEM 175	FISHER UK

Other apparatus and equipment are listed thus: Sieves, pipette, beakers, measuring cylinder, conical flasks, volumetric flasks, test tubes, filter cloth, filter paper, thermometer, stirring rod, stop watch and spatula.

3.2 Experimental Procedure

The production of ethanol from sawdust involves four experimental stages. This includes: The isolation of cellulose, hydrolysis of the cellulose, fermentation of the hydrolysate and the distillation of the fermented product (ethanol).

3.2.1 Isolation of Cellulose

3.2.1.1 *Milling*

The sawdust sample was milled and size reduction was carried out with 250μm sieve to get a homogeneous size. The size reduction was to effect efficient acid accessibility to the cellulose structure, increase the effective contact area for reaction.

3.2.1.2 *Removal of extractives*

Waxes, tannins, oil and fat resin were removed using diethyl ether. Ten gram of sawdust sample was treated with 20ml of diethyl ether in a 500ml beaker for two hours. The resulting residues were washed thoroughly with distilled water, filtered and dried (Akpan *et al.*, 2005).

3.2.1.3 *Lignin isolation*

Twenty milliliter of 14M H_2SO_4 was added to the washed residue and stirred to isolate the lignin. The hemicelluloses and cellulose were dissolved leaving lignin as a hard precipitate. The mixture was filtered and the filtrate was treated with 25ml of 0.1M sodium hydroxide, which precipitate cellulose. The precipitate was washed thoroughly with distilled water, filtered and dried (Akpan *et al.*, 2005).

3.2.2 Acid Hydrolysis of Cellulose

The effect of different concentrations of sulphuric acid on the hydrolysis of cellulose to glucose were determined by hydrolyzing the isolated cellulose from sawdust sample with different acid concentrations of 1.5M, 2.5M, 3.5M, 4.5M, and 6M. Fifty milliliters each of different acid concentrations was added to different isolated cellulose obtained from sawdust in conical flasks and they were put in a water bath at a controlled temperature of 50°C for three hours. This process was also repeated for temperatures of 60°C and 70°C so as to determine the best acid concentration for the hydrolysis. At thirty minutes intervals, some quantities of the

hydrolyzed sample were withdrawn and filtered. The resulting filtrates were analyzed for the glucose (sugar) using Spectrophotometer with a wave length of 540nm. The concentrations of the glucose were read from the standard calibrated curve for sugar prepared using the same wave length and they were recorded.

Fifty milliliters of the optimum acid concentration was added to another five different sets of isolated cellulose from the sawdust sample in 250ml conical flasks and the flasks were put in a water bath separately, at different controlled temperatures of 40, 50, 60, 70 and 80°C. This was to determine the effect of temperature on the acid hydrolysis and the results were recorded. The whole processes were repeated with temperature that has the optimum yield of sugar and the resulting hydrolysate (fermentation substrate) was stored for fermentation process (Akpan *et al.*, 2005).

3.2.2.1 Preparation of Standard Calibrated Curve for the Determination of Glucose Concentration

One hundred and fifty milligram of the standard D-glucose was dissolved in 100ml of distilled water. This was designated the standard D-glucose solution. The solution of 0.25, 0.5, 0.1, 1.25, and 1.5mg glucose per ml by dilution of the stock glucose solution was prepared using distilled water (Ceirwyn, 1998).

3.2.2.2 Preparation of DNS Reagent

One gram of DNS (Dinitrosalicylic acid) was dissolved in 20ml of 2M NaOH with warming and vigorous stirring. Ten grams of sodium potassium tartrate was dissolved in 50ml of distilled water (colour stabilizer). The two solutions were mixed together (Ceirwyn, 1998).

3.2.3 Preparation of media culture for *S. cerevisia* and *Z. mobilis*

Saccharomyces cerevisia was isolated from tiger-nut water with SDA (Sabouraud dextrose agar) medium and was sub-cultured many times in slant bottles to get a pure isolate. This was further inoculated into PDB (potato dextrose broth) medium and allowed to grow for two days. It was then stored in the refrigerator for further used (Ogundana, 1989).

Zymomonas mobilis was isolated from palm wine using standard solid medium which was sub-cultured many times in slant bottles. The media constituents include 50g of yeast extract, 20g of agar, 20g of glucose and 1litre of distilled water with pH 6.8. The medium was treated with antidione to inhibit *Z. mobilis* growth before autoclaving at 121^0C for 15minutes. The *Z. mobilis* was further inoculated anaerobically at 37^0C for 24hours. This was stored in the refrigerator for further use (Ogundana, 1989; Obire, 2005).

3.2.4 Fermentation of Hydrolysate

Two drops of concentrated antifoam was added to the Substrate medium and poured into the reactor. The medium was sterilized by heating up to 90^0C for 1.5hr and cooled to room temperature. The fermenter was also sterilized. Twenty five milliliter of the isolated medium culture (*S. cerevisia*) was introduced into the fermentable substrate. The magnetic stirrer was hooked to the top of the reactor and lid to the reactor was secured with cotton wool. The magnetic stirrer rate was set at 500-600rpm while the temperature was maintained at 25^0C. This process was repeated using *Z. mobilis.* Samples were withdrawn at intervals of one hour with a syringe and the concentrations of sugar, alcohol and biomass were analyzed for the first four hours. Then, the withdrawals were done for 24hours for the subsequent 5days. 2ml of the sample was put into the cuvette and the biomass concentrations were analyzed using the Spectrophotometer at the wavelength of 630nm with water as blank. Ethanol in the sample was

analyszed at wavelength of 540nm using the standard curve prepared for ethanol concentration (Wang *et al.*, 2004; Akpan *et al.*, 2005).

3.2.5 Distillation of Bioethanol

The ethanol was obtained at 78.3^0C using alcohol distillation apparatus. The set-up includes, the round bottom flask containing the fermented sample. This was placed in the heating mantle and the mouth fixed to the condenser. A collection flask was placed at the end of the set-up. Rubber pipes or hose were connected to the condenser to supply water from the tap for cooling the condenser and letting water out of the condenser simultaneously. As the vapor condenses, the condensed vapor was collected into the collection flask at the other end of the distillation set-up as the distillate (ethanol).

The distillate was further purified by the use of lime (calcium oxide). Lime, a basic oxide, when added to the ethanol, absorbed the water to form calcium hydroxide, an alkaline solution. This was separated from ethanol by further distillation which leaves absolute ethanol (Akpan *et al.*, 2005).

CHAPTER FOUR

4.0 EXPERIMENTAL RESULTS

4.1 Results

The experimental results obtained and their analyses are presented in this chapter. This involves the effect of acid concentrations on the hydrolysis, effect of temperatures on the hydrolysis, effects of two different microorganisms on the fermentation performance and the kinetic parameters estimated.

Table 4.1: Result of Proximate analysis of sawdust sample

Component	Content (%, w)
Moisture	5.8
Ash	6.5
Crude fibre	50.3
Carbohydrate	25.0
Vitamin C	0
Fat	11.5
Protein	0.86

Figure 4.1 shows the concentration of glucose from hydrolysis with time at different acid concentrations but at a constant temperature of 50°C. The glucose yields at this temperature (50°C) increase with time at different acid concentrations from 1.5M to 6M for one hundred and eighty minute.

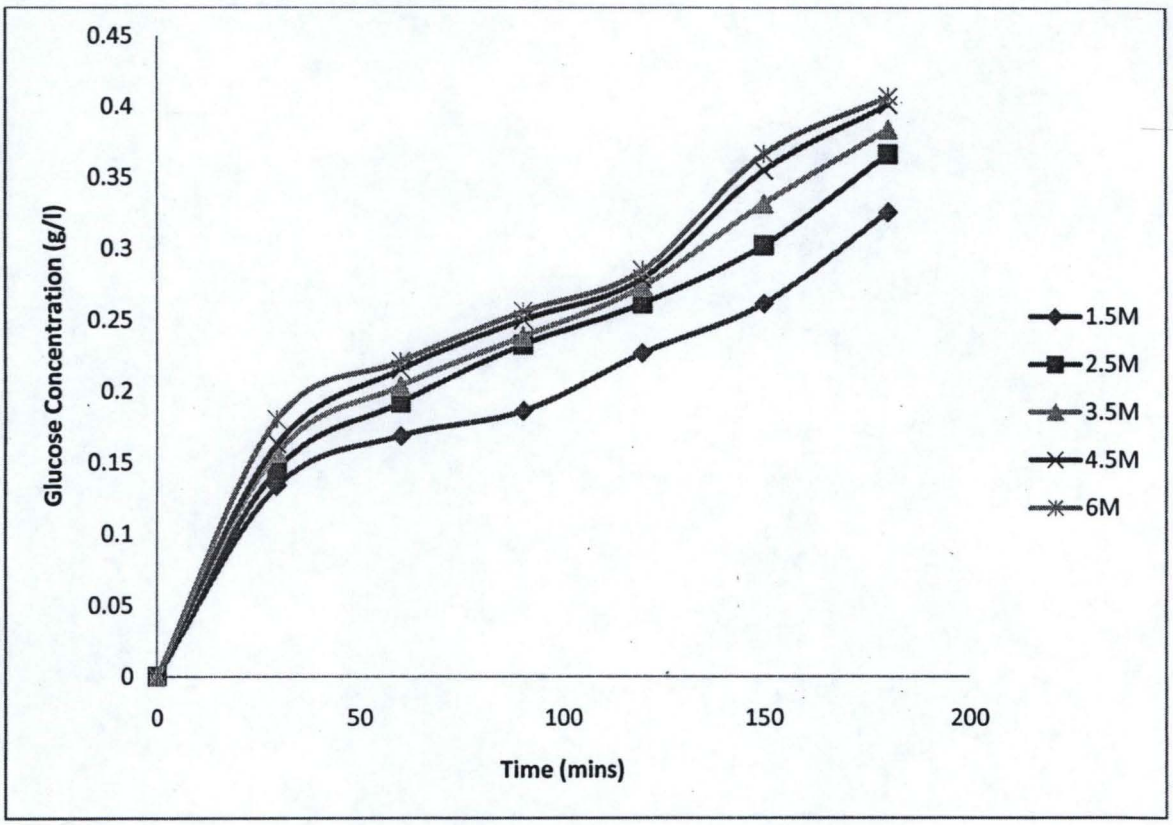

Figure 4.1: Effect of different acid concentrations on the hydrolysis at 50°C

Figure 4.2 shows the concentration of glucose from hydrolysis with time at different acid concentrations but at a constant temperature of 60°C. The glucose yields at this temperature (60°C) increase with time at different acid concentrations from 1.5M to 6M for one hundred and eighty minute.

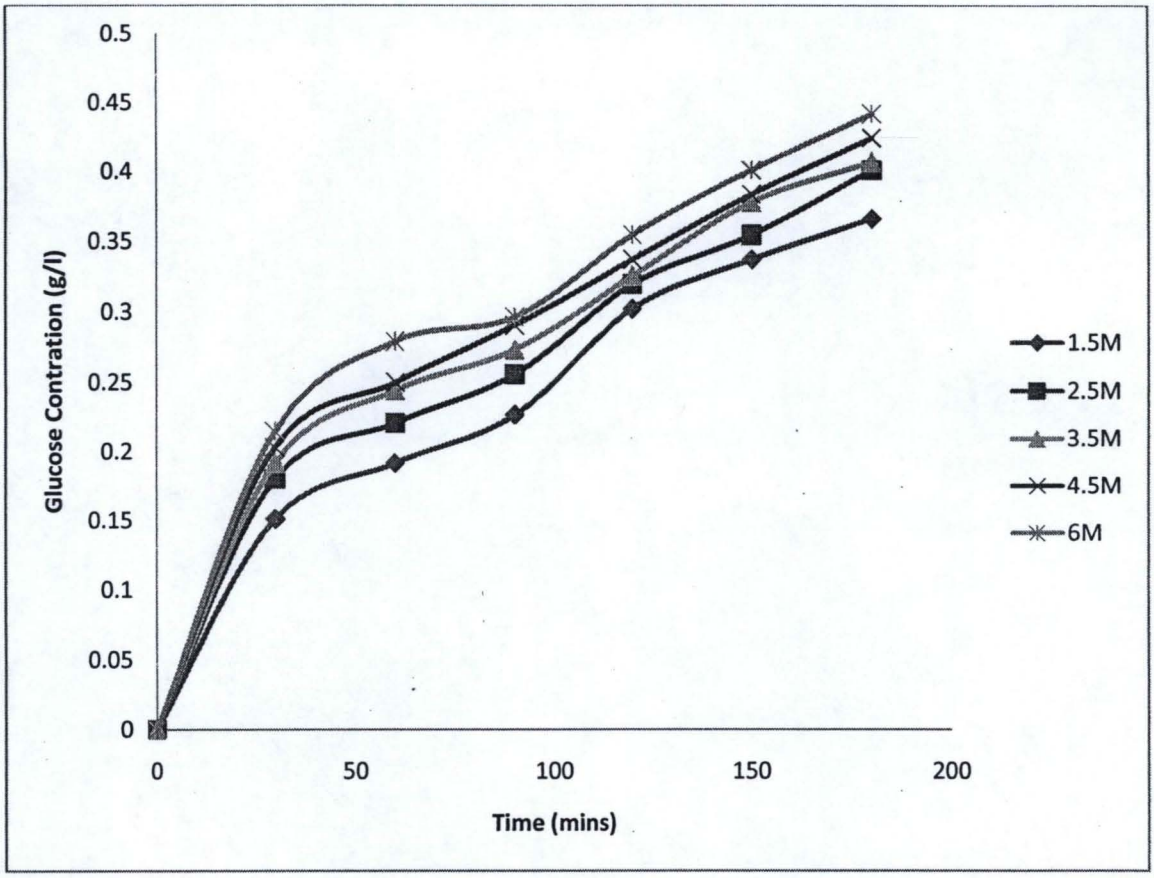

Figure 4.2: Effect of different acid concentrations on the hydrolysis at 60°C

Figure 4.3 shows the concentration of glucose from hydrolysis with time at different acid concentrations but at a constant temperature of 70°C. The glucose yields at this temperature (70°C) increase with time at different acid concentrations from 1.5M to 6M for one hundred and eighty minute.

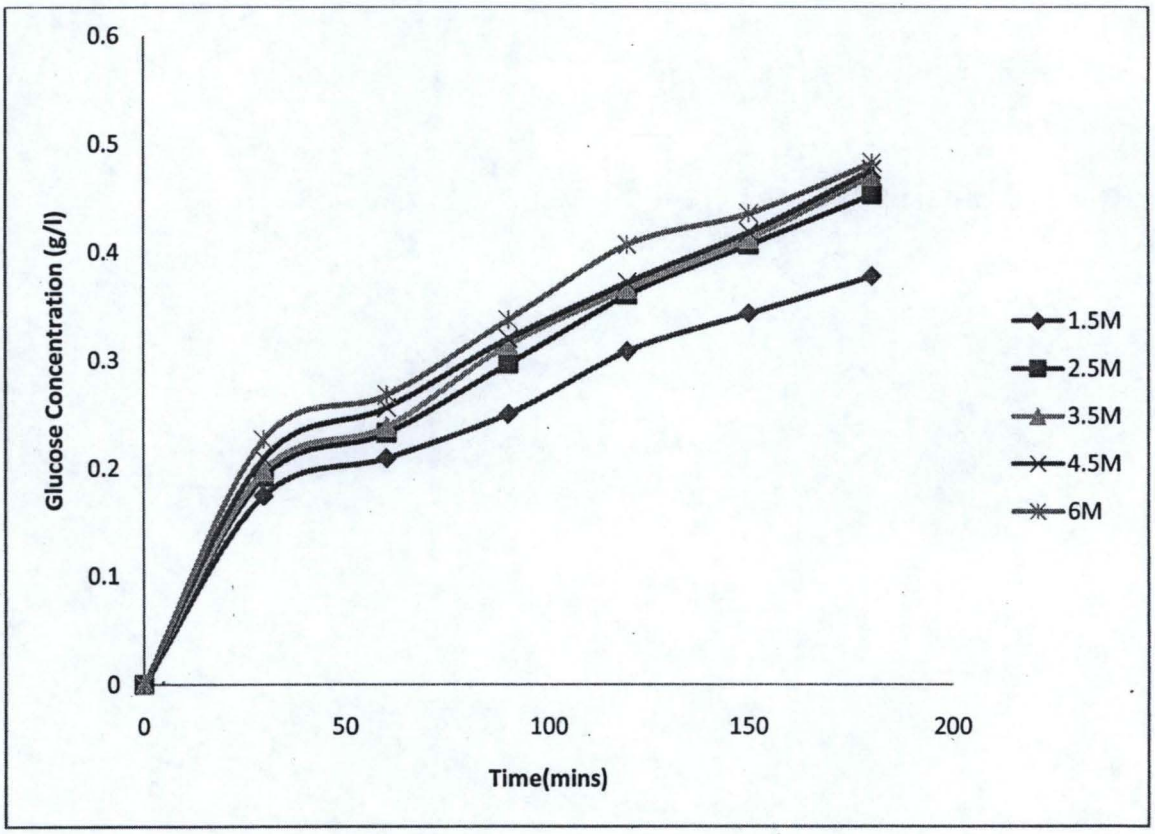

Figure 4.3: Effect of different acid concentrations on the hydrolysis at 70°C

The effect of different temperatures on glucose concentration from the hydrolysis at a constant acid concentration of 6M H_2SO_4 with time is showing increase in glucose concentration as the temperature increases. This is shown in Figure 4.4

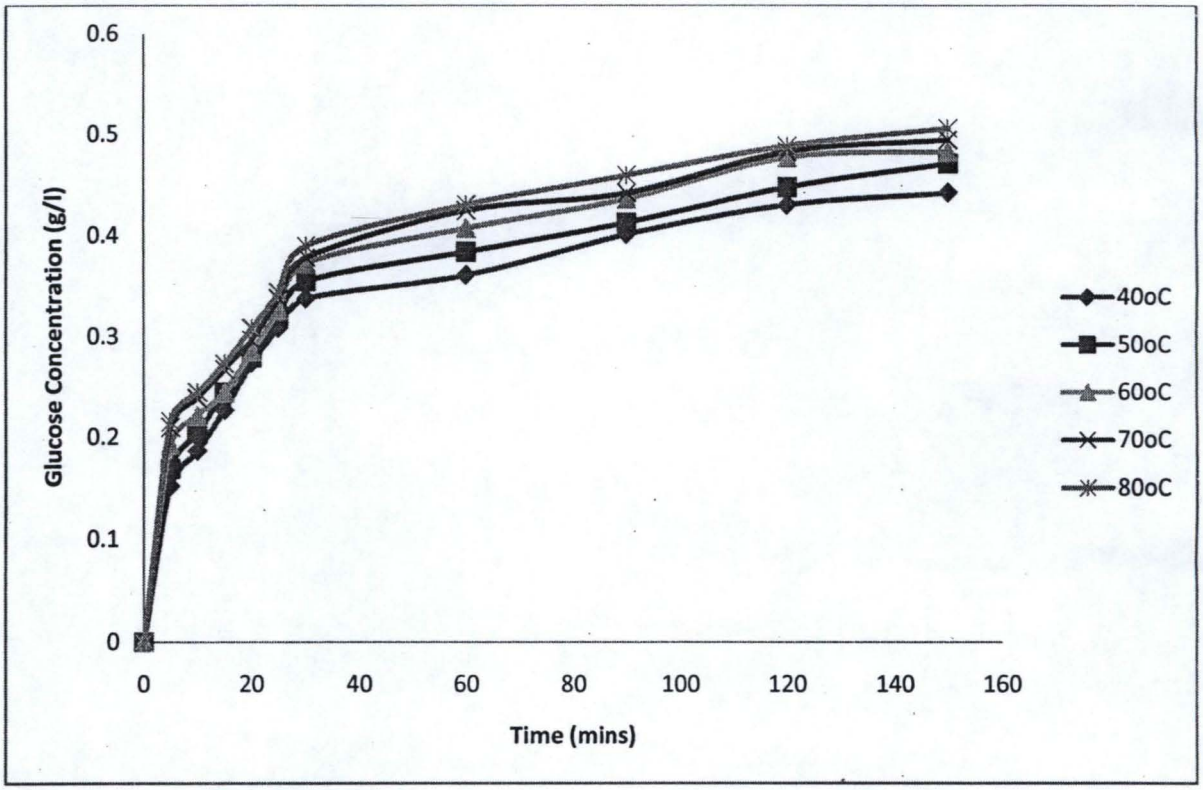

Figure 4.4: Effect of temperatures on glucose concentration for acid hydrolysis at constant acid concentration of 6M

Figure 4.5 shows the plot of Glucose concentration (g/l) against time at different temperatures for the determination of rate constants (K) as the gradients of each curve, depicting zero order kinetics.

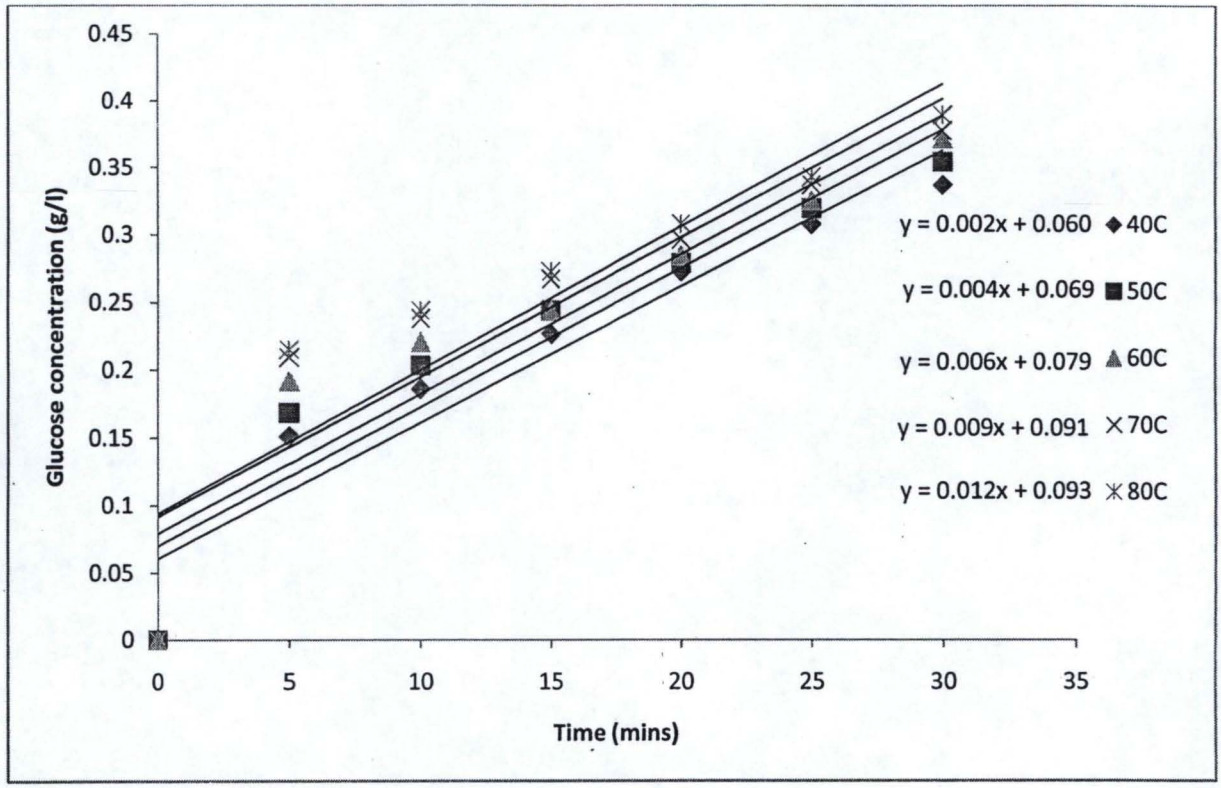

Figure 4.5: Glucose concentration (g/l) versus Time (mins) at different temperatures

Table 4.6: Kinetic parameters for the acid hydrolysis

Temp(^{o}k)	K(min^{-1})	1/T(10^{-3})(k^{-1})	(-LnK)(min^{-1})
313	0.002	3.19	6.215
323	0.004	3.10	5.521
333	0.006	3.00	5.116
343	0.009	2.92	4.711
353	0.012	2.82	4.422

The plot of -InK against the reciprocal of the temperatures which described the Arrhenius equation for the deduction of activation energy and exponential factor is shown in Figure 4.6.

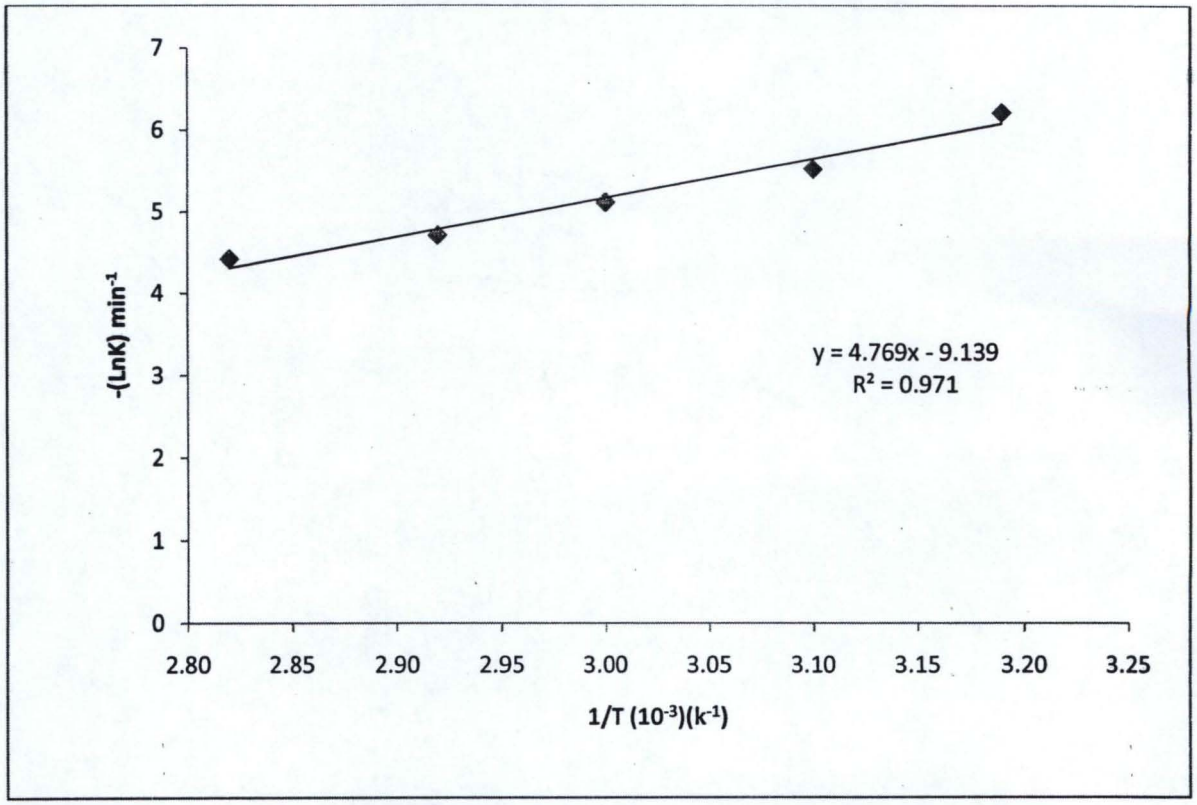

Figure 4.6: A Plot of -InK against the reciprocal of the temperatures (1/T).

Figure 4.7 shows the effect of biomass, ethanol and glucose concentration at different time intervals using *Saccharomyces cerevisia* during the fermentation process.

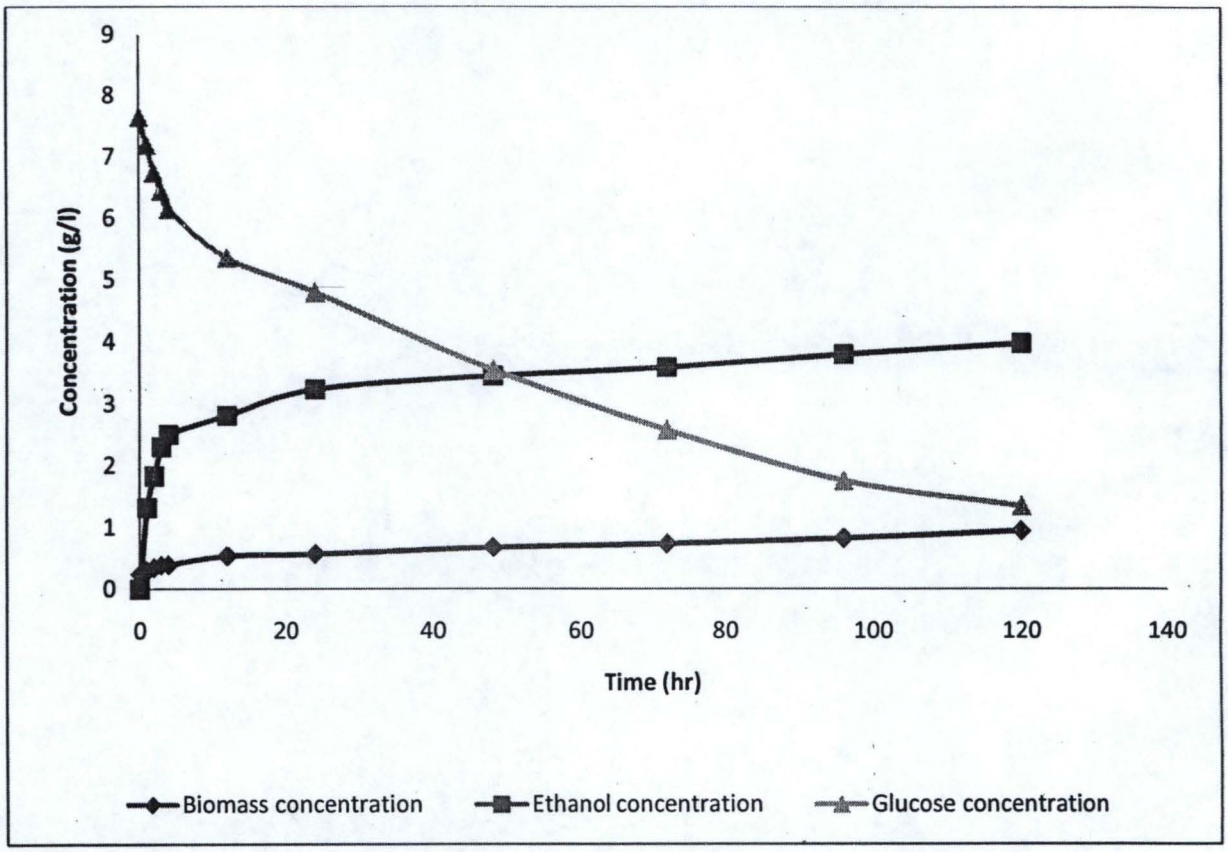

Figure 4.7: Biomass, ethanol and glucose concentration at different time intervals using *Saccharomyces cerevisia*

Figure 4.8 shows the effect of biomass, ethanol and glucose concentration at different time intervals using *Zymomonas mobilis* during the fermentation process.

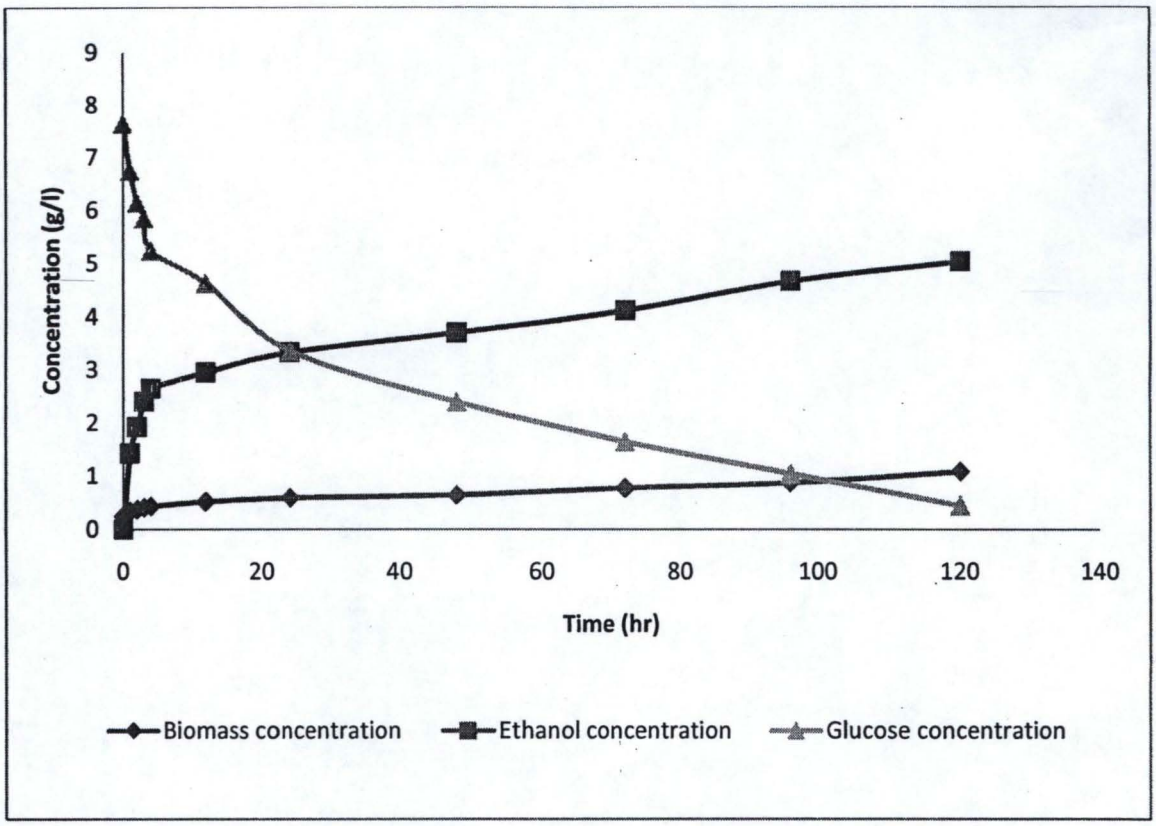

Figure 4.8: Biomass, ethanol and glucose concentration at different time intervals using *Zymomonas mobilis*

Table 4.9: Kinetic parameters estimated from the experimental data on different microorganisms, *Sacchsromyces cerevisia* and *Zymomonas mobilis.*

Kinetic parameter	*Saccharomyces cerevisiae*	*Zymomonas mobilis*
$\mu_m(h^{-1})$	0.0395	0.0385
$X_m(g/l)$	5.5182	5.4718
$Y_{p/x}(g/g)$	3.3949	3.6439
$\Delta t(h)$	4.8214	4.6350
$Y_{x/s}(g/g)$	0.1996	0.1992
$m(h^{-1})$	0.1325	0.1328

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion of Results

Sawdust was used to produce glucose through hydrolysis and subsequently the hydrolysate was fermentated with *Saccharomyces cerevisiae* and *Zymomonas mobilis* to produce bioethanol. In the process of fermentation, the microorganisms fermented the substrate (i.e sawdust hydrolysate) to bioethanol.

The effects of different acid concentrations of 1.5, 2.5, 3.5, 4.5, and 6M H_2SO_4 during acid hydrolysis on the concentration of glucose produced are shown on Figures 4.1, 4.2, and 4.3 respectively. These Figures show that concentration of glucose increased with time and with increase in acid concentration. These results agreed with that of Megawati *et al.*, (2010), Akpan *et al.*, (2005) and Aderemi *et al.*, (2008). The glucose yield was highest at the acid concentration of 6M H_2SO_4 compared to the other concentrations used.

The effect of temperatures on acid hydrolysis at constant acid concentration of 6M H_2SO_4 which is the optimum is shown in Figure 4.4. This shows that glucose yield increases with increase in temperature and with time. This result also agreed with that of Megawati *et al.*, (2010) and Akpan *et al.*, (2005). Though glucose yield increases with temperature, it is important to note that it may denature at temperature above its boiling point. Therefore, temperature above 80°C is not recommended.

A zero order kinetic was used to describe the acid hydrolysis based on integral method of analysis of the experimental data obtained with rate constants (K) at varying temperatures shown on Table 4.6. The plot of Glucose concentration (g/l) against time at different temperatures for the determination of rate constants (K) as the gradients of each curve depicting zero order

kinetics is shown in Figure 4.5. The dependence of reaction rate on temperature was evaluated using Arrhenius equation as described by equation 2.6. The activation energy and exponential factor were determined by plotting the natural logarithm of the reaction rate constants against the inverse of temperatures which gave a straight line with a slope and intercept as shown in Figure 4.6. The estimated activation energy from the slope and the exponential factor from the intercept are 39.652 KJ/mol and $9.345 \times 10^3 min^{-1}$ respectively. This agrees with the report of Yuan *et al.*, (2004) with activation energy of 36.61 KJ/mol and exponential factor of $2.013 \times 10^2 min^{-1}$. But comparing the activation energy and exponential factor obtained in this present work with those earlier reported by Megawati *et al.*, (2010) of 64.35 KJ/mol for activation energy and 1.58×10^5 min^{-1} for exponential factor, it can be observed that the value of the activation energy is lower. This difference in the activation energy may be attributed to the facts that different species of substance have difference in their physical and chemical compositions and also there may be different binding energy and that the temperatures strongly influence total sugar (glucose) concentration (Megawati *et al.*, 2010).

During fermentation, the amount of ethanol produced and glucose used were determined using different microorganisms, *Saccharomyces cerevisiae* and *Zymomonas mobilis* as shown in Figures 4.7 and 4.8 respectively. The difference in fermentation performance of the two microorganisms in cell growth rate, ethanol production, and sugar consumption using sawdust were investigated. Both microorganisms used during fermentation process, shows increase in ethanol production and decrease in the amount of glucose yield. This is due to the fact that, during fermentation process, the microorganisms utilized the glucose as a source of carbon and energy, whereas ethanol is produced as a result (Akpan *et al.*, 2005). The rate of ethanol production depends on both the amount of fermentable sugar available (substrate concentration)

and the number of microorganisms (*Saccharomyces cerevisiae* and *Zymomonas mobilis*) feeding on the substrate. Therefore, the higher the number of microorganism that fed on the available sugar, the higher the quantity of ethanol produced (Ekumankama *et al.*, 2001). Also, the amount of fermentable sugar consistently decreased with time because they do not replicate and as microorganisms feed on them, they are converted into ethanol hence, the decrease in concentration. It was noticed that the microorganisms multiply with time at a faster rate with *Zymomonas mobilis* as shown in Figure 4.8 compared to *Saccharomyces cerevisiae* as shown in Figure 4.7. As the substrates are being used, they deplete while the cells multiply to a time when there will be an insufficient substrate to sustain the cells, and then there will be a stationary phase of growth where a dynamic balance exists between the growth and death of cells. Therefore, it was observed that the yield of ethanol produced during fermentation with *Zymomonas mobilis* is higher than with *Saccharomyces cerevisiae* resulting in 5.05 g/l and 3.98 g/l respectively. These observations are consistent with the report of Akandi and Mudi, (2005), and Megawati *et al.*, (2010).

The fermentation kinetic parameters were estimated based on experimental data using mathematical software (MATLAB 7.0) with equations 2.17, 2.19, and 2.21. These equations are non-linear kinetic model of Monod's modified logistic equation for the fermentation of bioethanol using microorganisms. The estimated fermentation kinetic parameters include: Yeast maximum specific growth rate (μ_m) and maximum biomass concentration (X_m), the yield coefficient of the product ($Y_{p/x}$) and lag time (Δt), the yield coefficient of biomass on substrate ($Y_{x/s}$) and maintenance coefficient (m), respectively. The estimated values of these parameters are given in Table 4.9.

The bioethanol produced from fermentation process was distilled by applying a simple batch distillation process using Ethanol Distillation Apparatus. This was achieved at the boiling point of 78.3°C. The percentage yield of ethanol obtained using *Zymomonas mobilis* was 82.2% which is higher than 64.8% obtained using *Saccharomyces cerevisiae*. This is because *Zymomonas mobilis* can ferment both glucose and fructose better with higher yields than *Saccharomyces cerevisiae*. This report conforms to other literatures earlier mentioned (Megawati *et al.*, 2010; Galbe *et al.,* 2002). Slight difference in yield may be as a result of the difference of raw materials or feedstock used.

5.2 Conclusion

From the results of this research work, it is obvious that bioethanol could be produced from sawdust that constitute waste nuisance in our society and can be converted using *Saccharomyces cerevisiae* and *Zymomonas mobilis*.

The yield of glucose during acid hydrolysis increased with increase in acid concentration and with time. The optimum acid concentration of 6M H_2SO_4 was used at varying temperatures, giving the highest yield at 80°C in 150 minutes. The hydrolysis process was found to follow zero order kinetics based on integral method of analysis of the experimental data obtained with activation energy of 39.652 KJ/mol. During fermentation process, the amount of ethanol produced and glucose used were determined and a non-linear kinetic model based on the logistic equation of yeast growth, growth- associated production of ethanol with a lag time, and consumption of sugar for biomass formation and maintenance coefficient were applied for the estimation of the kinetic data using two microorganisms namely, *Saccharomyces cerevisiae* and *Zymomonas mobilis*. The kinetic fermentation parameters obtained with *Saccharomyces*

cerevisiae are the yeast maximum specific growth rate (μ_m), maximum biomass concentration (X_m), the yield coefficient of ethanol on biomass ($Y_{p/x}$), lag time (Δt), the yield coefficient of biomass on substrate ($Y_{x/s}$) and maintenance coefficient (m) which were calculated to be $0.0395h^{-1}$, 5.5182g/l, 3.3949g/g, 4.8214hrs, 0.1996g/g and $0.1325hr^{-1}$ respectively whilst the corresponding values for *Zymomonas mobilis* were $0.0385h^{-1}$, 5.4718g/l, 3.6439g/g, 4.6350hrs, 0.1992g/g and $0.1328hr^{-1}$ respectively. The percentage yield of ethanol obtained using *Zymomonas mobilis* was 82.2% and is higher than 64.8% for *Saccharomyces cerevisiae.* The results obtained indicated that the non-linear kinetic model of Monod's modified logistic equation could predict optimum fermentation performance using sugar in sawdust as the substrate.

5.3 Recommendation

Due to the fact that the present trend of sources of energy is geared towards renewable sources, the contribution of bioethanol in this direction has become imperative and can be fostered through the following recommendations:

- The culturing of viable microorganisms that can potentially increase the production of bioethanol from agricultural feedstock (green feedstock) should be embarked upon by research institutes.
- More research on various agricultural wastes that have the potentials of producing alternative source of fuel such as bioethanol should be worked on.
- For the purpose of promoting research to make it interesting for researchers in the field of biotechnology, a biotechnology laboratory within the department (Chemical Engineering) should be equipped by the university.

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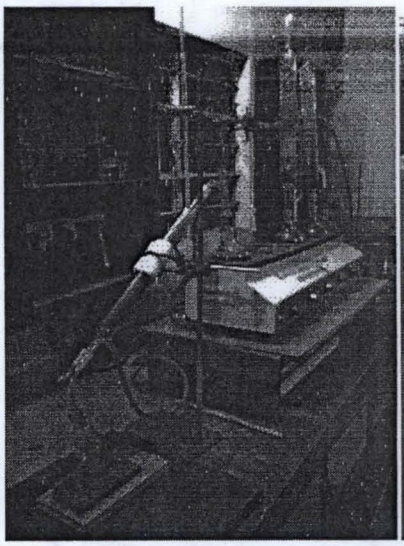
APPENDIX

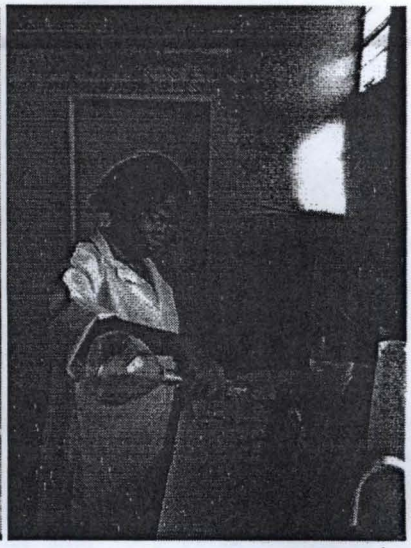
Experimental set-up

Distillation Apparatus

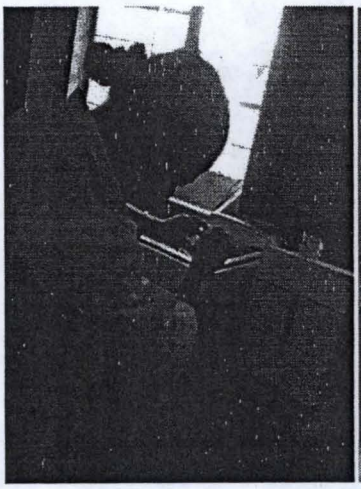


Preparing acid

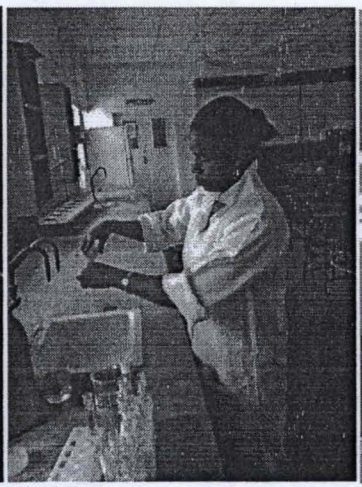
Preparing samples



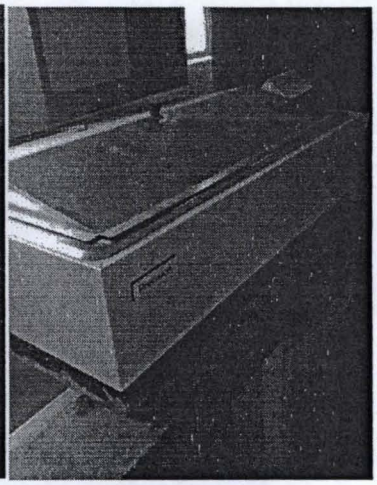
Withdrawing samples



Analyzing samples



A Water bath



Spectrophotometer, Autocalve, Digital weighing balance and Inoculation hood

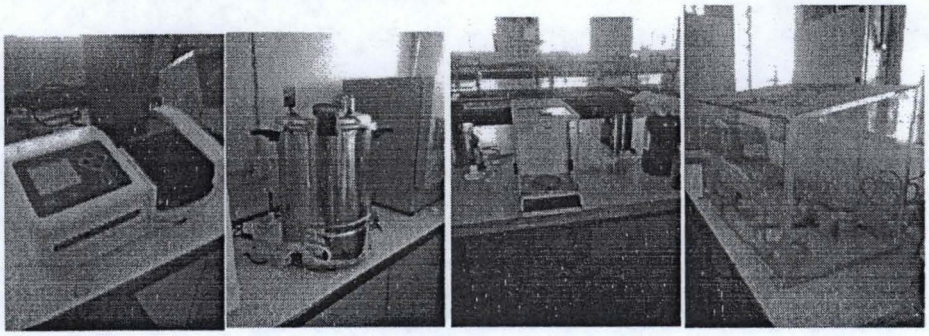


Figure 4.9 shows the plot of Absorbance against Glucose Concentration for Calibration Curve for Standard D-glucose solution

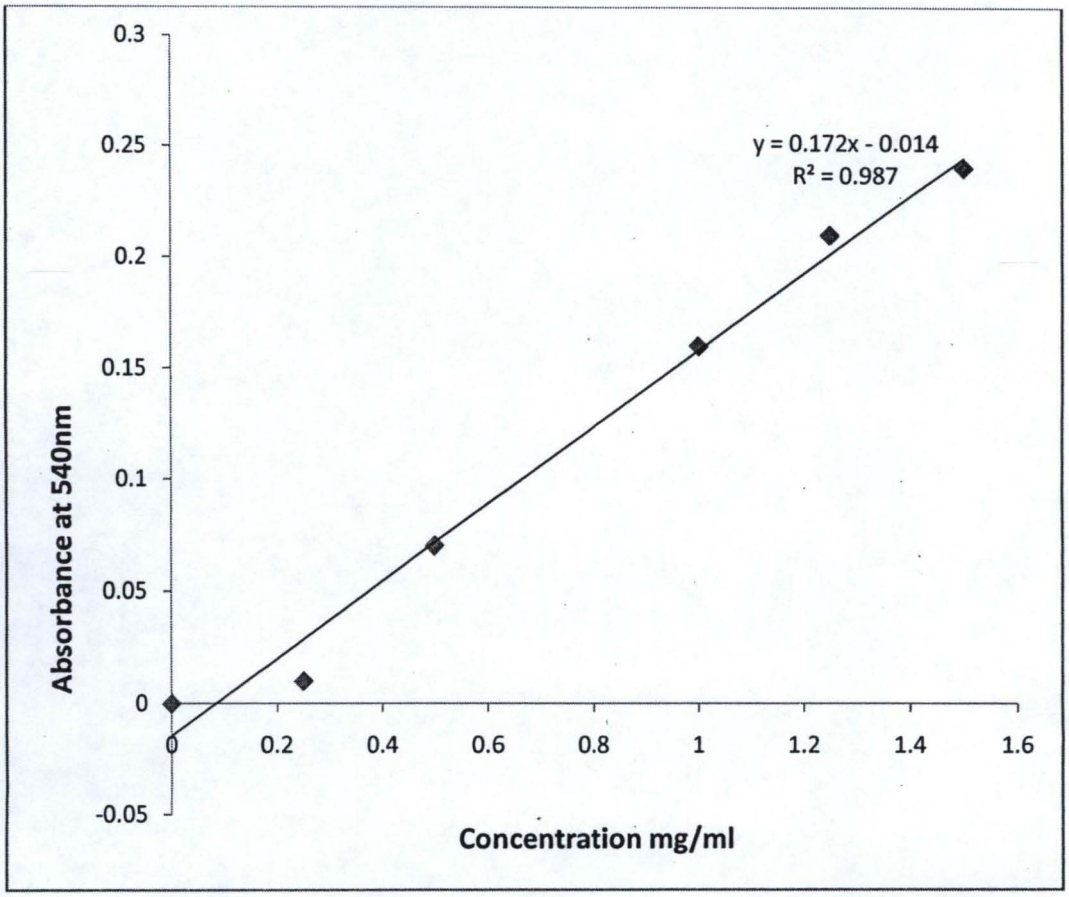


Figure 4.9: Plot of Absorbance against Concentration for Calibration Curve for Standard D-glucose solution

Table 4.2: Glucose yield (g/l) for acid hydrolysis of sawdust at 50°C using different acid concentrations

Time(mins)	1.5M	2.5M	3.5M	4.5M	6M
0	0	0	0	0	0
30	0.134	0.145	0.157	0.163	0.180
60	0.169	0.192	0.203	0.215	0.221
90	0.186	0.233	0.238	0.250	0.256
120	0.227	0.262	0.273	0.279	0.285
150	0.262	0.302	0.331	0.355	0.366
180	0.326	0.366	0.384	0.401	0.407

Table 4.3: Glucose yield (g/l) for acid hydrolysis of sawdust at 60°C using different acid concentrations

Time(mins)	1.5M	2.5M	3.5M	4.5M	6M
0	0	0	0	0	0
30	0.151	0.180	0.192	0.203	0.215
60	0.192	0.221	0.244	0.250	0.279
90	0.227	0.256	0.273	0.291	0.297
120	0.302	0.320	0.326	0.337	0.355
150	0.337	0.355	0.378	0.384	0.401
180	0.366	0.401	0.407	0.424	0.442

Table 4.4: Glucose yield (g/l) for acid hydrolysis of sawdust at 70°C using different acid concentrations

Time(mins)	1.5M	2.5M	3.5M	4.5M	6M
0	0	0	0	0	0
30	0.174	0.192	0.198	0.209	0.227
60	0.209	0.233	0.238	0.256	0.267
90	0.250	0.297	0.314	0.320	0.337
120	0.308	0.360	0.366	0.372	0.407
150	0.343	0.407	0.413	0.419	0.436
180	0.378	0.453	0.471	0.477	0.483

Table 4.5: Effect of temperatures on acid hydrolysis with glucose yield (g/l) at constant acid concentration of (6M)

Time(mins)	40°C	50°C	60°C	70°C	80°C
0	0	0	0	0	0
5	0.151	0.169	0.192	0.209	0.215
10	0.186	0.203	0.221	0.238	0.244
15	0.227	0.244	0.244	0.267	0.273
20	0.273	0.279	0.285	0.297	0.308
25	0.308	0.320	0.326	0.337	0.343
30	0.337	0.355	0.372	0.378	0.390
60	0.360	0.384	0.407	0.424	0.430
90	0.401	0.413	0.436	0.442	0.459
120	0.430	0.448	0.477	0.483	0.488
150	0.442	0.471	0.483	0.494	0.506

Table 4.7: Biomass, ethanol and glucose concentration at different time intervals using *Sacchsromyces cerevisia*

Time(hr)	Biomass concentration (g/l)	Ethanol concentration (g/l)	Glucose concentration (g/l)
0	0.25	0	7.65
1	0.30	1.32	7.20
2	0.35	1.83	6.75
3	0.39	2.30	6.45
4	0.40	2.50	6.15
12	0.54	2.81	5.36
24	0.57	3.23	4.80
48	0.68	3.45	3.55
72	0.74	3.59	2.58
96	0.83	3.80	1.76
120	0.95	3.98	1.35

Table 4.8: Biomass, ethanol and glucose concentrations at different time intervals using *Zymomonas mobilis*

Time(hr)	Biomass concentration (g/l)	Ethanol concentration (g/l)	Glucose concentration (g/l)
0	0.25	0	7.65
1	0.34	1.43	6.75
2	0.38	1.93	6.15
3	0.41	2.41	5.85
4	0.43	2.65	5.25
12	0.52	2.96	4.63
24	0.59	3.34	3.37
48	0.65	3.71	2.40
72	0.78	4.13	1.65
96	0.88	4.68	1.05
120	1.09	5.05	0.45

Table 4.10: Calibrated Curve for Standard D-glucose solution

Absorbance at 540nm	Concentration (mg/ml)
0	0
0.01	0.25
0.07	0.50
0.16	1.00
0.21	1.25
0.24	1.50