EVALUATION OF DIFFERENT METHODS OF BIO-ETHANOL

PRODUCTION, USING PROCESS SYNTHESIS.

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A Research project Submitted to the department of Chemical Engineering, in partial fulfillment of the requirements or the award of the Bachelor of Engineering (B.eng) degree in Chemical Engineering. School of Engineering and Engineering Technology, Federal University of Technology, Minna.

NOVEMBER, 2007

DECLARATION

I do hereby declare that this project is my original work and has never been submitted else where before.

David Olubusayo Obaro	Date

CERTIFICATION

This is to certify that this research project was fully supervised, moderated and approved by the following under listed person as presented by DAVID OLUBUSAYO OBARO 01/11587EH on behave of chemical engineering department for the award of the degree of bachelor of engineering (B. eng) in school of engineering and engineering technology.

Project supervisor

Engr. Habibu Hutman

27/11/2007

Date

Head Of Department

Dr. M.O Edoga

Date

External Examiner

Date

DEDICATION

This research Project is humbly dedicated to my Daddy and Mummy for the many sacrifices they have made on my behalf.

ACKNOWLEDGEMENT

My most profound gratitude to God almighty for giving me the health, patience and wisdom to complete this work.

My special thanks to my parents for their indispensable support throughout my study programme.

I also very much appreciate my supervisor for guiding me through my work with love and patience.

ABSTRACTS

The two common methods of acid hydrolysis of cellulosic biomass (dilute and concentrated acid hydrolysis) have been compared from the data obtained from the results. The method of production that has the most potential for cheap bio- ethanol production was analyzed. Sugars get degraded when dilute acid hydrolysis was used and so affected the yields of ethanol produced. Concentrated acid hydrolysis in its own sense, does not degrade the sugars and so enable better yield of bioethanol to be produced. The major contribution in this thesis was that different methods of producing bio-ethanol from cellulosic biomass (corn cobs) were successfully carried out although not without hitches. One of the limitation encountered was in availability of required heating element (230^0c), instead, kerosene stove was used to depolymerize the biomass to monomeric sugar. After comparing the result, the result of both methods of production, concentrated acid hydrolysis produced better yields than dilute acid hydrolysis with an average percentage (%) yield difference of 18.16%.

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CHAPTER 1

1.1 GENERAL INTRODUCTION

Ethanol is a clean, colourless, flammable oxygenated hydrocarbon with the chemical formula C_2H_5OH. Ethanol is produced from biological sources known as biomass. This biomass feedstock's may include agricultural crops and agricultural residues, trees, grasses, animal wastes, municipal solid wastes and indeed all organic materials that capture and store-solar energy. This wealth of stored energy can be extracted by a combination of physical, biological and chemical processes. During photosynthesis, plants combine carbondioxide from the air and water from the ground to form carbohydrates, which form the building block biomass.

In current times, the important of alternative energy sources has become even more necessary not only due to the continuous depletion of limited fossil fuel stock but also for the safe and better environment, with an inevitable depletion of the worlds energy supply, there has been an increasing world-wide interest in alternative sources of energy (wyman, 1999; lynd, 2004; Herrera 2004; Herrera 2006;).

The main objective of this work is to evaluate different methods of ethanol production, showing the most economically viable method. This work is necessitated by the fact that yields during production is low due to inadequate methods of production, e.g. In a chemical composition of raw materials and simulated ethanol production process as shown in the table1 below

Raw material	Cellulose hexosans	Hemicellulose Pentosans	Lignin	Ethanol yield	References
Corn cobs	42(H)	39(p)	14	0.358	Kuhad and Singh 1993
Rice straw	32(H)	24(p)	13	0.248	Kuhad and Singh 1993
Groundnut shell	38(H)	36(P)	16	0.327	Kuhad and Singh 1993
Rice hulls	36(H)	15(P)	19	0.265	Kuhad and Singh 1993
Saw dust	559(H)	14(P)	21	0.305	Olsson and hagadal 1996
Sorghum straw	33(H)	18(P)	15	0.240	Kuhuad and sigh 1993

By this fact, this work focuses on different methods and means of improving yield, comparism and evaluation of different methods of ethanol production is the focus of this research work. The ethanol yields, from different methods of ethanol production shall also be considered in this research work.

The ethanol yields and processes economics along with the technical maturity and environmental benefits of using ethanol blend fuel are the key parameters that determine the feasibility of bio-ethanol production (Nguyen and saddier, 1991). The important issue that needs to be addressed affirmatively is that of the global fuel needs. Research efforts are needed to design and improve the process, which would produce sustainable and economically feasible transportation fuel.

The steps or processes involved in the production of fuel ethanol consist of five basic unit operations.

1. Feedstock preparation
2. Decrystallization/hydrolysis reaction
3. Solids/liquids filtration
4. Separation of the acid and sugars
5. Fermentation of the sugars
6. Product purification

The process separates the biomass into two main constituent's cellulose and hemicelluloses, (the main building block of plant life) and lignin (the glue that holds the building blocks together. Conversion of the cellulose and hemicelluloses to sugars ferments them and purifies the fermentation liquids into products. After the biomass feedstock (corn cobs) are cleaned and ground. To reduce the particles sizes. Then they undergo acid hydrolysis. Two common methods for converting cellulose to sugar are dilute acid hydrolysis and concentrated acid hydrolysis, both of which uses tetraoxosulphate (VI). Dilute acid hydrolysis occurs in two stages to take advantage of the differences between hemi-cellulose and cellulose. The first stage is performed at low temperature to maximize the yields from the hemi-cellulose and the second at higher temperature. This stage is optimized for hydrolysis of the cellulose portion of the feed stocks; concentrated acid hydrolysis uses a dilute acid pretreatment to separate the hemi-cellulose and cellulose. The biomass is then dried before the addition of concentrated Tetraoxosulphate VI acid. Water is added to dilute the acid and then heated to release the sugars, producing a gel that can be separated from residue solids. Column chromatography is used to separate the acid from the sugars.

Both the dilute and concentrate acid processes have several drawback's. Dilute acid hydrolysis of cellulose tends to yield a large amount of by products. Concentrated acid hydrolysis forms fewer by products but since they are performed at high temperature, they both degrade the sugars, reducing the carbon sources and ultimately lowering the ethanol yields.

1.2 AIM/OBJECTIVE

To evaluate different methods of bio-ethanol production showing the most economically viable method

1.3 SCOPE OF WORK

Comparing bioethanol production methods from acid depolymerization of cellulosic biomass and then showing the most viable method.

CHAPTER TWO

2.0 LITERATURES REVIEW

The lignocellulose biomass comprises of cellulose, hemi-cellulose and lignin (Hayn et al, 1993). Cellulose is a linear, crystalline homo-polymer with a repeating unit of glucose strung together by beta 1, 4 glucosidic linkages. The structure is rigid and harsh treatment is required to break it down (Gray et al, 2006). Hemi-cellulose consists of short, linear and highly branched chains of sugars. In contrast to cellulose, which is a polymer of only glucose, a hemi-cellulose is a hetero-polymer of D-xylose, D-glucose, D-galatose, D-mannose and L-arabinose (Saha et al, 2006).

With these levels of differences between cellulose and hemi-cellulose, the requirement of treating both the same way to produce sugars will definitely lower the yields, Hence the need for specialized and innovative pretreatment methods.

Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate to monomeric sugars can be achieved more rapidly and with greater yield (sun and chang, 2002). The term macroscopic and microscopic size and structure is used to qualify reduction in size to powdered form of the feedstock (maize cobs) used in this work. Size reduction is mostly done by grinding to fine particles. This is due to the following reasons;

1. Finely powdered samples are more homogenous and can be sub-sampled with greater precision and accuracy if carefully mixed.
2. Finely powdered samples are easier to dissolve because they present a large surface area of contact. i.e., volume rate to any solvent or reagent used in dissolution.
3. Finely size particles present good surface area for reaction to take place.

Since the cellulose and hemi-cellulose contained in the maize cobs cannot be treated the same way, a need to separate cellulose and hemi-cellulose is very important. During dilute acid hydrolysis, hemi-cellulose fraction is de-polymerized at lower temperature than the cellulose fraction to separate cellulose fractions from the hemi-cellulose fractions.

After pretreatment, there are two types of processes to hydrolyze the feedstock into monomeric sugar constituents required for fermentation into ethanol. The hydrolysis methods most commonly used are acid (dilute and concentrated) and enzymatic. To improve the enzymatic hydrolytic efficiency, the lignin hemi-cellulose network has to be loosened for the better amenability of cellulose to residual carbohydrate fraction for sugar recovery. Dilute acid treatment is employed for the degradation of hemi-cellulose leaving lignin and cellulose network in the substrate. Other treatments are alkaline hydrolysis or microbial pretreatment with white-rot fungi (phaenerochate chrysosporium, cyathus stercoreus, cythus bullari and pyenoporous cinnabarinus .etc) which preferably act upon lignin leaving cellulose and hemi-cellulose network in the residual portion. However during both treatment, a considerably amount of carbohydrate are also degraded, hence the carbohydrate recovery is not satisfactory for ethanol production.

There are two types of acid hydrolysis process commonly used- dilute and concentrated acid hydrolysis. The dilute acid process is conducted under high temperature and pressure and has reaction time in the range of seconds or minutes. The concentrated acid process uses relatively mild temperature, but at high concentration of Tetraoxosulphate VI acid and a minimum pressure involved which only creates by pumping the materials from vessel to vessel. Reaction times are typically much longer than for dilute acid process.

2.1 DILUTE ACID HYDROLYSIS

In dilute acid hydrolysis, the hemi-cellulose fraction is de-polymerized at lower temperature than the cellulosic fraction. Dilute tetaoxosulphate IV acid is mixed with biomass to hydrolyze hemi-cellulose to xylose and other sugars. Dilute acid is interacted with the biomass and the

slurry is held at temperature ranging from 120-220^0 C for a short period of time. Thus hemi-cellulose fraction of plant cell wall is de-polymerized and will lead to the enhancement of cellulose digestibility in the residual solids (Wigam, 2002; Sun and Chang, 2002; Dien et al, 2006; Saha et al, 2005).

Dilute acid hydrolysis has some limitations. If higher temperatures (or longer residence time) are applied, the hemi-cellulose derived monosaccharide will degrade and give rise to fermentation inhibitors like furan compounds, weak carboxylic acids and phenolic compounds (Oisson and Hahn-Hagerdal, 1996; Klinke et al, 2004; Larsson et al, 1999).

These fermentation inhibitors are known to affect the ethanol production performance of fermenting microorganisms (Chandel et al, 2006). In order to remove the inhibitors and increase the hydrolysis fermentability, several chemicals and biological methods were used. These methods include over liming (Martinez et al, 2000), charcoal ion exchange (Nilvebrant, 2001) detoxification with laccase (Martin et al, 2002) and biological detoxification (Lopez et al, 2004). The detoxification of acid hydrolysis has been shown to improve their fermentability; however the cost is often higher than benefits achieved (Palmavist and Hahn-Hagerdal, 2000; Von sivers and Zoochi, 1996).

Dilute acid hydrolysis is carried out in two stages: first stage and two-stage.

2.1.1 FIRST-STAGE DILUTE ACID HYDROLYSIS

The lignocellulose material is first contacted with dilute Tetraoxosulphate VI acid (0.75%) and heated to approximately 50°C, followed by transforming to the first stage acid impregnator where the temperature is raised to 190°C. Approximately 80% of the hemi-cellulose and 29% of the cellulose are hydrolyzed in the first reactor. Hydrolysate is further incubated at a lower temperature for a residence time of 2hrs to hydrolyze most of the oligosaccharides into monosaccharide followed by the separation of solid and liquid fractions. The solid material is again washed with plentiful of water to maximize sugar recovery. The separated solid material is sent to second stage acid hydrolysis reactor.

2.1.2 TWO STAGE DILUTE ACID HYDROLYSIS

In two-stage dilute acid hydrolysis process, first biomass is treated with dilute acid at relatively mild condition during which the hemi-cellulose fraction is hydrolyzed and the second stage is normally carried out at higher temperature for de-polymerization of cellulose into glucose.

The liquid phase containing the monomeric sugars is removed between the treatments, thereby avoiding degradation of monosaccharide formed. It is very important to avoid monosaccharide degradation products for improving ethanol yield. (Sanchez et al, 2004) carried out the two stage dilute acid hydrolysis using Bolivian straw material (Paja brava). In first stage, paja brava was pretreated with steam followed by dilute Tetraoxosulphate VI acid (0.5 or 1.0%) hydrolysis between temperatures 170^0C and 230^0 C for a residence time 3 and 10 minutes.

The higher yield of hemi-cellulose derived sugar were formed at a temperature of 190°C, and a reaction time of 5-10mm, whereas in second stage hydrolysis, considerably higher temperature (230°C) was found for hydrolysis of remaining fraction of cellulose.\

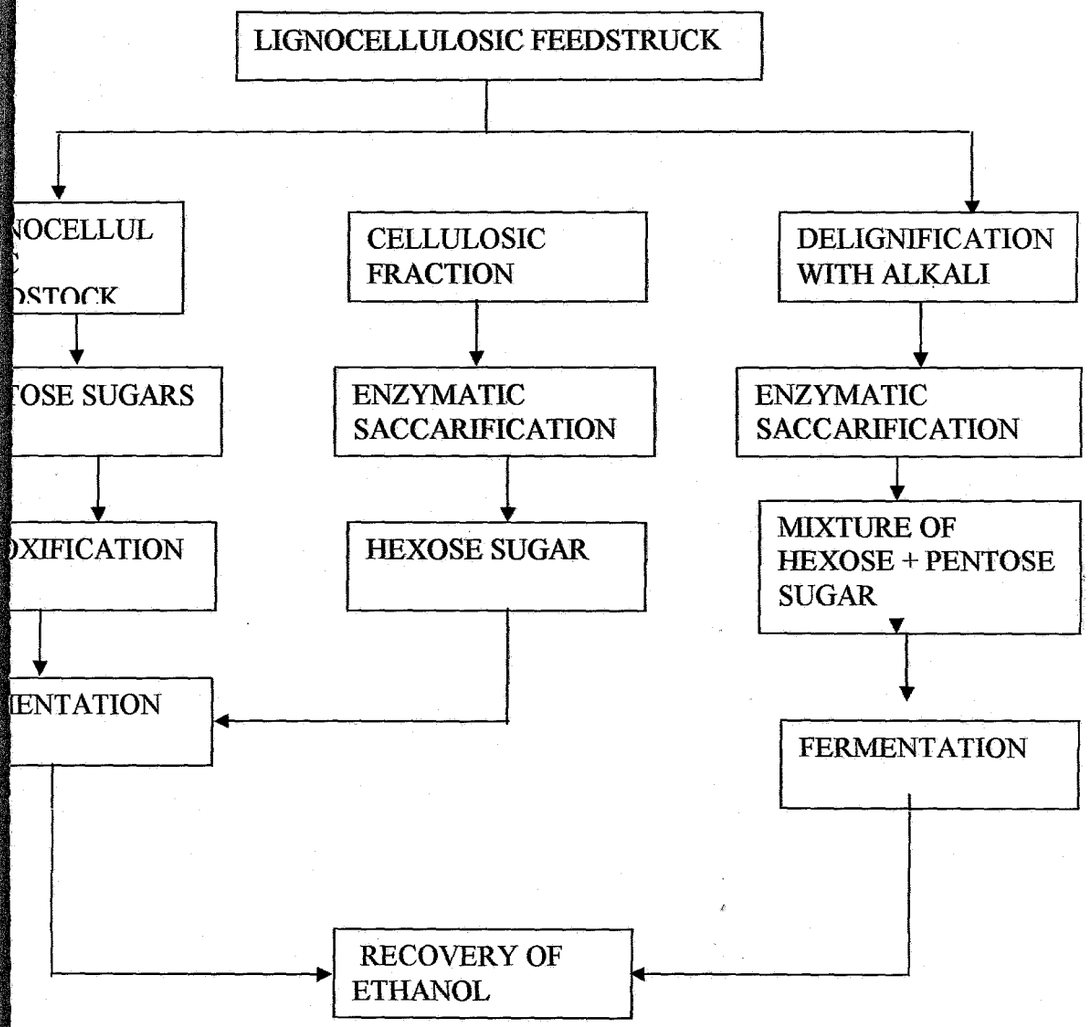

Figure 1. Summary dilute acid hydrolysis and separate fermentation of pentose and hexose's sugars

2.1.2.1 CONCENTRATED ACID HYDROLYSIS

This method uses concentrated tetraoxosulphate VI acid, followed by a dilution with water to dissolve and hydrolyze the substrate into sugar constituents. This process provides complete and rapid conversion of cellulose to glucose and hemi-cellulose to xylose and other sugars with a little degradation. The concentrated acid process uses 70% Tetraoxosulphate VI acid at 40-50°C for 2 to 4 hours in a reactor. The low temperature and pressure will lead to minimize the sugar degradation. The hydrolyzed material is then washed to recover the sugars.

In the next step, the cellulosic fraction has to be de-polymerized. The solid residue from first stage is de-watered and soaked in 30-40% Tetraoxosulphate VI acid for 50mins at 100^0 C for

further cellulose hydrolysis. The resulting slurry mixture is pressed to obtain second acid sugar stream (approximately 18% sugar, 30% acid of ratio (1.8:3).

Both the sugar streams from two hydrolysis steps are combined and maybe used for subsequent ethanol production. (Iranmahboob et al, 2002) performed the concentrated acid hydrolysis of mixed wood chips and found the maximum sugar recovery (78-82% theoretical yields) was achieved at concentrated tetraoxosulphate vi acid concentration (26%) for 2 hours of residence time.

Primary advantage of concentrated process is the potential for high sugar recovery efficiency. About 90% of both cellulose and hemi-cellulose fraction gets de-polymerized into their monomeric fraction. The acid and sugar syrup are separated via column chromatography or ion exchange and then acid is reconcentrated through multiple effects evaporators. The remaining lignin rich solids are collected and optionally palletized for fuel generation

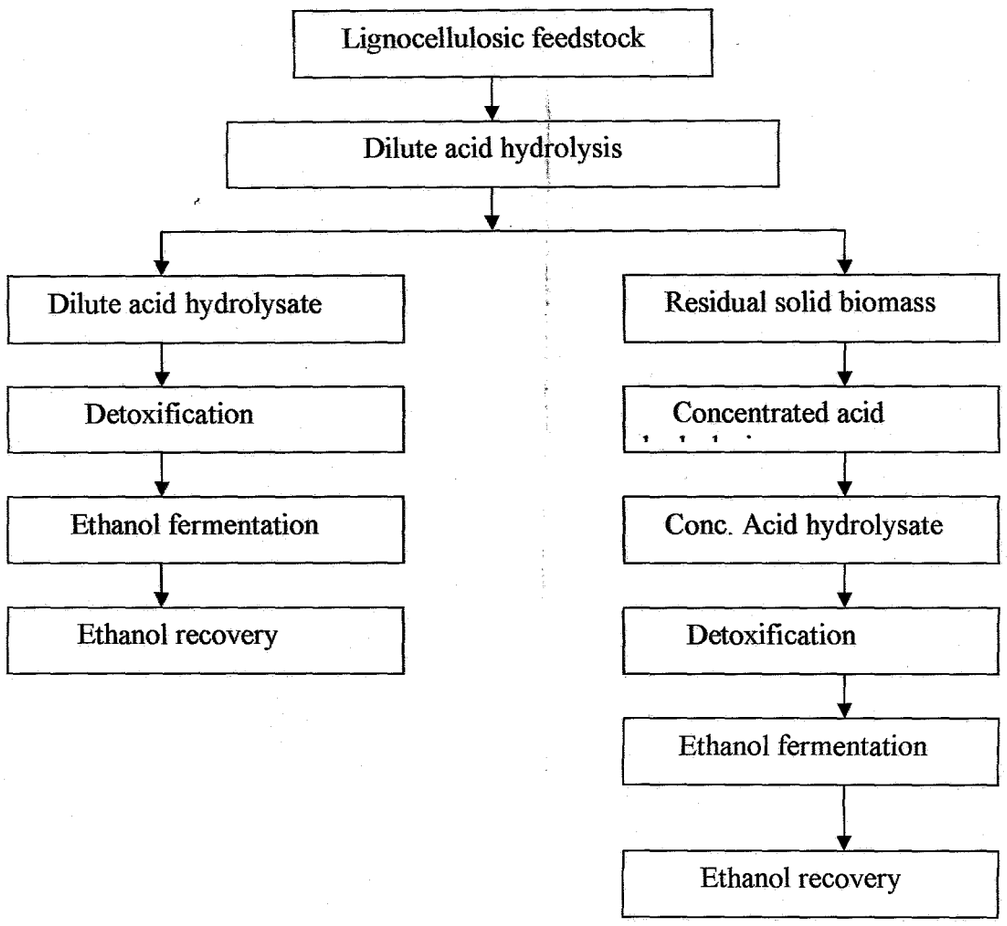

Figure 2: Concentrated acid hydrolysis and separate pentose and hexose sugars fermentation

2.1.4 COMBINED UTILIZATION OF BOTH CONCENTRATED AND DILUTE ACID HYDROLYSIS

Each of these methods discussed have great limitations which directly compromise the yield of sugar. Since yields of this sugar s are very important for the production of ethanol, a need to annex the potential of both method and discard their limitations is very important. Therefore, a combined utilization of both dilute and concentrated acid hydrolysis in different steps of hydrolysis may be useful. This is because sugars degrade with increase in temperature.

During two stage dilute acid hydrolysis this increase in temperature needed to depolymerize cellulose to glucose. Therefore instead of using high temperature during two stage dilute acid hydrolysis, a concentrated acid can be used at mild temperature. This will help to maintain the required sugar formation without degradation. This method will possibly improve yields of bio-ethanol production. It can also help to minimize energy consumption which is in contrast to both dilute and concentrated acid hydrolysis which requires high energy consumption. These combined benefits are capable to make bio-ethanol a more affordable renewable energy source.

2.1.1.4 ENZYMATIC HYDROLYSIS

The acid or fungal pretreated lignocelluloses can be saccharified enzymatically to get fermentable sugars (Ghose and Bisaria, 1979; Kuhad et al, 1997; Iitoh et al, 2003; Tuckur et al, 2003).

Bacteria and fungi are the good source of cellulase, hemicellulase that could be used for hydrolysis of preteated lignocelluloses.The enzymatic cocktail are usually mixtures of several hydrolytic enzymes comprising of cellulase. Xylanases, hemicellulase from bacteria and fungi sources have continued been isolated and regular effort have been made for the improved production of enzymatic liters (Aro et al, 2005; Foreman et al, 2003).

However, the cellulase was produced at a concentration too low to be useful. There is a, group of microorganisms (clostridium, cellulomass trichoderma, penicillium, neurospora fusarium aspergillum etc). Showing a high cellulolytic cellulose and xylan along with fermentation of

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

2.1.5 SEPARATE HYDROLYSIS AND FERMENTATION

Enzymatic hydrolysis performed separately from fermentation step is known as separate hydrolysis and fermentation (shf) (Sreenath et al, 2001; Wingren et al, 2003). The separation hydrolysis and fermentation offers various processing advantages and opportunities. It enables enzymes to operate at higher temperature for increased performance and fermentation organisms to operate at moderate temperatures optimizing the utilization of sugars.

2 .1.6 SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

The most important process improvement made for enzymatic hydrolysis of biomass is the introduction of simultaneous saccharification and fermentation which has been improved to include the co-fermentation of multiple sugar substrates (Sreenath et al, 2001; Wingren et al, 2003).

This approach combined the cellulase enzymes and fermenting microbes in one vessel, this enables a one step process of sugars production and fermentation into ethanol. Simultaneous saccharification of carbon polymer, cellulose to glucose, hemicelluloses to xylose and L. arabinous, and fermentation will be carried out by recombinant yeast or the organism which has the ability to utilize both C_5 and C_6 sugars.

According to Alkasrawi et al, 2006) the mode of preparation of yeast must be carefully considered in simultaneous saccharification and fermentation (ssp) designing.

A more robust strain will give substantial process advantages in terms of higher solid loading and possibly to recirculate the process stream, which results in increased energy demand and reduce fresh water utilization demand in process. Adaptation of yeast of the inhibitors present in the medium is an important factor for consideration in the design of ssf process.

More resently, (Kroumov et al, 2006) demonstrated an unstructured model of simultaneous saccharification and fermentation of starch to ethanol by genetically modified strain saccharomyces cereviseae YPB-G, using two hierarchy level of concept. In first concept, a mechanism of enzymatic hydrolysis of starch to glucose by combined action of two enzymes (alpha – amylase and glucoamylase) secreted by recombinant yeast and the second concept was the enzymatic degradation of starch to glucose by microorganism.Simultaneous saccharification and fermentation combines enzymatic hydrolysis with ethanol fermentation to keep the concentration of glucose low. The accumulation of ethanol in the fermenter does not inhibits cellulose action as much as high concentration of glucose, so, ssf is good strategy for increasing the overall rate of cellulose to ethanol conversion (Lin and Tanaka, 2006).

Simultaneous saccharification and fermentation gives higher ethanol yield while requiring low amounts of enzymes because end products inhibition from cellulose and glucose formed during enzymatic hydrolysis is relieved by the yeast fermentation (Banat et al, 1998). However, it is not feasible for simultaneous saccharification and fermentation to meet all challenges at industrial level due to its low rate of cellulose hydrolysis and most microorganisms employed for ethanol fermentation can not utilize all the sugars derived after hydrolysis.

To overcome these problems, the cellulolytic enzymes cocktail should be more stable in wide range of P.H and temperatures. Also the fermenting organism (yeast or bacteria) should be able to ferment a wide range of C_5 and C_6 sugars. Recently (Matthew et al, 2005) has found some promising ethanol producing bacteria viz recombinant E.coli koll, klebsiella oxytoca and zymomonas mobiles for industrial exploitation. Simultaneous saccharification and fermentation process has now improved after including the cofermentation of multiple sugar substrates present in the hydrolysate. This new variant of simultaneous saccharification and fermentation and simultaneous saccharification of co fermenter are preferred over shf, since both operations can be performed in the same tank resulting in low cost, higher ethanol yield and shorter processing time (Wright et al, 1988).

The most upgraded form of biomass to ethanol conversion is consolidated bioprocessing (CBP). Featuring cellulose production, cellulose hydrolysis and fermentation in one step. Is a highly

integrated approach with outstanding potential (Lynd et al, 2005).it has potential to provide the lowest route cost of biological conversion of cellulosic biomass to ethanol with high rate and desired yields

2.1.7 DIRECT MICROBIAL CONVERSIONS (DMC)

Direct microbial conversion is a method of converting cellulosic biomass to ethanol in which both ethanol and all required enzymes are produced by a single microorganism. The potential advantage of direct microbial conversion is that a dedicated process step for the production of cellulose enzymes is not necessary. Cellulase enzymes production (or procurement) contributes significantly to the cost involved in enzymatic hydrolysis process. However, direct microbial conversion is not considered in the leading process alternative. This is because there is no robust organism available that can produce cellulase or other cell wall degrading enzymes in conjunction with ethanol with a high yield. (Singh and coma, 1991) found that strain fusarlum oxysporum have the potential of converting not only D-xylose, but also cellulose to ethanol in one step process.

Distinguishing features of fusarlum oxyporum for ethanol production in comparism to other organisms are identified. This include of advantage in situ cellulase production and cellulose fermentation, pentose fermentation and the tolerant of sugars and ethanol. The main disadvantage of fusarlum oxysporum is its slow conversion rate of sugars to ethanol as compared to yeast

2.1.8 FERMENTATION

Bioconversion of various sources into ethanol by different microorganism has been summarized in Table 2. The sugar syrup obtained after cellulosic hydrolysis is used for ethanol fermentation. The ability to ferment pentose along with hexose is not wide spread among microorganism (Toivolla et al, 1984); sacchacromyces cerevieiae is capable of converting only hexose sugars to ethanol. The most promising yeast that have the abiliy to use both C_5 and C_6 sugars are (Pichia Stipilis, Candida, Shehatae, and Pachysoln, Tannophilus). However ethanol production from

sugar derived from starch and sucrose has been commercially dominated the yeast saccharomyces cereviceaes (Lin and Tanaka, 2006) thermo tolerant yeast could be more suitable for ethanol production at the industrial level. In high temperature process energy savings can be achieved through a reduction in cooling costs. Considering this approach (stree et al, 1999) develop solid-state fermentation system for ethanol production. From sweet sorghum and potato employing a thermo tolerant saccharomyces cereviseae strain (V53). It is interesting to know at this point that sacchromyeces cereviseaes (yeast) contains an enzyme called *invertase* which act as a catalyst and helps to convert the sucrose sugars into glucose and fructose (both $C_6H_{12}O_6$). The chemical reaction is shown below

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + \underset{\text{water}}{H_2O} \xrightarrow[\text{catalyst}]{\text{invertase}} C_6H_{12}O_6$$

The fructose and glucose sugars then react with another enzyme still in the yeast called Zymae which is also contains in the yeast to produce ethanol and carbon dioxide.

The chemical reaction is shown below

$$\underset{\text{Fructose/glucose}}{C_6H_{12}O_6} \xrightarrow[\text{catalyst}]{\text{Zymase}} \underset{\text{ethanol}}{2C_2H_5OH} + 2CO_2$$

This means Yeast = inverase + Zymase

Researchers are now focusing on developing i.e, recombinant yeast, which can greatly improve the ethanol production yield by metabolizing all form of sugars and reduce the cost of operation. In this contention the first researcher s have made efforts by following two approaches. The first approach has been to genetically modify the yeast and other natural ethanologens additional pentose metabolic pathways. The second approach is to improved ethanol yields by genetic engineering in microorganisms that have the ability to ferment both hexoses and pentoses (Jefrees and Jin, 2000; Dienetal 2003; Katheria et al, 2006). (Jeffries and Jin, 2004) compiles the recent developments happened towards the genetic engineering of yeast metabolism and concluded that strain selection through mutagenesis adaptive evolution using qualitative metabolism models may help to further improve their ethanol production rates with increased

productivities (Piscur et al, 2006) showed the recent developments in comparative genetics and bioinformatics to elucidate the high ethanol production mechanism from saccharmyces species. Though new technologies have greatly improved bioethanol production yet there are still a lot of problems that have to be solved. The major problems include maintaining a stable performance of genetically engineered yeast in commercial scale fermentation operation (Ho et al 1998;1999), developing more efficient pro-treatments technologies for lignocellulosic biomass, and integrating optimal component into economic ethanol production system (Dien et al, 2000). Sridhar and co-workers (2002) made an effort to improve the thermo-tolerance of yeast treating them with UV radiation

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

2.2 BATCH FERMENTATION

Traditionally ethanol has been produced batch wise. At presnt, nearly all the fermentation ethanol industry uses batch method. In batch fermentation, the microorganism works in high substrate concentration initially and a high product concentration initially (Elsson and Hangerdal, 1996). The batch process is a multi-vessel process, allows flexible operation and easily controls the process. Generally, batch fermentation is characterized by low productivity with an intensive labour (Shama 1788) for batch fermentation, elaborate preparatory procedures are needed, and because of the discontinuous start up and shut down operation, high labour cost are incurred. This inherent disadvantage and low productivity offered by the batch process have led many commercial operations to consider the other fermentation methods.

2.2.1.1 FED BATCH FERMENTATION

In fed batch fermentation, the microorganism works at low substrate concentration with an increasing ethanol concentration during the course of fermentation process. Fed batch cultures

often provides better yield and productivities than the batch culture for the production of microbial metabolites (Schugeri, 1987)

Keeping the low feed rate of substrate solution containing high concentration of fermentation inhibitors such as furfural, hydroxymethyl furfural and phenolics, the inhibitory effect of those compounds to yeast has been reduced.

Complete fermentation of an acid hydrolysate of spruce, which was strongly inhibiting in batch fermentation, has been achieved without any detoxification treatment (Taherzadeh, 1999). The productivity in batch fermentation is limited by the feed rate which, in turn is limited by the cell mass concentration (Lee and Chang, 1987; Palm Qvist et al, 1996). Ideally the cell density should be kept at a level providing maximum ethanol production and yield.

2.2.1.2 CONTINUOUS FERMENTATION

Continuous fermentation can be performed in different kind of bio-reactors- stirred tank reactors (single or series) or plug flow reactors. Continuous fermentation often gives a higher productivity than batch fermentation but at low dilution rates which offers highest productivities. (Alexander et al, 1989) studied the effect of shift in temperature and aeration in steady state continuous culture of C. shehatae to determine the effect of ethanol on xylose metabolism. The accumulation of ethanol exerted a delay inhibitory effect on the specific rate of substrate utilization. Continuous operation offers ease of control and is less labour intensive than batch operation. However contamination is more serious in this operation.

A high cell density of microbes in the continuous fermenter is locked in the exponential phase which allows high productivity and overall short processing of 4-6 hours as compared to the conventional batch fermentation (24-60h), this results in substantial savings in labour and minimizes investment cost by achieving a given production level with a much smaller plant

ERIAL	PETREAATMENT & SACCHARIFICATION	FERMENTATION CONDITION	MICROOGANISM	REFERENCES
ne	Dilute acid hydrolysis	BATCH	C shehatae	Chandel etal 2006
straw	Dilute acid, enzymatic hyrolysis	SSF, SHF	Ecoli FBK5	Shaha etal 2005
aws	Auto hydrolysis	Batch	C. shahatae Ncim3501	Abbi etal 1996
m	Steam explosion & enzymes	SSF	Kluyvermyces maxianus (lectiasts)	Balzsteros ztal 2004
over	Steam, Enzymatic	Fed batch	S. cereviseae Tmb3400	Ohgren etal 2006
husk	Steam, Enzymatic	SSF	S. cereviseae	Palmarola etal 2005
flower	Steam, Enzymatic	BATCH	S. cereviseae van ellipsoideus	Sharma etal 2002
cane	Alkaline H2O2	SSF	S. cereviseae wrrl-y-132	Krishna etal 2001
bran	Dilute acid, enzymatic hydrolysis	BATCH	S. cereviseae	Palmarola etal 2005
nut	Acid hydrolysis	BATCH	S. cereviseae	Akpan etal 2005
firbes	Liquid hot H2O	SSF, SHF	S. cereviseae FPL- 702	Sreenath etal 2001
	Acid hydrolysis	Continuous immobilized	P. SHPITIS R	Perekh etal 1987

		cells		
ust	Acid hydrolysis	Batch, continuous up flow Reaction	Clostridium thermosacchrorolytham	Liu etal 1988
	Acid hydrolysis	Continuous stirred tank reactor	P. STIPHIS- NRRL 1/24	Qureshi etal 1991
	Steam & explosion enzymatic	SSF, SHF	S. cereviseae	Cantarella etal 2004
	Acid hydrolysis	Batch	S. cereviseae	Johanssen etal 2001
	Dilute acid hydrolysis	Fed batch	S. cereviseae	Taherzedeh 1999
	Steam	Batch	E-coli koli	Olsson etal 1995
ava	Dilute acid hydrolysis two stage	Batch	C shehatae, p. stipitis	Sanche et al 2004;
rnia es	Dilute acid hydrolysis	Batch, fed batch	Pachysolen tannphilus, p. stipitis	Nigam 2002;
rum	Dilute acid hydrolysis	Batch, continous co-immobilized cells	P. stipitis Ncim 3498	Guta, 2006'
neum	Enzymatic	SSF	S diastaticus zymonnase mobilis	Amuthgandam, ashekhran, 2001;
a	Starch liquefaction	Continuous	S. diasticus zymonnase mobilis	Amutha and

n		immobilized cells		gumoshekaran
e ce		SSF	S. cereviseae ATCC 24702	ngadi and correlal 1992
apple	Juice extraction	Continuous immobilized cells	S. cereviseae ATTCC 24553	Nigam 2000
pal aste	Acid pretreatment	Batch	S. cereviseae	Mtul and nakamura 2005
ial	----------	SSF	K. Maxianus S. Cereviseae	kadar etal 2004

TABLE 2: SUMMARY REVIEW OF LITERATURES.

CHAPTER 3

3.0 METHODOLOGY

3.1 MATERIAL AND EQUIPMENT USED IN BIOETHANOL PRODUCTION

Feedstock utilized passed trough several batch processes (unit operations) to achieve a final product-bioethanol.

Equipments /Apparatus

1. 1000ml conical flask (reactor)
2. 1000ml measuring cylinder.
3. Thermometer
4. Heating element (kerosene stove)
5. 1000ml beaker
6. Column chromatograph
7. Centrifuge vi
8. Distillation column (simple).
9. Azeotropic distillation column
10. Funnel
11. pH meter

Materials and chemicals

1. Filter paper
2. 98% concentrated tetraoxosulphate vi acid
3. Hydrated lime [$Ca(OH)_2$]
4. Yeast (cereviseaes saccharomyces)
5. Benzene
6. Distilled water

3.1.1 PRETREATING FEEDSTOCK /RAW MATERIAL

The feedstock (corn cobs) after drying properly to about 10% moisture content was taken to the mill for size reduction. The purpose of milling is to break up the corn cobs to small particle size i.e Size that could create the required surface area for reaction to occur. After milling, the grinded feedstock is then sieve to a size of 250microns. This is to obtain a homogeneous size.

3.1.2 DILUTE ACID HYDROLYSIS

10g of feedstock was weighed first and charged into a conical flask. 387ml of diluted acid of 0.78% concentrations was added to the biomass. Stirring was maintained as the reactor and its content was heated to 50°C for 20 minutes. The stirring was maintained to allow equal distribution of heat and for the uniformity of the content. The temperature of the mixture was increased to 190°C for 10minute. The hydrolysate which contains xylose and hexoses was obtained after filtration. The resulting biomass was washed with plentiful of water to optimize sugar and then filter again. The sugar syrup obtained was incubated at 75°C for 2hours to convert the oligosaccharide to monosaccharide.This monosaccharide obtained was poured into an air tight container to prevent unwanted enzymatic transformation of the sample. The first stage dilute acid hydrolysis helps to enhance cellulose digestibility in the residual solid (Nigannnn, 2002; Sun and charge, 2002; Dire et al, 2006; Saha et al, 2005). This makes it less difficult for cellulose to be depolymerized in the two stage dilute acid hydrolysis.

3.1.3 TWO STAGE DILUTE ACID HYDROLYSIS.

The resulting biomass 4g was than pretreated with steam to open up the microstructure of the biomass. Dilute hydrogentetraoxosulphateVI acid of (1.0%concentration) was added to the biomass (4g) in the ratio 1:25 acid to biomass at a temperature of 190^{0}C for 5 minutes. After filtering, the resulting biomass was then hydrolysed again with thesame acid concentration (1.0 % acid concentration) at a temperature of 230 °C for 3minutes. The resulting sugar syrup was added together and then pured into another air tight container.

This process or steps was repeated for 20, 30 and 40grams

3.1.4 CONCENTRATED ACID HYDROLYSIS

After pretreatment of the feedstock, 70% concentration of hydrogentetraoxosulphate VI acid in a ratio of 1:25 acid to /biomass was poured into the reactor containing the biomass and controlled at a temperature of 45°C for 30minutes. Water was added to dilute the acid to about 25%. The mixture was now maintained at 50°C for the next 3hours. After 3hours, the mixture was then filtered to remove the residue from sugar.

3.1.5 COLUMN CHROMATOGRAPHIC SEPERATION (sugar/acid isolation)

The use of column chromatography was employed for this operation. A column in form of a hollow long pipe was packed with silica gel, wool was then placed on top of the gel so that the surface of the gel does not get disrupted when elating. The sample (sugar/acid mixture) was poured on the wool and then an eluting solvent (Benzene) was used to wash down the sample. Sugar was washed down first and then the acid. They were both collected into different containers. The acid can be re-concentrated for economical reasons. The sugar was tested with a pH meter to observe if it could accommodate microbes (yeast). The p.H meter reading was 3.7, yeast cannot ferment at this p.H so then, CA $(OH)_2$ (hydrated lime was added to increase the p.H to 4.5.

3.1.6 FERMENTATION

Once pH 4.5 was attained, yeast was mixed with water and poured into the sugars. The set-up was left to ferment for three days after which it was sent to the distillation column for distillation. Two types of distillation were employed. First the steam distillation and then the azeotropic distillation.

3.17 STEAM DISTILLATION

This method was employed to concentrate ethanol in ethanol – water system. This was possible because ethanol – water system operate within the physical laws that state that different materials boils at different temperatures.

The system in figure 2 is typical for the separation of a two component feed (e.g. ethanol–water) consisting of ideal or nearly ideal component into a relatively pure overhead product (ethanol) containing the lower boiling component and a bottom product containing primarily the higher boiling component of the original feed. Though in practical sense, pure ethanol was not obtained as the product component, this is because some fraction of water tends to behave like ethanol at certain stage during distillation to form azeotropes

3.1.8 AZEOTROPIC DISTILLATION

Since a distillate of 98% or 99% concentration is desired, an azeoteopic distillation was employed after the simple distillation. This azeotropic distillation is a special method of multicomponent distillation was used for the separation of binary mixtures that are difficult to separate by ordinary fractionating. Azeotropic distillation usually include the use of a third component called an entrainer which lower the boiling point of one of the component mixture so that the relative volatility becomes low and thus separation can be enhanced. For this particular unit, the entrainer used was benzene.

Instead of using azeotropic distillation, an absorption column called the molecular sieve can also be used for this purpose to dehydrate the ethanol further to concentration of 99%.

CHAPTER FOUR

4.1 RESULTS

S/Number	Mass of corn cobs (g)	Volume of sugars obtained (ml)	Yield of ethanol 200 proof 98% 9(ml)	Volume of acid used(ml)
1	10	525	9.50	271.25
2	20	1055	18.80	542.00
3	30	1575	28.5	813.75
4	40	2100	35.00	1084.3

Table 3: CONCENTRATED ACID HYDROLYSIS

S/ number	Mass of corn cobs (g)	Volume of sugar obtained (ml)	Yield of ethanol 200 proof 98% (ml)	Volume of acid used (ml)
1	10	587	6.50	7.25
2	20	11125	13.00	14.30
3	30	1761	19.00	21.75
4	40	2384	25.50	29.00

Table 4: dilute acid hydrolysis.

DISCUSSION OF RESULTS

From the results obtained in table 3 and 4 for both concentrated and dilute acid hydrolysis respectively show that increase in biomass always results to increase in volume of sugars obtained and consequent increase in ethanol produced. The ethanol yield/ biomass for both

methods of hydrolysis are not proportional. Concentrated acid hydrolysis seems to produce more ethanol per unit biomass compared to dilute acid hydrolysis. This is not far fetched from the fact that since a high temperature is needed to depolymerize or hydrolyse cellulose in the two stage hydolysis some sugars may have been degraded to form fermentation inhibitng substances/ chemicals such as furfural, syringaldehyde and vanillin. All these factors put together have effects on ethanol yield.

Concentrated acid hydrolysis from an economic stand point is more effective compared to dilute to acid hydrolysis, it produced more ethanol yield compared to dilute hydrolysis in an average yield difference of 18.16%. Concentrated acid hydrolysis does not also requires a lot of energy input compared to dilute acid hydrolysis and therefore able to maintain high sugar concentration with little or no degradation. Concentrated acid hydrolysis also has its limitation; this acid recovered can be reconcentrated in an additional unit. This makes the process more complex and ambiguous.

CHAPTER FIVE

5.1 RECOMMENDATION AND CONCLUSION

The objective of this research work is to evaluate different methods of bio – ethanol production and then showing the most economically viable method .This was achieved by producing ethanol from cellulosic biomass using the two different methods under investigation (dilute and concentrated acid hydrolysis). The result obtained from production was compared for both methods. The method that has the most potential to produce cheap ethanol fuel was then noted.

Concentrated acid hydrolysis from the result produced more yields than dilute acid hydrolysis. Although dilute acid hydrolysis more yields of sugar, but in terms of sugar concentration concentrated acid hydrolysis contains more sugar concentration. This is because some of the sugars in the dilute acid hydrolysis method were degraded by heat to produce ethanol inhibiting substances. These substances are capable of lowering the activities of saccharomyces cereviceae on the sugar to produce ethanol. This reduces the volume of ethanol produced. Concentrated hydrolysis on the other hand contains little or no degradation and so saccharomyces cereviceaes is able to ferment the sugars effectively to produce ethanol of better quantity.

Some equipment that was needed this research work were not available, therefore values generated may have been affected in one way or another.

Some of the equipments are;

1. 230^0 C heating element
2. Steam gun
3. Molecular sieve

Despite equipment limitation, 98% 200 PROOF bioethanol was produced. This when blended with gasoline in a ratio of 9:1 gasoline to ethanol ratio can cut down the emission of green house gases by 20% (National renewable energy department NRED)

5.2 RECOMMENDATION

Concentrated acid hydrolysis should be strongly considered over dilute acid hydrolysis when planning an industrial production of bio-ethanol.

More attention and priority should be placed on research and development in the production of microbes that can depolymerize and ferment biomass in the same step.

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NOMENCLATURE

Y_1 = slope of concentrated acid hydrolysis graph

Y_2 = slope of dilute acid hydrolysis graph

APPENDIX

Comparing the % of ethanol yields

At 10g

% yield for concentrated hydrolysis (X_1) = $\frac{9.5}{16} \times 100 = 59.37\%$

% for dilute acid hydrolysis (X_2) = $\frac{6.5}{16} \times 100 = 40.62\%$

Difference in % yield = 59.37 – 40.62 =18.70%

At 20g $X_1 = \frac{18.8}{31.8} \times 100 = 59.12\%$

$X_2 = \frac{13}{31.8} \times 100$ 40.88%

Difference in %yield = 59.12 – 40.88 = 18.24%

At 30g $X_1 = \frac{28.5}{47.5} \times 100 = 60\%$

$X_2 = \frac{19}{47.5} \times 100 = 40\%$

Difference in % yield = 60-40 20%

At 40g

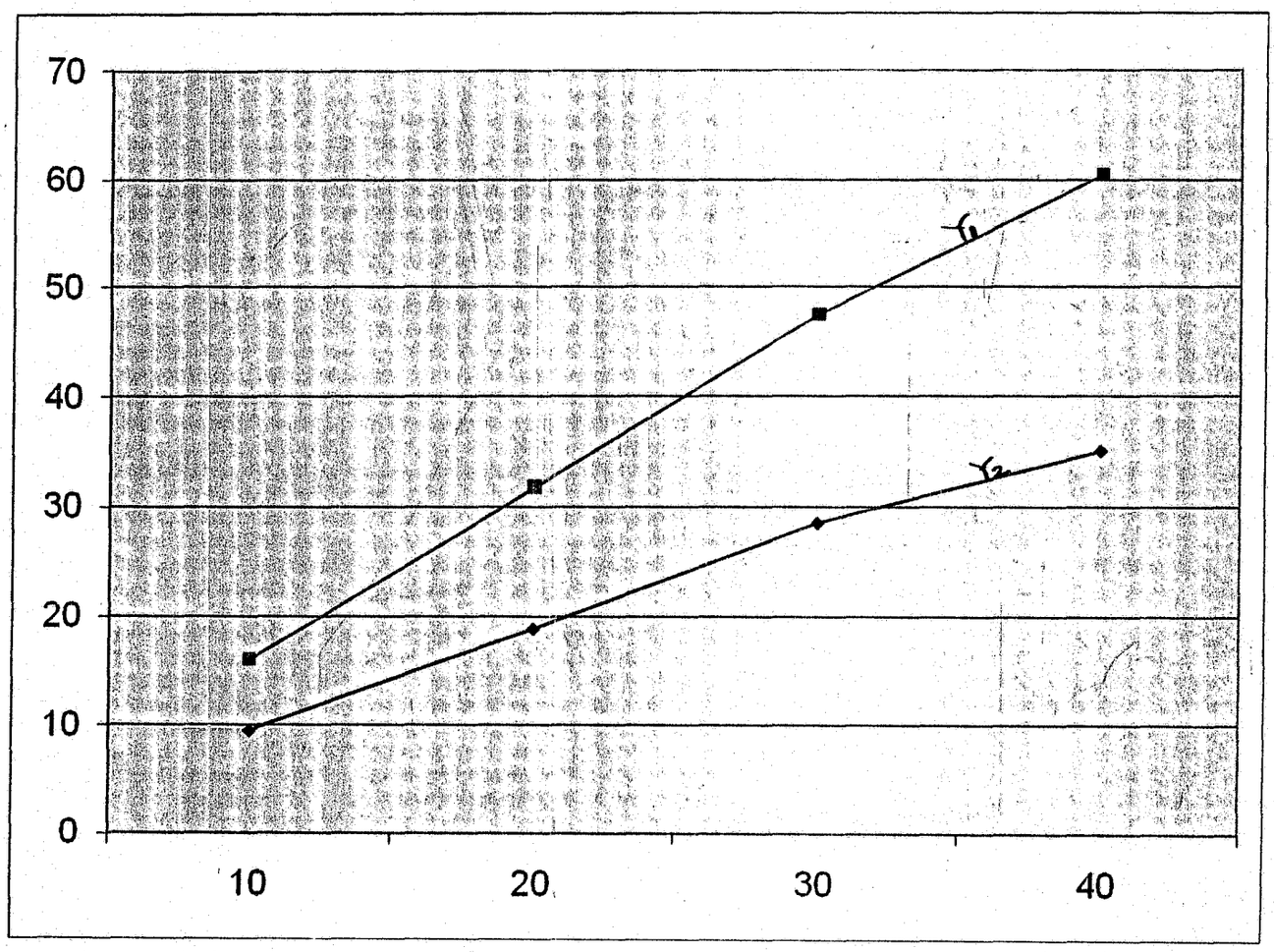
$X_1 = \frac{35}{60.5} \times 100 = 557.85\%$

$X_2 = \frac{25.5}{60.5} \times 100 = 42.15\%$

Difference in % yield = 57.85 – 42.15 = 15.70%

Average difference in yield = $\frac{18.70+18.24+20+15.70}{4} = 18.16\%$

A GRAPH COMPARING ETHANOL YIELD (mL)/BIOMASS (g) OF CONCENTRATE METHOD (Y_1) AGAINST DILUTE ACID HYDROLYSIS METHOD (Y_2)



Slope $(Y_1) = \frac{35.00 - 9.50}{30} = 0.85$ mL/g

Slope $(Y_2) = \frac{25.50 - 6.50}{30} = 0.633$ mL/g

$Y_1 - Y_2 = 0.85 - 0.63 = 0.22$mL/