KINETIC STUDIES ON GLUCOSE PRODUCTION FROM ELEPHANT GRASS

(*Pennisetum purpureum*)

BY

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DECLARATION

I Jibril Abdullahi declare that I did this work entitled " Kinetic studies on glucose Production from Elephant Grass (*Pennisetum purpureum)*" in the Department of Chemical Engineering, Ahmadu Bello University, Zaria, under the supervision of Prof. B. O. Aderemi, Dr. O.R. Momoh and Dr. Ebenezer Ojo. The information derived from the literature has been duly acknowledged in the text and a list of references provided. To the best of my knowledge, no part of this work was previously presented for another degree or diploma at this or any other institution, elsewhere.

…….. ……..

Jibril Abdullahi Date

CERTIFICATION

This dissertation entitled "KINETIC STUDIES ON GLUCOSE PRODUCTION FROM ELEPHANT GRASS (*Pennisetum purpureum)*" by JIBRIL ABDULLAHI meets the regulations governing the award of the degree of Masters of Science in Chemical Engineering of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to my wife Mrs Naima Abdullahi Jibril and our daughter Miss Jibril Hikmat Abdullahi as they continue to be the most important part of my life.

ACKNOWLEDGEMENTS

Praises and glory belong to the almighty God the most high who out of his bounties made this work possible and realizable.

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ABSTRACT

To utilize the economic benefits of elephant grass (*pennisetum purpereum*) and exploit its potential as biomass for glucose production, the kinetic model for the production of glucose from the grass was investigated at the optimum conditions of hydrolysis (pH 4.5, temperature 45 ^{o}C, time 24 hours). The grass was characterized, pretreated and then hydrolyzed using *Aspergillus niger*. The effects of particles size (75 - 600 μm), substrate concentration (5 – 100 g/l) and cell loading (0.1 – 1.0 g) were also investigated. Optimum hydrolysis conditions gave the yield of 82.6 % (w/w) glucose. The kinetic model rate expression was tested for the process based on the Michaelis – Menten's kinetics and the kinetic parameters were evaluated from the Lineweaver – Burk plots. The Kinetic parameters – maximum rate of reaction, r_{max}, and Michealis Menten's constant km – obtained are $0.25*10^{-4}$ $g.l^{-1}.s^{-1}$ and 2.85 $g.l^{-1}$ respectively. The model was developed and its reliability was tested with the experimental data. Comparison between the experimental data and those predicted from the rate model indicate good agreement with a correlation coefficient of 0.996.This shows reliability of the model in the design of bioreactor that could be used in the production of glucose from elephant grass.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Description	unit
Cs	Initial substrate concentration	gl^{-1}
r_p	Production rate	$gl^{-1}s^{-1}$
r	Initial rate of reaction	$gl^{-1}s^{-1}$
K_m	Michealis Menten's constant	gl^{-1}
r_{max}	Maximum rate of reaction	$gl^{-1}s^{-1}$

Abbreviation	Description
A.niger	*Aspergillus niger*
SEM	Scanning electron microscopy
DM	Dry Matter
ADF	Acid Detergent Fiber
NDF	Neutral Detergent Fiber
CP	Crude Protein
CF	Crude Fiber
Ash	Crude minerals in the sample
NFE	Nitrogen free extract

CHAPTER ONE

1.0 INTRODUCTION

Glucose is a ubiquitous energy source in biological system s (Peter *et al*,. 2016). As a simple sugar, glucose is a monosaccharide used in nature as a monomer to create polysaccharides such as starch and cellulose in plants and glycogen in animals. Hence, production of glucose from the natural sources via hydrolysis is more of depolymerization reactions. However, while starch is readily depolymerized to give glucose, cellulosic degradation is more challenging, due to its strong β – 1,4 glycosidic bond when compared to the α – 1,4 glycosidic bond in starch (Peter *et al.,* 2016).

According to Liming and Xueling (2004), commercial and cost-effective production of biofuels from biomass remains a daunted task. Although, a lot of breakthrough has been achieved in the field of biomass conversion, the actualization of commercially viable biofuels from second generation biomass is still pending. Researchers are recently, able to produce biofuels from elephant grass in a one-pot simultaneous saccharification and fermentation processes, but the quantification of glucose, an intermediate product formed during fermentation is a critical step to improve biofuel yield (Wen *et al.,* 2004).

Traditionally, starch from edible crops such as corn, potato, sugar cane, cassava etc., have been the main substrate for glucose production. However, its competition within the food chain compels seeking alternative sources i.e grasses, agricultural wastes, algae etc. among which grasses and lignocellulosic agricultural wastes have been found most promising (Peter *et al.* 2016). Nonetheless, the major setbacks in the development of cellulosic conversion technology are associated primarily with the complex nature of cellulose structure and its resistance to

degradation. Other difficulties include the limited number of commercially available enzymes capable of digesting cellulose and the high requirement of enzyme loading coupled with the long period usually needed to attain an appreciable conversion level (Luo *et al.*, 1997). These factors make it difficult to run biological systems in a continuous mode.

Attempts at addressing the aforementioned problems have led investigators to use soluble cellulose (Duff *et al*., 1996), filter paper (Wen *et al*., 2004), sawdust and animal solid wastes (Gregg and Sadder, 1996), with impressive results. Kaur *et al.* (1998), reported studies on enzymatic hydrolysis of rice straw using *Trichoderma reseei* cellulase. Also, the effect of cross-linked *Aspergillus niger* cellulase on the hydrolysis of rice hull has been reported by Sharma *et al.* (2001). Vlesenko *et al.* (1997) studied the enzymatic hydrolysis of pretreated rice straw. Aderemi *et al.* (2008), reported studies on the kinetics of glucose production from rice straw by A*spergillus niger.* Also, Aiyejagbara *et al.* (2015), reported production of bioethanol from elephant grass by simultaneous saccharification and fermentation.

However, systematic studies on glucose production from elephant grass and evaluation of the relevant kinetic parameters are the principal focus of the present investigation.

1.1 PROBLEM STATEMENTS

While much attention has been given to lignocellulosic materials from agricultural wastes in the production of glucose by researchers, little attention has been given to elephant grass, despite its availability and sustainability.

Although some researchers produced bioethanol from elephant grass in a one-pot simultaneous saccharification and fermentation processes, the quantification of the intermediate product (glucose) formed during fermentation, which is a critical step necessary to improve bioethanol yield, was not given adequate attention.

1.2 AIM AND OBJECTIVES

AIM

The aim of this work is to study the kinetics of the production of glucose from elephant grass using enzymatic hydrolysis.

OBJECTIVES

The objectives set out to achieve this work are as stated below:

- To characterize the elephant grass and verify its suitability as a substrate for glucose production.
- To evaluate the effect of ammonia pretreatment on elephant grass as a substrate for glucose production.
- To evaluate the effects of pH, temperature, particles size, substrate concentrations and cells loading on glucose yield.
- To evaluate the kinetic parameters

1.3 JUSTIFICATION

Elephant grass which is cheap and available is utilized for economic benefits and the use of direct cells (*Aspergillus niger*) capable of secreting enzymes needed for biochemical reactions minimizes cost of production. *Aspergillus niger* was chosen due to its ubiquitous nature and its capacity to release more β-glucosidase enzyme, which is responsible for cleaving cellobiose into glucose in hydrolysis, than other cells.

1.4 SCOPE

This work covers the studies on the kinetics of production of glucose from elephant grass through raw material characterization, pretreatment, substrate analysis, enzymatic hydrolysis, products analysis, optimum conditions investigation and kinetic parameters evaluation.

CHAPTER TWO

2.0 LITERATURE REVIEW

Biomass a non-fossil fuel source is made up of complex molecules present in plants and animals. It is considered as a rich source of organic products, which have a characteristic chemical composition of Carbon C, Hydrogen H, Oxygen O, and Nitrogen N (Prasenjit and Paresh, 2015). However, until now, much of the work has reported on the conversion of plant-derived biomass into chemicals. Naturally, plant biomass is produced during the photosynthesis pathway using water, carbon dioxide and sunlight and is classified into two categories, namely, edible and non-edible. For example, wheat, rice, corn, potato etc. are made up of a polysaccharide, starch and are considered as edible biomass or a first generation raw material (for the synthesis of fuels and chemicals) (Carlo *et al.*, 2008). Starch is composed of a mixture of linear polysaccharide, amylose (homopolymer of D-glucose linked via a α-1,4 glycosidic bond) and branched polysaccharide, amylopectin (homopolymer of D-glucose linked via a linear α-1,4 glycosidic bond and branched α-1,6 glycosidic bond) (Carlo *et al.,* 2008). Non-edible biomass, for example crop waste or wood, is called lignocellulosic biomass or lignocelluloses and is considered as a second generation raw material. Lignocelluloses have a composition of 45% cellulose (homopolymer or homopolysaccharide of D-glucose linked via a β-1,4 glycosidic bond), 25% hemicellulose (heteropolymer or heteropolysaccharide of several C_5 and C_6 sugars linked via various bonds), 20% lignin (amorphous 3D network polymer of several aromatic monomers), some macro and micro nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, manganese, copper, boron, zinc, chloride and molybdenum) and extractives (fats, fatty acids, resins, tannins, volatile oils, proteins etc.) (Carlo *et al.,* 2008).

2.1 Elephant grass (*Pennisetum purpureum*)

Elephant grass is a major tropical grass. It is one of the highest yielding tropical grasses. It is a very versatile specie that can be grown under a wide range of conditions and systems: dry or wet conditions, smallholder or larger scale agriculture. It is a valuable forage and very popular throughout the tropics, notably in cut-and-carry systems (Mennetje, 1992; FAO, 2015).

2.1.1 Morphology

Elephant grass (Ciyawan gamba) is a robust, rhizomatous, tufted perennial grass. It has a vigorous root system, developing from the nodes of its creeping stolons (Duke, 1983). The culms are coarse, perennial, and may be up to 4-7 m in height, branched above. Elephant grass forms dense thick clumps, up to 1 m across. The leaves are flat, linear, hairy at the base, up to 100-120 cm long and 1-5 cm wide, with a bluish-green colour. The leaf margin is finely toothed and the leaf blade has a prominent midrib. The inflorescence is a stiff terminal bristly spike, up to 15-20 cm in length, yellow-brown to purplish in colour. Spikelets are arranged around a hairy axis, and fall at maturity. Spikelets are 4-6 mm long and surrounded by 2 cm long plumose bristles. There is little or no seed formation. When seeds are present they are very small (3million see ds/kg) (Duke, 1983; Mennetje, 1992; Francis, 2004; CABI, 2014;). Elephant grass is very similar in appearance to sugarcane (*Saccharum officinarum*) but its leaves are narrower and its stems are taller (DAFF, 2014).

2.1.2 Utilization

Elephant grass is very important forage in the tropics due to its high productivity. It is particularly suited to feed cattle and buffaloes. Elephant grass is mainly used in cut-and-carry systems ("zero grazing") and fed in stalls, or made into silage or hay. Elephant grass can be grazed, provided it can be kept at the lush vegetative stage: livestock tend to feed only the

younger leaves (FAO, 2015). Elephant grass, as implied by its name, is an important source of forage for elephants in Africa (Cook *et al.,* 2005*).*

Elephant grass is a multipurpose plant. The young leaves and shoots are edible by humans and can be cooked to make soups and stews (Burkill, 1985). The culms can be used to make fences, and the whole plant is used for thatch. It is considered a potential second generation energy source crop in the USA (EPA, 2015). Leaf and culm infusions are reported to have diuretic properties (Duke, 1983). Elephant grass has several environmental applications. It can be used to make mulch and to provide soil erosion control. It is a weed controller and, in Africa, it has been reported to be used as a trap plant in push-pull management strategies to fight against stemborers in maize crops (Khan *et al.,* 2007).

Many cultivars of elephant grass have been developed worldwide to suit local conditions and there is a wide range of habits, yield potential and nutritive value. "Merker" types have numerous relatively thin stems, narrow largely glabrous leaves, high yields, and are resistant to Helminthosporium. Dwarf cultivars ("Merkeron" and "Mott", developed at the Tifton Station in 1955 and 1988 respectively) are leafy and of high feed value (Cook *et al.,* 2005). Elephant grass has the capability to exchange alleles with other Pennisetum species, and several hybrids have been developed. Hybrids of pearl millet (*Pennisetum glaucum*) and elephant grass ("King grass", "Pusa Giant", "Bana grass", "Florida" and others) benefit from the desirable characteristics of pearl millet such as vigour, drought resistance, disease tolerance, forage quality and seed size, whereas elephant grass provides rusticity, aggressiveness, perennity, palatability and high DM yield (Timbo *et al.,* 2010).

2.1.3 Distribution

Elephant grass originated from sub-Saharan tropical Africa (Clayton *et al.,* 2013). It has been introduced as forage into most tropical and subtropical regions worldwide. It was introduced into the USA in 1913, in the 1950s into Central and South America and the West Indies, and in the 1960s into Australia. It is commonly naturalized and sometimes becomes invasive (CABI, 2014). Elephant grass is mainly found from 10 °N to 20 °S. It is often regarded as a weed in crops, along roadsides, waterways, wetlands, floodplain, swamps, forest edges, disturbed areas and wastelands (Francis, 2004; CABI, 2014). It can withstand drought conditions and is a pioneer species in arid lands such as the Galapagos Islands (CABI, 2014).

Elephant grass is a summer growing grass that grows from sea level up to an altitude of 2000 m (Francis, 2004). It does well in places where temperatures range from 25 °C to 40 °C (FAO, 2015) and where annual rainfall is over 1500 mm. It stops growing below 15 °C and is sensitive to frost, though it can regrow from the stolons if the soil is not frozen (Duke, 1983). Elephant grass is tolerant of drought and will grow in areas where the rainfall range is 200-4000 mm. Elephant grass is not tolerant of flooding and prefers well-drained soils. With poor drainage, it is best grown on raised beds (Göhl, 1982). It does better on rich, deep soils, such as friable loams, but can grow on poorly drained clays, with a fairly heavy texture, or excessively drained sandy soils with a pH ranging from 4.5 to 8.2 (Duke, 1983; Cook *et al.*, 2005; FAO, 2015). Elephant grass is a full sunlight species that can still produce under partial shade but does not withstand complete shade under a dense tree canopy (Francis, 2004).

2.1.4 Elephant grass yields

Elephant grass produces very few seeds and is mostly propagated vegetatively through stem cuttings consisting of at least 3 nodes, 2 of which are buried in rows. Row width ranges from 50 to 200 cm and distance within rows is between 50 and 100 cm (Mennetje, 1992). After planting,

elephant grass grows vigorously and can reach 4 m in 3 months. Elephant grass is fast growing and has a high annual productivity that depends on the climatic conditions, especially temperature and rainfall (Artus-Poliakoff *et al.,* 1991; Aroeira *et al.,* 2005). Elephant grass requires high levels of fertilizer and a regular water supply (Mennetje, 1992). Yields range from 20 to 80 DM/ha/year under high fertilizer inputs (Francis, 2004; Skerman and Riveros, 2005). With no or inadequate fertilizer, yields are in the range of 2-10 DM/ha/year (Bogdan, 1977). Cuttings can be made at 45-90 day intervals, depending on location (FAO, 2015).

2.1.5 Environmental impact

Weed and soil erosion control

Elephant grass is a pioneer species that competes very efficiently with weeds (D'Antonio *et al.,* 1992; FAO, 2015). In the Philippines, it has been used to control Imperata cylindrica . In Nigeria. elephant grass has been used as mulch (25 cm layer) for weed control, for water storage and to reduce soil losses on slopes (Francis, 2004; Adekalu *et al.,* 2007). Elephant grass develops a vigourous root system that may help to prevent river bank erosion. Planted as hedgerows, elephant grass makes fences and provides effective windbreaks for crops and houses. It is used for erosion control and forage production in alley-cropping systems of agroforestry (Magcale-Macandog *et al.,* 1998).

2.1.6 Second generation energy crop

In the USA, the Environment Protection Agency agreed that elephant grass could be used as a source of biofuel under the Renewable Fuel Standard Program provided producers respect the Risk Management Plan for early detection and rapid response to potential spread (EPA, 2015).

Elephant grass is a promising lignocellulosic feedstock with various applications due to its rapid

biomass accumulation in temperate climates. Trials indicate that Elephant grass provides relatively high yields (double that of corn), requires limited fertilizer, few other inputs and adds significant amounts of organic matter to the soil (EPA, 2015).

2.1.7 Benefits of elephant grass

It is high yielding, environmentally friendly, low maintenance, annual growing circle, no pesticides or fertilizers, increases wild life biodiversity, eligible for cross compliance, long life span, and excellent game (NREL, 2010). Figure 2.1 shows the view of elephant grass.



Figure 2.1 Elephant grass (FAO, 2015)

2.2 Elephant grass constituents

Like any other lignocellulosic material, the main constituents of elephant grass are cellulose, hemi-cellulose and lignin (Jorgensen *et al.*, 2007)

. Table 2.1 shows the composition of some lignocellulosic materials.

Table 2.1: Biomass composition of some lignocellulosic materials

Lignocellulosic materials	% Cellulose	% Hemicellulose	% Lignin
Corn cobs	45	35	15
Cotton seeds	80 - 90	5 - 20	0
Grasses	35 - 50	25 - 40	10 - 30
Hardwood	45-50	24 – 40	18 - 30
Leaves	15-20	80 – 85	0
News paper	40-55	25-40	15-30
Nutshell	25-30	25-30	30-40
Paper	85-99	0	0 - 15
Primary waste water solids	85	-	-
Softwood stems	45-50	25-35	25-35
Sorted refuse	60	20	20
Swine waste	60	28	-

Wheat straw	30	50	15

Table 2.2 also shows the comparison of percentage composition of elephant grass with some grasses.

Table 2.2 Comparison of percentage composition of constituents of grasses

Grass	% Cellulose	% Hemicellulose	% Lignin
Elephant	50 – 60	23 – 32	8 - 11
Switch	45	31.4	12
Coastal bermsemueda	25	35.7	6.4
Others	25 – 40	30 – 50	10 - 30

2.2.1 Cellulose

Cellulose is the major polymeric component of plant material and is the most abundant polysaccharide on Earth (Liming and Xueling, 2004). It is one of the three major components of all plant cell walls with two other components; hemicelluloses and lignin. The digestive systems of man and most other animals (except ruminants) do not contain the necessary enzymes (cellulase) for hydrolyzing β – glucosidic linkages. However, cellulases are found in ruminants,

various insect, fungi, bacteria and algae (Lee, 1992). Tremendous quantities of waste cellulose materials exist which are classified as municipal wastes, industrial wastes and agricultural wastes. The agricultural wastes represent the largest class of cellulosic waste while a few of these agro–waste are fed to some farm animals, most are being burnt, buried or otherwise discarded indiscriminately, thus causing environmental pollution. Proper treatments could change them from liabilities to assets (Vlesenko *et al*., 1997). Figure 2.2 shows a single unit of cellulose.

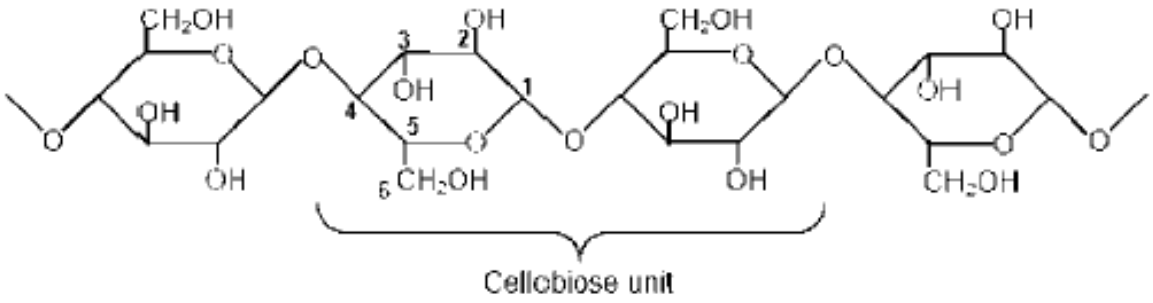


Figure 2.2: cellulose chain (George and Benson, 2005)

Cellulose is the main structural constituent in plant cell walls and is found in an organized fibrous structure. The structure of cellulose is shown in Figure 2.2. This linear polymer consists of D-glucose subunits linked to each other by β-(1,4)-glycosidic bonds. Cellobiose is the repeat unit established through this linkage (George and Benson, 2005). The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into microfibrils. Hemicelluloses and lignin cover the microfibrils (George and Benson, 2005). Fermentable D-glucose can be produced from cellulose through the action of either acid or enzymes breaking the β-(1,4)- glycosidic linkages (Lee, 1992). Cellulose in biomass is present in both crystalline and amorphous forms. Crystalline cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains form amorphous cellulose. Cellulose is more susceptible to enzymatic degradation in its amorphous

form (Vlesenko *et al*., 1997).

2.2.1.1 Cellulose Chemistry

Despite its simple chemical composition, cellulose exists in a number of crystalline and amorphous topologies. Its insolubility and heterogeneity make native cellulose a recalcitrant substrate for enzymatic hydrolysis. Microorganisms meet this challenge with the aid of a multi-enzymatic system. Specific enzymes act in synergy to elicit effective hydrolysis (Lee and Fan, 1982).

2.2.2 Hemicellulose

The main feature that differentiates hemi-cellulose from cellulose is that hemi-cellulose has branches with short lateral chains consisting of different sugars. These monosaccharides include pentoses (xylose, rhamnose, and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (e.g., 4-methylglucuronic, D-glucuronic, and D-galactouronic acids) (Tiangfu, 2014). The backbone of hemicellulose is either a homopolymer or a heteropolymer with short branches linked by β-(1,4)-glycosidic bonds and occasionally β-(1,3)-glycosidic bonds. Also, hemicelluloses can have some degree of acetylation, for example, in heteroxylan. In contrast to cellulose, the polymers present in hemicelluloses are easily hydrolyzable. These polymers do not aggregate, even when they co-crystallize with cellulose chains (Tiangfu, 2014).

Recent literature articles (Lucilia *et al*., 2014; Sharma, 2014) have demonstrated that xylitol (hydrogenation product of xylose) and L-arabinose can serve as functional sugar additives or

replacements for high-fructose corn syrup-derived foods and beverages (Tiangfu, 2014). It has been shown that the addition of 2-3 wt % of L-arabinose into sucrose can reduce glucose uptake by about 70 % while maintaining a similar sweetness level to sucrose. As a result, these C_5 sugar derivatives can serve as a health food resource (Tiangfu, 2014). The utilization of biomass for high value food or pharmaceutical applications like these can greatly improve the comprehensive economics of lignocellulose conversion and facilitate the likelihood of process commercialization (Tiangfu, 2014). The dehydration of C_5 sugars can yield furfural, which is currently commercially produced as a platform chemical having applications ranging from solvents to resins and fuel additives (Perez *et al.*, 2002). Figure 2.3 shows the structure of a single unit of hemicellulose.

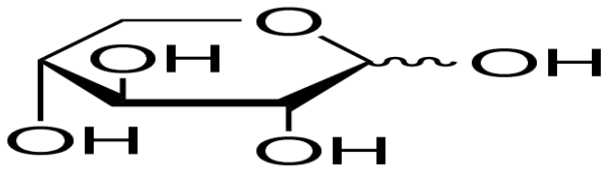


Figure 2.3: Hemicellulose structure (Tiangfu, 2014)

2.2.3 Lignin

Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack (Perez *et al.*, 2002). Three phenyl propionic alcohols exist as monomers of lignin: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (*p*-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol). Alkyl-aryl, alkyl-alkyl, and

aryl-aryl ether bonds link these phenolic monomers together. In general, herbaceous plants such as grasses have the lowest contents of lignin, whereas softwoods have the highest lignin contents (Perez *et al.,* 2002). Lignocellulosic biomass typically contains 50% - 80% (dry basis) carbohydrates that are polymers of five carbon and six carbon sugar units. Most carbohydrates can be processed either chemically or biologically to yield biofuels such as ethanol. Employment of enzymes for the hydrolysis of lignocellulose is considered the prospectively most viable strategy to offer advantages such as higher yields, low energy requirements, mild operating conditions, and environmentally friendly processing. The structural compositions of lignocelluloses are however resistant to direct enzymatic hydrolysis of cellulose (Beguin and Aubert, 1994). Therefore, pre-treatment step is invariably required to render the cellulose amenable to enzymatic action prior to the enzymatic hydrolysis, by breaking down the shield formed by lignin and hemicelluloses, disruption of the crystalline structure and reducing the degree of polymerisation of cellulose. Although pre-treatment has been viewed as one of the expensive processing steps within the conversion of biomass to fermentable sugar, the pre-treatment is also believed to have great potential for the improvement of efficiency and reduction of cost (Himmel *et al.*, 1994).

Lignin is a highly cross-linked phenolic polymer and the robust aromatic C-O-C ether bonds present throughout make it very difficult to deconstruct into monomeric constitutes. Therefore, efficient methods to depolymerize lignin are still a major goal. On the other hand, its aromatic-based structure gives lignin a high heating value. Lignin can potentially be upgraded into transportation fuels by hydrogenation and/or hydro deoxygenation. A study by Lercher (2013), found a method to quantitatively depolymerize lignin to its monomeric constitutes using a combination of base (as a catalyst) and boric acid (as a capping agent). Later, the same group

also reported that using a dual-function catalyst system, comprised of Pd/C and H_3PO_4, can be used to hydrogenate the monomer phenols in an aqueous media to form cycloalkanes, an ideal fuel. Dumesic (2014), found in the processing steps to convert cellulose and hemicellulose to chemicals and fuels, a solvent derived from lignin is particularly interesting for use in biphasic reaction systems in which the organic phase is the lignin-based phenol and the aqueous layer is the reactive media containing the carbohydrates and catalyst. The inner site produced furanics (5-hydroxymethylfurfural, furfural) or lactones (gamma-valero lactone, GVL) can be selectively extracted into the organic phase, making the process sustainable with both reactants and organic solvent being derived from lignocellulose (Perez *et al.*, 2002). Figure 2.4 shows the complex large molecular structure of lignin containing cross-linked polymers of phenolic monomers.

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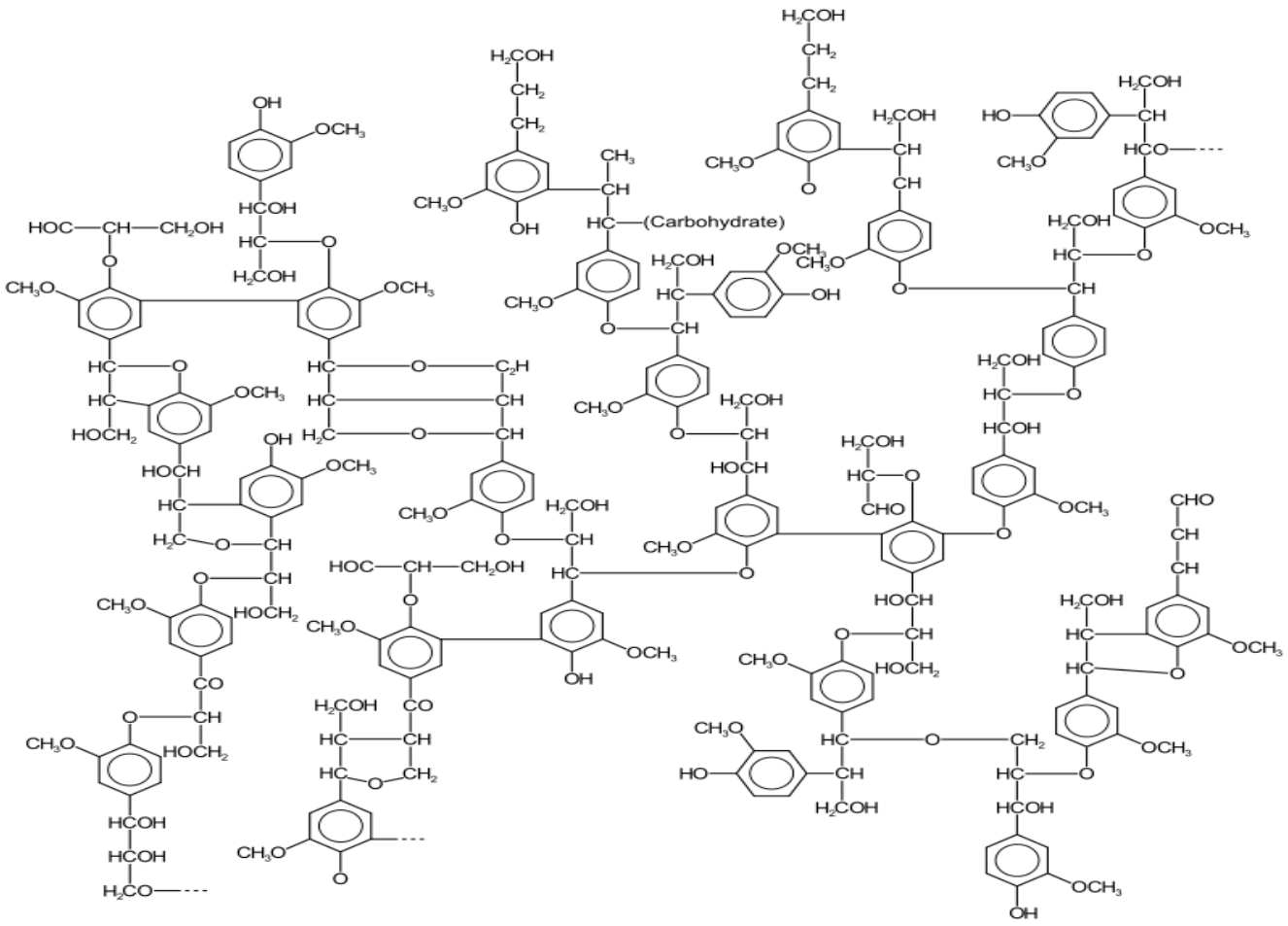


Figure 2.4: Lignin structure (Perez *et al.*, 2002)

These three main components of elephant grass are combined in Figure 2.5 according to percentage composition.

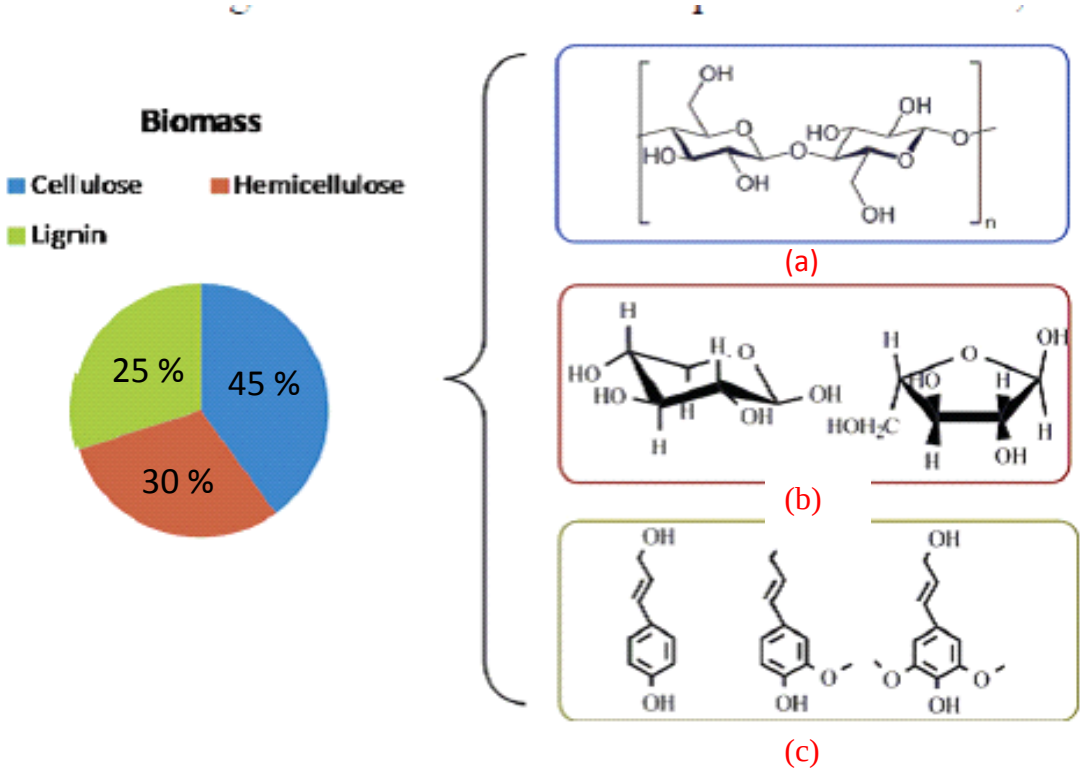


Figure 2.5. Lignocellulose biomass composition: (a) cellulose, (b) hemicellulose, and (c) lignin (Tillman *et al.*, 2010)

2.3 Elephant grass pre-treatment

The beneficial effects of pre-treatment of lignocellulosic materials have been recognized for a long time (Himmel *et al.*, 1994). The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic material. Pretreatment must meet the following requirements:

1. Improve the formation of sugars or the ability to subsequently form sugars by hydrolysis.

2. Avoid the degradation or loss of carbohydrate.

3. Avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes.

4. Must be cost-effective.

5. Must be environmentally friendly (Himmel *et al.*, 1994).

Pretreatment methods can be roughly divided into different categories: physical (milling and grinding), physicochemical (steam pre-treatment/autohydrolysis, Ammonia fiber explosion, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these. The following pretreatment technologies are potentially cost-effective for lignocellulosic biomass conversion to fuels and chemicals (Sun and Cheng, 2002).

2.3.1 Physical pre-treatment.

Mechanical comminution

Comminution of lignocellulosic materials through a combination of chipping, grinding, and/or milling can be applied to reduce cellulose crystallinity. The size of the materials is usually 10-30 mm after chipping and 0.2-2 mm after milling or grinding (Sun and Cheng, 2002). Vibratory ball milling was found to be more effective than ordinary ball milling in reducing cellulose.

2.3.2 Physicochemical pre-treatment.

Steam explosion

Steam explosion is the most commonly used method for the pre-treatment of lignocellulosic materials. In this method, biomass is treated with high pressure saturated steam, and then the pressure is suddenly reduced, which makes the materials undergo an explosive decompression

(Kabel *et al.,* 2007). Steam explosion is typically initiated at a temperature of 160-260 ^{0}C (corresponding pressure, 0.69-4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure (Kabel *et al.,* 2007). The process causes hemicelluloses degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. Removal of hemicelluloses from the microfibrils is believed to expose the cellulose surface and increase enzyme accessibility to the cellulose microfibrils (Kabel *et al.,* 2007). Lignin is removed only to a limited extent during the pretreatment but is redistributed on the fiber surfaces as a result of melting and depolymerization/repolymerization reactions (Parveen *et al.,* 2009). The removal and redistribution of hemicellulose and lignin increase the volume of the pre-treated sample. Rapid flashing to atmospheric pressure and turbulent flow of the material causes fragmentation of the material, thereby increasing the accessible surface area. Depending on the severity of the pre-treatment, some degradation of the cellulose to glucose can take place. Water acts as an acid at high temperatures. Addition of SO_2 (or H_2SO_4) or CO_2 [typically 0.3-3% (w/w)] in steam explosion can decrease time and temperature, effectively improve hydrolysis, decrease the production of inhibitory compounds, and lead to complete removal of hemicellulose (Parveen *et al.,* 2009). The factors that affect steam-explosion pretreatment are residence time, temperature, chip size, and moisture content. Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short residence time (270 ^{0}C, 1 min) or lower temperature and longer residence time (190 ^{0}C, 10 min) (Parveen *et al.,* 2009). The advantages of steam-explosion pretreatment include the low energy requirement compared to mechanical comminution and no recycling or environmental costs. The conventional mechanical methods require 70 % more energy than steam explosion to achieve the same particle size reduction (Hotzapple *et al.,* 1991). Steam explosion is recognized

as one of the most cost-effective pretreatment processes for hardwoods and agricultural residues, but it is less effective for softwoods. Limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin-carbohydrate matrix, and generation of compounds that might be inhibitory to microorganisms used in downstream processes (Parveen *et al.,* 2009).

Ammonia fiber explosion (AFEX)

Ammonia fiber explosion is a physicochemical pre-treatment process in which lignocellulosic biomass is exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is suddenly reduced (Holtzapple *et al.,* 1991). The AFEX process is very similar to steam explosion. In a typical AFEX process, the dosage of liquid ammonia is 1-2kg of ammonia/kg of dry biomass, the temperature is 90^0C, and the residence time is 30 min. AFEX pretreatment can significantly improve the fermentation rate of various herbaceous crops and grasses (Holtzapple *et al.,* 1991). During pretreatment only a small amount of the solid material is solubilized; that is, almost no hemicellulose or lignin is removed. The hemicelluloses is degraded to oligomeric sugars and deacetylated, which is most likely the reason that the hemicellulose is not soluble. The structure of the material is changed, resulting in increased water holding capacity and higher digestibility. Over 90% hydrolysis of cellulose and hemicellulose was obtained after AFEX pre-treatment of bermuda grass (approximately 5% lignin) and bagasse (15% lignin) (Holtzapple *et al.,* 1991). However, the AFEX process was not very effective for biomass with higher lignin content. The ammonia fiber explosion pretreatment simultaneously reduces lignin content and removes some hemicelluloses while decrystallizing cellulose. It can have a profound effect on the rate of cellulose hydrolysis. The cost of ammonia, and especially of ammonia recovery, drives the cost of the AFEX pretreatment (Holtzapple *et al.,*

1991).

Aqueous ammonia soaking (AAS)

Ammonia has desirable characteristics as a pretreatment reagent in that it is an effective swelling reagent for lignocellulosic materials and is highly selective for reactions with lignin over those with carbohydrates (Chang, 2005). Ammonia pretreatment works by breaking apart crystalline cellulose and acetyl linkages (Gollapalli *et al.,* 2002). Pretreatment using ammonia has the potential to be efficient because ammonia is highly volatile, which suggests that it could be easily recycled (Chang, 2005). Dale *et al*. (1986) suggested that residual amounts of ammonia following pretreatment may enhance the hydrolysis and subsequently, fermentation due to increased nitrogen content. Kim and Lee (2005) pioneered soaking in aqueous ammonia as a means of pretreating corn stover. More recently, Kim *et al*. (2007) found a pretreatment of barley hull with 15 wt% aqueous ammonia at 75 ^{o}C for 48 hours at a 1:12 solid to liquid ratio removed 66% of lignin and retained the xylan and glucan fractions. Isci *et al*. (2009) explored different liquid to solid ratios and soaking durations for switchgrass by operating a soaking and rinsing system. Using aqueous ammonia as a pretreatment reagent at ambient conditions retains the cellulose approach to increase the hydrolysis yield and subsequent fermentation yield and simplify the bioconversion scheme (Isci *et al*., 2009).

2.3.3 Chemical pre-treatment

Acid hydrolysis

Concentrated acids such as H_2SO_4, and HCl have also been used to treat lignocellulosic materials Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive, hazardous, and thus require reactors that are resistant to corrosion, which makes

the pretreatment process very expensive. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible (Galbe and Zacchi, 2007).

Dilute-acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. High temperature in the dilute-acid treatment is favorable for cellulose hydrolysis. Two types of dilute-acid pretreatment processes are typically used: a high-temperature (T > 160 ^{0}C), continuous-flow process for low solids loadings (weight of substrate/weight of reaction mixture) 5-10 %, and a low-temperature (T < 160 ^{0}C), batch process for high solids loadings (10-40 %) (Galbe and Zacchi, 2007). Although dilute-acid pretreatment can significantly improve cellulose hydrolysis, its cost is usually higher than those of physicochemical pretreatment processes such as steam explosion or AFEX. Neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes. Dilute-acid pretreatment is also known to have a negative influence on the enzymatic hydrolysis of biomass. In an article, Selig *et al.* (2007) reported the formation of spherical droplets on the surface of residual corn stover following dilute-acid pretreatment at high temperature. They suggested that the droplets formed were composed of lignin and possible lignin-carbohydrate complexes. It was demonstrated that these droplets were produced from corn stover during pretreatment under neutral and acidic pH at and above 130 ^{o}C and that they can deposit onto the surface of residual biomass. The deposition of droplets produced under certain pretreatment conditions (acidic pH, T > 150 ^{o}C) and captured on pure cellulose was shown to have a negative effect on the enzymatic saccharification of the substrate (Galbe and Zacchi, 2007).. Therefore, it is extremely important to carefully examine the appropriate dilute-acid pretreatment of lignocellulose biomass species. It has been shown that materials that have been subjected to acid hydrolysis can be harder to ferment because of the presence of toxic substances (Galbe and Zacchi, 2007). Furthermore, acid

pretreatment results in costly materials of construction, high pressures, neutralization and conditioning of hydrolysate prior to biological steps, slow cellulose digestion by enzymes, and nonproductive binding of enzymes to lignin (Galbe and Zacchi, 2007)..

Alkaline hydrolysis

Some bases can be used for the pretreatment of lignocellulosic materials, and the effect of alkaline pretreatment depends on the lignin content of the material (Hotzapple *et al.,* 1991). Alkali pretreatment processes utilize lower temperatures and pressures than other pretreatment technologies. Alkali pretreatment can be carried out at ambient conditions, but pretreatment times are in the order of hours or days rather than minutes or seconds. Compared with acid processes, alkaline processes cause less sugar degradation, and many of the caustic salts can be recovered and/or regenerated (Sun and Cheng, 2002). Sodium, potassium, calcium, and ammonium hydroxides are suitable alkaline pretreatment agents. Of these four, sodium hydroxide has been studied the most. However, calcium hydroxide (slake lime) has been shown to be an effective pretreatment agent and is the least expensive per kilogram of hydroxide. It is possible to recover calcium from an aqueous reaction system as insoluble calcium carbonate by neutralizing it with inexpensive carbon dioxide; the calcium hydroxide can subsequently be regenerated using established lime kiln technology (Sun and Cheng, 2002).

2.3.4 Biological pretreatment.

Most pretreatment technologies require expensive instruments or equipment that have high energy requirements, depending on the process (Sun and Cheng, 2002). In particular, physical and thermo chemical processes require abundant energy for biomass conversion. Biological treatment using various types of rot fungi, a safe and environmentally friendly method, is

increasingly being advocated as a process that does not require high energy for lignin removal from a lignocellulosic biomass, despite extensive lignin degradation (Sun and Cheng, 2002). In biological pretreatment processes, microorganisms such as brown- white- and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials. Brown rots mainly attack cellulose, whereas white and soft rots attack both cellulose and lignin. Lignin degradation by white-rot fungi occurs through the action of lignin-degrading enzymes such as peroxidases and laccase. These enzymes are regulated by carbon and nitrogen sources. White-rot fungi are the most effective for biological pretreatment of lignocellulosic materials. Hatakka (1983), studied the pretreatment of wheat straw by white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in 5 weeks. Akin *et al*. (1995) also reported the delignification of Bermuda grass by white-rot fungi. The biodegradation of Bermuda grass stems was improved by 29-32%, after 6 weeks, using *Ceriporiopsis subvermispora* and by 63-77% using *Cyathus stercoreus*. Advantages of biological pre-treatments are low energy requirement and mild operation conditions. Nevertheless, the rate of biological hydrolysis is usually very low, so this pretreatment requires long residence times (Sun and Cheng, 2002). Figure 2.6 shows the pretreatment process.

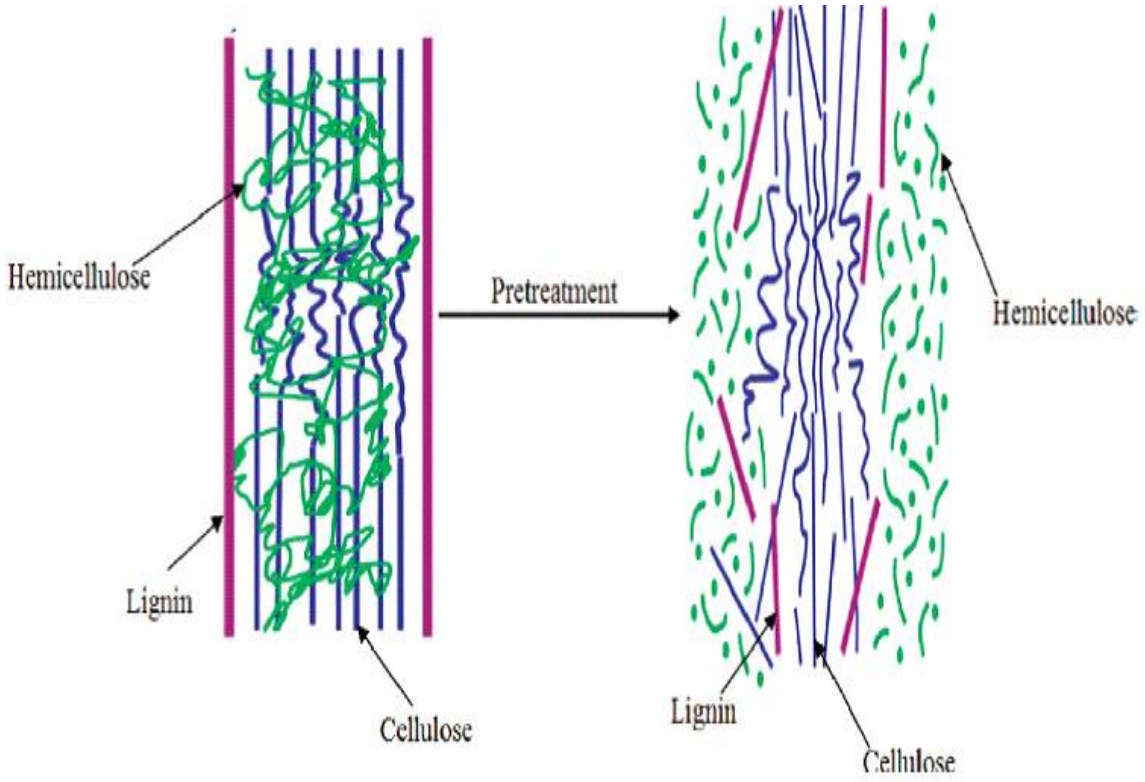


Figure 2.6: Effect of pretreatment on a typical lignocellulosic substrate (Kristensen, 2009)

2.4 Major lignocellulose conversion routes

Various approaches have been studied in order to produce renewable fuels and chemicals from lignocellulosic biomass (Tiangfu, 2014). Based on the temperature range under which the lignocellulose is processed and deconstructed, conversion routes can be classified into three major categories. From low temperature to high temperature, these three pathways involve:

(1) depolymerization into monomers, typically under 200 °C.

(2) pyrolysis and liquefaction, roughly around 500 °C.

(3) gasification, above 800 °C.

2.4.1. Depolymerization of biomass into monomers

When the monomers are obtained, subsequent chemical or biological catalysis can be performed to selectively convert these monomers to important platform chemicals (Carraher and Charles, 2010). This approach can be advantageous because each fraction of the lignocellulose can be converted via processes specifically developed to operate efficiently for each particular component. However, one of the disadvantages of this tandem cascade approach is the costly pretreatment and depolymerization steps needed to liberate monomers of each fraction (Carraher and Charles, 2010). Additionally, the multiple steps required during the process will also add to the cost. From a systems level, an effective and compatible network of conversion strategies and catalysts is necessary to improve the overall yield on a carbon basis for each monomer (Carraher and Charles, 2010).

2.4.2. Fast pyrolysis and liquefaction

Fast pyrolysis and liquefaction are two common methods used to directly convert raw lignocellulose into a mixture of liquid intermediates (Huber and Dumesic, 2014). Pyrolysis refers to the rapid heating of biomass to 400-600 °C under an inert atmosphere to yield bio-oil, incondensable gases, and char. The composition of bio-oil is very complicated with possibly more than 300 compounds including a high content of water. As such, the separation of components represents a significant hurdle. Bio-oil can later be stabilized and upgraded to useful chemicals and fuels by strategies including esterification or ketonization among others. In order to address the issue of low selectivity, recent efforts by (Huber and Dumesic, 2014) have explored catalytic fast pyrolysis for the production of aromatics and fuels. Liquefaction processes are conducted in the condensed phase under lower temperatures and higher pressures. Both pyrolysis and liquefaction produce a complex and unstable liquid product that can be further stabilized and upgraded to resemble conventional crude oil. However, due to its poor

selectivity to any single chemical species and resultant separation difficulties, thermochemical methods have a higher feasibility for producing fuels rather than chemicals (Huber and Dumesic, 2014). The advantage in thermochemical conversion lies in the benefit, from a logistical perspective, of allowing the operation of a distributed infrastructure. Smaller, and more numerous, satellite pyrolysis or liquefaction facilities can be used to convert low density biomass into bio-oil, which can then be transported to a larger centralized processing/upgrading facility. Upgrading of such bio-oil has been extensively studied by Dumesic (2013), and has been reviewed in several recent articles.

2.4.3 Gasification

Gasification of lignocellulose under temperatures above 800 °C results in the formation of syngas, consisting of CO, CO_2, H_2O, and H_2. Syngas can undergo conventional Fischer-Tropsch (FT) synthesis to produce hydrocarbons as liquid transportation fuels or be fed to the existing petrochemical infrastructure (Heidenreich, 2015). Syngas can also be converted to methanol, which can be used to make hydrocarbons and olefins for the polymer industry. These large-scale, technically mature strategies can produce fuels and chemicals that are compatible with the current infrastructure, but the disadvantage of this pathway is that the production of hydrocarbons and methanol from biomass remains relatively expensive and requires a large capital investment (Heidenreich, 2015). Overall, these three major strategies to convert lignocellulose have their own advantages and disadvantages. According to the speciation of biomass and targeted molecules, different routes (or combination of routes) may be devised to best suit the biomass stream of interest (Heidenreich, 2015). The economical production of chemicals and fuels will depend on the selectivities of the catalytic conversion process. In general, the concept of producing platform chemical intermediates that can be subsequently

converted to a range of products is particularly interesting. The current review will focus on the production of glucose from a lignocellulose material as the feedstock (Heidenreich, 2015).

2.5 The cellulase (enzyme) system

Although cellulose is a homopolymer (Kristensen, 2009), a number of enzymes are needed to degrade it. These can be divided into the following three types. These three groups of enzymes work synergistically to degrade cellulose by creating new sites for each other and preventing product inhibition. Endoglucanases (EG), which hydrolyse internal β-1,4-D-glucosidic linkages randomly in the cellulose chain; Cellobiohydrolases (CBH, also known as exoglucanases), which progress along the cellulose line and cleave off cellobiose units from the ends; β-glucosidases (BG), which hydrolyze cellobiose to glucose and also cleave off glucose units from cello-oligosaccharides (Kristensen, 2009). The concerted action of cellulases ensures an efficient hydrolysis and diminishes product inhibition (Kristensen, 2009). A simplified diagram of enzymatic hydrolysis can be seen in Figure 2.7. In total, *Trichoderma (T.) reesei* produces two Cellobiohydrolases (CBHs,) five Endoglucanases (EGs) and two β-glucosidases (BGs). Several of these apparently redundant enzymes have been shown to exhibit synergy by either hydrolyzing different ends of the cellulose chain or exhibiting different affinities for different sites of attack. Cellulases are classified in glycosyl hydrolase families based on their sequence homology and hydrophobic cluster analysis according to the carbohydrate active enzymes (CAZy) classification system (Norhidayu, 2014).

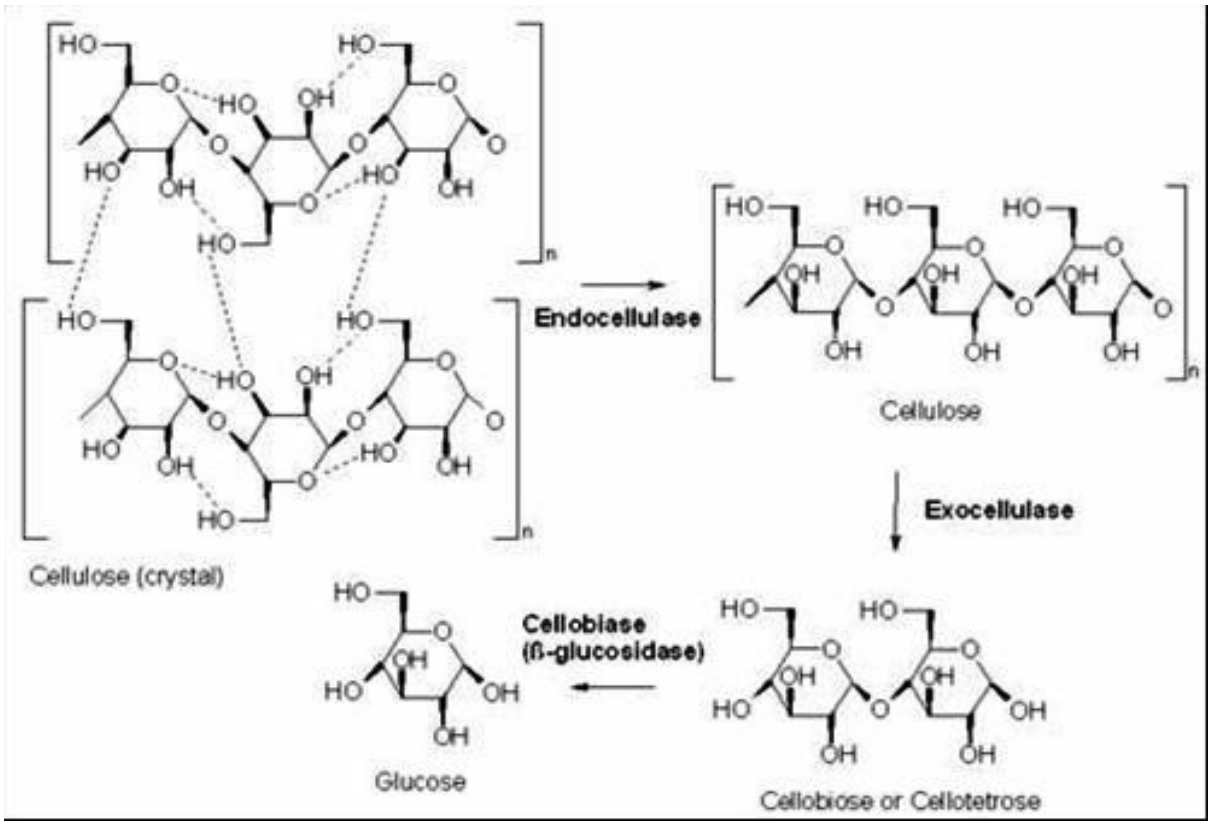


Figure 2.7 Action of enzymes on cellulose conversion to glucose (Enzymeindia, 2008)

2.6 *Aspergillus niger*

Aspergillus niger is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found (Anderson *et al.*, 2014). *Aspergillus niger* is ubiquitous in nature. It is geographically widely distributed, and has been observed in a broad range of habitats because it can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation (Wood *et al.*, 2013).

The spores are widespread, and are often associated with organic materials and soil. The primary uses of *A. niger* are for the production of enzymes and organic acids by fermentation. *A. niger* is also used to produce organic acids such as citric acid and gluconic acid

(Lynn, 2015).

In *A. niger,* there are two associated intra- and extracellular glycoproteins enzymes to target the -2, 1 linkage: inulinase and invertase (Lynn, 2015). These enzymes exhibit corresponding hydrolytic activities towards sucrose but differ in their specificities for higher-molecular-weight oligosaccharides and fructans of the inulin type. The potential of microorganisms to produce enzymes is vastly exploited for industrial purposes. *A. niger* ATCC 20611 (Wood *et al.*, 2013), efficiently utilizes wide a variety of inexpensive nutritional compounds to produce several intra- and extracellular enzymes. The productivity of many fermentation processes is affected by different parameters such as nutritional (medium composition) and physicochemical (agitation rates, pH value, inoculum size, and temperature) conditions to which the microorganism is exposed. The optimal design of culture media is an important aspect to be considered in the development of a fermentation process (Wood *et al.,* 2013). The formulation of media containing complex nutrients is generally preferred for large-scale fermentations, since it leads to the development of cost-effective processes that support maximum product yield. In the initial formulation of the medium in batch culture, an effort is made to understand the best source of carbon and energy, as well as the regulatory aspects of the enzyme (Lynn, 2015). A microscopic view of *Aspergillus niger* is shown in Figure 2.8.

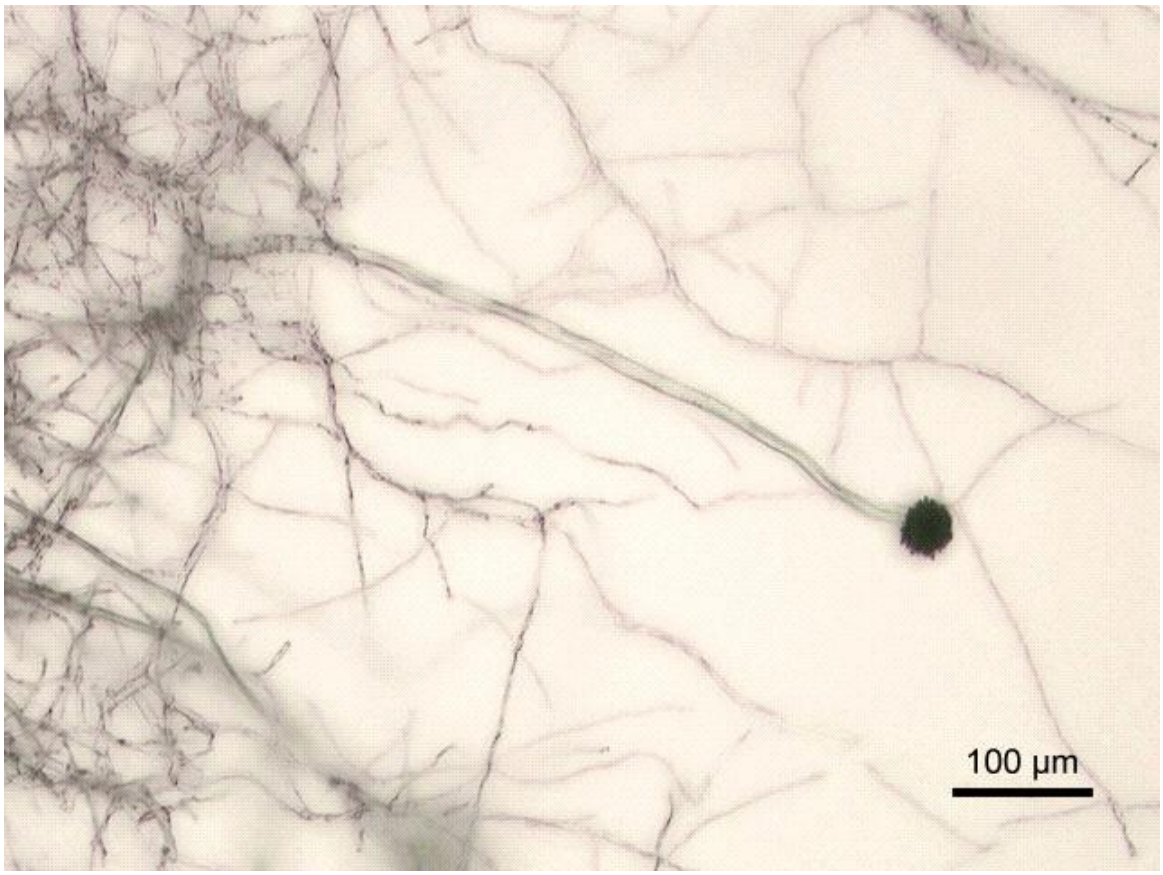


Figure 2.8: Microscopic view of *Aspergillus niger* (Lynn, 2015)

According to Anderson *et al.* (2014), the bioconversion of lignocellulosic materials into fermentable sugar requires a multienzymatic system cellulases, xylanases and other accessory enzymes. This enzymatic cocktail is one of the most costly inputs affecting the economic viability of the biochemical routes for biomass conversion into biofuels and other chemicals (Percival *et al.,* 2006). In this process, cellulases act synergistically on the hydrolysis of cellulose: endogluconases, exogluconases or cellobiohydrolases act directly on the cellulose fiber, while β-glucosidase (BGL) hydrolizes oligosaccharides and cellebiose into glucose (Macrelli *et al.,* 2012). The adequate proportion of each of these enzymes is crucial for an efficient hydrolysis, since the end product of CBH activity, which is cellobiose is a product inhibitor for these enzymes (Anderson *et al.,* 2014). Thus, β-glucosidase (BGL) activity is

important towards reducing inhibition effects. More importantly, cellobiose needs to be further degraded into the simple sugar glucose before it can be utilized by conventional yeasts for ethanol fermentation (Macrelli *et al.,* 2012).

Most commercial cellulases are produced by filamentous fungi of the genera *Trichoderma* and *Aspergillus* (Percival *et al.,* 2006). However, the amount of β-glucosidase (BGL) secreted by *Trichoderma* is not sufficient for an efficient biomass conversion. Since the extent of cellulose conversion into glucose is dependent upon the amount of active β-glucosidase (BGL) enzymes (Percival *et al.,* 2006). On the other hand, *Aspergillus* strain, such as *Aspergillus niger*, has been used for β-glucosidase (BGL) production that is often used as a supplementation of commercial enzymatic cocktails. Besides, *A. niger* is prized in the industry for its high fermentation capacity (Anderson *et al*., 2014).

2.7 Enzymatic hydrolysis

According to Kristen (2009), cellulases distinguish themselves from most other classes of enzymes by being able to hydrolyse an insoluble substrate. To facilitate sufficient contact and orientation between the catalytic domain (CD) and the substrate, CBHs and EGs have a cellulose-binding domain (CBD - or the broader term carbohydrate-binding module, CBM). The CBD is connected to the catalytic domain with a glycosylated flexible linker (Kristen, 2009). This modular feature of cellulases is critical to their ability to dock with and degrade crystalline cellulose. CBDs have little impact on the activity of cellulases on a soluble substrate (such as carboxymethyl cellulose), but removal of the CBD from the enzyme significantly impairs the hydrolysis of crystalline cellulose, demonstrating the importance of CBDs. CBDs of cellobiohydrolases are able to move laterally along the cellulose chain while the CD cleaves off cellobiose units. Interestingly, CBDs appear to have a disruptive effect on cellulose fibrils, an

ability that is still being discussed (Kristen, 2009). Furthermore, different CBDs appear to promote hydrolysis at different sites on cellulose (Kristen, 2009). However, despite extensive research on the mechanisms of CBDs, such as how the aromatic residues of the CBD are able to interact with the cellulose crystal structure, little is known about their ability to desorb from the substrate and re-attach (Kristen, 2009). Due to the insoluble nature of native cellulose and anchoring of CBDs, cellulases primarily work in a two-dimensional environment involving the uni-directional movement of cellobiohydrolases along the cellulose chain. Thus, with this lack of parameters normally used for evaluating enzyme kinetics (substrate concentration, freely diffusing enzymes) it is not surprising that the synergistic degradation of lignocellulose does not follow classic Michaelis-Menten kinetics. The understanding of mechanisms involved in the interfacial solid-liquid hydrolysis system is further complicated by a number of factors that include the heterogeneous nature of lignocellulose (Kristen, 2009). Even when working with model substrates (e.g. filter paper), the interaction between enzyme system and substrate creates innumerable factors, which influence the rate and extent of the hydrolysis. These factors often cannot be fully isolated, even under lab conditions. Much research has gone into cellulase kinetics, resulting in various models. Unfortunately, few have been able to extensively predict limiting factors or process design optima through kinetic models (Kristen, 2009).

2.8 Glucose

The name glucose is from the Greek word for ‘sweet’, which is 'glukus.' Glucose is a monosaccharide, which is another term for a simple sugar (Zhai, 2014).

Glucose is a sugar with the molecular formula $C_6H_{12}O_6$. The name "glucose" (/□ɡ lu□ kœ z/, /□ɡ lu□ kœ s/) comes from the Greek word γλυκός, meaning "sweet wine, must". The suffix "-ose" is a chemical classifier, denoting a carbohydrate. It is also known as grape sugar. With 6

carbon atoms, it is classed as a hexose, a sub-category of monosaccharides (Zhai, 2014).

α-D-glucose is one of the 16 aldosestereoisomers. The D-isomer (D-glucose), also known as dextrose, occurs widely in nature, but the L-isomer (L-glucose) does not. Glucose is made during photosynthesis from water and carbon dioxide, using energy from sunlight (Bhaduri, 2015). The reverse of the photosynthesis reaction, which releases this energy, is a very important source of power for cellular respiration (Bhaduri, 2015). Glucose is stored as a polymer, in plants as starch and in animals as glycogen, for times when the organism will need it. Glucose circulates in the blood of animals as blood sugar. Glucose can be obtained by hydrolysis of carbohydrates such as milk, cane sugar, maltose, cellulose, glycogen etc. It is however, manufactured by hydrolysis of cornstarch by steaming and diluting acid (Bhaduri, 2015).

Identification

Both forms of glucose have a molecular formula of $C_6H_{12}O_6$, molecular mass of glucose is 180.16 g mol and are sweet-tasting, white, crystalline powders with a melting point of 153 to 156°C (307--312°F). Glucose exists mostly in the cyclic chair form, but it can exist in chain form (Zhai, 2014). Figure 2.9 shows a single molecular structure of glucose.

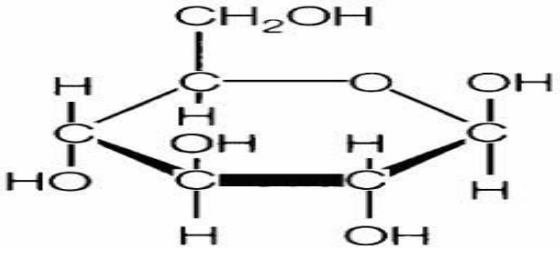


Fig.2.9 Structure of glucose molecule (Zhai, 2014)

The common primary raw materials for the production of glucose are corn, rice, sorghum, wheat and cassava (Aschengreen *et al*., 1979). Recently, the Agricultural waste and grasses have being focused as the alternative method to produce glucose.

Agricultural wastes are materials left in an agricultural field or orchard after the crop has been harvested. These residues include stalks and stubble (stems), leaves, and seed pods which contain the largest source of cellulose such as banana stem, rice straw and pineapple leaf (Bhaduri, 2015). By using fermentation method, glucose can be produced as the intermediate product. Fermentation begins as the growing population of microorganisms produces enzymes to break two-molecule sugars into single molecule sugars and then convert the single molecule sugars into the commercial chemicals and byproducts (Aschengreen *et al*., 1979).

2.8.1 Dextrose equivalent

Dextrose equivalent (DE) is a measure of the amount of reducing sugars present in a sugar product, relative to dextrose (glucose), expressed as a percentage on a dry basis (Zhai, 2014). For example, a maltodextrin with a DE of 10 would have 10 % of the reducing power of dextrose (which has a DE of 100). Maltose, a disaccharide made of two glucose (dextrose) molecules, has a DE of 52, correcting for the water loss in molecular weight when the two molecules are combined (180/342) (Zhai, 2014). Sucrose actually has a DE of zero even though it is a disaccharide, because both reducing groups of the monosaccharides that make it are connected, so there are no remaining reducing groups. For solutions made from starch, it is an estimate of the percentage of reducing sugars present in the total starch product (Aschengreen *et al*., 1979).

In all glucose polymers, from the native starch to glucose syrup, the molecular chain begins with a reducing sugar, containing a free aldehyde. As the starch is hydrolyzed, the molecules become

shorter and more reducing sugars are present (Aschengreen *et al*., 1979). Because different reducing sugars (e.g. fructose and glucose) have different sweetness, it is incorrect to assume that there is any direct relationship between DE and sweetness (Aschengreen *et al*., 1979).

The DE describes the degree of conversion of starch to dextrose. Starch is close to zero, glucose/dextrose is 100 (percent), dextrins vary between 1 and 13, maltodextrins vary between 3 and 20 glucose syrups contain a minimum of 20% reducing sugars.

The DE gives an indication of the average degree of polymerisation (DP) for starch sugars. The rule of thumb is DE × DP = 120. The standard method of determining DE is the Lane-Eynon titration, based on the reduction of copper(II) sulfate in an alkaline tartrate solution, an application of Fehling's test (Zhai, 2014).

2.8.2 Uses of glucose

Glucose is widely used as sweetener in food and beverage (Zhai, 2014). As a sweetener, Glucose can be used in a wide variety of industries including: food production, beverage, pharmaceutical, agriculture/animal feed, and various other industries (Shawn, 2014).

2.8.2.1 Glucose in food production

Glucose is widely used as nutritional supplement and sweetener in food production (Shawn, 2014). It is also used as nutritional supplement in infant formula to improve nutrition value. As sweetener, glucose is used in confectionery to provide cool taste; in bakery to enhance yeast growth and improve appearance (Shawn, 2014).

2.8.2.2 Glucose in beverage

Glucose is used as nutritional supplement in functional beverages to provide energy (Shawn, 2014). Glucose syrups have a well established role as key ingredients in the beverage industries (Jackson, 2016). Because of their competitive price and specific functional properties they are now used extensively in numerous applications across the beverage industries (Jackson, 2016).

2.8.2.3 Glucose in pharmaceuticals

In the pharmaceutical industries, glucose is widely used as nutritional supplement in oral tablets; and as buffering agent (injection grade) in injection (Shawn, 2014). Glucose is used in the pharmaceutical industries as a precursor to make vitamin C (L-ascorbic acid) in the Reichstein process, to make citric acid, gluconic acid and polylatic acid (Renee, 2017). It is also an ingredient that sweetens liquid medicines such as syrups and tonics. It is also used to coat tablets for ease of swallowing and as granulating agent (Renee, 2017).

2.8.2.4 Glucose in agriculture/animal feed

Glucose is widely used as Buffering agent veterinary medicine and nutrition supplement in Agriculture/Animal Feed to enhance nutrition value (Shawn, 2014). A recent advance in feed technology and animal nutrition is the development of sugar syrup, an intermediary product of sugar refining as energy feed for livestock (Zhai, 2014).

2.8.2.5 Glucose in other industries

Glucose is widely used as antioxidant in leather processing and as fermentation substrate in production of various vitamins, amino acids and other organic products (Shawn, 2014). It provides the carbohydrates for fermentation in yeast products (Renee, 2017).

Glucose is an indispensable substrate in fermentation processes to produce biofuels (Shawn, 2014).

2.9 Process optimization

An optimization process is any kind of process that systematically comes up with solutions that are better than the solution used before (Yudkowsky, 2017). More technically, this kind of process is one that performs searches in a large search space, hitting small, low probability targets. When this process is gradually guided by some agent into some specific state, through searching specific targets, we can say it prefers that state.

For an industrial hydrolysis process, hydrolysis medium and hydrolysis process conditions play a critical role, because they effect the formation, concentration and yield of a particular hydrolysis end product, thus effecting the overall process economics (Bibhu *et al.,* 2007). Therefore it is important to consider the optimization of hydrolysis medium and process conditions in order to maximize the profits from the process (Schmidt, 2005). There are many challenges associated with optimization of a process, it is laborious, expensive, open ended and time consuming process involving many experiments (Bibhu *et al.*, 2007). In bioprocess industry it is often needs to conduct optimization experiments because new mutants and strains are continuously being introduced. In hydrolysis process, optimization, different combinations and sequence of process conditions and medium components are needed to be investigated to determine the growth condition that produces the biomass with the physiological state best constituted for product formation (Stanbury *et al*., 1997). Table 2.3 shows the various types of optimization techniques used in process optimization. In this work, the one-factor-at-a-time optimization method was used considering its advantage.

Table 2.3: Types of optimization techniques and their advantages and disadvantages

Optimization technique	Advantages	Disadvantages
Borrowing	It is simple easy and requires no mathematical skill	There are too many options for a given process
Component replacing	Useful for screening	Does not consider the component interaction
Bilogical mimicry	Gives an idea about different micro and macro elements required	Does not consider the component interaction
One-factor-at-a-time	The individual effect of medium components and process condition can be seen on graph	Interactions between the component are ignored
Factorial design	It allows for the estimation of the effects of each factor and interactions	Requires mathematical skill
Response surface Methodology	Visualizations of results by 3D graphs and predicts models	Requires mathematical skill
Artificial neural network	Large numbers of data are easily handled	Requires mathematical skill
Fuzzy logic	It has the ability to tolerate highly variable data	Requires high mathematical skill
Genetic algorithms	Systematic and steady improvement of factors	Requires high mathematical skill

Source: (Bibhu *et al.* 2007)

2.10 Process reaction kinetics

Chemical kinetics is the study of chemical processes and reactions. This includes analysis of conditions that affect speed of a chemical reaction, understanding reaction mechanisms and transition states, and forming mathematical models to predict and describe a chemical reaction.

The field of chemical kinetics was developed from the law of mass action, which was formulated in 1864 by Peter Waage and Cato Guldberg (Helmenstine, 2017). The law of mass action states that the speed of a chemical reaction is proportional to the amount of reactants. Experimental data is used to find reaction rates, from which rate laws and chemical kinetics rate constants are derived by applying the law of mass action. Rate laws allow for simple calculations for zero order reactions, first order reactions, and second order reactions.

The rate of a zero-order reaction is constant and independent of the concentration of reactants. rate = k

The rate of a first-order reaction is proportional to the concentration of one reactant: rate = k[A]

The rate of a second order reaction has a rate proportional to the square of the concentration of a single reactant or else the product of the concentration of two reactants. rate = $k[A]^2$ or k[A][B]

Rate laws for individual steps must be combined to derive laws for more complex chemical reactions. For these reactions: There is a rate-determining step that limits the kinetics.

In enzymatic reactions, the rate of reaction is best described by the Michealis – Menten's reaction kinetics (Lee, 2002). Where r_{max} (the maximum reaction rate) and k_m (rate constant) are the kinetic parameters, which are needed to be experimentally determined and Cs is substrate concentration.

2.11 Spectrophotometer working principles

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution (Atkins *et al.,* 2006). The basic principle is that each compound absorbs or transmits light over a certain range of wavelength (Atkins *et al.*, 2006). This measurement can also be used to measure the amount of a known chemical substance (Atkins *et al.*, 2006). Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications (Atkins *et al.*, 2006).

Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a certain range of wavelength. A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected (Price *et al.*, 1997). Depending on the range of wavelength of light source, it can be classified into two different types:

UV-visible spectrophotometer: uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum (Price *et al.*, 1997).

IR spectrophotometer: uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum (Irwin, 1995).

In visible spectrophotometry, the absorption or the transmission of a certain substance can be determined by the observed color. For instance, a solution sample that absorbs light over all visible ranges (i.e., transmits none of visible wavelengths) appears black in theory. On the other hand, if all visible wavelengths are transmitted (i.e., absorbs nothing), the solution sample appears white. If a solution sample absorbs red light (~700 nm), it appears green because green is

the complementary color of red. Visible spectrophotometers, in practice, use a prism to narrow down a certain range of wavelength (to filter out other wavelengths) so that the particular beam of light is passed through a solution sample (Irwin, 1995).

Figure 2.10 illustrates the basic structure of spectrophotometers. It consists of a light source, a collimator, a monochromator, a wavelength selector, a cuvette for sample solution, a hotoelectric detector, and a digital display or a meter.

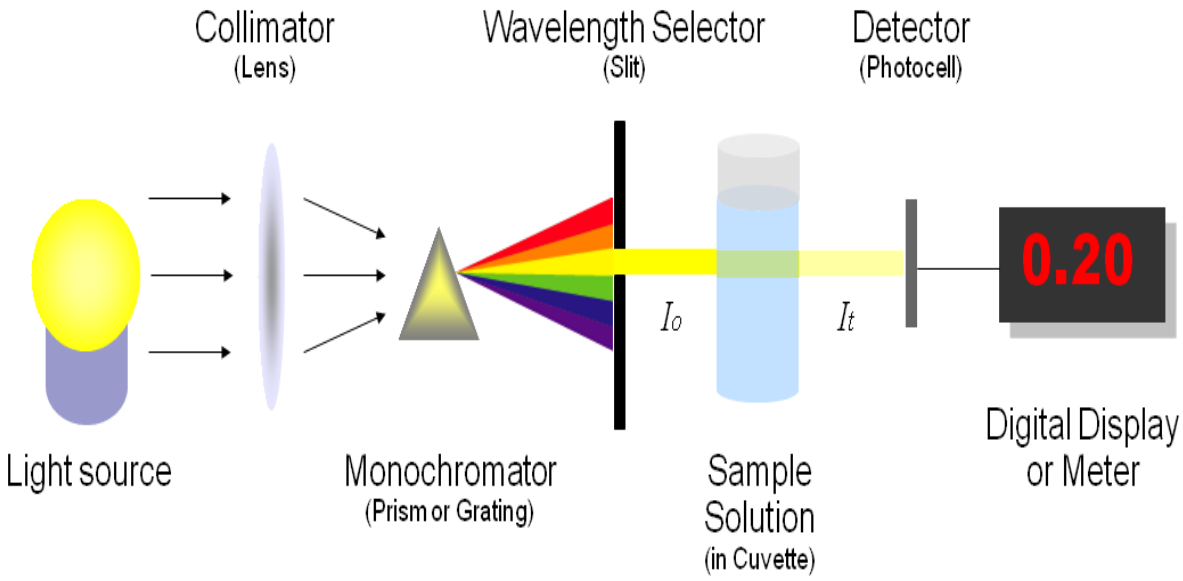


Figure 2.10: Basic structure of spectrophotometers (Gore, 2000)

A spectrophotometer, in general, consists of two devices; a spectrometer and a photometer. A spectrometer is a device that produces, typically disperses and measures light. A photometer indicates the photoelectric detector that measures the intensity of light (Gore, 2000).

Spectrometer: It produces a desired range of wavelength of light. First a collimator (lens) transmits a straight beam of light (photons) that passes through a monochromator (prism) to split

it into several component wavelengths (spectrum) (Gore, 2000). Then a wavelength selector (slit) transmits only the desired wavelengths, as shown in Figure 2.10.

Photometer: After the desired range of wavelength of light passes through the solution of a sample in cuvette, the photometer detects the amount of photons that is absorbed and then sends a signal to a galvanometer or a digital display, as illustrated in Figure 2.10.

CHAPTER THREE

3.0 Materials and Methodology

In this chapter, materials, equipment and instruments used to achieve the desired aim of this work are presented in tables 3.1 – 3.3, including their specifications, sources and manufacturers. Figure 3.1 also shows the block diagram indicating process routes taken to transform the elephant grass into glucose.

3.1 List of Materials

Table 3.1: List of materials

Material	Specification	Source	Manufacturer
Elephant grass		NAPRI, Ahmadu Bello University Zaria	
Sodium acetate	Analar grade	Steeve Moore Chemical Company Ltd. Layin zomo Zaria	Vaibhav Industries Inc.
Standard glucose	Analar grade	Department of Chemistry Ahmadu Bello University Zaria	Sigma aldrich
Aqueous ammonia solution	Analar grade	Steeve Moore Chemical Company Ltd. Layin zomo, Zaria	Sigma aldrich
Distilled water.	pH 7	Department of Chemical Engineering Ahmadu Bello University, Zaria	Sigma aldrich
Aspergillus niger	cultured	Department of microbiology Ahmadu Bello University, Zaria	
DNS reagent		Steeve Moore Chemical Company Ltd. Layin zomo, Zaria	Research –Lab Fine Chem Industries
Hydrochloric acid	Analar grade	Department of Biochemistry Ahamadu Bello University Zaria	Amcor Limited

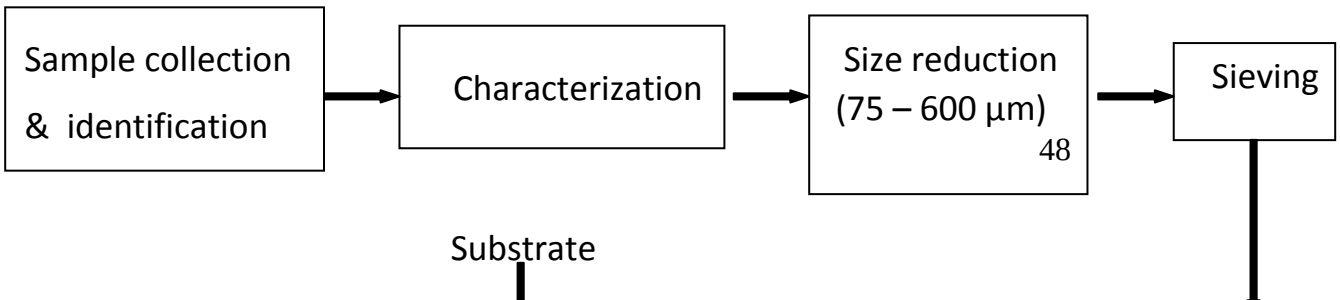
3.2 List of Equipment

Table 3.2: List of equipment

Material	Specification	Source	Manufacturer
Weighing balance	(ae Adam pw 184)	Department of Chemical Eng'g. Ahmadu Bello University Zaria	Contech Instrument Ltd. Mumbai
Cooled incubator	(Gallenkamp model)	Department of Biochemistry Ahmadu Bello University Zaria	Gemini BV Laboratory equipment
Digital pH meter	(Jenway 3150)	Department of Biochemistry Ahmadu Bello University Zaria	Qingdao Tlead Int'l Co. Ltd.
Oven	(Gallenkamp model)	Department of Biochemistry Ahmadu Bello University Zaria	Environmental equipment Co. Ltd.
Magnetic hot plate stirrer regulator	(Gallenkamp model)	Department of Chemical Eng'g Ahmadu Bello University Zaria	Remi Elektrotechnic Ltd.
Laboratory mill	(Thomas wite model 4)	Institute for Agricultural Research Ahmadu Bello University Zaria	Thomas scientific equipment
Spectrophotometer	(WPA 5050, U.S.A)	Department of Chemical Engineering Ahmadu Bello University Zaria	BWB Technologies Inc.
Thermostatic water bath	(HL/BT1)	Department of Chemical Engineering Ahmadu Bello University Zaria	Laboratory Instruments Inc.

3.3 List of instruments

S/No	Instrument	Specification & sensitivity	Manufacturer
1	Immersion thermometer.	(0 – 100 ^{o}C)	Brannan Thermometers and Instruments UK
2	Beaker	(250 ml ± 10 ml)	Jencons Glass Industries
3	Funnel	(90 ml ± 5 ml)	Gonotec-Osmometer Inc.
4	Spatula	(stainless steel)	Guilin Wookpecker Medical Instrument Co. Ltd
5	Filter paper		Filtros Anoia, SA Inc.
6	Mixing tubes	(20 ml ± 2 ml)	Ace glass Inc. USA
7	Aluminium foil		Amcor Ltd. Australia
8	Measuring cylinder	(100 ml ± 5 ml)	Ace glass Inc. USA



Aspergillus niger

Analysis

Figure 3.1: Block diagram for glucose production process from elephant grass

3.4 Sample collection, identification and characterization

Samples of grasses were collected from National Animal Production and Research Institute NAPRI) Zaria provinces. Samples were identified as *Pennisetum purpureum* at Biological Sciences Laboratory, Ahmadu Bello University Zaria and were separated into three – the leaves, the inner (soft) stem and the outer (hard) stem. The three samples were dried at 55 °C in an oven (Gallenkamp model) for 48 hours, ground to small particles and later sieved using different meshes (75 µm – 600 µm).

Biomass contents of the three samples were analyzed according to the method of United States National Renewable Energy Laboratory (NREL, 2010) adapted by Animal Science Biochemical Laboratory, Institute of Agricultural Research (IAR) Ahmadu Bello University Zaria. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Lignin (L) and other fractions of the grass were determined. Hemicellulose content of biomass was determined the difference between NDF and ADF, and cellulose content of biomass was determined from the difference of 100 and aomount of Lignin and hemicellulose (100 – Lignin – Hemicellulose). The stem of the elephant grass was selected for the conversion.

3.4.1 Characterization procedures

3.4.1.1 Determination of moisture content

A clean aluminium dish was dried in an oven at 105 ^{o}C for one hour, and was placed into desiccators to cooled and weighed. The dish was handled with metal tongs, then about 40 g of the sample was weighed and put into the weighed dish and reweighed, then placed in an oven at 105 ^{o}C overnight to dry to a constant weight. The dish and sample was cooled in a desiccator and weighed quickly as possible to prevent moisture absorption. The moisture content was calculated as

$$\%\ ash\ content = \frac{loss\ in\ weight\ on\ drying}{weight\ of\ sample\ before\ drying} * 100 \quad (3.1)$$

Triplicate determinations were carried out on the sample.

The % Dry Matter was calculated as 100 – % Moisture content (AOAC, 2016).

3.4.1.2 Determination of ash

The sample was ignited at 600 ^{o}C in a Muffle Furnace to turn off all organic materials. The inorganic material which does not volatilise at that temperature is called ash (AOAC, 2016).

A clean crucible was placed into a Muffle Furnace at 600 ^{o}C for one hour. The crucible was transferred from the furnace to a desiccator and cooled to room temperature and weighed.

A 2.0 g portion of the sample was put into the crucible and reweighed. Then the crucible and its content were placed into a muffle furnace at a temperature of 600 ^{o}C at 12 hours to obtain whitish-grey ash. Thereafter, the crucible was transferred into a desiccator and cooled to room temperature and weighed as quickly as possible to prevent moisture absorption. Triplicate determinations were performed on the sample and the mean value recorded (AOAC, 2016).

$$\%\ lipid = \frac{weight\ of\ oil}{weight\ of\ sample} * 100 \quad (3.2)$$

3.4.1.3 Determination of ether extract (oil)

The sample was treated with light Petroleum, boiling range of 40-60 ^{o}C to extract the ether extract (oil), the solvent was distilled off and the dried extract was weighed (AOAC, 2016).

Zero point five grammes of the dried sample was weighed and wrapped in a filter paper and placed into the extraction thimble. The thimble was placed inside the Soxhlet extractor; dried, tarred solvent flask was placed in position beneath, with about 180 cm^3 of solvent and connected to the condenser. The heating rate was adjusted to give a condensation rate of 3 drops/second and extraction was carried out for 6 hours. The thimble was removed and the ether reclaimed using the apparatus. The completion of the removal of ether was on a boiling water bath and dry flask at 105 ^{o}C for 30 minutes, allowed to cool in a desiccator before weighing to constant weight. Triplicate determinations were carried out on the sample (AOAC, 2016).

3.4.1.4 Determination of crude protein

The sample was digested in sulphuric acid to breakdown organic matter and reduce nitrogenous compounds to ammonium compounds. Ammonia was liberated by boiling with sodium hydroxide; the steam was distilled into boric acid solution and determined titrimetrically. During the digestion phase, tablets containing potassium sulphate and mercuric oxide were added. The potassium sulphate raises and controls the temperature of the reaction, while the mercuric oxide acted as a catalyst. During the digestion process, the following reaction occurs

$$CH_3NH_2 + 4H_2SO_4 \rightarrow CO_2 + 3SO_2 + 4H_2O + NH_4HSO_4 \quad (3.3)$$

For the distillation phase, the following reaction occurs

$$NH_4HSO_4 + 2NaOH \rightarrow NH_3 + Na_2SO_4 + 2H_2O \quad (3.4)$$

(AOAC, 2016).

Half a gramme of the sample was weighed and put into 100 cm^3 digestion flask. 30 cm^3 of

concentrated sulphuric acid were added into a kjeldahl digestion flask. The content in the Kjeldahl digestion flask was heated gently to 105 ^{o}C (by means of electric element) and then shaken vigorously for 2 hours with intermittent swirling of the flask to ensure even digestion and prevent over heating of the content. At this stage of digestion, the reaction mixture turns black due to the dehydrating action of the sulphuric acid and formation of free carbon. Thereafter, it was allowed to cool and 10 cm^3 of distilled water was added to dissolve the crystals that were formed and poured into a 100 cm^3 volumetric flask and the volume was brought to 100 cm^3 with distilled water (AOAC, 2016). A 10 cm^3 aliquot of the diluted solution was pipetted into a Markham distiller and 10 cm^3 of 40 % Sodium hydroxide (NaOH) solution added. The solution was distilled and the liberated ammonia trapped in a 100 cm^3 conical flask containing 10 cm^3 of 4 % boric acid and 2 drops of methyl red indicator, distillation was continued until the indicator colour changes from pink to green. When 50 cm^3 of the distillate was collected in the green boric acid and indicator solution, the distillate was then titrated with 0.02 N Sulphuric acid which gave a grey-mauve end point. The volume of the acid used as well as the blank was noted. Triplicate determinations were carried out on the sample (AOAC, 2016).

% Total nitrogen was calculated as

$$\%\, N_2 = \frac{(sample\ titre - blank\ titre) * normality\ of\ acid * 1402 * 10}{weight\ of\ sample\ (g) * aliquot} * 100 \qquad (3.5)$$

% Crude Protein = % N_2 * 6.25, where 6.25 is a conversion factor

3.4.1.5 Determination of crude fibre

It involves sequential treatment of the sample with boiling solutions of Sulphuric acid and Sodium hydroxide. The residue was separated by filtration, washed, dried, weighed and ashed. The loss of weight resulting from ashing corresponds to the crude fibre present in the sample (AOAC, 2016).

Two grammes of the sample was weighed and put into a 600 cm^3 beaker and 200 cm^3 of 0.128 N sulphuric acid solution was added and the mixture boiled for 30 minutes with the use of labconco heating mantle. The hot solution was quickly filtered and the insoluble matter washed with hot water several times to ensure that it was free of acid. The residue was transferred into a beaker and 200 cm^3 of hot Sodium hydroxide solution was added, and the mixture boiled for another 30 minutes and the solution was filtered quickly, washed with hot water until it was free of base. The insoluble residue obtained was then washed with acetone and collected into a clean crucible and dried in an oven at 105 ^oC to constant weight. The dried residue was weighed in a crucible as quickly as possible, after cooling in a desiccator to avoid moisture absorption. The crucible and its content were incinerated in a Muffle Furnace to ash at 600 ^oC for 3 hours. The crucible and ash content was allowed to cool in a desiccator and reweighed (AOAC, 2016).

. The fibre content was calculated as:

$$\%\ Crude\ fiber = \frac{weight\ of\ ashes}{weight\ of\ sample} * 100 \qquad (3.6)$$

3.4.1.6 Determination of nitrogen free extract

The nitrogen free extract (NFE) was calculated by difference as:

% NFE = 100 – (% CP + % CF + % EE + % Ash)

where, CP = Crude Protein, CF = Crude Fibre, EE = Ether Extract (Oil) (AOAC, 2016).

3.4.1.7 Determination of Hydrogen Cyanide (HCN)

Alkaline titration method (AOAC, 2016)

Ten grammes of ground sample was soaked in a mixture of 200 cm^3 of distilled water and 10 ml of othophosphoric acid. The mixture was left for 12 hours to release all bounded hydrocyanic

acid. A drop of antiforming agent (paraffin) and anti-bumping agent was added and the solution distilled until 150 cm^3 of the distillate was collected. 20 cm^3 of the distillate was taken into a conical flask and diluted with 40 cm^3 of distilled water. 8.0 cm^3 0f 0.6 M ammonium hydroxide (NH_4OH) and 2 cm^3 of 5 % (W/V) potassium iodide (KI) solution were added. The mixture was titrated with 0.02 M silver nitrate ($AgNO_3$) solution using a micro burrete until a faint but permanent turbidity was obtained.

3.5 The Pretreatment

One hundred grammes of the selected sample was soaked in boiled water and allowed to cool, then filtered and dried at 55 ^oC for 48 hours. This was done simply to remove the soluble free sugar content of the grass and to check for the possibility of the removal of lignin. The filtrate was analyzed for the soluble sugar present. The dried sample was weighed and later separated into two portions. Ten grammes of one of the portions to be used as treated substrates were added to 100 ml of dilute NH_4OH solution at 25 ^oC for 24 hours. This step was repeated using concentrated NH_4OH solution. The sample was filtered and washed repeatedly with distilled water until pH of 7 was attained. The substrate was then dried in an oven at 55 ^oC to a constant dry weight for 48 hours.

Scanning Electron Microscopy (SEM) was used for the four samples (i.e The untreated, the hot water treated, the dilute ammonium hydroxide solution treated and the concentration ammonium hydroxide solution treated), to check the effect of pretreatment before the hydrolysis.

3.6 Hydrolysis Steps

Aspergillus niger was obtained from Department of Microbiology, Ahmadu Bello University, Zaria and stored in a refrigerator for a week for continuous growth.

In a typical run, 2 g of the substrate was placed in a beaker containing 100 ml of 0.1 M solution of sodium acetate at pH 4.5. The temperature of the magnetic stirrer (Sharmond model) was set at constant temperature 45 °C. 0.1g *A. niger* species was added. Samples were being withdrawn at intervals of 30 minutes, within 24 hours reaction time for analyses. The concentrations of glucose in the samples were determined by following the method of Lee and Fan (1982) and Lee (1992) which uses a Randox glucose kit and colorimeter (Model WPA 5001, U.S.A.) at 540 nm.

3.6.1 Effect of reaction time

The effect of reaction time on glucose concentration was investigated at 8 hours, 16 hours, 24 hours, 48 hours and 72 hours. Other reaction variables were kept constant and the experiment was repeated in triplicate.

3.6.2 Effect of pH

The effect of pH on glucose concentration was investigated by carrying out the hydrolysis at 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 using Sodium acetate buffer solution (Omemu *et al.* 2005). All other conditions were kept constant as described in this session. Reaction time was 24 hours.

3.6.3 Effect of temperature

The effect of temperature on glucose concentration was investigated by carrying out the experiment at 30 ^{o}C, 35 ^{o}C, 40 ^{o}C, 45 ^{o}C, 50 ^{o}C, 55 ^{o}C and 60 ^{o}C. Other fermentation parameters were held constant with reaction time, 24 hours (Omemu *et al*. 2005).

3.6.4 Effect of substrate particle size

The particle sizes of the elephant grass (both treated and untreated) were selected by passing it through different sizes of standard sieves after crushing. Fractions of particle sizes of 75 μm,

100-150 μm, 151 - 250 μm, 251 – 350 μm, 351-450 μm, 451-550 μm and 551-600 μm were used in the hydrolysis. Other conditions were kept constant with the reaction time, 24 hours. Analyses were made and the optimum particle size range was reported.

3.6.5 Effect of substrate concentration

The effect of the amount of substrate on glucose concentration was investigated by using substrate initial concentrations of 5 g/l, 10 g/l, 20 g/l, 40 g/l, 60 g/l, 80 g/l and 100 g/l. Other reaction conditions were kept constant (Aderemi *et al.* 2008).

3.6.6. Effect of cell loading

The effect of *A.niger* concentration was investigated using 0.1 g, 0.2 g, 0.3 g, 0.4 g, 0.5 g, 0.8 g and 1.0 g with other reaction conditions kept constant (Highina *et al*. 2011). All runs were repeated three times and the mean value of each set of runs was reported, accordingly.

3.7 Determination of glucose concentrations

3.7.1 Preparation of the control sample

The spectrophotometer was plugged and turned on by pressing the ON button. It was then allowed to warm up for 5 minutes. After it must have warmed up, the wavelength dial was set to 540 nm. This is a wavelength of light that gets absorbed readily by the pigment in the glucose solution. With no vial in the spectrophotometer, the light path was closed, therefore no light was transmitted, the needle was set to read infinity (∞) on the absorbance scale. A cuvette (a small test tube that is 12 mm in diameter and 100 mm in length) was filled with Blank sample (distilled water).

The cuvette containing Blank sample was inserted into the sample chamber. As this was done,

the light path opened. This blank solution did not contain any glucose solution, and so the absorbance was set to zero using the right knob. The blank sample is the control sample.

3.7.2 Preparation of glucose standard curve

Several dilutions of a glucose standard solution that has an initial concentration of 50 g/l of glucose was prepared. Four large mixing tubes were labelled A, B, C, and D. 50 ml of distilled water was pipette into each of the large mixing tubes. 50 ml of glucose standard solution was added to mixing tube A, and then covered with aluminium foil and thoroughly mixed. 50 ml of standard dilution A, was added into mixing tube B, covered with aluminium foil and thoroughly mixed. 50 ml of standard dilution B, was added into mixing tube C, covered with aluminium foil and thoroughly mixed. 50 ml of standard dilution C, was added into mixing tube D, covered with aluminium foil and thoroughly mixed. The concentration of glucose in each tube was calculated using the following equation $V_1C_1 = V_2C_2$ (Samer, 2009).

Where V_1 = volume of the stock solution

C_1 = the concentration of the stock solution

V_2 = the final diluted volume (volume of stock solution and diluent)

C_2 = the final diluted concentration.

2 ml of the prepared dilute solutions was pipette from each of the tubes into another tubes labelled E, F, G, H and I. 2 ml of dinitrosalicylic acid (DNS) reagent was added into each tube making 1:1 DNS – solution ratio. The contents of the test tubes were heated in a boiling water bath for five minutes and the solution turned into dark orange-red colour. The test tubes were cooled to room temperature, after taking them out of the water bath. 10 ml distilled water was

added to each test tube and was thoroughly mixed (Lee, 1992). 2 ml of the final solution was taken from each test tube into different cuvettes and each cuvette was placed in the sample chamber of the spectrophotometer. The intensity of the dark orange red colour of the samples was observed and recorded at 540 nm as the absorbance (Lee, 1992). Graph of the absorbance versus glucose concentration was plotted using Microsoft Excel. Concentrations of the produced glucose were determined using the plotted graph.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Introduction

In this chapter, experimental results obtained are explicitly presented in tables, figures and plates. Due to the large number of anticipated experimental runs, some preliminary studies were done to ascertain the practical significance, if any, of delignification on glucose yield. Similarly, the particle size of elephant grass required for optimum reaction rate in favor of the targeted product was also determined. Effect of operation conditions such as pH and temperature were also determined. The kinetics were investigated based on Michelis-Menten's kinetics and a model is established based on the obtained kinetic parameters.

4.2 Elephant grass constituents analysis

Table 4.1 shows the fiber fractions and proximate compositions of elephant grass. It could be seen that the stem contains more lignin than the leaves, and this is due to the fact that the stem protecting to the other parts of the plant. The difference in neutral detergent fiber (NDF) and acid detergent fiber (ADF) gives the value of the hemicellulose contents of the plant (NREL, 2010). The various values of ADF and NDF presented in Table 4.1 indicates that the leaves contain more hemicellulose than the stem of the plant. In terms of proximate compositions, the leaves contain more crude protein than the stem, while the entire plant has very little oil making it difficult for biodiesel production. The stem also has more carbohydrate contents than the leaves, which shows that the stem will give more sugar than the leaves.

Table 4.1 Fiber fractions of elephant grass

Description	% Lig.	% DM	% ADF	% NDF	% C.P	% C.F	% Oil	% Ash	% NFE
Inner Stem	8.05	75.72	29.66	53.36	2.79	10.24	0.40	3.42	83.15
Outer Stem	10.78	91.64	49.88	82.04	1.36	27.76	0.18	9.76	68.60
Leaves	7.88	58.36	4.62	71.38	6.29	16.84	1.95	2.10	65.16

LEGEND: Lig. = Lignin DM = Dry Matter ADF = Acid Detergent Fiber
NDF = Neutral Detergent Fiber C.P = Crude Protein C.F = Crude Fiber
NFE = Nitrogen Free extract

In Table 4.2, it could be seen that the inner stem has the highest cellulose contents (68.25 %) followed by the inner stem with 57.06 %. Thus, the stem was chosen for the hydrolysis since it contains more cellulose than the leaves. Perhaps, this explains why most researchers often use the stems for biofuel production. For example, Aiyejagbara *et al.* (2015), in their work used elephant grass stem to produce bioethanol by simultaneous saccharification and fermentation and obtained 78 % yield.

Table 4.2 Experimental values for elephant grass composition before pretreatment

Description	% Lignin	% Hemicellulose	% Cellulose
Inner Stem	8.05	23.70	68.25
Outer Stem	10.78	32.16	57.06
Leaves	7.88	66.86	25.26

4.3 Effect of pretreatment on glucose yield

Table 4.3 shows the contents of cellulose, hemicellulose and lignin of the stem after

pretreatment. The results show that when concentrated ammonium hydroxide is used for pretreatment more lignin was removed than when dilute ammonium hydroxide was used. This is due to the fact that concentrated ammonium hydroxide reacts more readily with lignin than the dilute ammonium hydroxide. The concentrated ammonia soaked large amount of lignin compare to the dilute ammonium hydroxide, thereby did more work of exposing the cellulose for enzymes action than the dilute ammonium hydroxide.

Scanning Electronic Microscopy (SEM) analyses were made for the grass sample both before and after pretreatment and the results are as shown in Plate 4.1:

Table 4.3 experimental values for elephant grass composition after pretreatment

	Conc. NH_4OH			Dilute NH_4OH		
Description	% Lig.	% Hemicel.	% Cellulose	% Lig.	% Hemicel.	% Cellulose
Inner stem	1.24	23.70	68.25	5.41	23.70	68.25
Outer stem	2.78	32.16	57.06	8.02	32.16	57.06
Leaves	0.88	66.86	25.26	4.38	66.86	25.26

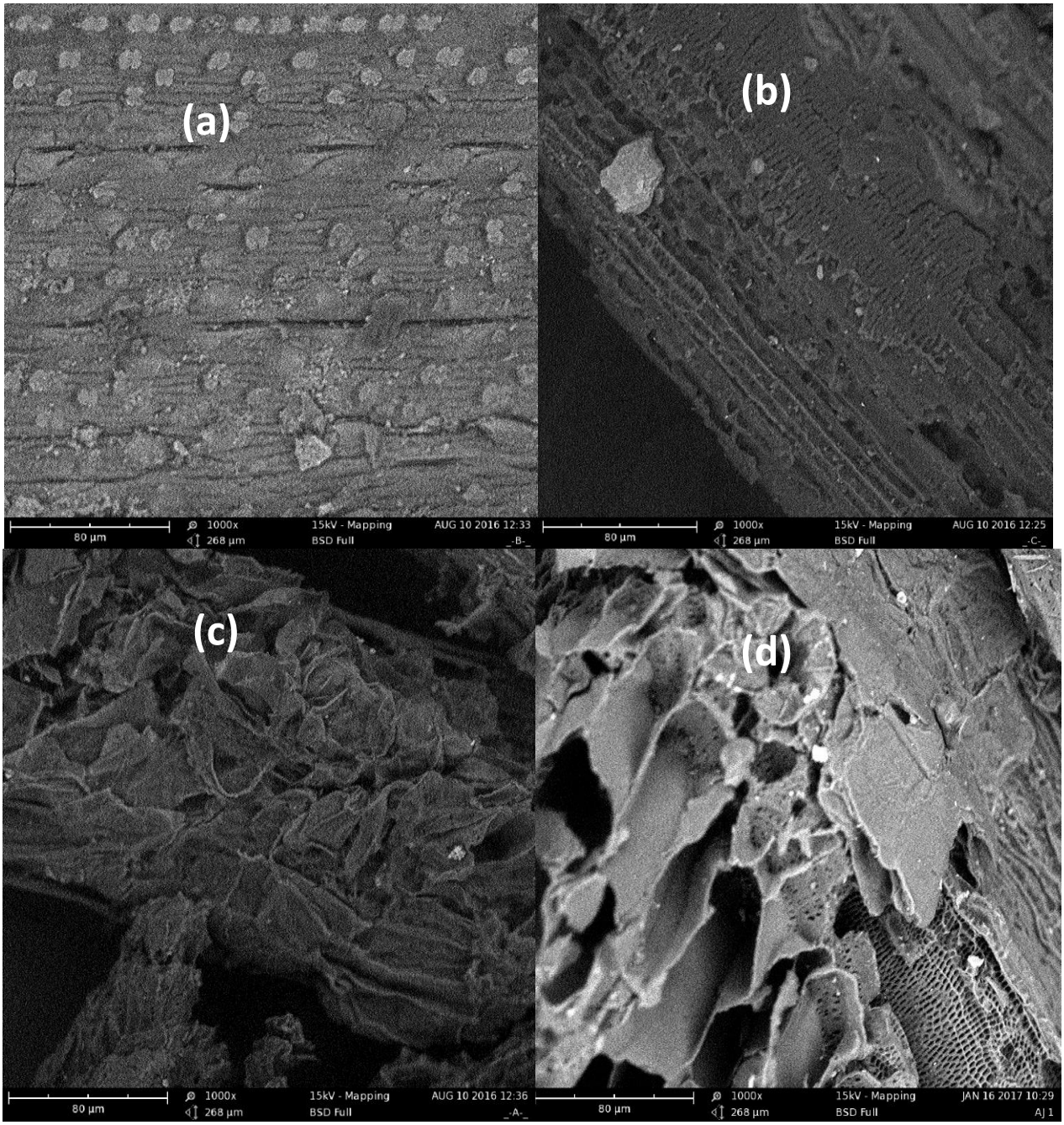


Plate 4.1: Scanning Electron Microscopy (SEM) view: (a) Untreated (b) Treated with hot water

(c) Treated with dilute ammonium hydroxide (d) Treated with concentrated ammonium hydroxide.

Plate 4.1 (a) shows the untreated sample of elephant grass. It could be seen that lignin accumulated in layers providing shield for the cellulose from enzymes attack. In plate 4.1 (b), a sample of elephant grass treated with hot water is displayed. Here, lignin was partially dispersed, even though the aim was to remove the soluble free sugar contents of the elephant grass, it also served as means of pretreatment but nor effective, because very little lignin was removed. Also plate 4.1 (c) displayed the sample of elephant grass treated with dilute ammonium hydroxide. In this case, more of lignin was removed but little cellulose was exposed, this may be due to the weakness of the dilute solution. Plate 4.1 (d) shows clearly that when elephant grass was treated with concentrated ammonium hydroxide, more lignin was removed and dispersed, exposing the cellulose for enzymes attack.

It has been established that when cellulose is exposed to enzymes attack in the appropriate conditions high yield of glucose is expected (Alexander *et al*. 2013).

4.4 Enzymatic hydrolysis

After the hydrolysis process, glucose concentrations and yields were determined and some parameters were varied and the results obtained with the variation of each parameter are as presented in Figures 4.1 – 4.7

4.4.1 Effect of reaction time on glucose yield

The effect of reaction time as well as pretreatment on glucose yield was investigated. In Figure 4.1, the glucose yield increased with increase in reaction time, but at a certain time no significant changes were observed. It could be seen that at 8 hours only 23.86 %, 25.57 % and 26.10 % yields were obtained for the untreated, dilute ammonium hydroxide treated and concentrated

ammonium hydroxide treated substrates, respectively. When the reaction time was extended to 24 hours the yields were 77.19 %, 80.09% and 82.56 %. But at 48 and 72 hours there were no significant changes observed, likely due to product inhibition by cellobiohydrase CBH) as the reaction proceeds. This indicates that the reaction could be quenched at 24 hours, since no more yield could be obtained after this time. This result also indicates that critical evaluation should be done when deciding for pretreatment since, the difference in yield is not much, considering the amount of money that could be invested for pretreatment.

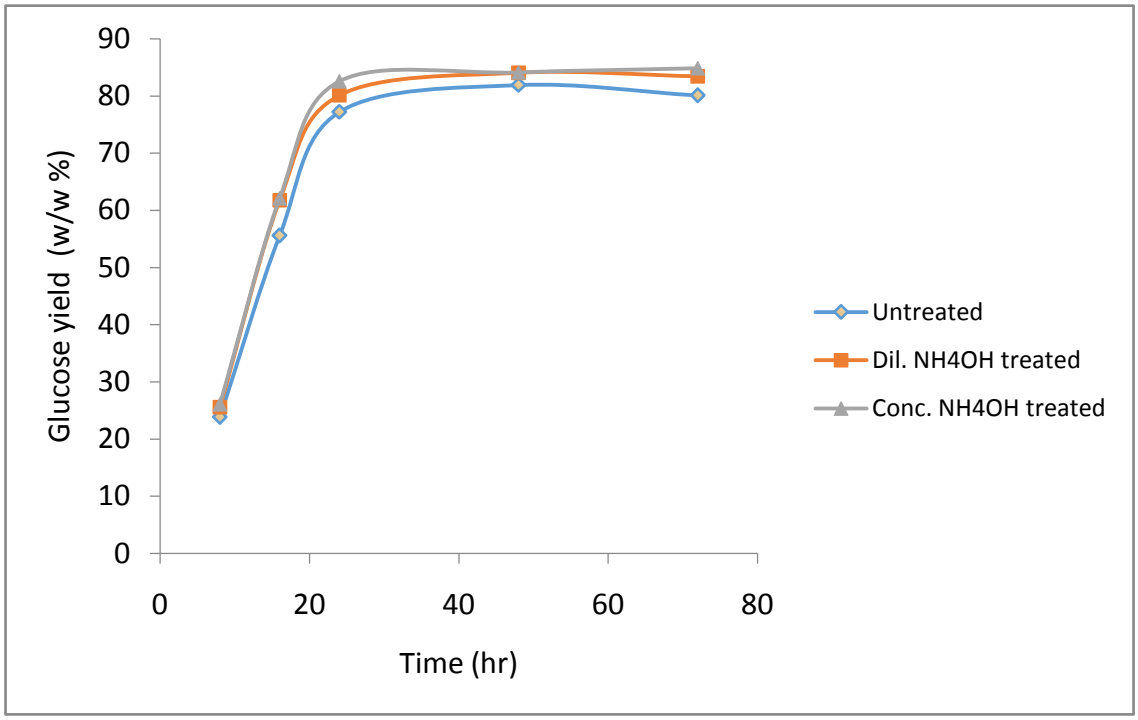


Figure 4.1: Effect of reaction time on glucose yield

(@ 45 ^{o}C, pH 4.5, 100 – 150 µm, 20 g/l substrate concentration, 0.1 g cell loading)

4.4.2 Effect of pH on glucose yield

The effect of pH on glucose yield was investigated as shown in Figure 4.2. The pH of 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 were used in this investigation. Other conditions kept constant are as shown at the foot note of Figure 4.3. From the result, it could be deduced that as the pH

increased from 3.5 to 4.5, the glucose yield increased to a maximum of 82.6 %. Increase in pH to 5.0 also give a reasonable yield of 80.12 %. But further increase in pH to 5.5 resulted in decrease in the yield of glucose. At pH 6.5, there was a drastic decrease in glucose yield indicating that neutral medium does not favour the reaction. The decrease in glucose yield is due to the fact that the cells are more active in acidic medium (pH of 3.5-5.0 for *A. niger* cells), the increase in pH of the medium away from the more acidic medium results in decrease in glucose yield. The reported pH tolerance range for *A. niger* is 3.5-7.0 (Rezael *et al.* 2009). Moreso, metabolic processes are generally temperature and pH dependent. Another reason for the decrease in glucose yield may be due to product inhibition by the enzymes secreted by the A*spergillus niger*. Similar result was reported by Aderemi *et al.* (2008) in hydrolysis of rice straw to produce glucose in which the optimal pH range that gave the highest yield of glucose was 4.5 - 5.0.

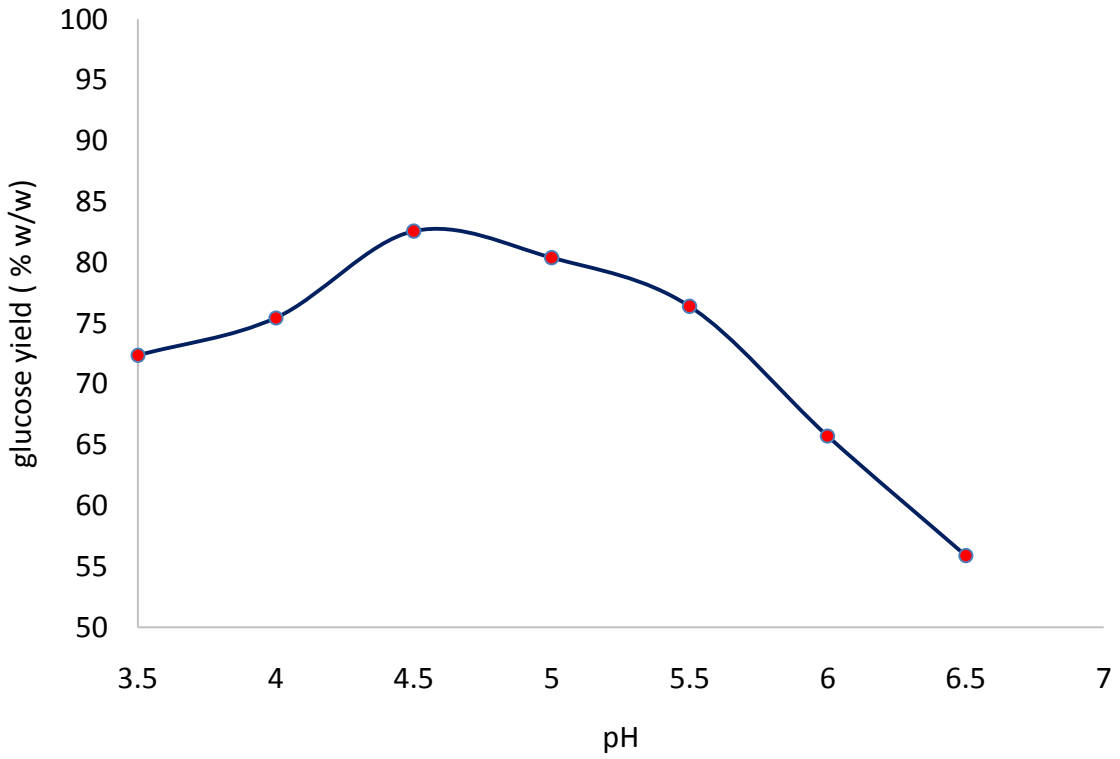


Figure 4.2: Effect of pH on glucose yield

(@ 24 hours, 45 ^{o}C, 100 – 150 μm, 20 g/l substrate concentration, 0.1 g cell loading)

4.4.3 Effect of temperature on glucose yield

Figure 4.3 shows the result of the effect of temperature on glucose yield. This result shows the optimum temperature range of 40 – 50 °C is needed to achieve the best elephant grass conversion to glucose. This is in agreement with the result obtained from the hydrolysis of animal manure lignocellulosic at 50 ^{o}C as reported by Wen *et al*. (2004) and pretreated apple pomace at 40 ^{o}C reported by Alexander *et al.* (2013). It also agrees with the result reported by Aderemi *et al.* (2008) in their work, the kinetic of glucose production from rice straw in which the temperature range was 45 – 50 ^{o}C. Highina *et al* (2011) also reported optimum glucose yield at 45 ^{O}C, in hydrolysis of wheat straw by *Aspergillus niger*.

It could be observed from the curve that lower temperatures like 30 ^{o}C and 35 ^{o}C favour more formation of glucose than higher temperatures (i.e 55 ^{o}C – 65 ^{o}C). This might be due to the fact that enzyme activity and other chemical reaction in the cells are not favoured at those temperatures. It also indicates that denaturing of the cells occurs at high temperatures beyond the optimum rendering the cells less active.

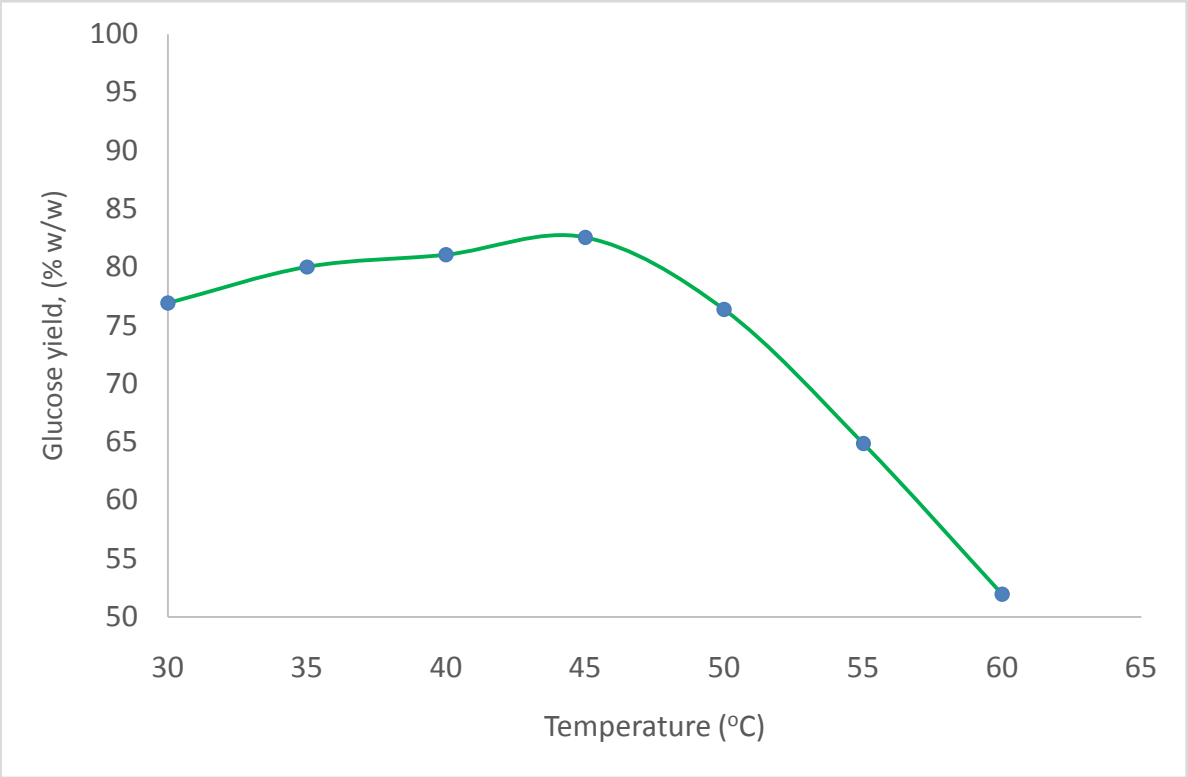


Figure 4.3: Effect of Temperature on glucose yield

(@ 24 hours, pH = 4.5, 100 – 150 µm, 20 g/l substrate concentration, 0.1 g cell loading)

4.4.4 Effect of particles size on glucose yield

Figure 4.4 shows how glucose yield varies with changes in particles size ranges. It can be seen from the result that the particle size range of 100 µm – 150 µm gave the highest glucose yield of 82.56 %. But as the particles size ranges increase from 151 µm – 600 µm, glucose yield decreases continuously from 82.56 % to 34.37 %,. This implies that as the particle size increases beyond 150 µm, the surface area available for enzymatic attack decreases. Also agitation during stirring was more effective with smaller particles size than the larger particle sizes. Moreso, reaction rate is proportional to diffusion, where as fine particles diffuse more rapidly than coarse particles, the reaction rate was faster with fine particles than with coarse particles, hence, more yield expected from fine particles than from coarse particles. A slight deviation from this

phenomenon was observed when the particles size was further reduced below 100 µm. This might be due to product inhibition on the enzymes released by the cells.

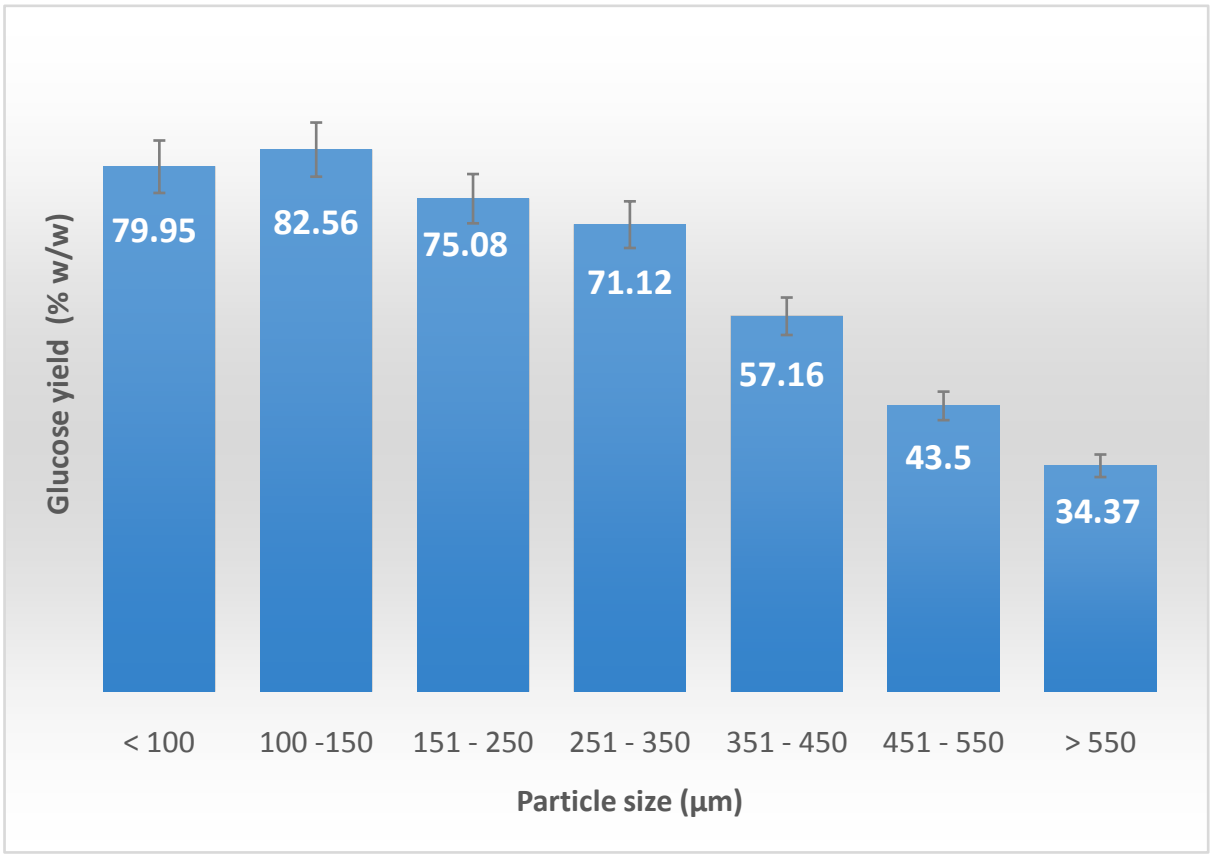


Figure 4.4: Effect of particle size on glucose yield

(@ 24 hours, 45 ^{o}C, pH = 4.5, 2 g substrate concentration, 0.1 g cell loading)

4.4.5 Effect of amount of substrate on glucose concentration

Different initial amounts of substrate (elephant grass) were used to investigate how substrate amount varies with product concentration. The amounts of substrate used as indicated in Figure 4.5 were 0.5 g, 1.0 g, 2.0 g, 4.0 g, 6.0 g, 8.0 g and 10.0 g. Other reaction conditions were kept constant. It is clear from the result that glucose concentration increased from 3.6 g/l to 36.34 g/l as the amount of substrate increased from 0.5 g to 10.0 g. This result is expected since more substrate gives more product. Moreso, continuous excretion of β-glucosidase by *A. niger*

prevents possible product inhibition in the process. This is responsible for the linear relationship between the amount of substrate and glucose concentration, because, if product inhibition occurs at some points, glucose yield will decrease even with increase in amount of substrate.

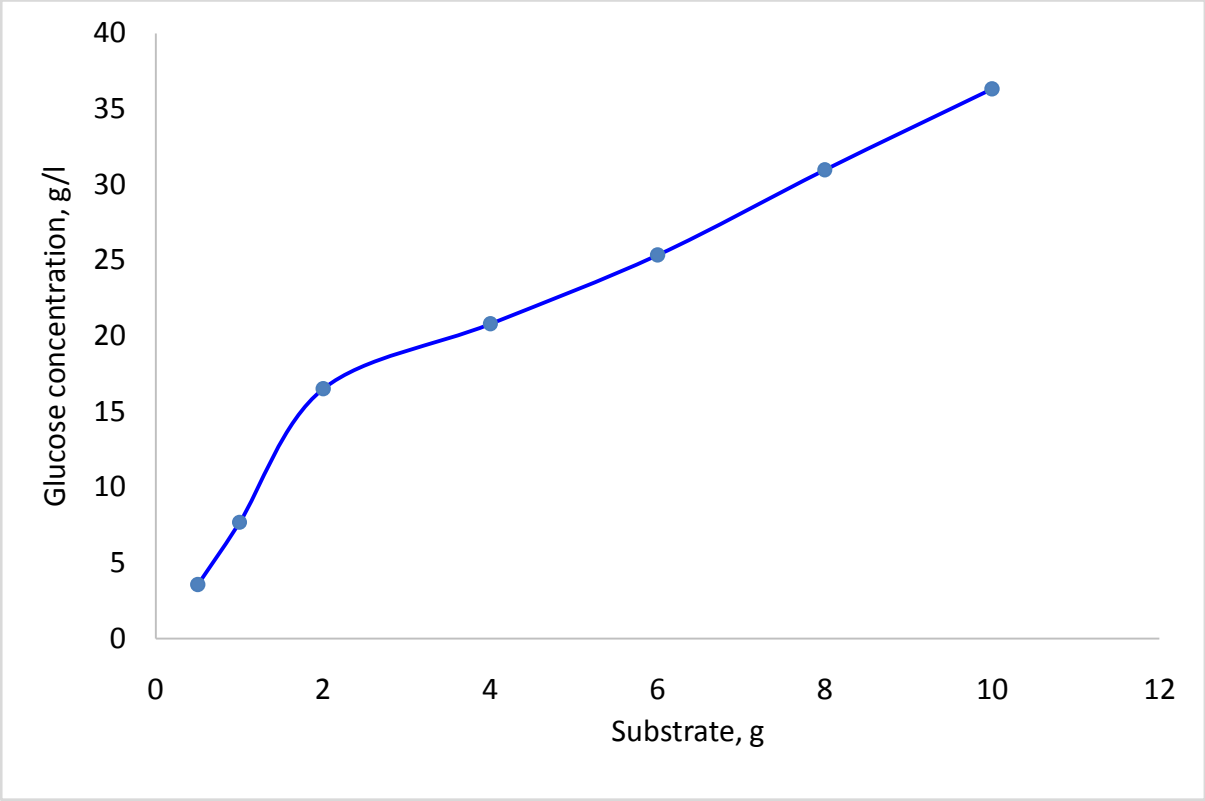


Figure 4.5: Effect of amount of substrate on glucose concentration

(@ 24 hours, 45 ^{o}C, 100 – 150 µm, pH = 4.5, 0.1 g cell loading)

4.4.6 Effect of substrate concentration on glucose yield

Figure 4.6 shows how glucose yield is affected with various substrate concentrations. At the initial stage, the yield increased from 71.3 % to 82 .6 % as substrate concentration increased from 1 mol/l to 6 mol/l. This might be due to inadequate substrate for enzymes attack, thus the cells would rather feed on the product formed for their maintenance, thereby causing the low percentage yield. But the results became stable as the substrate concentration increased from 6 mol/l to 29 mol/l, the product yield drop from 82.6 % to 36.3 %. This decrease in product yield is expected since percentage yield is calculated on weight basis. At high substrate concentration,

the enzymes are saturated, since they had enough to feed on, thus, at this point, reaction rate does not depend anymore on substrate concentration indicating zero order reaction. These results conform to the one reported by Alexander *et al.* (2009) in the hydrolysis of apple pomace to produce glucose. It also agrees with the results reported by Norhidayu (2014) in the hydrolysis of banana stem to produce glucose using stain B.

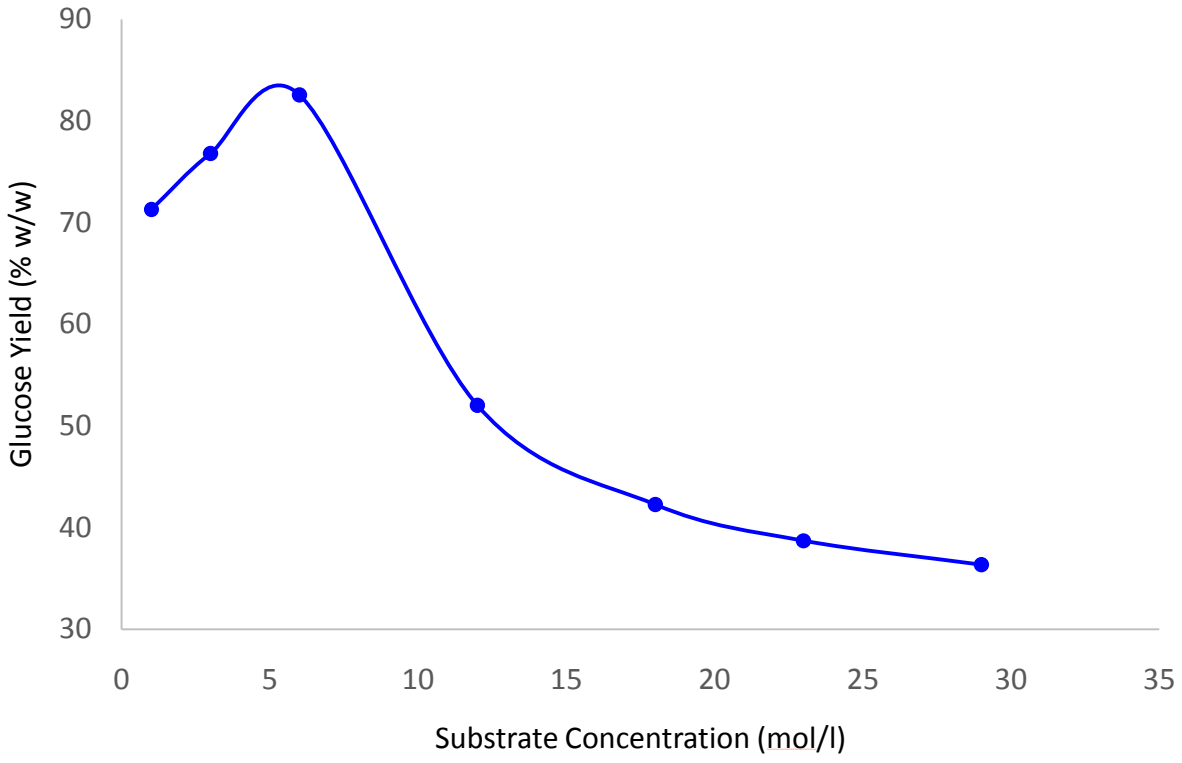


Figure 4.6: Effect of substrate concentration on glucose yield

(@ 24 hours, 45 ^{o}C, 100 – 150 µm, pH = 4.5, 0.1 g cell loading)

4.4.7 Effect of cell loading on glucose yield

The effect of *Aspergillus niger* concentration on glucose yield was investigated, while keeping the other parameters constant as indicated in the footnote of Figure 4.7. The result shows that as *A. niger* concentration increases from 0.1 g to 1.0 g, glucose yield increases from 82.6 % to 90 %

after 24 hours of hydrolysis. Similarly, as the cell loading increased, the glucose concentration increased proportionally. This may be due to continuous excretion of enzymes by the cells into the solution. Unless, substrate or product inbition occurs at some points, the cell-substrate concentrations relationship remains linear. This result agrees with that reported by Aderemi *et al.* (2008) and Aiyejagbara *et al.* (2015).

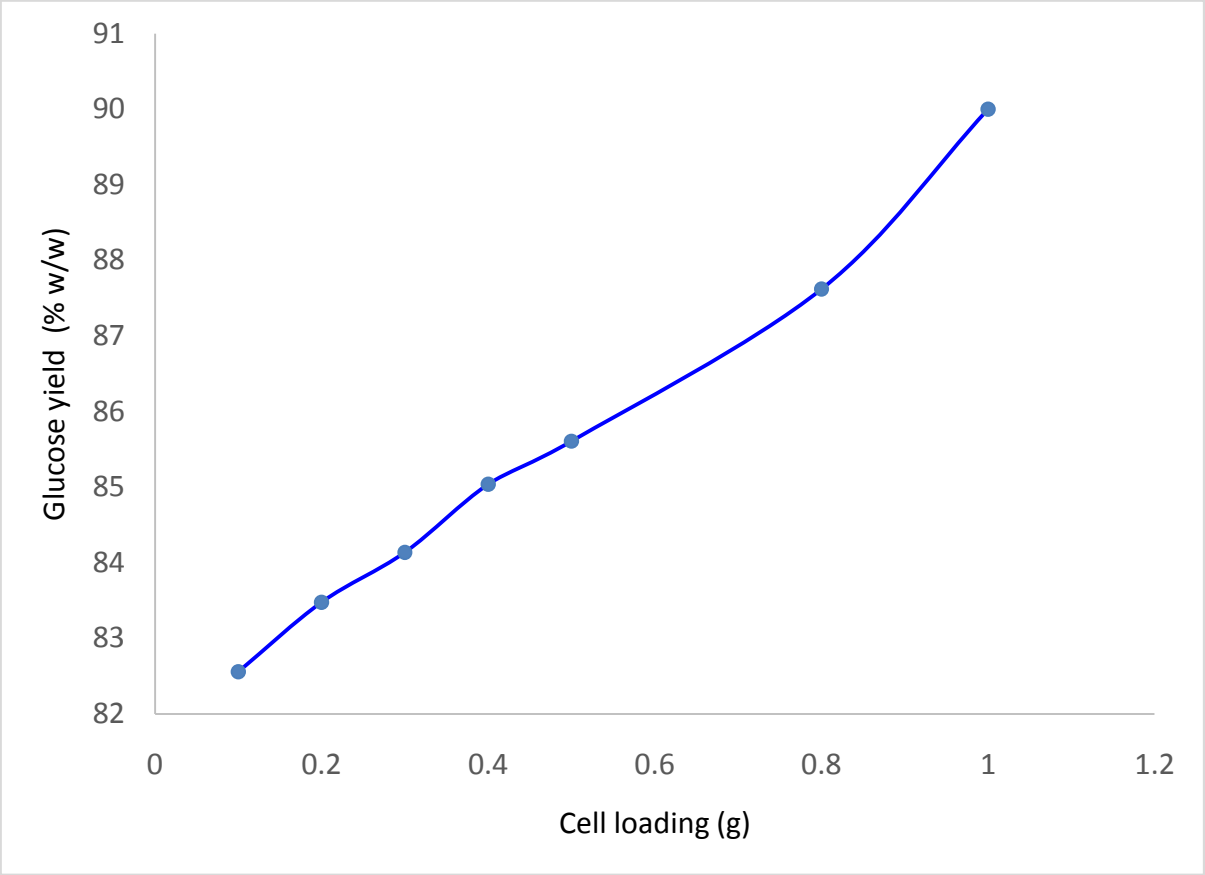


Figure 4.7: Effect of cell loading on glucose yield
(@ 24 hours, 45 ^{o}C, 100 – 150 µm, pH = 4.5, 20 g/l substrate concentration)

4.5 Evaluation of kinetic parameters

The modeling in this section is based on the following observations and assumptions:

1. The cells were introduced at their exponential growth phase.

2. The model is targeted to capture only the first segment (initial rate) of the concentration-time curve.

3. Glucose is the main product of interest.

4. A pseudo homogeneous system is being assumed throughout based on solid concentration of 20 g/l

5. The reaction was viewed as enzymes been excreted by the cells into the solution, enabling the overall system to be treated as that of enzyme-substrate kinetics.

Consequently, treatment of kinetic results by graphical differentiation of curves using the initial rates method (Aderemi *et al.* 2008), afforded the plot of initial rates at different levels of substrate initial concentrations as shown in Figure 4.8.

From this curve, it can be observed that the initial reaction rate is proportional to the substrate concentration (that is, first order reaction) when the substrate concentration is in the low range.

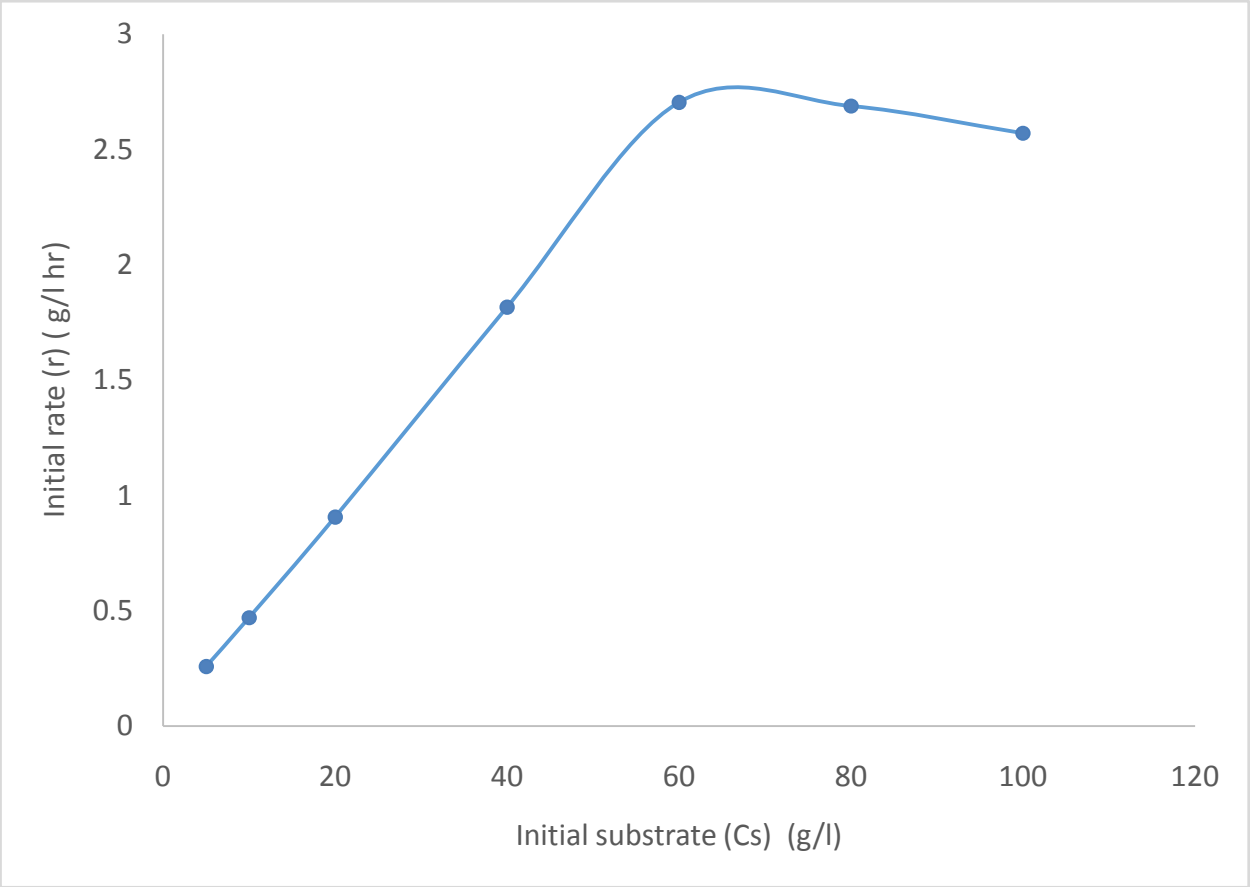


Figure 4.8: Initial rate versus initial substrate concentration plot

At low concentration of substrate, there is a steep increase in the reaction rate with increasing substrate concentration. The catalytic sites of the enzymes secreted by the cells are probably empty, waiting for substrate to bind, for much of the time, and the rate at which product is formed is limited by the concentration of substrate which is available (Imai *et al*. 2004).

Also from the curve, it can be seen that, as the concentration of substrate increases, the reaction rate approaches a constant value indicating that the enzymes become saturated with substrate. As soon as the catalytic sites of the enzymes are empty, more substrate is available to bind and undergo reaction. The rate of formation of product now depends on the activity on the enzymes and not the substrate concentration. That is, the reaction rate changes gradually from first order

to zero order. This form of behavior is commonly described by the Michaelis-Menten kinetic expression:

$$r_p = \frac{r_{max}\, C_s}{K_M + C_s} \tag{4.1}$$

Where r_{max} (the maximum reaction rate) and k_m (rate constant) are the kinetic parameters, which are needed to be experimentally determined and Cs is substrate concentration.

Applying the Line weaver – Burk method to linearize the rate expression by inverting equation (4.1), yields

$$\frac{1}{r_p} = \frac{1}{r_{max}} + \frac{K_M}{r_{max}}\left(\frac{1}{C_s}\right) \tag{4.2}$$

A plot of the reciprocal of the initial rate r_p versus the reciprocal of the initial substrate concentration Cs is expected to yield a straight line with an intercept $1/r_{max}$ and the slope K_M/r_{max}. A plot of this using the generated experimental data in this work is shown in Figure 4.9 from which the kinetic parameters (r_{max} and K_M) were estimated as $0.25*10^{-4}$ gl^{-1} s^{-1} and 2.85 gl^{-1}, respectively.

The maximum reaction rate found, conform to the values $0.1910 – 1.7766 * 10^{-4}$ gl^{-1} s^{-1} reported by Imai *et al.* (2004), when pure enzyme was used on a pure cellulose in a mixed cellulose system, at a 100% *Aspergillus niger* system (that is no *Trichorderma viride* present).

The reaction rate constant K_M was also found comparable to the value 2.75 gl^{-1} as reported by Imai *et al.*, (2004) for hydrolysis of a soluble cellulose (CMC) using the synergetic effects of combined cellulases from *Trichoderma viride* and *A. niger*. K_M represents the strength of binding or affinity of the substrate for the enzyme. Tightly bound substrates have a low K_M and loosely bound substrates have a high K_M. Also the K_M is that concentration of substrate at which half the

active sites of the enzyme are filled. This results shows that the K_M is relatively low, hence, the activity of the enzyme will be at constant rate regardless of the variation in substrate concentration.

Also, when K_M is so large compare to Cs, the reaction rate will be first order, and will definitely depend on substrate concentration. On the other hand, when K_M is so small compare to Cs, the reaction rate, r_p will be equal to the maximum reaction rate, r_{max}, hence, the reaction is zero order independent of substrate concentration.

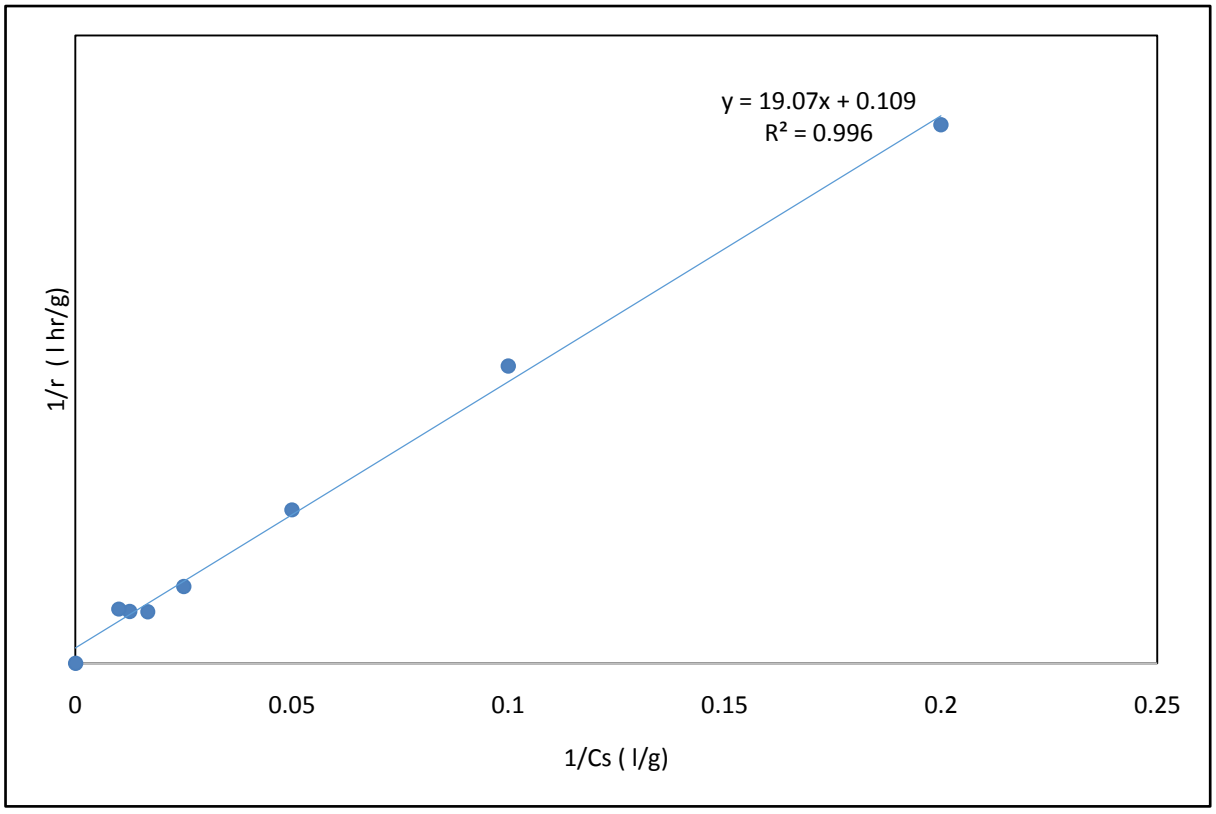


Figure 4.9: Line-Weaver Burk plot

Based on the evaluated kinetic parameters, the model equation is given as

$$r_p = \frac{0.25 \text{ X } 10^{-4}\, C_s}{2.85 + C_s} \qquad (4.3)$$

The consistency of this model equation was tested with the generated data to evaluate its reliability, statistically. Different initial substrate concentrations were used to evaluate the values of rates using the predicted model.The correlation coefficient (r^2) of the two variables was calculated using this equation:

$$r = \frac{N\sum XY - (\sum X)(\sum Y)}{\sqrt{\left[\left(N\sum X^2 - (\sum (X)^2)\right)\left(N\sum Y^2 - (\sum (Y)^2)\right)\right]}} \quad (4.4)$$

Where r = correlation coefficient; x = experimental rate values; and y = predicted rate values

After the evaluation, the r^2 was found to be 0.996 indicating fit relationship and good agreement.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

(1) Elephant grass stem contains relatively high amount of cellulose (57 -65 %) and lignin (8 – 11 %) when compared to the leaves which contains 25 % and 7.88 % respectively.

(2) Concentrated ammonium hydroxide removes more lignin from a lignocellulosic material than dilute ammonium hydroxide when used for pretreatment.

(3) Enzymes secreted by *Aspergillus niger* in the enzymatic hydrolysis of cellulose are more active at the following optimum conditions: pH 4.5, temperature 45 ^{o}C, particles size range 100 – 150 μm, substrate concentration 20 g/l and cell loading 0.1 g with 82.6 % glucose yield.

(4) In the hydrolysis of elephant grass to glucose, the kinetic parameters of the reaction are r_{max} = $0.25*10^{-4}$ $gl^{-1}s^{-1}$ and K_M = 2.85 gl^{-1} as obtained; and the kinetic model established for the process is

$$r_p = \frac{0.25 \text{ X } 10^{-4}\, C_s}{2.85 + C_s}$$

This model equation was found to be consistent with the experimental data with correlation coefficient (r^2) of 0.996.

(5) The present study showed that elephant grass is effective for the production of fermentable sugar which can be further processed to bioethanol and other valuable industrial chemicals.

5.2 Recommendations

Based on the results obtained and the limitations faced while conducting this research the following recommendations are made:

1. Pretreatment should be eliminated from the conversion processes to avoid unnecessary cash spending on investment, since elephant grass is found to have low lignin contents and the hydrolysis does not actually depend on pretreatment.

(2) Further research on downstream processing of the obtained glucose should be conducted to improve the product in terms of concentration and purity.

(3) Design expert software should be used for optimization to reduce the level of intervals of uncertainty of process parameters at reduced cost.

(4) A systematic studies should be done on the leaves, since it contains more hemicellulose, which is a source for the production of furfural (a very important Industrial chemical).

(5) Further research on the rate of cells growth in the conversion of elephant grass to glucose using A*spergillus niger* should be conducted.

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APPENDICES

APPENDIX I

Table A.1.1: glucose concentrations with time and effect of pretreatment

(@ 45 ^{o}C, pH 4.5, 100 – 150 μm, 20 g/l substrate concentration, 0.1 g Cell Loading)

Time	Untreated (g/l)	Treated with dilute NH_4OH (g/l)	Treated with concentrated NH_4OH (g/l)
8.0	4.772	5.113	5.219
16.0	11.116	12.347	12.422
24.0	15.438	16.017	16.512
48.0	16.679	16.809	16.821
72.0	16.015	16.683	16.967

Table A.1.2: glucose yields with time and effect of pretreatment

(@ 45 ^oC, pH 4.5, 100 – 150 μm, 20 g/l Substrate concentration, 0.1 g Cell Loading)

Time	(Untreated) (%)	Treated with dilute NH_4OH (%)	Treated with concentrated NH_4OH (%)
8.0	23.86	25.57	26.10
16.0	55.58	61.74	62.11
24.0	77.19	80.09	82.56
48.0	81.89	84.05	84.11
72.0	80.08	83.42	84.84

APPENDIX II

Table A.2.1: Effect of pH on glucose concentration

(@ 24 hours, 45 ^oC, 100 – 150 μm, 20 g/l Substrate concentration, 0.1 g Cell Loading)

pH	Glucose Conc. (g/l)

3.5	14.471
4.0	15.090
4.5	16.512
5.0	16.076
5.5	15.275
6.0	13.148
6.5	11.175

Table A.2.2: Effect of pH on glucose yield

(@ 24 hours, 45 ^{o}C, 100 – 150 μm, 20 g/l Substrate concentration, 0.1 g Cell Loading)

pH	Glucose yield, %
3.5	72.36
4.0	75.45
4.5	82.56
5.0	80.38
5.5	76.38
6.0	65.74
6.5	55.88

APPENDIX III

Table A.3.1: Effect of temperature on glucose concentration

(@ 24 hours, pH 4.5, 100 – 150 μm, 20 g/l Substrate concentration, 0.1 g Cell Loading)

Temperature, ^{o}C	Glucose Conc., g/l

30	15.386
35	16.008
40	16.214
45	16.512
50	15.275
55	12.974
60	10.386

Table A.3.2: Effect of temperature on glucose yield

(@ 24 hours, pH 4.5, 100 – 150 μm, 20 g/l substrate concentration, 0.1 g cell loading)

Temperature (^{o}C)	Glucose yield, %
30	76.93
35	80.04
40	81.07
45	82.56
50	76.38
55	64.87
60	51.93

APPENDIX IV

Table A.4.1: Effect of particle size on glucose concentration

(@ 24 hours, pH 4.5, 45 ^{o}C, 20 g/l Substrate concentration, 0.1 g Cell Loading)

Particle size, μm	Glucose Conc., g/l
75	16.249
100 – 150	16.512

151 – 250	15.572
251 – 350	14.122
351 – 450	12.699
451 – 550	8.700
550 – 600	6.874

Table A.4.2: Effect of particle size on glucose yield

(@ 24 hours, pH 4.5, 45 ^{o}C, 20 g/l substrate concentration, 0.1 g cell loading)

Particle size, µm	Glucose yield, %
<100	79.95
100 – 150	82.56
151 – 250	75.08
251 – 350	71.12
351 – 450	57.16
451 – 550	43.5
550 – 600	34.37

APPENDIX V

Table A.5.1: Effect of amount of substrate on glucose concentration

(@ 24 hours, pH 4.5, 45 ^{o}C, 100 – 150 µm, 0.1 g cell loading)

Amount of substrate, g	Glucose conc., g/l
0.5	3.564

1.0	7.68
2.0	16.512
4.0	20.806
6.0	25.356
8.0	30.987
10.0	38.341

Table A.5.2: Effect of Substrate concentration on glucose yield

(@ 24 hours, pH 4.5, 45 ^{o}C, 100 – 150 µm, 0.1 g cell loading)

Substrate concentration, mol/l	Glucose yield, %
1	71.28
3	76.8
6	82.56
12	52.02
18	42.26
23	38.73
29	36.341

APPENDIX VI

Table A.6.1: Effect of cell loading on glucose concentration

(@ 24 hours, pH 4.5, 45 ^{o}C, 100 – 150 µm, 2 g substrate concentration)

Cell loading, g	Glucose Conc., g/l
0.1	16.512

0.2	16.695
0.3	16.827
0.4	17.008
0.5	17.121
0.8	17.524
1.0	18.001

Table A.6.2: Effect of cell loading on glucose yield

(@ 24 hours, pH 4.5, 45 ^{o}C, 100 – 150 μm, 2 g substrate concentration)

Cell loading, g	Glucose yield, %
0.1	82.56
0.2	83.48
0.3	84.14
0.4	85.04
0.5	85.61
0.8	87.62
1.0	90

APPENDIX VII

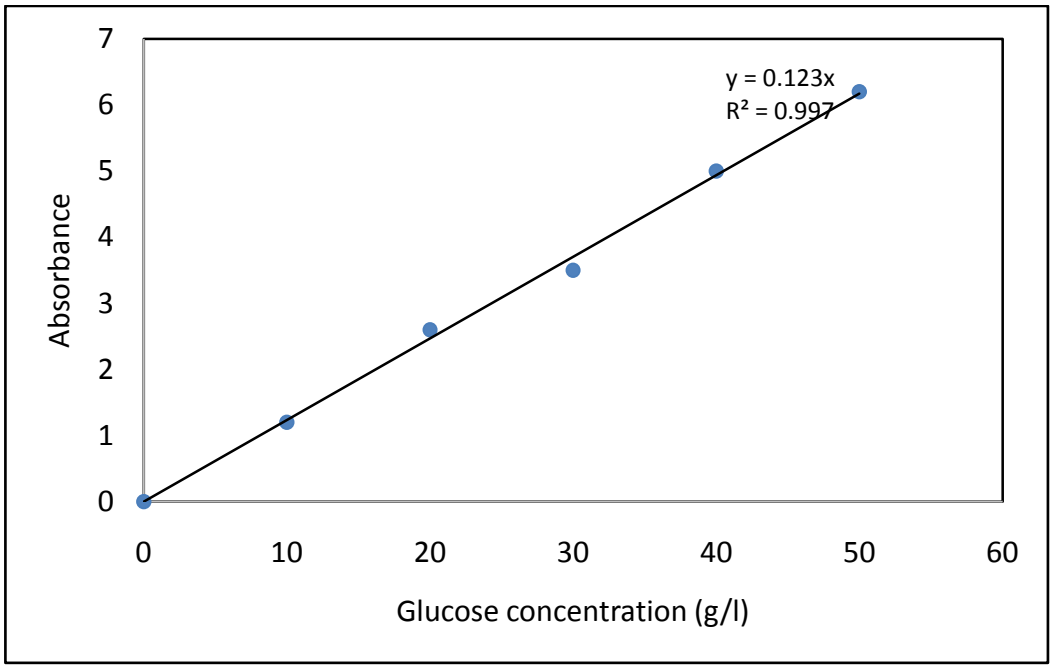


Figure A7.1: Standard glucose concentration curve

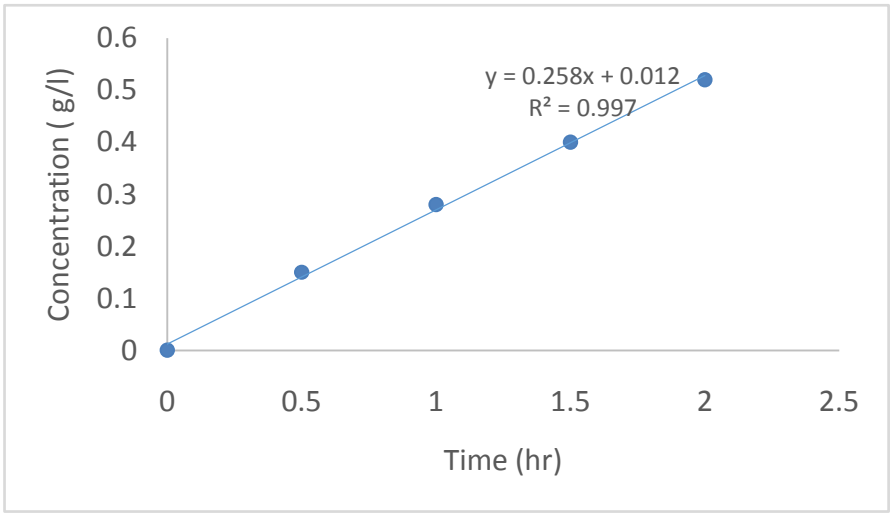


Figure A7.2: Concentration - Time Plot (Initial substrate, 0.5 g)

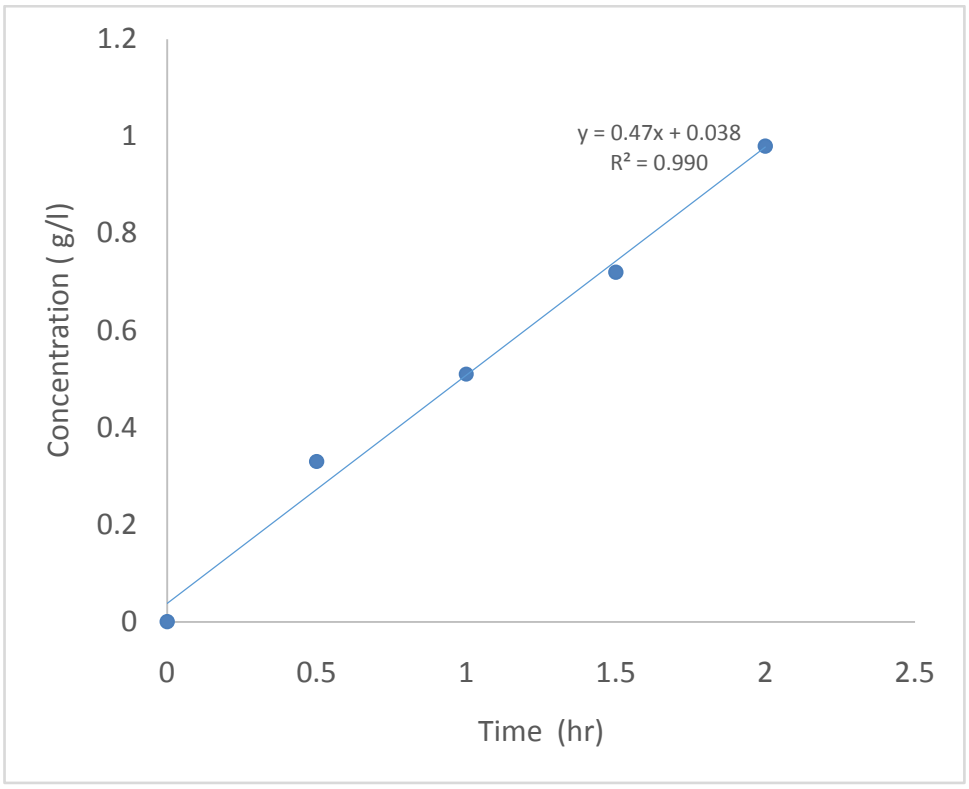


Figure A7.3: Concentration - Time Plot (Initial substrate, 1.0 g)

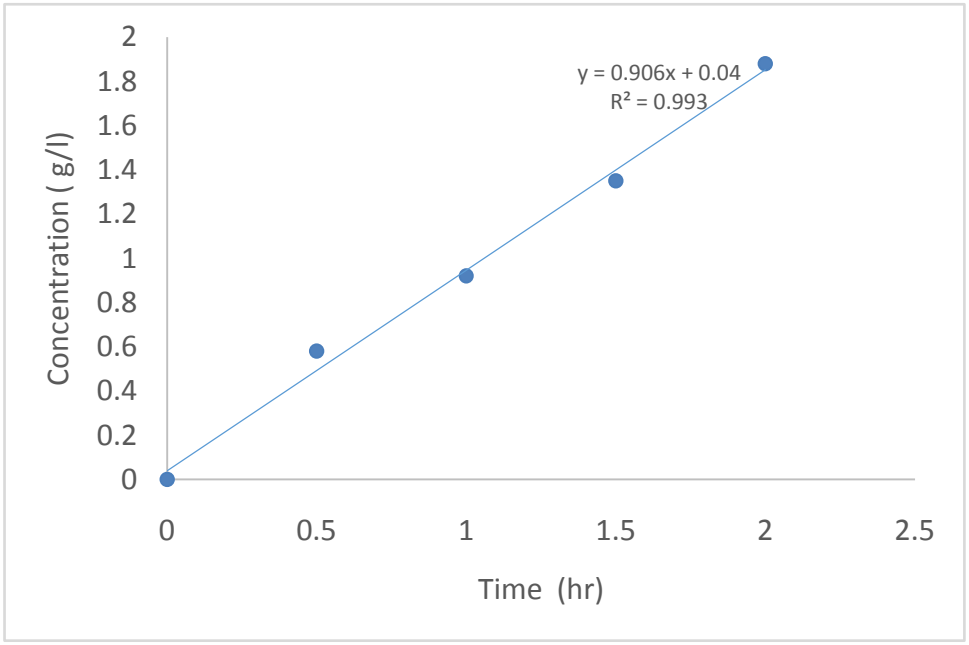


Figure A7.4: Concentration - Time Plot (Initial substrate, 2.0 g)

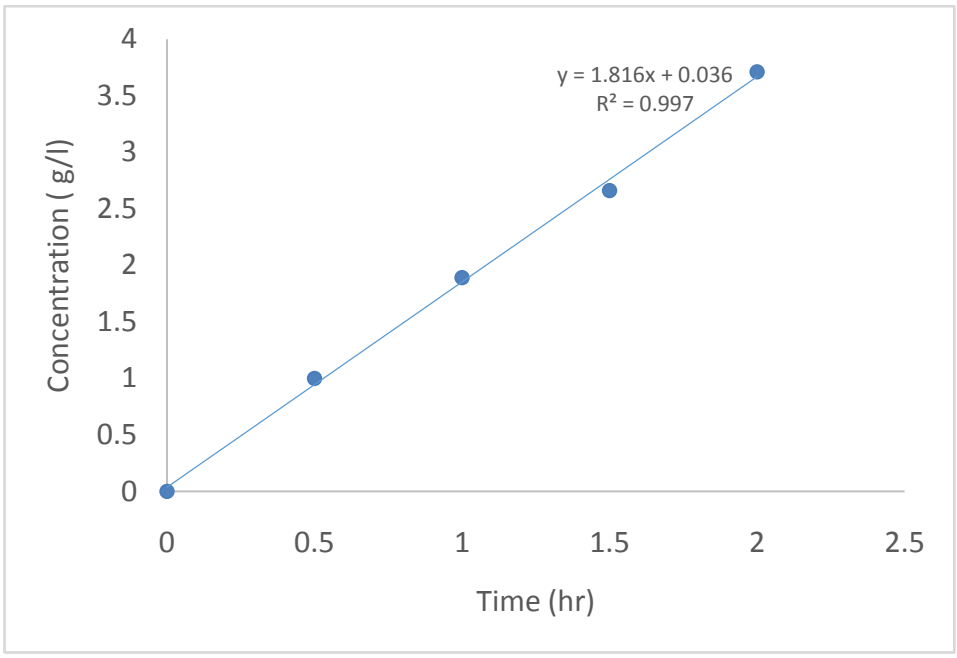


Figure A7.5: Concentration - Time Plot (Initial substrate, 4.0 g)

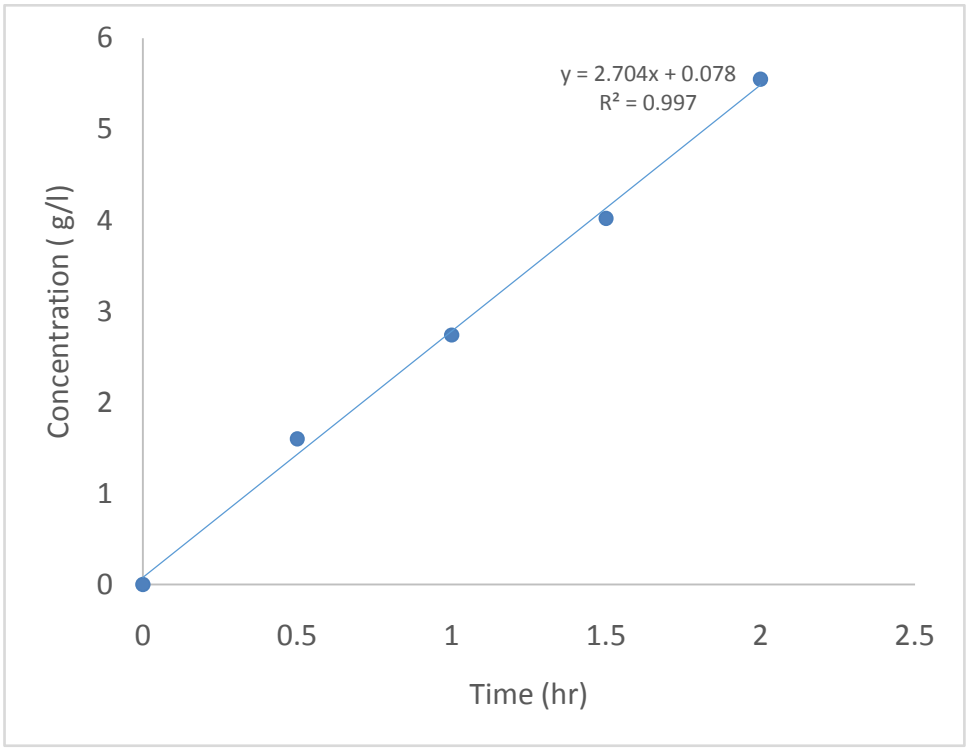


Figure A7.6: Concentration - Time Plot (Initial substrate, 6.0 g)

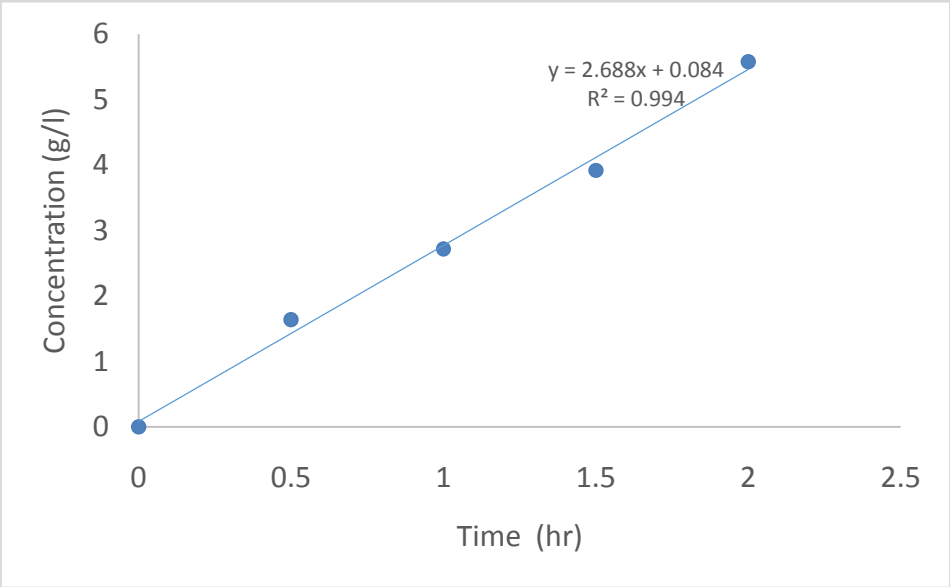


Figure A7.7: Concentration - Time Plot (Initial substrate, 8.0 g)

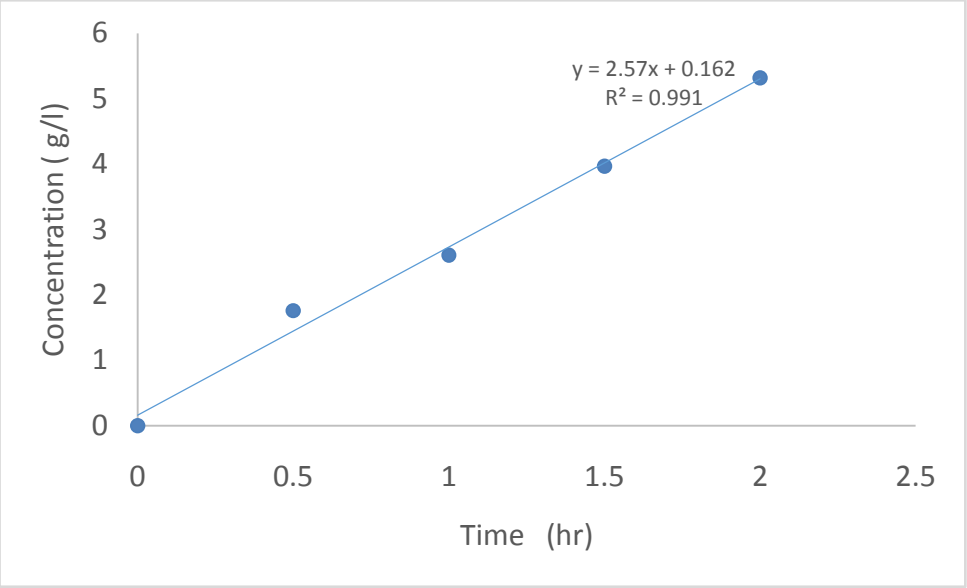


Figure A7.8: Concentration - Time Plot (Initial substrate, 10.0 g)