Optimization of pretreatment for enhanced saccharification for bio-ethanol production from groundnut shell using SSF and comparison of yield of free and immobilized *S.cerevisiae* for bio-ethanol production.

Project Report Submitted in partial fulfilment of the requirements for the degree of

Master of Technology
(Biotechnology)

Submitted by
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DECLARATION

I hereby declare that the project entitled "**Optimization of pretreatment for enhanced saccharification for bio-ethanol production from groundnut shell using SSF and comparison of yield of free and immobilized *S.cerevisiae* for bio-ethanol production**" is an authentic record of my own work carried out at School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, for the partial fulfilment of the award of Bachelors/Master of Technology in Biotechnology under the guidance of **MR. Himanshu singh**.This work is our original work and has not been submitted for any degree/diploma in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

Place: Zeeshan Qadir (11506931)

Date:

CERTIFICATE

This is to certify that **zeeshan Qadir shagoo** (11506931) have completed the project , entitled "**Optimization of pretreatment for enhanced saccharification for bio-ethanol production from groundnut shell using SSF and comparison of yield of free and immobilized *S.cerevisiae* for bio-ethanol production**"under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study. No part of the report has ever been submitted for any other degree at any University. The report is fit for submission and the partial fulfilment of the conditions for the award of M. Tech Biotechnology.

Date: Supervisor Signature:

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Zeeshan Qadir (11506931)

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

In the age of rapid industrialization, the requirement of Biofuel is increasing day by day. Numerous physicochemical, morphological and compositional variables obstruct the hydrolysis of cellulose present in biomass to sugars and other natural intensifies that can later be changed over to powers. The objective of pretreatment is to make the cellulose open to hydrolysis for transformation to energize. Different pretreatment strategies change the physical and chemical structure of the lignocelluloses biomass and enhance hydrolysis rates. The cost of the fuels can be minimized by using bioethanol which has good liquid fuel properties and can be mixed with liquid fuel. The retention time for the 2%NaOH free cell solution was 7.114 which is nearly same as that of the standard which means purity of the solution is same as that of the standard. The production of ethanol was seen higher in 2% NaOH equal to .461(v/v) in free cells than Immobilized cells.34 (v/v).

Keywords : Bioethanol, fermentation, yield, pretreatment, SSF, Temperature, pH.

CHAPTER ONE……..

……..INTRODUCTION

Introduction

Peanut or groundnut is one of the members in the family of legumes or beans. The peanuts were cultivated firstly in the areas of Paraguay. Being a herb plant it grows upto 50cm tall. The leaves are opposite, pinnate with four leaflets. Since our country being agriculturally rich, it is producing peanuts in large scale, but due to public unawareness very few of it is used as fuel and roughage and most of them are not using it in reasonable way. In fact the peanut shell is of much use. In recent years, many scientific researchers from overall world have been doing researches on comprehensive development and utilization of peanut shells, and have obtained great progress and acquired a certain economic benefits.

People often called peanut as poor man's nut and nowadays it is necessary as a food crop and oilseed. The botanical name for groundnut, Arachis hypogaea., is derived from two Greek words, Arachis meaning a legume and hypogaea meaning below ground, referring to the formation of pods in the soil. Groundnut is an upright or prostrate annual plant. Generally transported in the tropical, sub-tropical and warm temperate ones. Export rates have risen from $15 billion during 2009-10 to $37 billion during 2012-13.

The Ministry of Commerce, held the meeting in Hyderabad during the annual trade meet announced that t production of groundnut during Kharif 2013 is estimated to be 49 lakh tonnes in the major states of Gujarat, Rajasthan, Andhra Pradesh, Karnataka and Tamil Nadu which accounts for 90 percent of total groundnuts produced in India. Groundnut exports during 2013-14 are expected to be about 6 lakh tones.

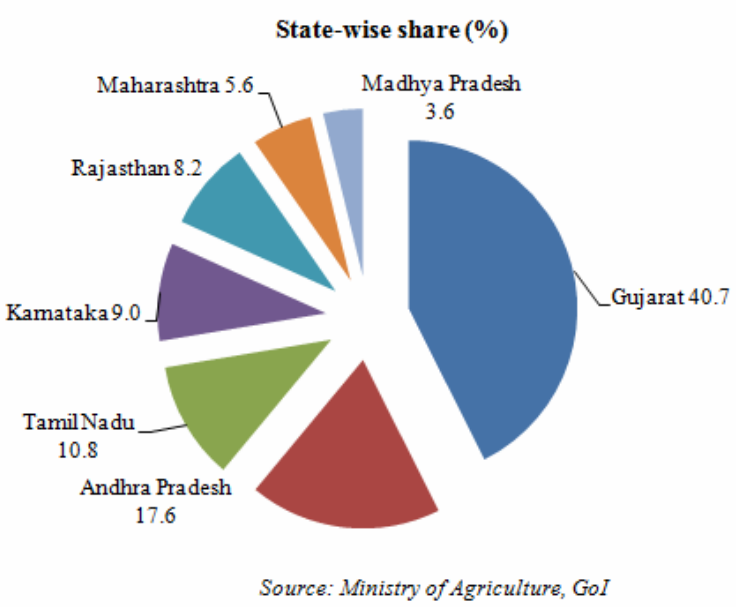


FIGURE 1: state wise production of groundnut

1.1. Composition of groundnut shells:- the functional components that are present in the groundnut shells contain cellulose (40%), lignin (26%), hemicelluloses (15%) and potassium oxide(K_2O), magnesium oxide (MgO), calcium oxide(CaO), and phosphorous oxide(PO).

TABLE 1.1 composition of GS shell (Kiran et.al 2011)

S.No.	Characterstics	Shell
1	Cellulose	65.5-79.3%
2	Hemicelluloses	10.1%
3	Carbohydrates	10.6-21.2%
4	Proteins	4.8-7.5%
5	Calcium	0.24-0.27%
6	Phosphorus	0.08-0.09%
7	Minerals	4.3%

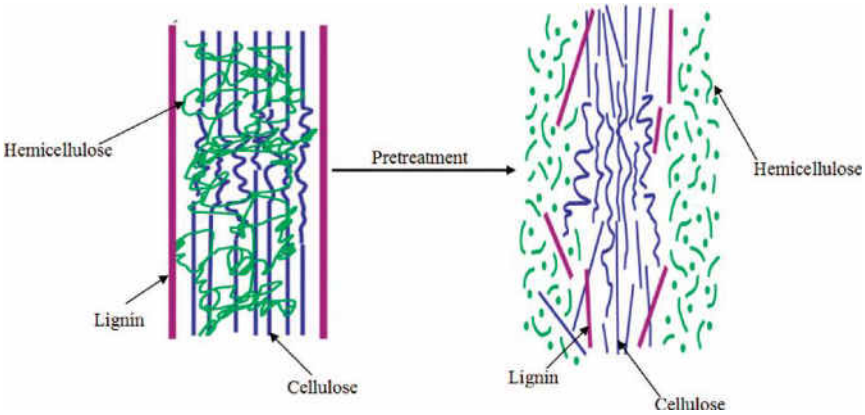


Figure 1.1 Groundnut shell biomass

1.2.Bioethanol

The continuous consumption of the fossil fuel and continuous increase in its price has stimulated us to find the alternative technologies to meet global demand of energy. As the result alternative sources like hydrogen, ethanol, and methane are considered increasingly to be the most promising alternative fuel. Ethanol is being considered to be the most promising fuel as it can be produced from agriculture wastes like sugarcane, potato, agro waste. Ethanol or ethyl alcohol is a colourless, flammable, mild toxic chemical with a perfume like odour and it is found in beverages like wine, beer etc. For the purpose of alcoholic beverages, ethanol is produced by fermentable sugars such as glucose. Bio ethanol is considered important renewable fuel and it contributes by reducing negative impacts that are generated to environment by the utilization of these natural fuels. Alcohol that is produced by microorganisms, such as *Saccharomyces cerevisiae*, by fermentation from biomass is known as bio ethanol. Bio ethanol is most widely used bio fuel for transportation in countries like United States and Brazil since 1980s.Ethanol has a much higher latent heat of vaporization than petrol.

Ethanol Fermentation:- Fermentation of carbohydrates to alcohol is one of the oldest process. Fermentation is a process of conversion of sugar to ethanol in absence of oxygen. This reaction is catalyzed by enzyme called zymases that is produced by yeast. The chemical reaction for conversion of glucose to ethanol is

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

1.3. *Tricoderma Reesei* :- it is a mesophilic filamentous fungi originally separated from solmon islands and was identified as *T.viride* later it was recognised as *T. reesei* and was a good producer of cellulose enzyme. Consequently strain development programes were initiated to modify these strains so as to increase cellulose productionfor examples RUT-C30 a hypercellulolytic strain was produced.

1.4. *Saccharomyces cerevisiae*: - It is a species of yeast which is a single celled eukaryotic organism and produces zymase which is an enzyme complex responsible for production of ethanol and carbon dioxide from sugars and its activity among various yeast strains changes. The optimum temperature for growth is 25^0C to 35^0C.

Class : *Saccharomycetes*

Order : *Saccharomycetales*

Family : *Saccharomycetaceae*

Genus : *Saccharomyces*

Species : *cerevisiae*

1.5. IMMOBILIZATION:- immobilization is a process by which cell or enzyme is attached to the inert material in order to limit its mobility so that it will be protected from the harsh environment like temperature, toxicity or pH. In freely suspended cell systems, continuous operation is restricted by flow rate as cells are conveyed in the effluent that leads to a decline or complete loss of cells. Cell immobilization underpins the cells advancing operation at higher flow rates. Immobilization is accomplished by different components. One of which is surface adsorption where cells actually cling to the surface of the material through electrostatic force. Yeast cells are adsorbed in the surface of NDC which is an exceptionally hydrophilic and solid material with a young's modulus practically identical to aluminum [1]. Immobilization of microbial cells for fermentation has been created to dispense with restraint brought on by high grouping of substrate and product, additionally to upgrade efficiency and yield of ethanol generation, recent work on ethanol generation in an immobilized cell reactor (ICR) demonstrated that production of ethanol utilizing Z.mobilis was multiplied [2]. The potential utilization of immobilized cells in fermentation process for fuel generation has been depicted previously. If in place microbial cells are specifically immobilized, the expulsion of

microorganisms from downstream product can be precluded and the loss of intracellular enzyme movement can be kept to a base level [3]

FIGURE 1.5 Immobization of *Saccharomyces cerevisiae*

Fermentation by immobilized cell has numerous specialized and economic advantages when contrasted with free cell system, for instance high fermentation rate, better substrate use, longer working life time, simplicity of partition to encourage their reuse and simple gather from the item, expanded bioreactor efficiency, decrease in cost of bioprocessing by taking out long and costly procedures of cell recovery and cell reuse, upkeep of high cell thickness per volume, porous to reactant and product, less restraint by product, no diminishment in the coveted biocatalytic movement of cell, protection against high shear damage, give great microenvironment to cell, less chances for defilement, high resistance to liquor, and so on[4,5].

CHAPTER TWO

TERMINOLOGY

Terminology

Aerobic organism:- the Organism that can live, grow and survive in the presence of environment that carries oxygen

Anaerobic organism:- the one that doesn't require oxygen for their growth and survival and may have negative impact on growth in the presence of oxygen.

Cellulases:- it is a complex of various enzymes that are responsible for breakdown of cellulose into monosaccharide like glucose. These reactions involve hydrolysis of glycosidic linkages in the cellulose, hemicelluloses etc

Chromatography (GAS chromatography):- the analysis that is done in order to separate various components in the sample in the vapor form without decomposition.

Distillation: - Distillation is a process of separation of liquids from a mixture of liquids by selective evaporation and condensation, more volatile liquid separates first.

Dinitrosalicylic acid (DNSA):- it is an aromatic organic compound that reacts with the reducing sugar in order to determine them by absorbing light at 540 nm .

FT-IR:- A technique which is used to detect the wide spectrum of solid, liquid or gas in the infra red region so that we can identify how much absorption is taking place.

Immobilization:- is process by which cell or enzyme is attached to the inert material in order to limit its mobility so that it will be protected from the environment temperature or pH.

Reducing sugar:- the sugar that has free aldehydic or ketonic group that undergoes reduction and forms an organic compound with the DNS acid.

Simultaneous Saccharification and Fermentation (SSF):- the process in which enzymatic hydrolysis and fermentation takes place simultaneously in the bioreactor.

Saccharification: - Saccharification is a process of hydrolysis of complex polysaccharides and non reducing sugar to simple reducing sugar.

Zymases:- is an enzyme complex responsible for production of ethanol and carbon dioxide from sugars.

CHAPTER THREE

REVIEW OF LITERATURE

REVIEW OF LITRATURE

Padmaja et.al. has used various agri-wastes like groundnut shells, rice straw etc by making use of various anaerobic bacteria for ex. Clostridium thermocellum and found 0.20-0.12 g ethanol per g of substrate degraded. The reaction is carried out by continuous sparging of nitrogen so as to make the system free from oxygen at 600C and pH of 7.5 the substrate is given mild treatment of alkali so as to enhance the yield and utilization of ethanol. Thus conversion to ethanol from treated and untreated wastes ranges between 65% and 42% respectively. Archana Mishra et al. performed enzymatic hydrolysis on rice straw and other agri wastes by *T.viride* which is treated with *S. cerevisiae* for fermentation. The yield of was found to be 55.27 mg/g of biomass and ethanol of the order of 17.54 mg/ml of substrate from 3:1 ratio of rice straw and vegetable waste after 9 days of incubation at 27 ^{0}C[6].

In the present study groundnut shell (GS), are readily available as a potential feedstock for production of fermentable sugars. Sodium sulfite was used to delignify the substrate and substrate released 670 mg/g of sugars after enzymatic hydrolysis (50_C, 120 rpm, 50 hrs) using commercial cellulase (Dyadic Xylanase PLUS, Dyadic Inc. USA). The groundnut shell hydrolysate after enzymatic hydrolysis (45.6 g/L reducing sugars) was fermented for production of ethanol with free and sorghum stalks immobilized cells of Pichia stipitis under submerged cultivation conditions. Immobilization of yeast cells on sorghum stalks were confirmed by scanning electron microscopy (SEM). In batch fermentation conditions ethanol production (17.83 g/L, yield 0.44 g/g and 20.45 g/L, yield 0.47 g/g) was observed with free and immobilized cells of P. stipitis respectively. immobilized cells while recycling showed a stable ethanol production (20.45g/L, yield 0.47 g/g) up to 5 batches followed by a gradual downfall in subsequent cycles[7].

In this study the groundnut shells were used as a substrate. They were first grinded and then physical and chemical treatment was done. Physical treatment done by steam explosion method. After this they were given chemical treatment with inorganic (0.25N HCl and 0.25N NaOH) chemicals and also organic (0.25N CH_3COOH and 0.25N lactic acid) to separate out lignin and cellulose. This structural change was confirmed after FTIR analysis. After the physical and chemical treatment and confirmation in structure and chemical change, the sample was set for saccharification following by fermentation. The microorganisms used for

fermentation were *Saccharomyces cerevisiae* and *Bacillus stearothermophilus*. The process was carried out for 16 days and after 16 days the solution was distilled and estimation of ethanol was done [8].

Rice husks and groundnut shells were treated with 3, 4 and 5% concentrations of dil. hydrochloric acid and dinitrosalicylic acid (DNS) method was used to determine th reducing sugar concentration**.** The production of bioethanol was determined by using potassium dichromate method. There was no significant difference seen in the yield of reducing sugar obtained at different treatments [9].

Silverstein et.al determined the effectiveness for conversion of various solid residues like cotton stalk to ethanol like sulfuric acid, sodium hydroxide, hydrogen peroxide, and ozone pretreatments for the conversion of cotton stalks to ethanol. There was significant lignin degradation after treating the solids with various pretreatments like hydrogen peroxide, sodium hydroxide and sulphuric acid and higher sugar availability. Sodium hydroxide pretreatment resulted in the highest level of delignification and cellulose conversion. Similarly Sulfuric acid pretreatment resulted in the highest xylan reduction[10].

The experiment resulted in concluding that enough glucose was produced by pretreating groundnut shell and maize cobs with sulphuric acid. The groundnut shell and maize cobs were present in the ratio of 1:3. The parameters that help in optimizing the ethanol production were 4.5M H_2SO_4 and temperature 80^{o}c. A significant amount of ethanol is produced if glucose is fermented with *S.cerevisiae* at specific condition. The research takes into consideration the chances of production of ethyl alcohol which is an important component in the beverage industry, as a solvent, antiseptic and fuel produced by fermentation of sugars [11].

The present study is focused on production of fuel ethanol by saccharification and fermentation of cellulosic substrates. The substrates that were utilized for ethanol were easily available and were less costly were groundnut shells and rice husk. Before starting the saccharification and fermentation experiments total sugars, reducing sugars and cellulose content were estimated Cellulose in Rice husk was 45% and that of groundnut shell was 65% [12].

This study was done in order to know how much yield of ethanol is produced by fungal organism like *A. niger* and *S. cerevisiae* that convert pentose and other carbon molecules. The result of the experiment performed reveals that Cellulosic material mostly groundnuts shell is a required substrate and can be exploited in industries for bioethanol production on a commercial scale as they are cheap and renewable. the data supports the conclusion that environmental impact associated with production of cellulosic biomass appears to be generally acceptable and can be positive[13].

M. Abdullahi, and J. J. Ijah et.al worked on ethanol production from maize cobs and groundnut shells taking them in different ratios and ethanol content was determined. Firstly sample was grinded to obtain the particle of size 300 micrometer average. After grinding, the sample was dried in hot air oven to remove the moisture. After this process, 10 gram of sample was mixed with 20 ml of diethyl ether. As benzyl alcohol is a component of lignin and is soluble in diethyl ether. Hence the cellulose bound to lignin is removed and become available for further process. Groundnut shells and Maize cobs were taken in different ratios and both acidic and basic treatment was given to it. Acidic and basic treatment was given by H_2SO_4 and NaOH respectively. After hydrolysis, the solution was set for fermentation and after the fermentation, when the ethanol estimation done, it was found that the best yield of ethanol was obtained from the ratio of maize cobs and groundnut were mixed in a ratio of 3:1[14].

The Cellulase activity estimation was done by DNS method by estimation of the presence of reducing sugar glucose. Crude enzyme after mixing with CMC and incubation, to stop the reaction, previously prepared DNS was added and boiled in water bath. Reducing sugars were estimated by taking O.D. at 540nm and from this result calibration curve of glucose was drawn to estimate the activity of cellulase enzyme production (Shoham et al., 1999). As the enzyme quantity, 1 unit of enzyme activity was estimated that is required for per minute 1 mol glucose in standard conditions (Muhammad et al., 2012) [15].

The study of cellulolytic bacteria*, Bacillus coagulans* and *Geobacillus stearothermophilus*, that were isolated from rice field and plated on CMC agar media were used to degrade the cellulose present in lignocellulosic materials such as groundnut shells, wheat bran etc after the chemical treatment with acid and base among these, for the degradation of lignin, 0.25N HCL showed better results. After chemical treatment, the sample was inoculated with these

organisms that helps in degradation of cellulose in various lignocellulosic materials. After the dialysis, SDS PAGE and FTIR, it was found that cellulase produced by *Geobacillus stearothermophilus* was found to be more specific than *Bacillus coagulans*[16].

The execution of Nata de coco (NDC) or coconut water extract and Calcium alginate (CA) as an immobilization medium for *Saccharomyces cerevisiae* cells are looked at regarding generation rate and transformation. *Saccharomyces cerevisiae* cells are immobilized in NDC and CA globules utilizing a cell suspension with a normal rough live cell density of 232.1288 ± 1.5387cells/mL. The biocatalysts NDC and CA are rushed into level aging reactors. A diffusive pump and complex is utilized to control the stream rate to a coveted stream rate of 9 mL/hr. Tests are gathered at regular intervals and tried for ethanol by gas chromatography and glucose focus by colorimetry. The normal relentless state emanating ethanol fixation, profitability and transformation in NDC are 5.093 % by volume, 52.329 mL/hr and 0.7779, respectively [17].

The immobilization of yeast on polyacrylamide gel prompts a productive immobilization of the yeast cells, the aftereffects of the analysis being tantamount with the ones got for the immobilization of yeast on calcium alginate. The benefit of utilizing immobilized yeast on polyacrylamide gel is that the granules are safer, and they keep their shape amid the aging handle. The immobilization of yeast on polyacrylamide gel is more productive in a constant maturation handle [18].

Continuous ethanol generation in an ICR was effectively done with high sugar focus. In batch fermentation, when the concentration of glucose was 50 g/l, significant substrate hindrance firmly happened. The benefit of immobilized cells reactor was that the substrate hindrance of substrate and item were not evident even with 150 g/l glucose arrangement in the new bolster. The ICR framework displayed a higher yield of ethanol creation (38%) contrasted with the cluster framework. The outcomes showed that the immobilization of *S.cerevisiae* has the limit not exclusively to use high grouping of sugar additionally to yield higher ethanol productivities over the span of continuous fermentation. The ethanol generation in ICR column was enhanced by 5-fold, as the glucose concentration was doubled from 25 to 50 g/l [19].

The present review expected to assess the impact of utilizing immobilized cell frameworks on mead generation. Our outcomes illustrate that the immobilization of yeasts in Ca-alginate did not adversely influence the fermentation procedure. Minor contrasts were recognized in the

fermentation time, despite the fact that higher convergences of reasonable cells were accomplished in immobilized frameworks [20].

The study investigated that *saccharomyces cerevisiae* cells were immobilized in calcium alginate and chitosan-secured calcium alginate dots and examined in the fermentation of glucose and sucrose for ethanol generation. The batch fermentation were completed in an orbital shaker and surveyed by observing the grouping of substrate and item with HPLC. Cell immobilization in calcium alginate dots and chitosan-secured calcium alginate beads permitted reuse of the beads in eight successive maturation cycles of 10 h each. The last convergence of ethanol utilizing free cells was 40 g L-1 and the yields utilizing glucose and sucrose as carbon sources were 78% and 74.3%, separately. For immobilized cells in calcium alginate globules, the last ethanol focus from glucose was 32.9 ± 1.7 g L-1 with a 64.5 ± 3.4% yield, while the last ethanol fixation from sucrose was 33.5 ± 4.6 g L-1 with a 64.5 ± 8.6% yield. For immobilized cells in chitosan-secured calcium alginate beads, the ethanol concentration from glucose was 30.7 ± 1.4 g L^{-1} with a 61.1 ± 2.8% yield, while the final ethanol concentration from sucrose was 31.8 ± 6.9 g L^{-1} with a 62.1 ± 12.8% yield [21].

The entire cell immobilization in ethanol concentration should be possible by utilizing normal bearers or through engineered bearers. These strategies have a similar motivation behind holding high cell fixations inside a specific characterized locale of space which prompts higher ethanol profitability. Lignocelluloses plant substance represents one of profoundly potential sources in ethanol creation. A few reviews have found that cellulosic substances can likewise be utilized as a characteristic transporter in cell immobilization by re-flowing pre-culture medium into a reactor. In this investigation, rice frames with no treatment were utilized to immobilize *Saccharomyces cerevisiae* through semi-strong state hatching joined with re-circulating pre-culture medium. In liquid batch fermentation framework with an underlying sugar convergence of 50 g/L, about 100% aggregate sugar was expended following 48 hours. This brought about an ethanol yield of 0.32 g ethanol/g glucose, which is 62.7% of the hypothetically proposed. By utilizing immobilized cells, fermentation procedure could be done quicker contrasted with the free cell shape with ethanol efficiency more than 2 times higher efficiency of 0.59 g/(L.h) is 2.3 crease higher than that of free cells which is 0.26 g/(L.h)m [22].

CHAPTER FOUR

RATIONALE OF STUDY

Future Research Priorities in Biomass to Ethanol

Biomass to ethanol will be technical and economical viable alternative to Ist generation ethanol, if appropriate conditions are developed. Current production problems hence determine immediate and future research priorities

Pretreatment is the first step in the ethanol production which accounts for 33% of the total cost. So we need better and cost efficient pretreatment techniques so that we can reduce the microbial contaminants that reduce the yield. Now in order to overcome these problems various membranes were used like ultra filtration microfiltration and nanofiltration or reverse osmosis.

The genetically recombinant modification of fermentative and cellulolytic microorganisms is allowed to increase the ethanol yield and productivity under the stress conditions of high production bioethanol processes. A new powerful biotechnological tool that is genetic engineering is essential for making new strategies for increasing the ethanol fermentation performance. Since fermentative microorganisms must be capable of surviving the high temperatures of SSF processes, further research is required. various altered genes have been introduced into the genome of these fermentative organisms and their heterologous expression of genes has been incorporated into *Z. mobilis* to extend its effectiveness toward other substrates. Further research is certainly required in optimizing biological pretreatment involving fungi (e.g., *T. reesei* and Basidiomycetes) that exhibit lignocellulolytic properties at low pH levels and high temperature. Improvement in each of these individual aspects is required to achieve high conversion and cost-effective biomass-to-bioethanol operations [23].

CHAPTER FIVE

OBJECTIVES

AIMS AND OBJECTIVES

1. To perform the various pretreatments like Alkali and Acidic to increase cellulose activity.

2. TO perform the FTIR analysis of groundnut shell and chemical treated sample.

3. To perform immobilization and to check its effect on glucose concentration and to compare with free cells

4. To determine the ethanol yield by GAS chromatography.

CHAPTER SIX

RESEARCH METHODOLOGY

6. Materials and Methods

6.1. Materials Required:-

Chemicals Used

1. 3,5-Dinitrosalisylic acid
2. Dextrose
3. Peptone
4. Agar
5. Yeast extract
6. Nutrient broth
7. NaOH
8. Rochelle's salt
9. H2SO4
10. Sodium thiosulphate
11. Potassium iodide
12. $K_2Cr_2O_7$
13. Phenol

Lab wares Used

1. Test tubes
2. Petri plates
3. Conical flasks
4. Beaker
5. Measuring cylinder
6. Round bottom flask
7. Condenser
8. Alcoholmeter

Instruments Used

1. Incubator
2. Autoclave
3. Hot air oven
4. Spectrophotometer
5. Refrigerator
6. Water bath

7. Laminar air flow
8. Rotary shaker

6.2 **Sample Collection:-** Groundnut shells were collected from the agriculture site of jalandhar which was used as a raw material.

Figure 6.2 sample collection

METHODS

6.3 **Pre- treatment**:-

6.3.1**Physical pre-treatment**: - Groundnut shells were dried in a oven to remove moisture and then grinded in a grinder and sieved to get uniform sized particles.

6.3.2**Chemical pre-treatment:-**Both acidic (with H_2SO_4) and basic(with NaOH)pre treatment was given to sample to separate cellulose bound to lignin.

Acidic Pre-treatment:- 20 grams of sample was taken and treated with H2SO4 with concentrations 1%, 1.5 & 2% separately for 12 hours at normal temperature and ph is set to neutral at 7

Basic treatment:- Similarly to acidic, basic treatment was also performed with NaOH with concentrations 1M for 1%, 1.5%, 2% separately for 12 hours at normal room temperature and ph is set at 7.

After acidic and basic treatment, the solution s washed with water and dried in a oven and stored fur further processes.

6.3.3 **FTIR Analysis:-**

All samples after acidic and basic treatment were sent for FTIR analysis . the Groundnut shell undergoes various physical and chemical changes after pretreatment which was confirmed by FTIR. Thus we can compare the structural changes that sample has undergone. This has been explained in the discussion.

6.3.4 **Enzymatic Treatment: -** After chemical treatment, 5gm of treated sample is taken in separate conical flasks and mixed with 100 broth and autoclaved .

The composition of 100 ml broth is:-

Yeast extract -0.25gm

KH_2PO_4 -2.0gm

$CACL_2 .2H_2O$ -0.21gm

Urea -0.21gm

$MgSO_4.7H_2O$ - 0.15gm

Peptone -1.0gm

$FeSO_4.7H_2O$ - 0.15gm

$MNSO_4.H_2O$ -1.6gm

$ZNSO_4.7H_20$ - 1.4gm

$COCL_2$ -2.0gm

The pH was adjusted to slightly acidic equal to 6 after adding the NaOH to the broth and the colour of the broth changed to purple. After autoclaving, the solution was inoculated with *Tricoderma reese*i aseptically inside laminar air flow hood to avoid contamination and covered and put on rotary shaker for cellulose reduction.

6.3.5 **Saccharification: -** Saccharification is a process of hydrolysis of complex polysaccharides and non reducing sugar to simple reducing sugar. Sample was inoculated with *tricoderma reesei* which produces cellulase enzyme that converts cellulose to glucose that is further used for fermentation.

Procedure:- After the broth was prepared the treated groundnut sample was added in the broth and inoculated with *T.reesei* and was kept for incubation in B.O.D incubator for 6 days so that *T.reesei* can degrade the cellulose to simple sugar(glucose).since *T.reesei* is capable of producing cellulase enzyme which helps in conversion.

6.3.6 Standard curve for glucose

1. We added 1 gm of glucose in 100ml of water.

2. Now we added glucose solution in 5 test tubes as .2,.4,.6,.8,1 ml separately and another test tube as blank solution

3. We add distilled water in the same test tubes so as to make the solution equal to 2 ml .

4. Then we added 1ml DNS reagent in each test tube except the blank solution.

5. The solution was kept in the water bath for 5- 15 min at 90^{o}c.

6. After that the test tubes were cooled to the room temperature and then added 9ml dist. Water in it and mixed well.

7. After that we perform spectrophotometric analysis at 540nm by taking 1ml from each test tube and observed their absorbance.

8. Then we plot graph between amount of glucose on X axis and absorbance on the Y axis and we get a straight line.[12]

6.3.7 DNS test:-

After 6 days of incubation, we inoculated treated sample with Saccharomyces cerevisiae for fermentation. For the determination of glucose content, we performed DNS test for this process, 3,5-dinitrosalicyclic acid (DNS)reagent was prepared in a brown coloured bottle so as to prevent it from light.

For preparation of 100 ml DNS reagent [24]

TABLE 6.3.7 **(**For preparation of 100 ml DNS reagent)

S.NO	Chemical	Amount in gram
1	3,5-dinitrosalicyclic acid	1gm
2	NaOH	8gm
3	Rochelle salt	30gm

1 gm of DNS is added in 20 ml 2N NaOH+30 gm Rochelle salt in 50 ml of H_2O +30 ml H_2O

1. We added 3 ml DNS reagent with the same amount of groundnut shell sample(NaoH and H_2SO_4 treated).
2. The Mixture was heated at 90 degrees for 20 minutes to change the colour to reddish brown.
3. Rochelle-salt solution (40%) was then added to stabilize the colour.
4. Since the solution was colourish that we had to perform dilution and so we added 4ml of distilled water to the 1 ml of solution.
5. After cooling it for some time at room temperature, we recorded the absorbance with the spectrophotometer at 540 nm.

6.3.8 **Fermentation: -** Fermentation of carbohydrates to alcohol is one of the oldest processes. Fermentation is a process of conversion of sugar to ethanol in absence of oxygen. Fermentation is catalyzed by zymase, an enzyme produced by yeast. A chemical reaction for conversion of glucose to ethanol is:-

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

Samples inoculated with *Saccharomyces cerevisiae* were kept for fermentation process for 7 days. After fermentation the Dns test was performed again to check the glucose content the results were observed at wavelength of 540nm with dilution factor = 3

6.3.9 METHOD OF IMMOBLIZATION

1. The microbial culture of *Saccharomyces cerevisiae* was centrifuged and wet mass of cells was determined.
2. The Cells were re-suspended in 500 µ l of distilled water and mixed with 50 ml of sodium alginate solution.
3. The mixture was filled in syringe and dropped in ice cold calcium chloride continuously stirred over stirred.

4. Beads thus produced were kept for some time more in solution.

Then the beads were inoculated in the medium containing the source material (groundnut shells). The flasks were incubated for 7 days in the B.O.D incubator at 37°c. After incubation was complete the absorbance was taken at 540nm by performing DNS test.

6.4 Distillation: - Distillation is a process of separation of liquids from a mixture of liquids by selective evaporation and condensation, more volatile liquid separates first. Fermentation alone does not produce the beverage containing alcohol content more than 11-15% because high content of alcohol destroys yeast cells so for production of beverages with high content of alcohol, the aqueous solution must distilled.

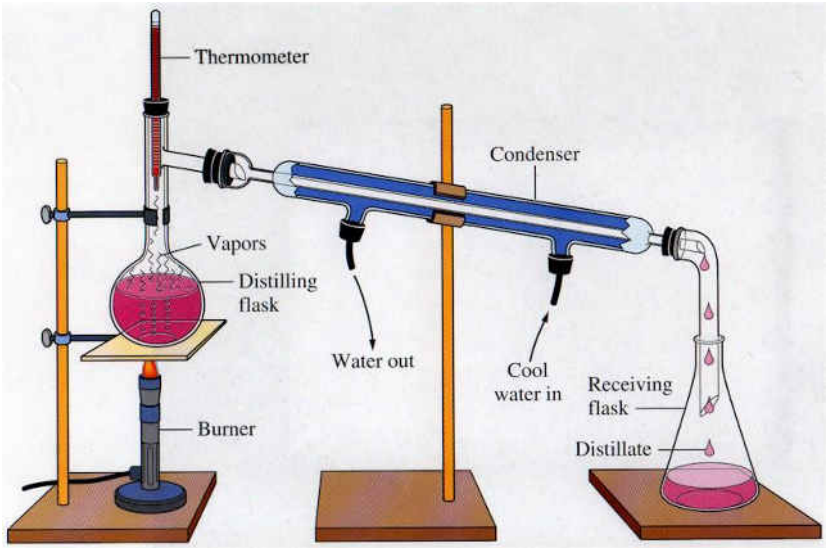


FIGURE 6.4 (DISTILLATION APPARATUS)

Applications of distillation:-

- In the fossil fuel industry, for obtaining various materials from crude oil for purpose of fuel and chemical feedstock.
- Fermented products are distilled for production distilled beverage having high alcohol content.

In field of industrial chemistry, wide range of crude liquid products formed by chemical synthesis is distilled for their separation

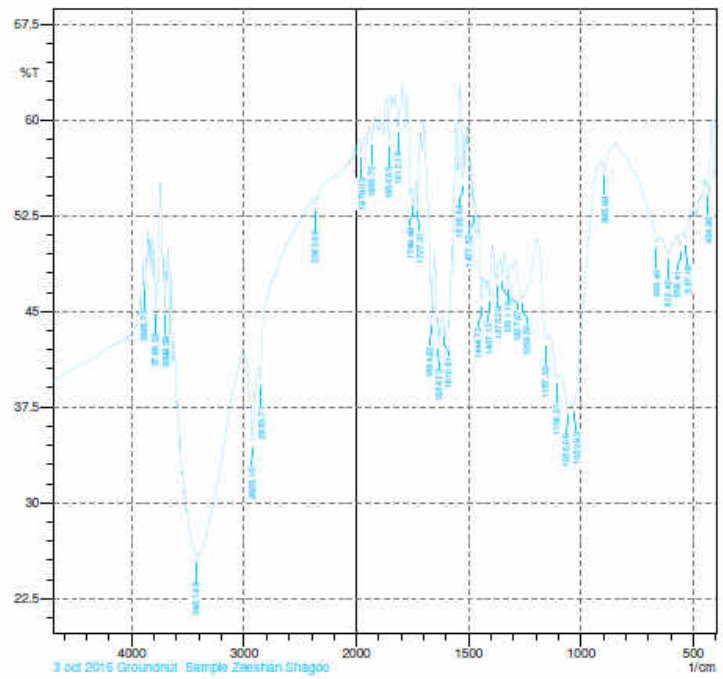
CHAPTER SEVEN

RESULTS AND DISCUSSION

FTIR Results

7.1(a) UNTREATED GROUNDNUT SHELL

UNTREATED GROUNDNUTSHELL



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	434.96	54.511	0.373	442.68	433.03	2.51	0.009
2	537.19	50.678	0.42	545.87	530.44	4.518	0.021
3	558.41	50.211	0.951	574.81	545.87	6.529	0.105
4	612.42	49.511	0.433	635.57	605.67	9.026	0.071
5	665.46	50.37	0.855	799.52	656.82	38.372	-0.224
6	895.96	56.139	0.969	911.4	846.78	15.668	0.12
7	1032.92	37.636	1.575	1042.56	911.4	42.761	-1.057
8	1056.06	37.388	1.064	1091.75	1042.56	20.418	0.28
9	1106.21	39.784	1.264	1139.97	1091.75	16.666	0.313
10	1157.33	42.73	2.903	1193.01	1139.97	18.13	0.626
11	1259.56	46.071	3.466	1294.26	1193.01	32.526	1.865
12	1327.07	47.206	1.864	1340.57	1312.6	8.828	0.175
13	1351.18	47.832	1.457	1358.9	1340.57	5.707	0.082
14	1375.29	47.466	0.731	1379.15	1364.68	4.583	0.023
15	1407.12	46.201	1.075	1410.01	1394.58	4.876	0.014
16	1444.73	45.803	3.732	1456.3	1437.98	5.743	0.247
17	1477.52	53.032	1.043	1481.36	1473.66	2.085	0.025
18	1528.64	55.346	4.7	1540.21	1523.82	3.823	0.276
19	1610.61	43.485	0.214	1618.33	1609.65	3.092	0.004
20	1634.73	42.544	1.547	1639.55	1630.87	3.134	0.05
21	1664.62	44.384	1.912	1670.41	1660.77	3.279	0.072
22	1727.31	53.33	3.119	1733.1	1713.81	4.892	0.211
23	1749.49	53.677	0.328	1753.35	1747.57	1.551	0.01
24	1812.18	59.507	0.576	1815.06	1806.4	1.927	0.015
25	1856.55	58.399	3.456	1867.16	1844.01	5.04	0.213
26	1933.7	58.461	0.266	1941.42	1931.77	2.214	0.002
27	1979.03	57.558	1.011	1990.6	1962.64	6.566	0.071
28	2362.88	53.366	0.561	2385.06	2349.38	9.642	0.07
29	2855.71	39.617	1.367	2870.17	2385.06	149.307	-10.782
30	2925.15	34.864	6.207	2994.59	2870.17	51.205	3.204
31	3431.48	25.836	0.349	3446.91	3422.8	14.068	0.063
32	3699.59	45.198	3.285	3708.27	3691.88	5.359	0.207
33	3788.32	45.268	2.615	3800.86	3778.68	7.297	0.245
34	3885.73	47.164	0.177	3887.66	3883.8	1.256	0.003

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FIGURE 7.1(a)(UNTREATED GROUNDNUTSHELL)

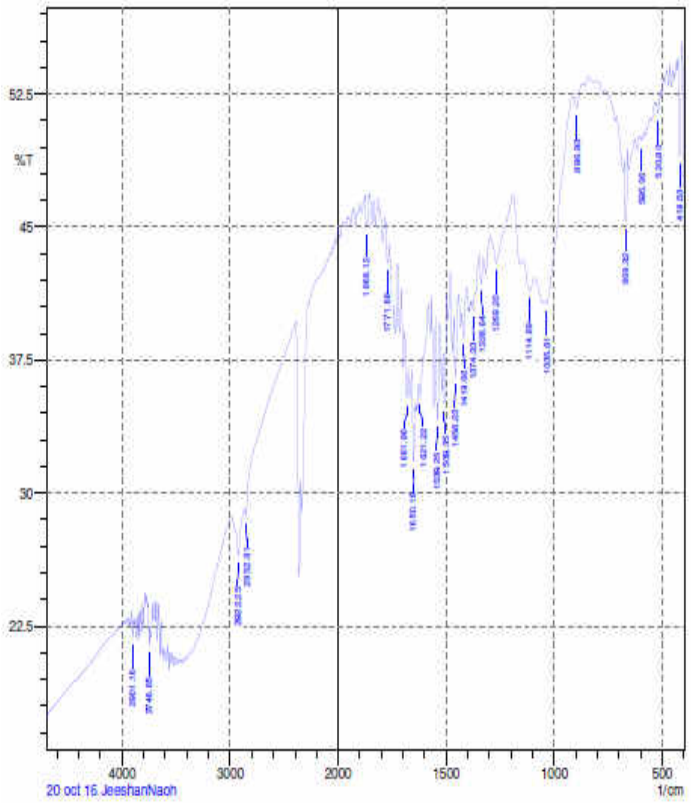
DISCUSSION

To confirm the degradation FTIR study was done which was in the range of 1100-1400 cm-^{1}, as comparison to non pretreated groundnut shell, (1100-1300 cm-1 wavenumber i.e. it depicts the lignin, cellulose and hemicellulose degradation.

In untreated Groundnut Shell the C-H peaks for cellulose lies in the wave number of 895cm^{-1}. The peaks in GS show C-H in cellulose and hemi-celluloses in 1375cm^{-1}. syringyl ring and C-O stretching in lignin and xylan at wave number 1,259 cm^{-1}. Aromatic skeletal

and C-O stretch at 1106 cm^{-1}. The lignin lies in spectrum peaks at 1477cm^{-1}, 1444 cm^{-1} 1528cm^{-1}, 1032cm^{-1}.

7.1(b) NaOH TREATED SAMPLE



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	399.28	46.745	0	409.89	399.28	2.995	-0.116
2	419.53	48.945	5.998	427.25	409.89	5.019	0.51
3	520.8	51.396	0.842	525.62	510.19	4.409	0.074
4	595.05	49.866	0.224	599.88	588.31	3.487	0.012
5	669.32	45.33	3.763	677.04	659.68	5.728	0.368
6	896.93	51.719	0.896	912.36	670.89	11.658	0.123
7	1035.81	40.643	1.006	1045.45	912.36	44.835	0.23
8	1114.89	41.38	1.202	1145.75	1067.89	21.773	0.337
9	1269.2	42.958	2.114	1296.21	1192.05	36.528	1.024
10	1338.64	41.793	1.601	1347.32	1329.96	6.457	0.163
11	1374.33	40.315	0.622	1379.15	1365.65	5.264	0.039
12	1419.66	38.734	2.001	1427.37	1407.12	8.092	0.227
13	1458.23	36.557	3.796	1464.96	1444.73	8.394	0.459
14	1509.35	35.043	3.959	1515.14	1497.78	7.507	0.514
15	1539.25	34.127	5.639	1549.86	1530.57	8.423	0.701
16	1621.22	35.445	0.934	1627.01	1614.47	5.583	0.06
17	1650.16	31.761	3.937	1661.73	1644.37	8.191	0.49
18	1681.98	35.348	2.525	1691.63	1675.23	7.17	0.29
19	1771.58	42.996	0.47	1783.25	1769.75	4.793	0.026
20	1868.12	45.014	1.777	1876.8	1859.44	5.898	0.172
21	2852.81	28.723	0.68	2865.35	2390.85	215.764	-6.592
22	2922.25	26.489	2.425	2995.55	2865.35	71.955	1.729
23	3746.85	21.399	1.41	3760.35	3742.99	11.295	0.268
24	3901.16	21.833	1.302	3910.8	3892.48	11.874	0.229

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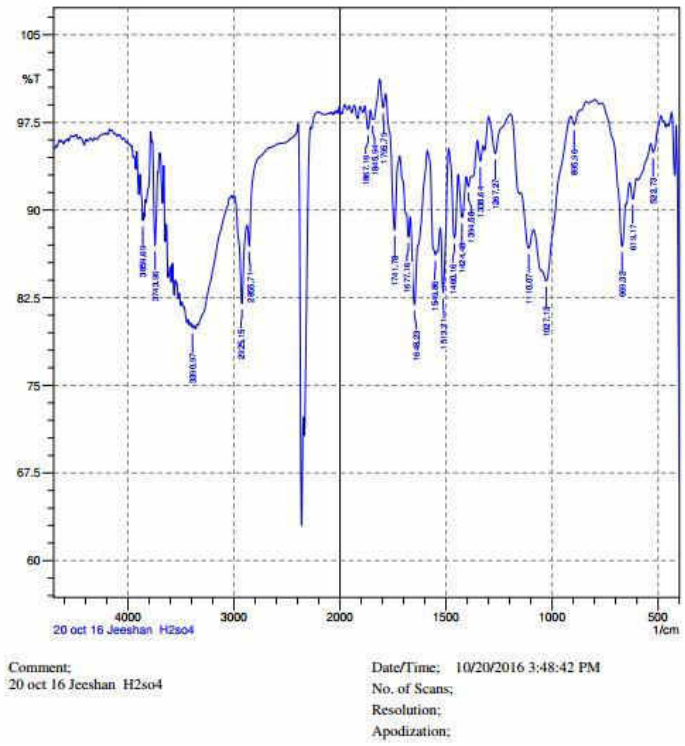
FIGURE 7.1(b)

The peak of C-H deformation in cellulose at 896.9 cm^{-1}. the peaks in GS shows C-H deformation in cellulose and hemi-celluloses at 1374 cm^{-1}. Syringyl ring and C-O stretching in lignin and xylan at wave number 1,269 cm^{-1}. The lignin degradation lies in spectrum peaks at 1458cm^{-1}, 1539 cm^{-1}, 1035cm^{-1}. Aromatic skeletal and C-O stretch at 1114cm^{-1}.

7.1(c) H_2SO_4 TREATED SAMPLE



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	522.73	94.948	1.277	533.34	486.08	0.788	0.136
2	619.17	90.942	1.586	633.64	601.81	1.192	0.121
3	669.32	86.896	5.002	765.77	651	3.044	0.348
4	895.96	97.337	0.887	912.36	868	0.407	0.075
5	1027.13	83.927	2.931	1048.35	912.36	5.968	0.508
6	1110.07	86.746	2.727	1148.65	1087.89	3.194	0.382
7	1267.27	94.832	3.224	1299.1	1195.91	1.435	0.574
8	1338.64	94.186	1.54	1352.14	1325.14	0.61	0.098
9	1394.58	92.001	0.865	1404.22	1379.15	0.85	0.044
10	1424.48	89.365	3.592	1441.84	1404.22	1.533	0.338
11	1460.16	87.606	6.337	1487.17	1441.84	1.824	0.637
12	1513.21	82.918	7.88	1526.71	1487.17	2.23	0.763
13	1549.86	86.167	1.627	1587.47	1543.1	2.06	0.177
14	1648.23	81.921	6.204	1668.48	1630.87	2.641	0.592
15	1677.16	87.706	1.835	1687.77	1668.48	1.005	0.081
16	1741.78	88.296	8.175	1785.18	1718.63	1.786	0.979
17	1795.79	98.775	1.66	1812.18	1785.18	0.038	0.105
18	1845.94	97.732	1.411	1855.58	1812.18	0.22	0.195
19	1867.16	96.903	1.803	1882.59	1855.58	0.26	0.11
20	2855.71	86.929	2.372	2875.96	2659.93	6.596	-1.277
21	2925.15	82.009	7.708	2995.55	2875.96	7.216	1.705
22	3390.97	79.927	0.31	3407.37	3378.43	2.791	0.026
23	3743.96	86.982	7.585	3770	3718.88	2.179	0.943
24	3859.69	89.103	1.334	3885.73	3852.94	1.361	0.079

FIGURE 7.1(c) (H_2SO_4 TREATED SAMPLE)

The peaks in GS shows C-H deformation in cellulose and hemi-celluloses at 1394.5 cm^{-1} ; C-H vibration in cellulose and C-O vibration in Syringyl derivatives at 1,319 cm^{-1} ; syringyl ring and C-O stretching in lignin and xylan at wave number 1,267 cm^{-1}; aromatic skeletal and C-O stretch at 1,110 cm^{-1}; C-O stretch in cellulose & hemicelluloses at wave number 1027cm^{-1} and C-H deformation in cellulose at 895.9 cm^{-1}. The lignin degradation lies in spectrum peaks at wave number of 1,599, 1,511, 1,467, 1,429, 1,157 and 1,054 cm^{-1} as reported by Adapa (25). Similar results for lignin degradation are in the range of 1549.83 cm-1, 1513 cm^{-1}, 1440cm^{-1}, 1424cm^{-1} and in the present study.

7.2(a) RESULTS FOR STANDARD GLUCOSE CURVE

Figure 7.2(a) standard glucose curve

7.2(b) GLUCOSE CONCENTRATION AND ITS ABSORBANCE

TABLE 7.2(b) absorbance of Glucose sample.

S.NO	Amount of glucose in ml	Absorbance
1	0 ml	0
2	.2 ml	.214
3	.4 ml	.412
4	.6 ml	.612
5	.8 ml	.715
6	1 ml	1.15

STANDARD GLUCOSE CURVE

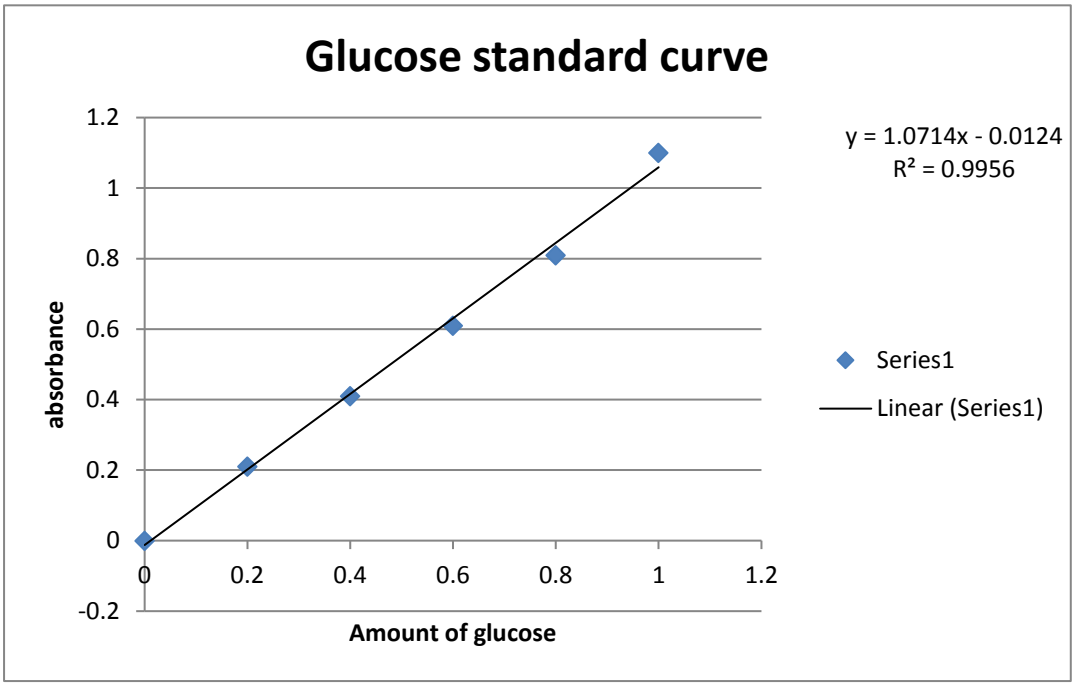


FIGURE 7.2(b) standard glucose curve

7.3 RESULTS FOR DNS TEST (FREE CELLS)

7.3(a) FOR NaOH TREATED

TABLE 7.3(a) (FOR NaOH TREATED) D.F = 3

Sample percentage (%)	Absorbance	glucose mg/ml
1%	.15	.45
1.5%	.24	.72
2%	.31	.93

7.3(b) FOR H_2SO_4 TREATED

TABLE 7.3(b) (FOR H2SO4 TREATED) D.F = 3

Sample percentage (%)	**Absorbance**	**glucose mg/ml**
1%	.11	.33
1.5%	.18	.54
2%	.27	.81

Comparison between ACID and BASE and its effect before fermentation

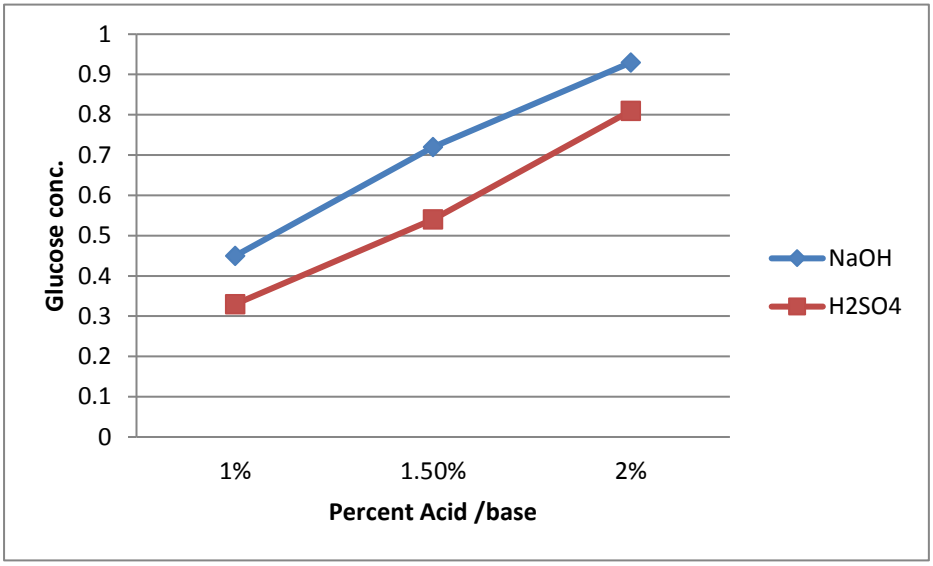


Figure 7.3(a)&(b) Comparison of H2SO4 and NaOH to show its effect on the Glucose conc.

7.4 RESULTS FOR DNS TEST AFTER FERMENTATION (FREE CELLS)

7.4(a) For NaOH

TABLE 7.4(a) (NaOH AFTER FERMENTATION) (D.F=3)

Percentage (%)	**Absorbance**	**glucose mg/ml**
1%	.18	.54
1.5%	.16	.48
2%	.06	.18

Figure 7.4(a) (NaOH TREATED AFTER FERMENTATION)

7.4 (b) FOR H_2SO_4

TABLE 7.4(b) (H_2SO_4 AFTER FERMENTATION) (DF=3)

Percentage(%)	Absorbance	glucose mg/ml
1%	.21	.63
1.5%	.18	.54
2%	.09	.27

COMPARISON AFTER FERMENTATION

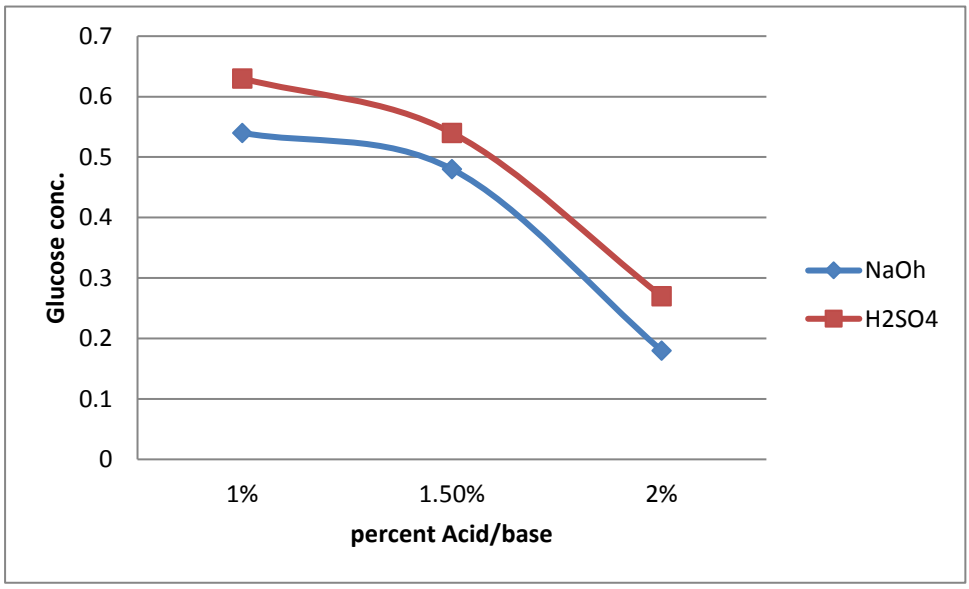


Figure 7.4(a)&(b) (**NaOH** and **H_2SO_4** AFTER FERMENTATION)

Figure 7.4(b) (**H_2SO_4** TREATED AFTER FERMENTATION)

After the chemical pretreatments were done the spectrophotometric results shows the increasing absorbance which finally leads to increased glucose concentration but after the fermentation was done by adding the *Saccharomyces cerevisiae* the DNS test was again performed which shows the decreased glucose concentration which implies that microorganism has utilizes the glucose as substrate and results in the ethanol production. The yield of ethanol will be slightly low because *Saccharomyces cerevisiae* is not able to convert pentose sugars efficiently because of the lack of initial metabolic pathways. The GAS chromatography will be performed in order to know the bioethanol yield.

7.5 RESULTS FOR DNS TEST (Immobilized Cells)

7.5(a) **FOR NaOH TREATED**

TABLE 7.5(a) (NaOH before FERMENTATION) **D.F=3**

Sample percentage (%)	Absorbance	glucose mg/ml
1%	.04	.12
1.5%	.09	.27
2%	.15	.45

7.5(b) **FOR H_2SO_4 TREATED**

TABLE 7.5(b) (H2SO4 before FERMENTATION) D.F = 3

Sample percentage (%)	Absorbance	glucose mg/ml
1%	.05	.15
1.5%	.10	.30
2%	.13	.39

Comparison of Glucose concentration before fermentation

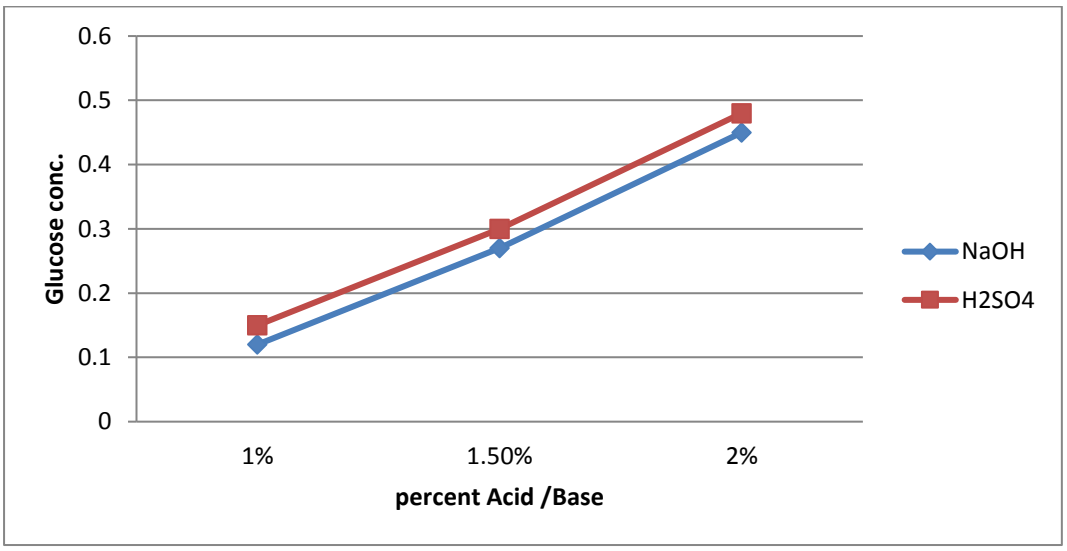


FIGURE 7.5(a)&(b) Comparison of Glucose concentration before fermentation

7.6 RESULTS FOR DNS TEST AFTER FERMENTATION (Immoblized cells)

7.6(a) **For NaOH**

TABLE 7.6(a) (NaOH after FERMENTATION) **D.F =3**

Sample percentage (%)	Absorbance	glucose mg/ml
1%	.30	.90
1.5%	.26	.78
2%	.18	.54

7.6(b) **FOR H2SO4**

TABLE 7.6(b) (H2SO4 after FERMENTATION) **D.F =3**

Sample percentage (%)	Absorbance	glucose mg/ml
1%	.27	.81
1.5%	.24	.72
2%	.15	.45

After fermentation glucose concentration

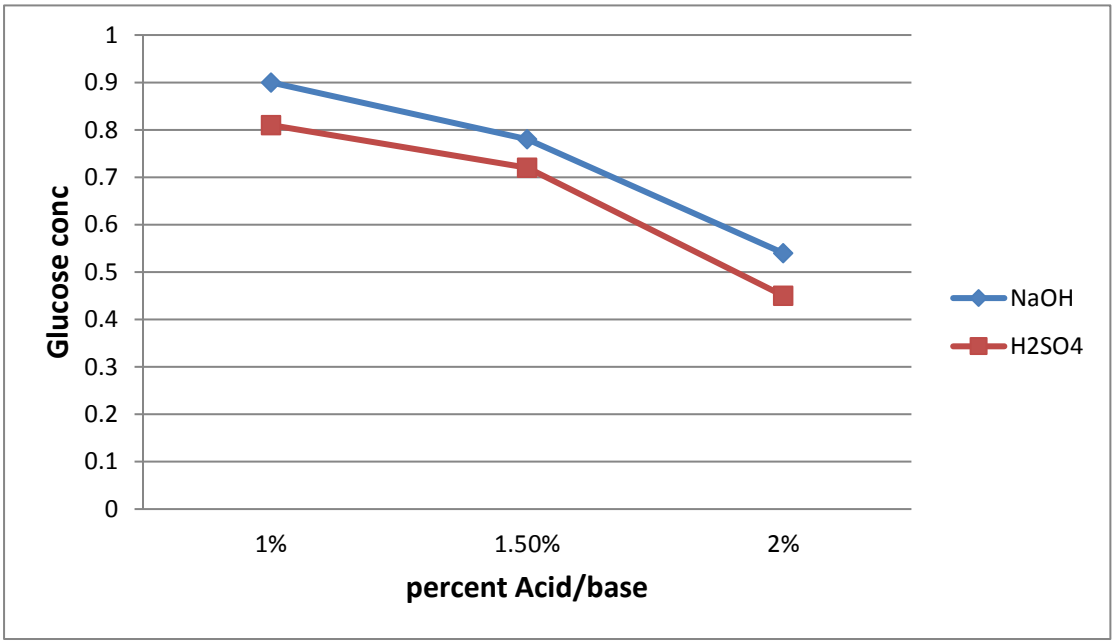


Figure 7.6(a)&(b) comparison of glucose conc. after fermentation of Immoblized cells

Figure 7.6(a)&(b) Immobilized cells after fermentation.

After the chemical pretreatments were done the spectrophotometric analysis shows that the absorbance of all the samples increases, which finally leads to increased glucose concentration but the increment was less as comapred to that of free cells. the fermentation was done by adding the immobilized *Saccharomyces cerevisiae* after that DNS test was again performed which shows the decrease in glucose concentration but the reduction in case of immobilized cells was not much seen in free cells because the cells were not able to utilize the glucose completely due to the mass transfer limitation, thus percentage of ethanol produced was decreased.

7.7 RESULTS OF GAS CHROMATOGRAPHY

7.7(a) the following results were shown after performing the gas chromatography

1) Standard ethanol solution

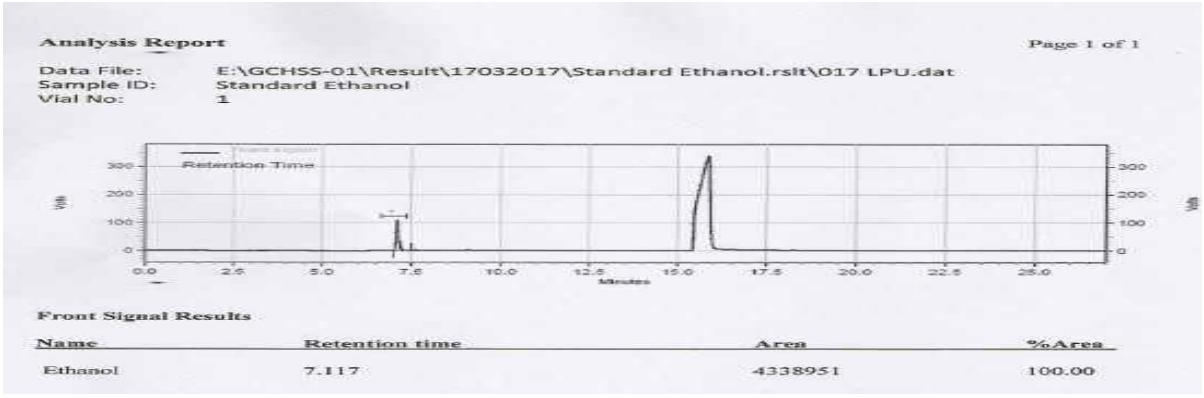


Figure 7.7.(a).1 GC Graph showing the peak for standard ethanol solution

2) Free cell Solution 2% H2SO4

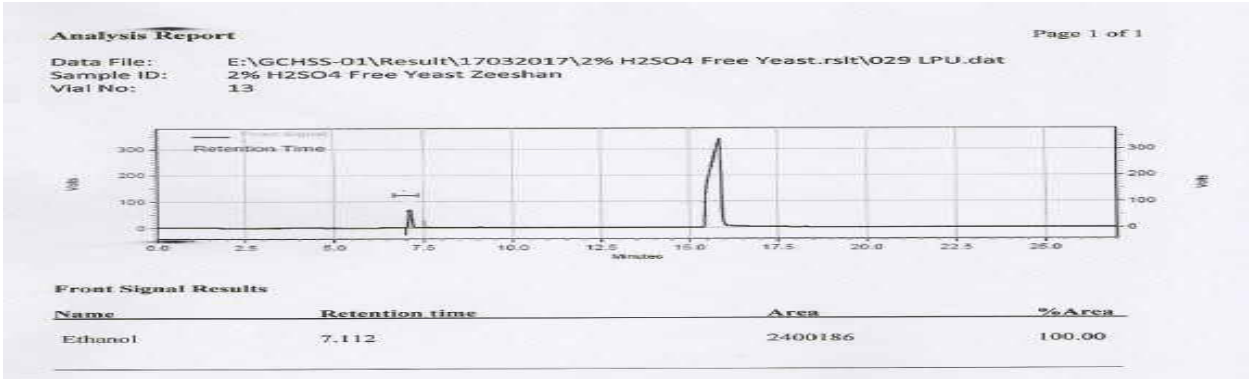


Figure 7.7.(a).2GC Graph showing the peak for 2% H2SO4 free cell ethanol solution

3) Free Cell Solution 2%NaOH

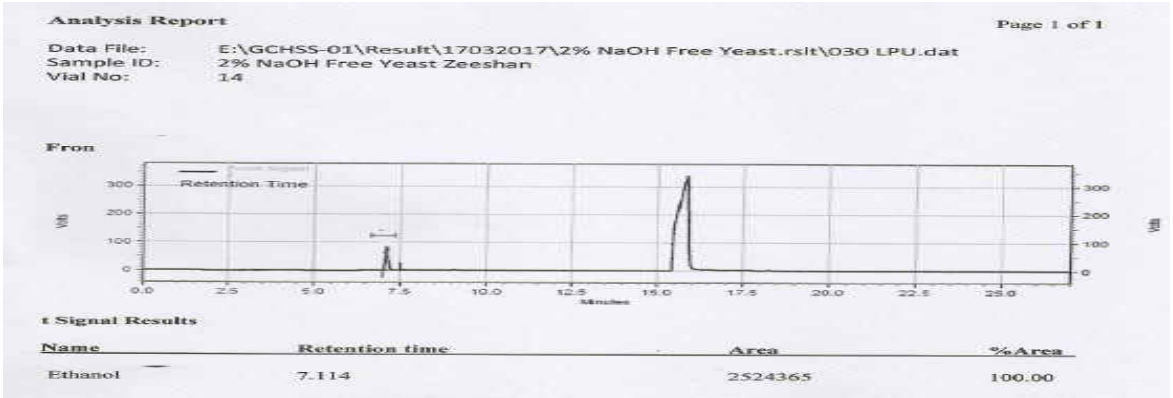


Figure 7.7.(a).3 GC Graph showing the peak for 2% NaOH free cell ethanol solution

Theses graphs depict that the retention time for the 2% H2SO4 free cell solution was 7.112 which is nearly same as that of the standard so the purity of solution (sample) is same as that of the standard. the ethanol was not produced so much but as compared to the immobilized cells the percentage was little bit more the reduction was due the reason that *Saccharomyces cerevisiae* was not able to utlilize the pentose sugar completely because of the lack of initial metabolic pathways. The retention time for the 2%NaOH free cell solution was 7.114 which is nearly same as that of the standard which means purity of the solution is same as that of the standard. The production of ethanol was seen higher in 2% NaOH than others which was due to the pretreatment by NaOH.

4) Immoblized Cell Solution 2% NaOH

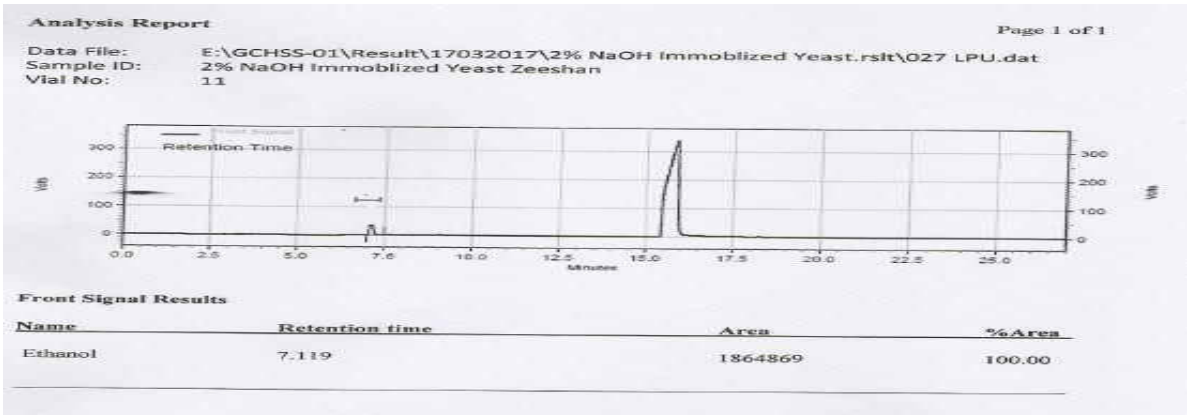


Figure 7.7.(a).4 GC Graph showing the peak for 2% NaOH Immoblized cell ethanol solution

5) Immoblized Solution 2%H2SO4

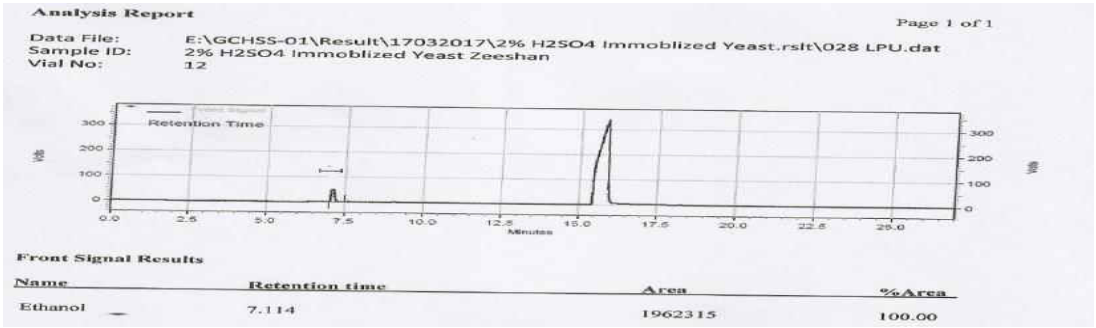


Figure 7.7.(a).5 GC Graph showing the peak for 2% H2SO4Immoblizrd cell ethanol solution

This graph depicts that the retention time for the 2% H2SO4 immobilized cell solution was 7.114 which is nearly same as that of the standard thus purity of the sample is same as that of the standard. Similarly the graph shows that the retention time for the 2% NaOH Immobilized cell solution was 7.119 which is nearly same as that of the standard suggesting that the purity

of sample is same as that of the standard The glucose utilization in both the cases was seen lesser as compared to that of the free cells due to which ethanol production was also less the reason is due to mass transfer limitation and less utilization of pentose sugars.

7.7.(b) **Ethanol yield by Gas Chromatography**

Table for Ethanol percentage 7.7.(b)

	Area of test	Vol of sample	Purity of Std	Vol of Standard	Area of std	Ethanol percent(v/v)
2%Naoh Immoblized	1864869	100	99.5	200	4338951	0.342118573
2%h2so4 Immoblized	1962315	100	99.5	200	4338951	0.359995478
2%h2so4 free	2400186	100	99.5	200	4338951	0.440324875
2%Naoh free	2524365	100	99.5	200	4338951	0.463106069

a) Free cells 2% NaOH and 2% H2SO4

In case of free cells the Area of 2% NaOH and 2% H2SO4 is different than that of the standard so by using the Area under peak the analyte concentration can be calculated since the area under peak is proportional to the analyte concentration, therefore by using concept of integration. Thus concentration of ethanol can be determined using calibration curve or relative response factor. The ethanol concentration was .4631(v/v) in case of NaOH (2%) and .4402(v/v) in case of H2SO4 (2%).

b) Immobilized Cells 2% NaOH and 2% H2SO4

In case of immobilized cells the area under peak of immobilized cells 2% NaOH and 2% H2SO4 is very small as that of standard, thus the ethanol concentration in immobilized cells is lesser than that of free cells by using the relative response factor.thus the ethanol concentration calculated was found to be .342 for 2% NaOH and .359 for 2% H2SO4.

CHAPTER EIGHT

CONCLUSION

CONCLUSION

The present study of bioethanol production from groundnut shell was through simultaneous saccharification and fermentation(SSF) which has revealed the feasibility of raising the theoretical yield of ethanol by using proper chemicals like Sulphuric acid, hydrocholoric acid at proper concentration and at optimum temperature. The DNS test shows that by increasing the Alkali or acidic concentration the glucose production also increases. Similarly after fermentation the microorganisms converts glucose to ethanol so glucose concentration decreases because *Saccharomyces cerevisiae* cannot convert pentose sugars to ethanol so the conversion to ethanol will be low.

The ethanol percentage was very less it might be due to the less degradation of the sample with the pre treatment techniques and non utilization of pentose sugars. Thus we need to develop some more efficient pre treatment methods like ion liquid pretreatments and fractionation which will enhance the production of cellulose.

Thus from the resulted obtained from gas chromatography the ethanol percentage was very less free cells as that of immobilized cells, the inference was obvious that the ethanol concentration rate was more in case of the free cells. The reason is that in case of immobilized cells the mass transfer limitations were there due to the presence of polymer formation made of calcium aliginate around yeast cells, which offers resistance to the transfer of glucose available to the cells, thus rate of conversion was less, which was not the case seen in free cells.

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CHAPTER NINE

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CHAPTER TEN

APPENDIX

APPENDIX

Mendals Medium

Chemicals	Weight in gm
Yeast extract	0.25gm
KH_2PO_4	2.0gm
$CACL_2.2H_2O$	0.21gm
Urea	0.21gm
$MgSO_4.7H_2O$	0.15gm
Peptone	1.0gm
$FeSO_4.7H_2O$	0.15gm
$MNSO_4.H_2O$	1.6gm
$ZNSO_4.7H_20$	1.4gm
$COCL_2$	2.0gm

DNS reagent:

S.NO	Chemical	Amount in gram
1	3,5-dinitrosalicyclic acid	1gm
2	NaOH	8gm
3	Rochelle salt	30gm

YPGA medium:

Material	Quantity
1. Yeast extract	0.3 gm
2. Peptone	0.5 gm
3. Glucose	1.0 gm
4. Agar	2.0 gm
5. Distilled Water	100 ml
6. PH	4.5 gm