PRODUCTION OF ETHANOL FROM BAGASSE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Bachelor of Technology

in

Chemical Engineering

By

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AMIT NAG

Department of Chemical Engineering
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CERTIFICATE

This is to certify that the thesis entitled,

"PRODUCTION OF ETHANOL FROM BAGASSE"

submitted by Ms. ROSALIN PRADHAN and Sri. AMIT NAG in partial fulfillments for the requirements for the award of Bachelor of Technology Degree in Chemical Engineering at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by them under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

Date: 2ND–May-2007

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ACKNOWLEDGEMENT

We would like to express our gratitude to Prof. P. Rath (Head of the Department) and Prof. G. Satpathy for their constant support and encouragement.

We wish to express our deep sense of gratitude and indebtedness to Prof. M. Kundu, Department of Chemical Engineering, N.I.T Rourkela for introducing the present topic and for her inspiring guidance, constructive criticism and valuable suggestion throughout this project work.

We would also like to thank Mr. Chandan Pattnaik and Miss Tarangini for their timely help and advice. We are also thankful to all staff members of Department of Chemical Engineering, NIT Rourkela.

Date: 2ND-May-2007

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ABSTRACT

PURPOSE OF THE PROJECT

Bio-fuel has been a source of energy that human beings have used since ancient times. Increasing the use of bio-fuels for energy generation purposes is of particular interest nowadays because they allow mitigation of greenhouse gases, provide means of energy independence and may even offer new employment possibilities. Bio-fuels are being investigated as potential substitutes for current high pollutant fuels obtained from conventional sources. The aim of the project is the production of low cost ethanol by using lignocellulosic materials basically the agro wastes like sugarcane bagasse, rice husk, wheat straw, corn fiber, crop residues, grasses and other materials like saw dust, wood chips, solid animal wastes etc.

BREIF DESCRIPTION OF THE EXPERIMENT

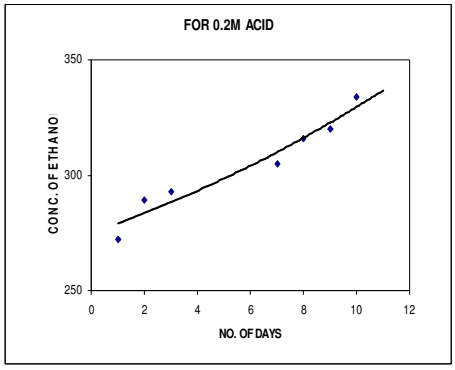
The raw material used for the experiment is bagasse. First the bagasse is chipped and grinded (may be upto powder form). Then this is taken for acid hydrolysis. Dilute sulphuric acid of 0.2M, 0.3M and 0.5M concentration were used in this process. For the acid hydrolysis,5 gms of bagasse was put in 100 ml of each of the concentrations and was allowed to soak for 1 day. Then it was auto-claved for half an hour and allowed to cool. The fermenting media was prepared.5 gms of yeast was added to the above media and kept in incubator for 1 day.10 ml of this media was added to each of the samples in asceptical (laminar flow hood) manner and placed in orbital shaking incubator. The ph was adjusted to 5 and the fermenting temperature was kept at 35^0C.Fermentation may take upto 10 days.

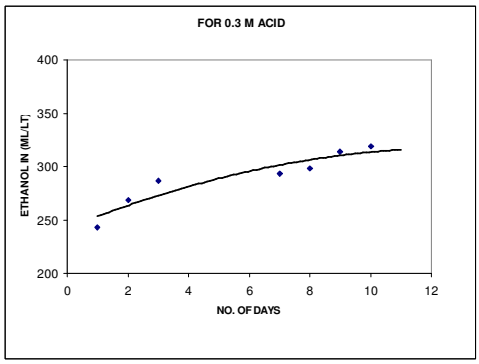
ANALYSIS OF ETHANOL AND SUGAR

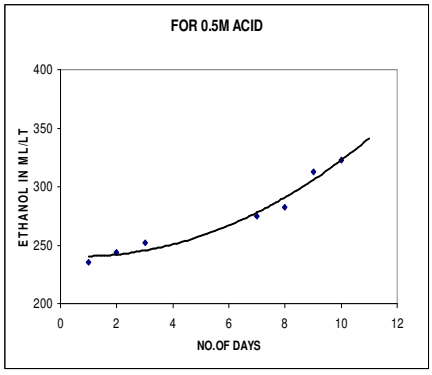
Sugar is analyised by DNS method. For ethanol analysis, after each interval of 24 hrs, 5 ml of the sample is taken and filtered. The filtrate is to be analyzed under UV-spectrophotometer. Quantification of ethanol was done by using standard ethanol.

RESULTS AND DISCUSSION

The ethanol concentration increased as the number of days increased. The following graphs show the plot between the ethanol conc.in ml/lt vs number of days for 0.2M, 0.3M and 0.5M.







The ethanol concentration decreased as the molarity of acid used (sulphuric acid) increased.The maximum concentration of ethanol obtained was 389.22 ml/lt of acid hydrolysed bagasse used.

Chapter 1

INTRODUCTION

1.1 INTRODUCTION

Bio-fuel has been a source of energy that human beings have used since ancient times. Increasing the use of bio-fuels for energy generation purposes is of particular interest nowadays because they allow mitigation of greenhouse gases, provide means of energy independence and may even offer new employment possibilities. Bio-fuels are being investigated as potential substitutes for current high pollutant fuels obtained from conventional sources.

The quest for alternative energies has provided many ways to produce electricity, such as wind farms, hydropower, or solar cells. However, about 40% of the total energy consumption is dedicated to transports and in practice requires liquid fuels such as gasoline, diesel fuel, or kerosene. These fuels are all obtained by refining petroleum. This dependency on oil has two major drawbacks: burning fossil fuels such as oil contributes to global warming and importing oil creates a dependency on oil producing countries.

Also it has been estimated that the decline in worldwide crude oil production will begin before 2010. They also predicted that annual global oil production would decline from the current 25 billion barrels to approximately 5 billion barrels in 2050. Because the economy in the US and many other nations depends on oil, the consequences of inadequate oil availability could be severe. Therefore, there is a great interest in exploring alternative energy sources)

Unlike fossil fuels, ethanol is a renewable energy source produced through fermentation of sugars. Ethanol is widely used as a partial gasoline replacement in the US. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since the 1980s. These gasoline fuels contain up to 10% ethanol by volume. As a result, the US transportation sector now consumes about 4540 million liters of ethanol annually, about 1% of the total consumption of gasoline. Recently, US automobile manufacturers have announced plans to produce significant numbers of flexible-fueled vehicles that can use an ethanol blend – E85 (85% ethanol and 15% gasoline by volume) – alone or in

combination with gasoline. Using ethanol-blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission. However the cost of ethanol as an energy source is relatively high compared to fossil fuels.

The aim of the present paper is the production of low cost ethanol cellulosic materials basically the agro wastes like sugarcane bagasse, rice husk, wheat straw, corn fibre, crop residues, grasses and other materials like saw dust. wood chips, solid animal wastes etc.

Chapter 2

LITERATURE REVIEW

2.1 CELLULOSIC FEEDSTOCKS

Cellulosic resources are in general very widespread and abundant. For example, forests comprise about 80% of the world's biomass. Being abundant and outside the human food chain makes cellulosic materials relatively inexpensive feedstocks for ethanol production. Cellulose is a remarkable organic polymer consisting of solely of units of glucose held together in a giant straight chain molecule,

Cellulosic materials are comprised of lignin, hemi cellulose and cellulose and are thus sometimes called lignocellulosic materials. One of the primary functions of lignin is to provide structural support for the plant. Thus, in general, trees have higher lignin contents then grasses. Unfortunately, lignin which contains no sugars encloses the cellulose and hemicellulose molecules, making them difficult to reach.

Cellulose molecules consist of long chains of glucose molecules as do starch molecules, but have a different structural configuration. These structural characteristics plus the encapsulation by lignin makes cellulosic materials more difficult to hydrolyze than starchy materials.

Hemi cellulose is also comprised of long chains of sugar molecules; but contains, in addition to glucose (a 6-carbon or hexose sugar), contains pentose (5-carbon sugars). To complicate matters, the exact sugar composition of hemi cellulose can vary depending on the type of plant.

Since 5-carbon sugars comprise a high percentage of the available sugars, the ability to recover and ferment them into ethanol is important for the efficiency and economics of the process.

The contents of the cellulose, hemicellulose and lignin in common agricultural residues and wastes are:

Lignocellulosic materials	Cellullose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA[b]	24–29
Swine waste	6.0	28	NA[b]
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

Table 2.1

The technological hurdles that are presented by the materials are:

- The separation of lignin from the cellulose and hemi-cellulose to make the material susceptible to hydrolysis.
- The hydrolysis of cellulose and hemi-cellulose takes place at different rates and over reaction can degrade the sugars into materials that are not suitable for ethanol production.
- The hydrolysis of these materials produces a variety of sugars. Not all of these sugars are fermentable with the standard yeast that is used in the grain ethanol industry. The pentose sugars are particularly difficult to ferment.

2.2 PHYSICAL PROPERTIES:

Ethyl alcohol under ordinary condition is volatile, flammable, clear, colourless liquid. Its odour is pleasant, familiar and characteristics as is its taste when suitable diluted with water. Otherwise its taste may be pungent

The physical and chemical properties of ethyl alcohol are primarily dependant upon hydroxyl group. This group imparts polarity to the molecule and also gives rise to hydrogen bonding. These two properties account for the abnormal physical behaviour of lower molecular weight alcohols as compared to hydrocarbons of equivalent weight. Infra red spectrographic studies have shown that, in the liquid state, hydrogen bonds are formed by the attraction of the hydroxyl hydrogen of one molecule and the hydroxyl oxygen of a second molecule. The net effect of this bonding is to make liquid alcohol behave as though it were largely dimerized. This behaviour is analogous to the behaviour of water, which however is more strongly bonded and appears to exist in liquid clusters of more than two molecules. The association of ethyl alcohol, it should be noted, is confined to the liquid state in the vapour state, this alcohol is monotheric.

The molecular association of liquid ethyl alcohol gives rise to an abnormally high boiling point and a high heat of vaporization. Trouton's constant for ethyl alcohol is 26.9 as compared to 21 for unassociated liquids. This constant is the entropy of vaporization at atmospheric pressure and is obtained by dividing the molecular heat of vaporization by the absolute temperature of the atmospheric boiling point.

Ethyl alcohol's polarity and association also manifest themselves in the non-ideal behaviour of many ethyl alcohol solutions and in the fact that ethyl alcohol forms a large no. of azeotropes. Many other examples of ethyl alcohol abnormalities may be found in the properties of ethyl alcohol solutions appearing in the literature. A summary of physical properties of ethyl alcohol is presented in Table

Table-2.2

Properties		Value
1) Freezing point, ^{O}C	=	-114.1
2) Normal boiling point, ^{O}C	=	78.32
3) Critical temperature, ^{O}C	=	243.1
4) Critical pressure, atm	=	63.0
5) Critical volume, 1/mole	=	0.167
6) Solubility in water at 20 ^{O}C	=	miscible
7) Flammable limits in air		
Lower % by volume	=	4
Upper % by volume	=	19
8) Auto-ignition temperature, ^{O}C	=	793
9) Flash point, open cup, ^{O}F	=	70.0
10) Specific heat, at 20^{O}C, cal/kg^{O}C	=	0.579
11) Thermal conductivity, at 20^{O}C	=	0.00170J/sec.cm^2 (^{O}C/cm)

2.3 CHEMICAL PROPERTIES

The chemical properties of ethyl alcohol are primarily concerned with the hydroxyl group, namely reactions involving dehydration, dehydrogenation, oxidation and esterification. The H_2 atom of the hydroxyl group can be replaced by an active metal, such as sodium, potassium and calcium with the formation of a metal ethoxide (ethylate) and the evolution of H_2 gas.

$$2C_2H_5OH + 2M \rightarrow 2C_2H_5OM + H_2$$

Sodium ethoxide can be prepared by the reaction between absolute ethyl alcohol and sodium or by refluxing absolute ethyl alcohol with anhydrous sodium hydroxide, as shown

$$CH_3CH_2OH + NaOH \rightarrow CH_3CH_2ONa + H_2O$$

The sodium ethoxide precipitates upon addition of anhydrous acetone. This strong base hydrolyses readily to give ethyl alcohol and sodium and hydroxyl ions

$CH_3CH_2O^-Na^+ + H_2O \rightarrow CH_3CH_2OH + Na^+ + OH^-$

Commercially water is removed by azeotropic distillation with benzene. Sodium ethoxide may also be prepared by reacting sodium amalgam with ethyl alcohol.

Sodium ethoxide is used in organic synthesis as a condensing and reducing agent. The reaction between sodium ethoxide and sulphur monochloride results in the formation of diethyl thiosulphate

$2CH_3CH_2ONa + S_2Cl_2 \rightarrow (CH_3CH_2)_2S_2O_2 + 2NaCl$

The commercial production of barbiturates (vernal, Barbital, luminal, amytal), ethyl ortho formate and other chemicals is dependent upon the use of sodium ethoxide.

Aluminium and magnesium also react to form ethoxides, but the reaction must be catalysed by amalgamating the metal (adding a small amount of mercury)

$6CH_3CH_2OH + 2Al \rightarrow 2(CH_3CH_2O)_3Al + 3H_2$

$2CH_3CH_2OH + Mg \rightarrow (CH_3CH_2O)_2Mg + H_2$

Ethyl alcohol also reacts with acid anhydrides or acid halides to give corresponding esters

$CH_3CH_2OH + (RCO)_2O \rightarrow RCOOCH_2CH_3 + RCOOH$

$CH_3CH_2OH + RCOCl \rightarrow RCOOCH_2CH_3 + HCl$

The direct conversion of ethyl alcohol to ethyl acetate as believed to take place via acetaldehyde and its condensation to ethyl acetate (Tischenko reaction)

$CH_3CH_2OH \rightarrow CH_3CHO + H_2$

$2\ CH_3CHO \rightarrow CH_3COOCH_2CH_3$

About a 26% yield of ethyl acetate is obtained using a copper oxide catalyst containing 0.1-0.2% thoria at a temperature of 350□.

Ethyl alcohol may be dehydrated intramolecularly to form ethylene or ethyl ether

$CH_3CH_2OH \rightarrow CH_2{=}CH_2 + H_2O$

$2CH_3CH_2OH \rightarrow CH_3CH_2OCH_2CH_3 + H_2O$

Generally both ethylene and ethyl ether a formed to some extent of the same time, both the conditions may be altered to favour one reaction or the other.

The dehydrogenation of ethyl alcohol to acetaldehyde may be obtained by a vapour phase reaction over various catalyst.

$CH_3CH_2OH \rightarrow CH_3CHO + H_2$

Ethyl alcohol reacts with sodium hypochlorite to give chloroform – the haloform reaction

$CH_3CH_2OH + NaOCl \rightarrow CH_3CHO + NaCl + H_2O$

$CH_3CHO + 3\ NaOCl \rightarrow CCl_3CHO + 3NaOH$

$CCl_3CHO + NaOH \rightarrow CHCl_3 + HCOONa$

Similarly bromoform($CHBr_3$) and iodoform(CHI_3) are obtained from sodium hypobromite and hypoiodite respectively. Ethyl alcohol is the only primary alcohol that undergoes this reaction.

Chapter 3

PRODUCTION METHODS

3.1 PRODUCTION METHODS

Processing of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation/purification. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its sub-microscopic chemical composition and structure so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields. Hydrolysis includes the processing steps that convert the carbohydrate polymers into monomeric sugars. Although a variety of process configurations have been studied for conversion of cellulosic biomass into ethanol, enzymatic hydrolysis of cellulose provides opportunities to improve the technology so that biomass ethanol is competitive when compared to other liquid fuels on a large scale.

Cellulose can be hydrolytically broken down into glucose either enzymatic ally by cellulases or chemically by sulfuric or other acids. Hemicellulases or acids hydrolyze the hemicellulose polymer to release its component sugars. Glucose, galactose, and mannose, six carbon sugars (hexoses), are readily fermented to ethanol by many naturally occurring organisms, but the pentoses xylose and arabinose (containing only five carbon atoms) are fermented to ethanol by few native strains, and usually at relatively low yields. While pentoses are not readily fermented, the ketose of xylose, xylulose, is converted to ethanol by *S. pombe*, *S. cerevisiae*, *S. amucae,* and *Kluveromyces lactic*. Xylose and arabinose generally comprise a significant fraction of hardwoods, agricultural residues, and grasses and must be utilized to make the economics of biomass processing feasible). Genetic modification of bacteria and yeast has produced strains capable of co-fermenting both pentoses and hexoses to ethanol and other value-added products at high yields.

The basic five stages of this process are:

1. A "pre-treatment" phase, to make the raw material such as wood or straw amenable to hydrolysis,
2. Hydrolysis, to break down the molecules of cellulose into sugars;
3. Separation of the sugar solution from the residual materials, notably lignin.

4. Yeast fermentation of the sugar solution;
5. Distillation to produce 99.5% pure alcohol.

3.2 Pretreatment of lignocellulosic materials

The effect of pretreatment of lignocellulosic materials has been recognized for a long time. The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost-effective. Physical, physico-chemical, chemical, and biological processes have been used for pretreatment of lignocellulosic materials.

3.2.1 Physical pretreatment

3.2.1.1. Mechanical comminution

Waste materials can be comminuted by a combination of chipping, grinding and milling 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. Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling. The power requirement of mechanical comminution of agricultural materials depends on the final particle size and the waste biomass characteristics.

3.2.1.2. Pyrolysis

Pyrolysis has also been used for pretreatment of lignocellulosic materials. When the materials are treated at temperatures greater than 300 °C, cellulose rapidly decomposes to produce gaseous products and residual char. The decomposition is much slower and less volatile products are formed at lower temperatures. Mild acid hydrolysis (1 N H_2SO_4, 97 °C, 2.5 h) of the residues from pyrolysis pretreatment has resulted in 80–85% conversion of cellulose to reducing sugars with more than 50% glucose. The process can be enhanced with the presence of oxygen. When zinc chloride or sodium carbonate is added as a catalyst, the decomposition of pure cellulose can occur at a lower temperature.

3.2.2. Physico-chemical pretreatment

3.2.2.1. Steam explosion (autohydrolysis):

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials. In this method, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160–260 °C (corresponding pressure 0.69–4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. Ninety percent efficiency of enzymatic hydrolysis has been achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips. 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 °C, 1 min) or lower temperature and longer residence time (190 °C, 10 min). Recent studies indicate that lower temperature and longer residence time are more favorable.

Addition of H_2SO_4 (or SO_2) or CO_2 in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds, and lead to more complete removal of hemicellulose. The optimal conditions of steam explosion

pretreatment of sugarcane bagasse have been found to be as following: 220 °C; 30 s residence time; water-to-solids ratio, 2; and 1% H_2SO_4.Sugar production was 65.1 g sugar/100 g starting bagasse after steam explosion pretreatment.

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 size reduction. 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 may be inhibitory to microorganisms used in downstream processes. Because of the formation of degradation products that are inhibitory to microbial growth, enzymatic hydrolysis, and fermentation, pretreated biomass needs to be washed by water to remove the inhibitory materials along with water-soluble hemicellulose. The water wash decreases the overall saccharification yields due to the removal of soluble sugars, such as those generated by hydrolysis of hemicellulose. Typically, 20–25% of the initial dry matter is removed by water wash.

3.2.2.2. Ammonia fiber explosion (AFEX)

AFEX is another type of physico-chemical pretreatment in which lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is swiftly reduced. The concept of AFEX is similar to steam explosion. In a typical AFEX process, the dosage of liquid ammonia is 1–2 kg ammonia/kg dry biomass, temperature 90 °C, and residence time 30 min. AFEX pretreatment can significantly improve the saccharification rates of various herbaceous crops and grasses. It can be used for the pretreatment of many lignocellulosic materials including alfalfa, wheat straw, wheat chaff, barley straw, corn stover, rice straw, municipal solid waste, softwood newspaper, kenaf newspaper, coastal Bermuda grass, switchgrass, aspen chips, and bagasse. The AFEX pretreatment does not significantly solubilize hemicellulose compared to acid pretreatment (to be discussed in the following

section) and acid-catalyzed steam explosion compared the steam and ammonia pretreatment for enzymatic hydrolysis of aspen wood, wheat straw, wheat chaff, and alfalfa stems, and found that steam explosion solubilized the hemicellulose, while AFEX did not. The composition of the materials after AFEX pretreatment was essentially the same as the original materials. Over 90% hydrolysis of cellulose and hemicellulose has been obtained after AFEX pretreatment of Bermuda grass (approximately 5% lignin) and bagasse (15% lignin). However, the AFEX process was not very effective for the biomass with high lignin content such as newspaper (18–30% lignin) and aspen chips (25% lignin). Hydrolysis yield of AFEX-pretreated newspaper and aspen chips was reported as only 40% and below 50%, respectively.

To reduce the cost and protect the environment, ammonia must be recycled after the pretreatment. In an ammonia recovery process, a superheated ammonia vapor with a temperature up to 200 °C was used to vaporize and strip the residual ammonia in the pretreated biomass and the evaporated ammonia was then withdrawn from the system by a pressure controller for recovery. The ammonia pretreatment does not produce inhibitors for the downstream biological processes, so water wash is not necessary. AFEX pretreatment does not require small particle size for efficacy.

3.2.2.3. CO_2 explosion

Similar to steam and ammonia explosion pretreatment, CO_2 explosion is also used for pretreatment of lignocellulosic materials. It was hypothesized that CO_2 would form carbonic acid and increase the hydrolysis rate. Researchers used this method to pretreat alfalfa (4 kg CO_2/kg fiber at the pressure of 5.62 MPa) and obtained 75% of the theoretical glucose released during 24 h of the enzymatic hydrolysis.

The yields were relatively low compared to steam or ammonia explosion pretreatment, but high compared to the enzymatic hydrolysis without pretreatment. They compared CO_2 explosion with steam and ammonia explosion for pretreatment of recycled paper mix, sugarcane bagasse, and repulping waste of recycled paper, and found that CO_2

explosion was more cost-effective than ammonia explosion and did not cause the formation of inhibitory compounds that could occur in steam explosion.

3.2.3. Chemical pretreatment

3.2.3.1. Ozonolysis:

Ozone can be used to degrade lignin and hemicellulose in many lignocellulosic materials such as wheat straw, bagasse, green hay, peanut, pine, cotton straw, and poplar sawdust. The degradation was essentially limited to lignin and hemicellulose was slightly attacked, but cellulose was hardly affected. The rate of enzymatic hydrolysis increased by a factor of 5 following 60% removal of the lignin from wheat straw in ozone pretreatment. Enzymatic hydrolysis yield increased from 0% to 57% as the percentage of lignin decreased from 29% to 8% after ozonolysis pretreatment of poplar sawdust. Ozonolysis pretreatment has the following advantages: (1) it effectively removes lignin; (2) it does not produce toxic residues for the downstream processes; and (3) the reactions are carried out at room temperature and pressure. However, a large amount of ozone is required, making the process expensive.

3.2.3.2. Acid Pretreatment

Concentrated acids such as H_2SO_4 and HCl have been used to treat lignocellulosic materials. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible

Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis. At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition. High temperature in dilute acid treatment is favorable for cellulose hydrolysis. Recently

developed dilute acid hydrolysis processes use less severe conditions and achieve high xylan to xylose conversion yields.

Achieving high xylan to xylose conversion yields is necessary to achieve favorable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials. There are primarily two types of dilute acid pretreatment processes: high temperature (*T* greater than 160 °C), continuous-flow process for low solids loading (5–10% [weight of substrate/weight of reaction mixture]), and low temperature (*T* less than 160 °C), batch process for high solids loading (10–40%). Although dilute acid pretreatment can significantly improve the cellulose hydrolysis, its cost is usually higher than some physico-chemical pretreatment processes such as steam explosion or AFEX. A neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes.

3.2.3.3. Alkaline Pretreatment

Some bases can also be used for pretreatment of lignocellulosic materials and the effect of alkaline pretreatment depends on the lignin content of the materials. The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds cross linking xylan hemicelluloses and other components, for example, lignin and other hemi cellulose. The porosity of the lignocellulosic materials increases with the removal of the cross-links. Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure. The digestibility of NaOH-treated hardwood increased from 14% to 55% with the decrease of lignin content from 24–55% to 20%. However, no effect of dilute NaOH pretreatment was observed for softwoods with lignin content greater than 26%. Dilute NaOH pretreatment was also effective for the hydrolysis of straws with relatively low lignin content of 10–18%. The combination of irradiation and 2% NaOH for pretreatment of corn stalk, cassava bark and peanut husk was used. The glucose yield of corn stalk was 20% in untreated samples compared to

43% after treatment with electron beam irradiation at the dose of 500 kg and 2% NaOH, but the glucose yields of cassava bark and peanut husk were only 3.5% and 2.5%, respectively.

Ammonia was also used for the pretreatment to remove lignin. Scientists described an ammonia recycled percolation process (temperature, 170 °C; ammonia concentration, 2.5–20%; reaction time, 1 h) for the pretreatment of corn cobs/Stover mixture and switch grass. The efficiency of delignification was 60–80% for corncobs and 65–85% for switch grass.

3.2.3.4. Oxidative delignification

Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of H_2O_2 .The pretreatment of cane bagasse with hydrogen peroxide greatly enhanced its susceptibility to enzymatic hydrolysis. About 50% lignin and most hemicellulose were solubilized by 2% H_2O_2 at 30 °C within 8 h, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45 °C for 24 h. Developers used wet oxidation and alkaline hydrolysis of wheat straw (20 g straw/l, 170 °C, 5–10 min), and achieved 85% conversion yield of cellulose to glucose.

3.2.3.5. Organosolv process

In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicellulose bonds. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol and triethylene glycol and tetrahydrofurfuryl alcohol. Organic acids such as oxalic, acetylsalicylic and salicylic acid can also be used as catalysts in the organosolv process. At high temperatures (above 185 °C), the addition of catalyst was unnecessary for satisfactory delignification. Usually, a high yield of xylose can be obtained with the addition of acid. Solvents used in the process need to be drained from the reactor, evaporated, condensed and recycled to reduce the cost. Removal of solvents from the

system is necessary because the solvents may be inhibitory to the growth of organisms, enzymatic hydrolysis, and fermentation.

3.2.4 Biological pretreatment

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, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials. Researchers studied the pretreatment of wheat straw by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in five weeks. Similar conversion was obtained in the pretreatment by *Phanerochaete sordida* 37 and *Pycnoporus cinnabarinus* 115 in four weeks. In order to prevent the loss of cellulose, a cellulase-less mutant of *Sporotrichum pulverulentum* was developed for the degradation of lignin in wood chips (also reported the delignification of Bermuda grass by white-rot fungi. The biodegradation of Bermuda grass stems was improved by 29–32% using *Ceriporiopsis subvermispora* and 63–77% using *Cyathus stercoreus* after 6 weeks.

The white-rot fungus *P. chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation. Both enzymes have been found in the extracellular filtrates of many white-rot fungi for the degradation of wood cell walls. Other enzymes including polyphenol oxidases, laccases, H_2O_2 producing enzymes and quinone-reducing enzymes can also degrade lignin. The advantages of biological pretreatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment processes is very low.

3.3. Hydrolysis of lignocellulosic materials

The cellulose molecules are composed of long chains of glucose molecules. In the hydrolysis process, these chains are broken down to "free" the sugar, before it is fermented for alcohol production. There are two major hydrolysis processes: a chemical reaction using acids, or an enzymatic reaction.

3.3.1 Acid hydrolysis

Acid hydrolysis has been investigated as a possible process for treating lignocellulosic materials such as wood chips (Silva 1996), rice straw (Almeida 1991), sugar beet pulp and wheat straw . According to Parisi (1989), the mineral acids act simply and rapidly as reaction catalyzers of polysaccharide fractions. Sugarcane bagasse can be hydrolyzed using dilute acid to obtain a mixture of sugars with xylose as the major component. However, in the hydrolyzate some by-products generated in the hydrolysis, such as acetic acid, furfural, phenolic compounds, or lignin degradation products, can be present. These are potential inhibitors of a microbiological utilization of this hydrolyzate.

Processes such as two-stage acid hydrolysis can be employed to produce xylose and glucose (Beck 1986). Treatment with dilute sulphuric acid at moderate temperatures (the first stage of acid hydrolysis) has proven to be an efficient means of producing xylose from hemicellulose. In the second stage more drastic reaction conditions are employed and glucose can be produced from cellulose hydrolysis (Gregg and Saddler 1995).

In general, acid treatment is effective in solubilizing the hemicellulosic component of biomass. Proper combinations of pH, temperature, and reaction time can result in high yields of sugars, primarily xylose from hemicellulose (Elander and Hsu 1995). Sulphuric acid is a catalyst for this reaction and, in this work, was used to study the hydrolysis of sugarcane bagasse hemicellulose. The effects of temperature, acid concentration and reaction time were studied, and the effectiveness of the hydrolysis was evaluated in terms of hemicellulose solubilization.

3.3.2 Enzymatic hydrolysis of cellulose

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes, which are highly specific. The products of the hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45–50 °C) and does not have a corrosion problem. Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora*, and *Streptomyces* can produce cellulases. *Cellulomonas fimi* and *Thermomonospora fusca* have been extensively studied for cellulase production. Although many cellulolytic bacteria, particularly the cellulolytic anaerobes such as *Clostridium thermocellum* and *Bacteroides cellulosolvens* produce cellulases with high specific activity, they do not produce high enzyme titres. Because the anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has focused on fungi.

Fungi that have been reported to produce cellulases include *Sclerotium rolfsii*, *P. chrysosporium* and species of *Trichoderma*, *Aspergillus*, *Schizophyllum* and *Penicillium*. Of all these fungal genera, *Trichoderma* has been most extensively studied for cellulase production.

Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase (EG, endo-1,4-D-glucanohydrolase, or EC 3.2.1.4.) which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-β-D-glucan cellobiohydrolase, or EC 3.2.1.91.) which degrades the molecule further by removing cellobiose units from the free chain-ends; (3) β-glucosidase (EC 3.2.1.21) which hydrolyzes cellobiose to produce glucose. In addition to the three major groups of

cellulase enzymes, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetylesterase, xylanase, β-xylosidase, galactomannanase and glucomannanase. During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented.

The factors that affect the enzymatic hydrolysis of cellulose include substrates, cellulase activity, and reaction conditions (temperature, pH, as well as other parameters). To improve the yield and rate of the enzymatic hydrolysis, research has focused on optimizing the hydrolysis process and enhancing cellulase activity.

3.4 Fermentation process

A variety of microorganisms, generally either bacteria, yeast, or fungi, ferment carbohydrates to ethanol under oxygen-free conditions. They do so to obtain energy and to grow. According to the reactions, the theoretical maximum yield is 0.5 kg ethanol and 0.49 kg carbon dioxide per kg sugar:

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2 \quad (1)$$

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

Methods for C6 sugar fermentation were already known (at least) 6000 years ago, when Sumerians, Babylonians and Egyptians began to perfect and describe the process of making beer from grain (starch). After it became possible to free the C6 sugars in lignocellulosic crops (end 19th century), conversion of the C5 sugars became interesting. They represent a high percentage of the available sugars, the ability to recover and ferment them into ethanol is important for the efficiency and economics of the process. Only in the 1980s research on xylose fermentation began to bear fruit, when a number of wild type yeast were identified that could convert xylose to ethanol. Bacteria have drawn special attention from researchers because of their speed of fermentation. In general, bacteria can ferment in minutes as compared to hours for yeast.

All microorganisms have limitations: either in the inability to process both pentoses and hexoses, the low yields of ethanol, or the co-production of cell mass at the cost of ethanol. Furthermore, the oxygen free condition of fermentation slowly exterminates the microorganism population. Therefore, in early processes, the different sugars were fermented in different sequential reactors. There is a tendency towards combining reaction steps in fewer reactors. When hydrolysis and fermentation reactions are connected directly, intermediate inhibitive products are avoided, and the yield is potentially higher. Also, genetic engineering and new screening technologies will bring bacteria and yeast that are capable of fermenting both glucose and xylose, although fermentation of xylose and arabinose remains problematic. Near-term fermentation using genetically engineered yeast or bacteria may even utilize all five of the major biomass sugars–glucose, xylose, mannose, galactose and arabinose. Mid- to long-term technology will improve the fermentation efficiency of the organism (yielding more ethanol in less time), as well as its resistance, requiring less detoxification of the hydrolysate.

The fermenting bacteria and yeast are grown in series of aerated seed reactors. These consume a side-streamed carbohydrate fraction (9% of the cleaned hydrolysate), and some protein nutrients. The consolidation of conversions in fewer reactors has impact on the total process integration,.

3.4.1 Various methods used for fermentation process

1.Simultaneous saccharification and fermentation

Bioprocessing of agro-industrial residues in SSF has often been found very efficient. There has been a wide-spread resurgence of SSF all over the world due to several advantages it offers, mainly on engineering aspects Numerous SSF processes have been developed in which bagasse has been used as the solid substrate. While in most of the processes, it has been used as the carbon (energy) source, in some processes it has been used as the solid inert support.

Bagasse has most commonly been used for the production of protein-enriched animal feed by SSF, employing yeasts and fungi. A number of reports have appeared on production of animal feed in recent years The C/N ratio and initial moisture were critical factors. Bagasse has been differentiated into four fractions of particle size (<1, 1–3 mm, 3–5 mm and 5–10 mm) with a view to enhancing its nutritive value as animal feed. Scientists have found varying degrees of degradation by white-rot fungi and also variation in in vitro cultivation of a fungal strain of *Streptomyces,* which resulted in 45% depletion of lignocelluloses. A patent was obtained on the application of bagasse, softened with alkali treatment, for feedstuff, fertilizer, and sweetener by cultivating *Enterococcus faecium* in SSF also reported feedstuff production from bagasse using two strains of *Pleurotus* sp.

Amongst the various enzymes produced in SSF of bagasse, cellulases have most extensively been studied. It is well established that the hydrolysis of the lignocellulosic residues using enzymes largely depends upon the cost of the production of cellulases. Application of bagasse in SSF for this purpose appears attractive.. A significant FPD activity was noted from *Pencillium. chrysogenum*, which, apart from the enzyme, also showed high levels of reducing sugars (glucose and xylose). It suggested an integral process for the production of ethanol, furfural, mixture of bagasse and wheat bran (4:1) for the production of cellulases. Scientists have used a suggested hydraulic pressing as a good technique to leach out the enzymes from the fermented matter. Modi et al. (1994) reported higher yields of cellulase from a strain of *Streptomyces* sp. HM29 when grown on bagasse instead of rice straw, rye straw or corncobs. The yields were comparable with those obtained from rice bran but lower than those from wheat straw, wheat bran, and newspaper.

Chapter 4

MATERIAL & EQUIPMENTS USED

4.1 MATERIALS USED

4.1.1 Chemicals Required

(i)	Dilute sulfuric acid	=	0.2M, 0.3M, 0.5M
(ii)	Concentrated NaOH	=	dropwise
(iii)	DNS	=	10 gm
(iv)	Phenol	=	2 gm
(v)	Sodium sulphite	=	0.5 gm
(vi)	Sodium hydroxide	=	10 gm
(vii)	Na-K tartarate (40%)		

4.2 EQUIPMENTS REQUIRED

4.2.1 Vertical autoclave

This equipment is primarily used for sterilization purpose. It is an enclosed space where steam bath is given to any equipment placed inside it. Water filled in it is heated by electric coils present at bottom. It has a vent at top, from where steam can be released to maintain the required pressure. For our case, sterilization is required after maintaining the pH and also for each filtration. Pressure around 2.02 kg/cm^2 is used. Once the pressure reaches 2.02 kg/cm^2 ,it is maintained for half an hour. The equipments are allowed to cool down before removing from the autoclave.

4.2.2 Laminar flow chamber

a) Firstly, the whole chamber is wiped on the inside by spirit solution or ethanol.

b) UV light is turned on with the shutter completely closed and left for 15 minutes. After switching off the UV, we again wait for 15 minutes.

c) Next the fan is turned on and shutter opened.

d) Any transfer taking place in these conditions should be done close to a Bunsen burner flame.

e) All flasks or jars should be properly capped inside the chamber itself.

4.2.3 Vacuum filtration

For this filtration, a filter having sintered base is fitted into a Buchner-type funnel which in turn is air tightly attached to a conical flask having a side outlet to provide vacuum. For every filtration, Whatman filter papers are attached onto the filter. Also after every filtration, the conical flask and filter media are cleaned and sterilized.

4.2.4 UV-Spectrophotometer

To analyze the samples, UV-Spectrophotometer is used. For our analysis, we use the particular wavelength measurement. The equipment consists of two cubettes, one of which acts as a reference while in the other cubette the sample is kept.
Once kept inside, a UV-light is used to know the absorbance of a particular sample at a particular wavelength.

4.2.5 Shaking incubator-

This instrument is used for fermentation. It is an enclosed space where given conditions are maintained at a particular rpm. Flasks are kept on a shaking platform moving at a particular rpm.

4.2.6 pH-meter

This instrument is used for measuring the pH of a given solution.

Chapter 5

PROCEDURE OF EXPERIMENT

5.1 PRETREATMENT OF BAGASSE

About 1 kg of post-harvest sugarcane bagasse is taken and dried to remove all the moisture present in it. This dried bagasse undergoes size reduction by the help of a grinder. Even the powdered form is used for hydrolysis.

5.2 HYDROLYSIS

The cellulose molecules are composed of long chains of glucose molecules. In the hydrolysis process, these chains are broken down to "free" the sugar, before it is fermented for alcohol production. Though hydrolysis is of many types, dilute acid hydrolysis is an easy and productive process. Also the amount of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis. Concentrated acid hydrolysis is not used as it is a hazardous and corrosive process and also acid has to be separated out after hydrolysis for the experiment to be feasible.

5.2.1 Steps involved in dilute acid hydrolysis are

1. 4 nos. of 250ml conical flasks were taken and to each of them 12.5 gm of grinded sugarcane bagasse is added.
2. Another flask of 500ml size is taken and to it 25 gm of grinded sugarcane bagasse is added carefully.
3. About 9.8gm of dilute sulfuric acid of concentration 0.2M is added to the 500ml flask.
4. 7.35gm of dilute sulfuric acid of concentration 0.3M is added to two of the 250ml flasks.
5. 12.25gm of dilute sulfuric acid of concentration 0.5M is added to the remaining two 250ml flasks.
6. All this samples are left to be soaked in dilute sulfuric acid for 24 hrs.
7. The bottles were then capped with the help of cotton plugs.

5.3 pH ADJUSTMENT

Before addition of any micro-organism to the above prepared samples, pH of these samples has to be adjusted. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 5-5.5 is maintained.

5.3.1 Steps involved in pH adjustment are

1. The 500ml flask containing 0.2M dilute sulfuric acid hydrolyzed bagasse is taken and its pH is checked with the help of a pH-meter.
2. As samples are acid hydrolyzed, a highly basic solution is added to bring the pH in the range of 5-5.5.
3. For this purpose, a highly concentrated NaOH solution is prepared by mixing water with Na pellets.
4. This NaOH solution is added dropwise to the 0.2M 500ml flask with constant stirring until the pH reaches to a range of 5-5.5.
5. If suppose the pH goes beyond 5.5, concentrated HCl acid is added dropwise to maintain the pH in the range.
6. The above steps are repeated for the 0.3M and 0.5M dilute sulfuric acid hydrolyzed bagasse.

After maintaining the pH, the samples are kept in a *Vertical autoclave* for ½ hr at 120^{o}C (around 2.02 kg/cm^{2} pressure) and allowed to cool.

5.4 FERMENTATION

5.4.1 Media preparation

For preparing 100ml media, we add

Sugar (Dextrose)	=	10 gm
Yeast extract	=	0.2 gm

Magnesium sulfate	=	0.04 gm
Ammonium sulfate	=	0.2 gm
Make-up water	=	100 ml

(In case Magnesium sulfate and Ammonium sulfate are not available 1gm of urea can be used).

5.4.2 Steps involved in fermentation are

1. To the above 100ml media, 0.5gm of yeast (*Saccharomyces cerevisiae*) is added in a 250ml conical flask.
2. This conical flask is then placed in a shaking incubator for 24 hrs.
3. 10ml of this medium is then added to each of the 5 autoclaved samples aseptically in a *Laminar flow chamber* .
4. The flasks are properly covered with cotton plug.
5. These flasks are then placed in the shaking incubator at a temperature of 35^{o}C and 120 rpm.
6. After 24 hrs, the flasks are covered with aluminium foils over the cotton plug.

5.5 FILTERING AND ANALYZING OF SAMPLES

At an interval of 24 hrs, 12 ml samples are taken in test-tubes from the flasks undergoing fermentation in the shaking incubator. These flasks are brought to the *Laminar flow chamber*, where 12 ml from each flask is taken out in aseptic conditions.

These 12 ml samples are then filtered to remove the biomass, leaving behind a mixture of reducing sugars and alcohol. *Vacuum filtration* is used for this purpose. The filtrate then goes for ethanol and sugar analysis with the help of *UV-spectrophotometer*.

In case of ethanol analysis, the filtrate is directly placed in *UV-spectrophotometer* and its absorbance is noted down at 197 nm wavelength. But in case of sugar analysis, DNS method is followed.

5.6 DNS METHOD

(i) For preparing 1 L of Dinitrosalicylic acid reagent solution, we need

DNS	=	10 gm
Phenol	=	2 gm
Sodium sulphite	=	0.5 gm
Sodium hydroxide	=	10 gm
Distilled water	=	makeup

(iii) Na-K tartarate (40%)

5.6.1 Procedure

(1) The above reagents were first prepared.

(2) 3 ml of reagent was added to 3 ml of glucose solution in a lightly capped test tube.

(3) The mixture was heated at 90^{o}C for 5-15 minutes till red-brown coloration is developed.

(4) 1 ml of 40% Na-K tartarate solution was added to stabilize the color.

(5) After the solution was cooled to room temperature in a water bath, the absorbance of the solution was recorded with the help of *UV-spectrophotometer* at 575 nm.

(6) The above procedure was repeated for each of the filtered samples and graph between optical density Vs concentration was plotted.

Chapter 6

RESULTS

6.1 STANDARD PLOT FOR ETHANOL

16 ml of absolute ethanol was mixed with 24ml of water and an absorbance of 0.8958nm was found in the UV-spectrophotometer. This was assumed to be 100 percent ethanol and the standard plot was drawn.

SL.NO.	Amount Of Ethanol (in ml)	Amount Of water (in ml)	Conc. Of Ethanol	Absorbance
1	0	3.0	0	0.000
2	0.3	2.7	10	0.187
3	0.6	2.4	20	0.225
4	0.9	2.1	30	0.294
5	1.2	1.8	40	0.376
6	1.5	1.5	50	0.419
7	1.8	1.2	60	0.611
8	2.1	0.9	70	0.665
9	2.4	0.6	80	0.730
10	2.7	0.3	90	0.803
11	3.0	0	100	0.896

Table-6.1

The 100 percent of this ethanol is actually the 40 percent concentration of absolute alcohol. Therefore the actual standard plot is given in the next table:

SL.NO.	Amount Of Ethanol (in ml)	Amount of Water (in ml)	Conc. Of Ethanol	Absorbance
1	0	3.0	0	0.000
2	0.3	2.7	40	0.187
3	0.6	2.4	80	0.225
4	0.9	2.1	120	0.294
5	1.2	1.8	160	0.376
6	1.5	1.5	200	0.419
7	1.8	1.2	240	0.611
8	2.1	0.9	280	0.665
9	2.4	0.6	320	0.730
10	2.7	0.3	360	0.803
11	3.0	0	400	0.896

Table-6.2

The graph of the standard plot is as follows:

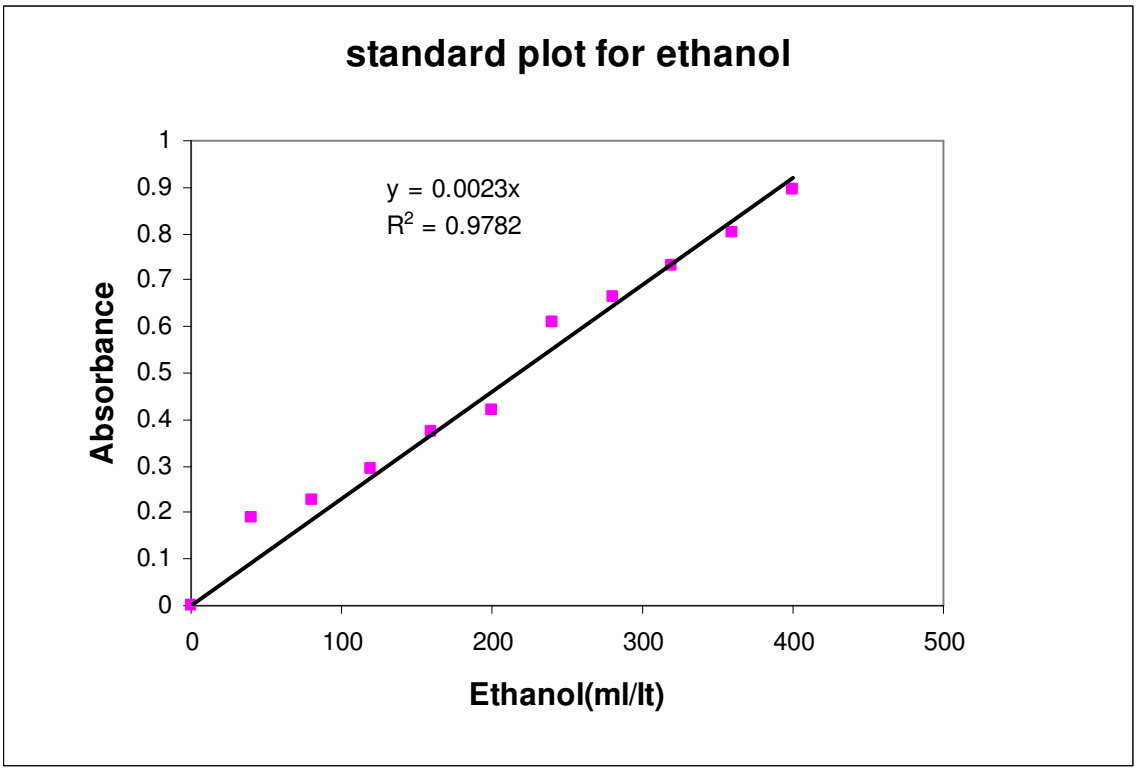


Figure-6.1

6.2 ANALYSIS OF ETHANOL FOR DIFFERENT DAYS

DAY 1 **Table-6.3**

Sl No.	Sample	Absorbance	
		210nm	197nm
1	0.2	0.6262	0.8475
2	0.3(I)	0.5346	0.7344
3	0.4(II)	0.582	0.7014
4	0.5(I)	0.5217	0.7164
5	0.6(II)	0.5618	0.7158

DAY 2 **Table-6.4**

Sl No.	Sample	Absorbance	
		210nm	197nm
1	0.2	0.6652	0.8556
2	0.3(I)	0.646	0.7564
3	0.4(II)	0.5881	0.7112
4	0.5(I)	0.5511	0.7223
5	0.6(II)	0.512	0.721

DAY 3 **Table-6.5**

Sl No.	Sample	Absorbance	
		210nm	197nm
1	0.2	0.6741	0.8667
2	0.3(I)	0.6567	0.7724
3	0.4(II)	0.6109	0.7692
4	0.5(I)	0.6609	0.7339
5	0.6(II)	0.5503	0.7312

DAY7 **Table-6.6**

Sl No.	Sample	Absorbance	
		210nm	197nm
1	0.2	0.7014	0.8802
2	0.3(I)	0.6705	0.7936
3	0.4(II)	0.6794	0.8114
4	0.5(I)	0.6676	0.7914
5	0.6(II)	0.5983	0.7723

DAY8 **Table-6.7**

Sl No.	Sample	Absorbance	
		210nm	197nm
1	0.2	0.7269	0.8823
2	0.3(I)	0.6799	0.8194
3	0.4(II)	0.6931	0.8242
4	0.5(I)	0.6956	0.8001
5	0.6(II)	0.6015	0.8121

DAY 9 **Table-6.8**

Sl No.	Sample	Absorbance	
		210nm	197nm
1	0.2	0.7358	0.8901
2	0.3(I)	0.7023	0.8226
3	0.4(II)	0.7416	0.8424
4	0.5(I)	0.7169	0.8253
5	0.6(II)	0.7215	0.8516

DAY 10 **Table-6.9**

Sl No.	Sample	Absorbance	
		210nm	197nm
1	0.2	0.7674	0.8952
2	0.3(I)	0.7233	0.8708
3	0.4(II)	0.7423	0.8533
4	0.5(I)	0.7367	0.8597
5	0.6(II)	0.7492	0.8555

6.3 COMPARISON OF ETHANOL CONCENTRATION WITH INCREASING NUMBER OF DAYS

FOR 0.2M ACID CONCENTRATION:

Table-6.10

SL.NO.	NO.OF DAYS	ETHANOL CONC.(ML/LT)
1	1	272.26
2	2	289.22
3	3	293.087
4	4	
5	5	
6	6	
7	7	304.96
8	8	316.04
9	9	319.91
10	10	333.65

The graph of the above table is as follows

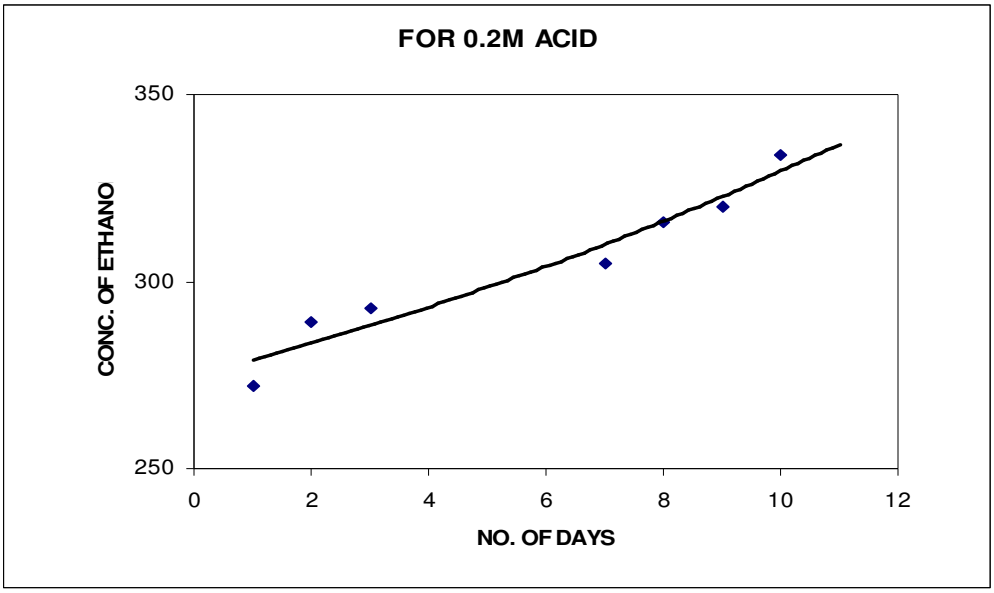


Figure 6.2

FOR 0.3 M ACID CONCENTRATION:

SL.NO.	NO.OF DAYS	ETHANOL CONC.(ML/LT)
1	1	242.74
2	2	268.28
3	3	286.44
4	4	
5	5	
6	6	
7	7	293.46
8	8	298.48
9	9	313.89
10	10	318.61

Table-6.11

The graph of the above table is as follows:

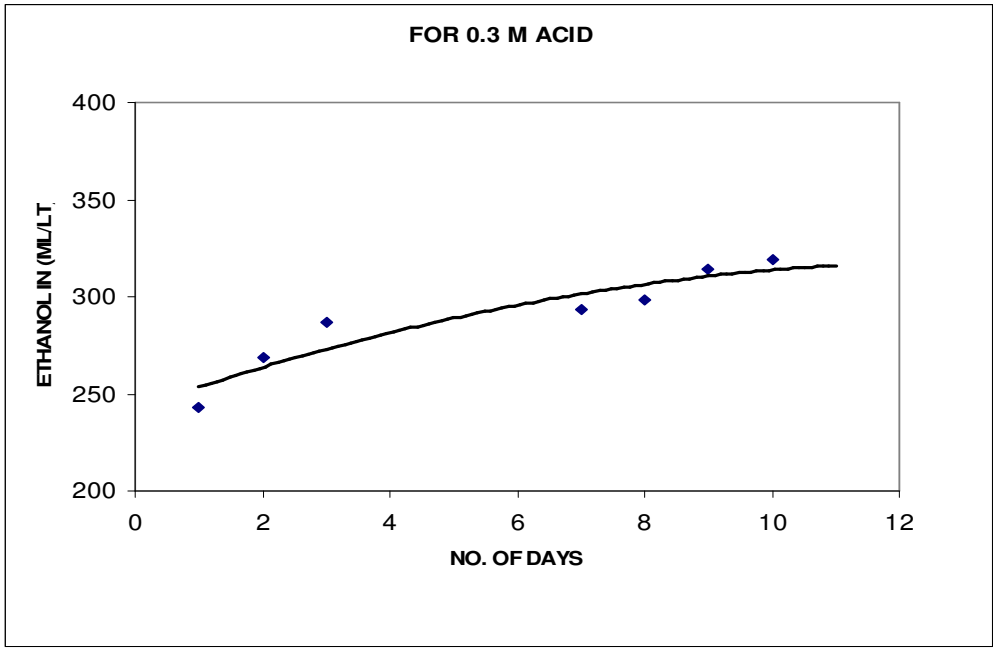


Figure-6.3

FOR 0.5 M ACID CONCENTRATION:

SL.NO.	NO.OF DAYS	ETHANOL CONC.(ML/LT)
1	1	235.54
2	2	243.98
3	3	252.435
4	4	
5	5	
6	6	
7	7	275.2
8	8	281.98
9	9	312.7
10	10	323.02

Table-6.12

The graph of the above table is as follows:

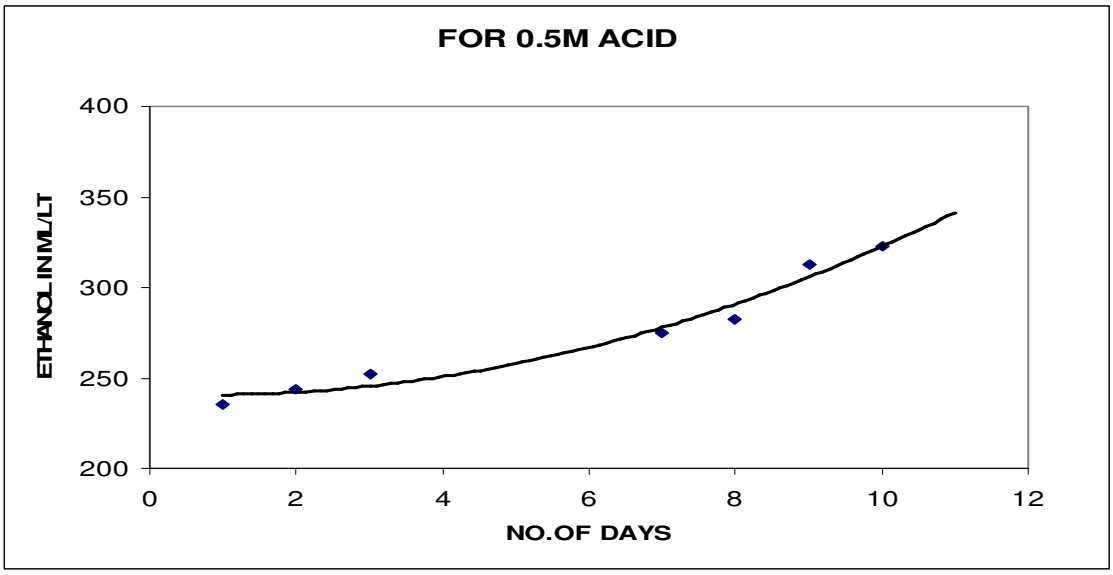


Figure-6.4

6.4 ANALYSIS OF SUGAR WITH INCREASING NUMBER OF DAYS

Sugar was analysed by DNS method. The method is explained previously. Initial amount of sugar was 25 mg in 100 ml of water. The standard plot of sugar is shown as follows:

Table-6.13

SL.NO.	Amount Of glucose (in ml)	Amount of Water (in ml)	Conc. Of Glucose (gms/lt)	Absorbance At 575 nm
1	0	3.0	0	0
2	0.3	2.7	0.025	0.0928
3	0.6	2.4	0.05	0.111
4	0.9	2.1	0.075	0.178
5	1.2	1.8	0.1	0.295
6	1.5	1.5	0.125	0.332
7	1.8	1.2	0.15	0.444
8	2.1	0.9	0.175	0.555
9	2.4	0.6	0.2	0.6483
10	2.7	0.3	0.225	0.7603
11	3.0	0	0.25	0.8425

The graph of the standard plot is shown below:

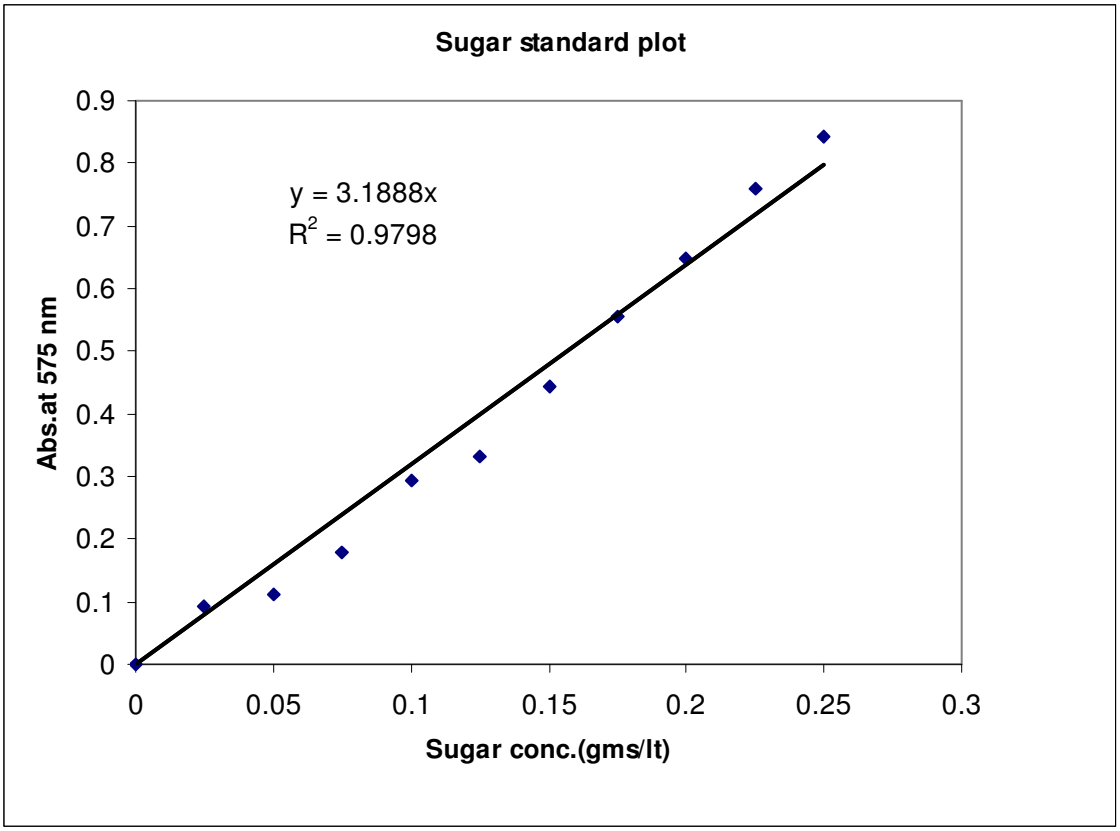


Figure-6.5

The concentration of sugar before fermentation and after fermentation with increasing number of days is given in the tabular and graphical form:

BEFORE FERMENTATION:

SL NO.	SAMPLE	ABS.AT 575 NM	CONC.OF SUGAR(GMS/LT) 75 DILUTIONS	ACTUAL CONC. (GMS/LT)
1	0.2	0.8313	0.2607	19.552
2	0.3	0.8014	0.2513	18.849
3	0.5	0.7083	0.2221	16.659

Table-6.14

AFTER FERMENTATION:

DAY 1 **Table-6.15**

SL NO.	SAMPLE	ABS.AT 575 NM	CONC.OF SUGAR(GMS/LT) 75 DILUTIONS	ACTUAL CONC. (GMS/LT)
1	0.2	0.8045	0.2523	18.922
2	0.3	0.7823	0.2453	18.400
3	0.5	0.6222	0.1951	14.634

DAY 2 **Table-6.16**

SL NO.	SAMPLE	ABS.AT 575 NM	CONC.OF SUGAR(GMS/LT) 75 DILUTIONS	ACTUAL CONC. (GMS/LT)
1	0.2	0.7523	0.2359	17.694
2	0.3	0.7292	0.2287	17.151
3	0.5	0.605	0.1897	14.229

DAY 3 **Table-6.17**

SL NO.	SAMPLE	ABS.AT 575 NM	CONC.OF SUGAR(GMS/LT) 75 DILUTIONS	ACTUAL CONC. (GMS/LT)
1	0.2	0.6875	0.2156	16.170
2	0.3	0.6525	0.2046	15.347
3	0.5	0.5823	0.1826	13.696

DAY 7 **Table-6.18**

SL NO.	SAMPLE	ABS.AT 575 NM	CONC.OF SUGAR(GMS/LT) 75 DILUTIONS	ACTUAL CONC. (GMS/LT)
1	0.2	0.3232	0.1014	7.602
2	0.3	0.3079	0.0966	7.242
3	0.5	0.3053	0.0957	7.181

DAY 8 **Table-6.19**

SL NO.	SAMPLE	ABS.AT 575 NM	CONC.OF SUGAR(GMS/LT) 75 DILUTIONS	ACTUAL CONC. (GMS/LT)
1	0.2	0.2911	0.0913	6.847
2	0.3	0.2886	0.0905	6.788
3	0.5	0.2784	0.0873	6.548

DAY 9 **Table-6.20**

SL NO.	SAMPLE	ABS.AT 575 NM	CONC.OF SUGAR(GMS/LT) 75 DILUTIONS	ACTUAL CONC. (GMS/LT)
1	0.2	0.1241	0.0389	2.919
2	0.3	0.1235	0.0387	2.905
3	0.5	0.1223	0.0384	2.876

DAY 10 **Table-6.21**

SL NO.	SAMPLE	ABS.AT 575 NM	CONC.OF SUGAR(GMS/LT) 75 DILUTIONS	ACTUAL CONC. (GMS/LT)
1	0.2	0.0122	0.0038	0.287
2	0.3	0.0053	0.0017	0.125
3	0.5	0.0013	0.0004	0.031

6.5 FOR DIFFERENT CONCENTRATION OF ACID, THE DECREASING TREND OF SUGAR WITH NO.OF DAYS IS SHOWN BELOW

FOR 0.2 M CONCENTRATION

SL.NO	NO.OF DAYS	SUGAR CONC.(GMS/LT)
1	0	19.552
2	1	18.922
3	2	17.694
4	3	16.17
5	4	
6	5	
7	6	
8	7	7.602
9	8	6.847
10	9	2.919
11	10	0.287

TABLE-6.22

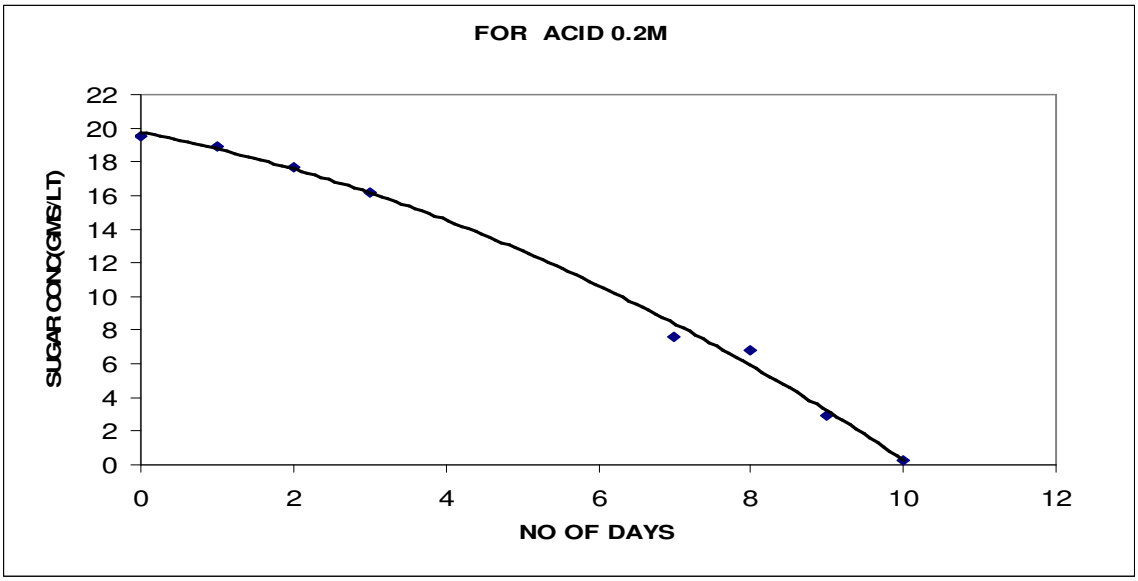


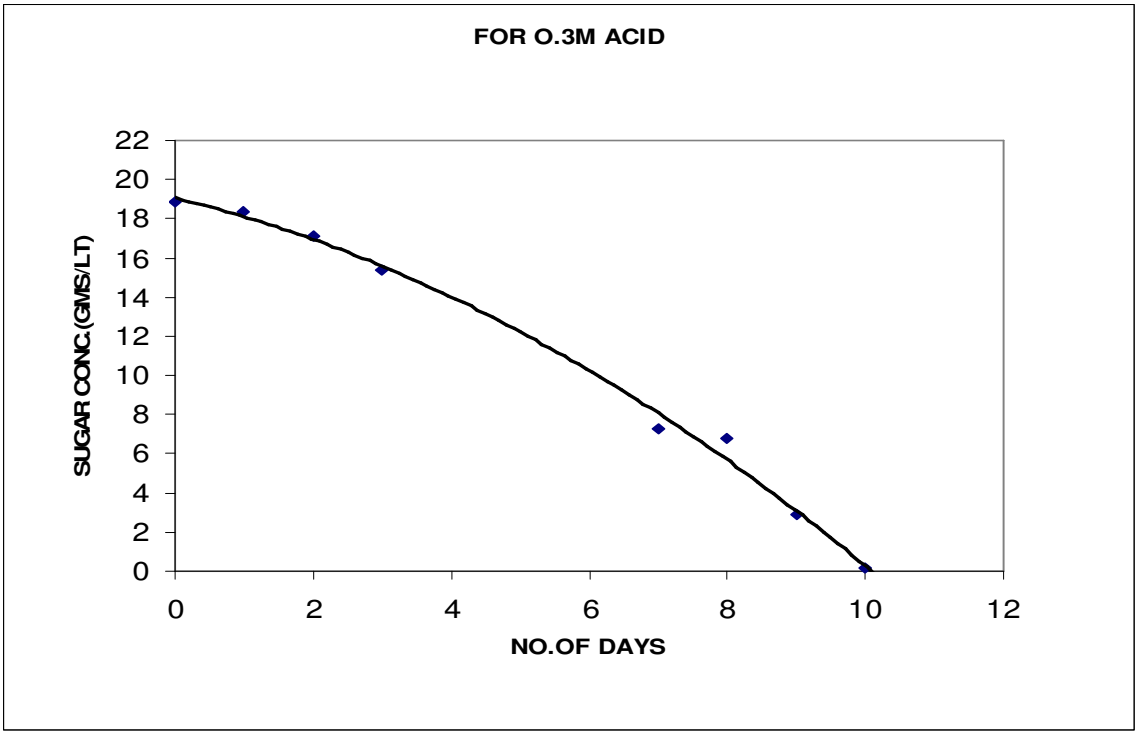
FIGURE-6.6

FOR 0.3 M ACID CONCENTRATIONS:

SL.NO	NO.OF DAYS	SUGAR CONC.(GMS/LT)
1	0	18.849
2	1	18.4
3	2	17.151
4	3	15.347
5	4	
6	5	
7	6	
8	7	7.242
9	8	6.788
10	9	2.905
11	10	0.125

TABLE-6.23

FIGURE-6.7

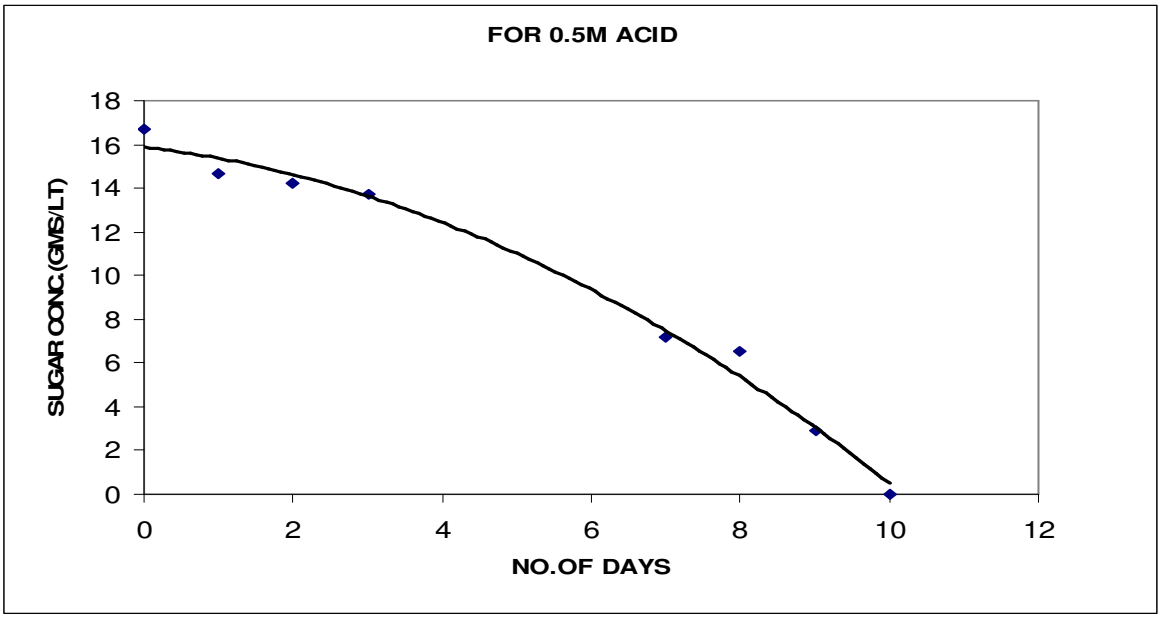


FOR 0.5 M ACID CONCENTRATION:

SL.NO	NO.OF DAYS	SUGAR CONC.(GMS/LT)
1	0	16.659
2	1	14.634
3	2	14.229
4	3	13.696
5	4	
6	5	
7	6	
8	7	7.181
9	8	6.548
10	9	2.876
11	10	0.031

TABLE-6.24

FIGURE-6.8


FOR 0.5M ACID
SUGAR CONC.(GMS/LT)
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
NO.OF DAYS

Chapter 7

DISCUSSION

7. DISCUSSION

1. The ethanol concentration in ml/lt of acid hydrolyzed bagasse increases with the increasing number of days while sugar concentration decreases. This shows that the sugar is being fermentated by the help of the micro organism yeast for the production of ethanol.

2. The amount of sugar is maximum on the first day after fermentation i.e.19.552 gms/lt and it is almost zero on the 10^{TH} day of fermentation.

3. The maximum concentration of ethanol is 323 ml/lt of acid hydrolyzed bagasse which was found on the 10^{TH} day of fermentation.

4. With increasing molar concentration of sulphuric acid, the concentration of ethanol and sugar decreases. More amount of ethanol and sugar was found for 0.2M than 0.3M and 0.5M for each day.

Chapter 8

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8. REFERENCES

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