Synthesis Of Cellulose And Its Application For Bioethanol Production And Wastewater Treatment

Submitted by

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Submitted for the partial fulfilment for the degree of

MASTER OF BIOPROCESS ENGINEERING
DEPARTMENT OF CHEMICAL ENGINEERING
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I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of her **Master of Bioprocess Engineering** studies**.**

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

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A thesis submitted in partial fulfillment of the requirements of the degree of Master of Bioprocess Engineering in Chemical Engineering

Examination Committee:

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Certificate

This is to certify that this thesis work entitled "Synthesis Of Cellulose And Its Application For Bioethanol Production And Wastewater Treatment" submitted by Ms. Preetha Ganguly is a bonafide thesis work carried out under my supervision and guidance and fulfilling the nature and standard required for the partial fulfilment of the degree of Master of Bioprocess Engineering in Chemical Engineering. The work embodied in this thesis has not been submitted elsewhere for a degree.

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Abstract

The aim of this research is utilization of lignocellulosic waste feedstock in biofuel generation and waste water treatment. Isolating the two important macromolecules of plant cell wall, lignin and cellulose microcrystal (CMC) from agro waste (peanut shell) and then evaluating their potential application in biofuel production and waste water treatment was investigated. In comparison different pre-treatments were also done on the shell waste then hydrolyzed and fermented for bioethanol production. From the pre-treated peanut shell waste the ethanol produced was 50%, whereas by direct cellulose extraction biofuel generated maximum was 19.13%. The results shows that about 36.5 g of cellulose were extracted from 100 gram of groundnut shell waste powder. Scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) were considered for the characterization of cellulose and lignin. Further the effect of different factors (dose, temperature, pH and substrate concentration) on the reducing sugar production was also estimated. In this research two microbial strains (*Zymomonas mobilis and Sacchromyces cerevisiae)* are taken for fermentation process in both aerobical and aerobical conditions..

In the second part of the study the cellulose left after hydrolysis, and the value-added products such as lignin and cellulose were investigated for their potential in dye removal from waste water. The study shows that the maximum amount of crystal violet dye was removed by the LCS in all the different parameters applied with about 99% removal.

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Signature Of Candidate.....

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Nomenclature

S.No	Abbreviation	Full Form
1	MCC	Microcrystalline Cellulose
2	CVD	Crystal Violet Dye
3	SEM	Scanning Electron Microscope
4	FTIR	Fourier Transform Infrared Spectroscopy
5	XRD	X –Ray Diffraction
6	LCS	Left Cellulose After Saccharification

CHAPTER 1

Bioethanol Production from Agricultural Waste:-Groundnut shell waste By Traditional Method

1 INTRODUCTION:-

1.1 Scenario of Sustainability, global energy

Depletion of present hydrocarbon deposits has become the main point for concerned from both fuel and the substantial point of view. United state Energy Information Administration submitted a report in 2006 stating that annual crude oil consumption all over the world will increase to 98.3 million barrel per day in 2015 and 118 million barrel per day in 2030. Rapid industrialization all over the world is increasing the demand of fuel. This industrial revolution is causing fuel crises and in turn affecting our environment. In order to meet the energy demands for such growing economy has been marked as one of the major challenges faced by the worldwide humanity (*Smalley et al 2003*).Moreover, plastics which are extensively used today are also products which are derivative products from the resources of petroleum (*Mosier et al., 2005*). Therefore an alternative to fossil fuel is needed to be investigated by the researchers.

The combustions of the fossil fuels like natural gases, coal, crude oil is the resource of most of the world energy. World Energy Council in year 2016 reported that "the world wide consumption of these fuel by 2015 have reached about 86%". If this current rate of consumption continues than the world will be deprived of nonrenewable resources within 120 years (International Energy Agency 2013). Furthermore, one of the major sources of greenhouse gases is the combustion of the fossil fuels whose negative impact on global warming and on climate changes are well reported and forecast . In order to reduce the GHG emission, and also to supplement the non-renewable resources of energy, clean fuel and low carbon alternative are being developed and utilized.

The uncertainty in supply of the fossil fuel , the excessive growing demand of energy , along with suitable policy predilection, federal authorization and associated allowances (*Guo,*

Song, and Buhain 2015; International Energy Agency 2015) are the main key drivers in the variegation of energy resources and gradual shift towards the recyclable energy . These renewable energy includes from wind, solar, biomass, nuclear, hydrothermal and geothermal which have unexpectedly grown over the last 15 years reported by World Energy Council 2016 and continuously appealing towards the investors, facilitating advancements in terms of infrastructure and technology.

1.1.2 Biomass Economy:-

Biomass economy, on the other hand, is characterized based on converting lignocellulosic biomass in biorefineries to various important products such as chemicals, bio-fuels, and substances, which have gained immense importance due to their dependence on the reusable resources of energy and also because it is an alternative to the crude oil. The energy which is derived from the conversion of recyclable organic matter from plant or animal sources to energy is referred as 'bioenergy'. It was reported in the year 2014, that worldwide total primary energy supply (TPES) was about 5.7 x 1020 J, 10 % out of which was accounted to be the bioenergy (International Energy Agency 2016; World Energy Council 2016). At the center of the biomass economy concept is the Biorefinery, and it can be defined as combining various biomass conversion processes, where a range of value added products are produced such as bio-fuels, energy, new chemicals and materials through combining various different technologies (*FitzPatrick et al., 2010*). Biorefineries are considered as eco-friendly comparable to the oil refineries. The various value added byproducts generated can be utilized to reduce biofuel generation price and also to improve industrial profitability.

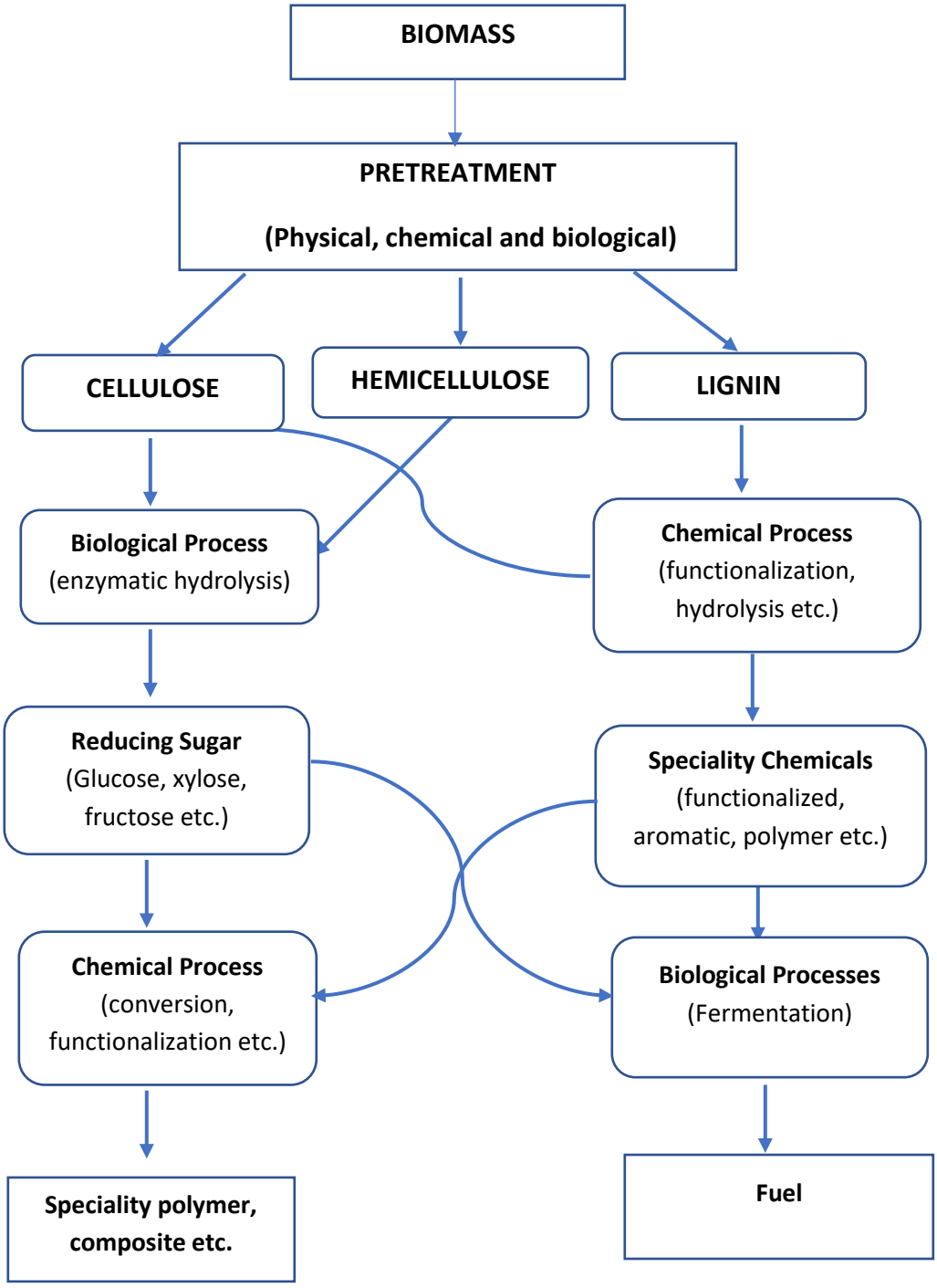
Biofuels can be liquid or gaseous derived from the lignocellulosic biomass. Major Liquid biofuel energy includes biobutanol, biodiesel, bioethanol and biopropanol, whereas biohydrogen and biomethane are some common forms of gaseous biofuels. By 2050 the contribution of biofuel is expected to 3 times critical especially in sector of transportation (*Eisentraut et al. 2011*).Bioethanol production is one of the good examples of biofuel concept, which has been utilized as a gasoline additive for three decades. In this approach, ethanol generation is dependable upon the sugar derived grains having starch such as corn

starch, sugarcane and sugar beet by the scarrification process. Hydrolyzation of sugar into its monomeric (glucose) units is done, which are then later fermented to bioethanol. However, in the first generation of bioethanol production the feedstocks used were food materials, and using food materials as source of raw materials for ethanol generation was ultimately creating a debate over fuel vs. food competition (*Mabee et al., 2011; Agbor et al., 2011; Vancov et al., 2012*).

Therefore, another biorefinery concept was developed and processed. In this study, fermentable sugar was obtained from the cellulose in lignocellulosic feedstock, which includes rice husk, cotton stalk, sugarcane bagasse, wood chips, saw dust, corn Stover, paper waste, switch grass etc., as cellulose is one of the most abundant polymer on this earth. However, this approach is still under expansion, because the extraction of cellulose part and then hydrolyzing into glucose unit is expensive with today's technology. In this theory, the lignocellulosic feedstock is firstly pretreated through chemical, physical and biological methods in order to bifurcate and extract cellulose. After the isolation of cellulosic part from lignocellulosic biomass it is further exposed to either chemical, enzymatic or biological scharrification to break the cellulose and to generate glucose monomers. Fermentable sugar produced finally is converted into biofuel via fermentation process. Ethanol from lignocellulosic biomass has gained immense importance because the feedstock is recyclable and cheap, adding to this in a report it has been estimated that over one billion tons of feedstock is available as a fuel raw material (*Li et al., 2010*).

One of the main problem related with the use of lignocellulosic raw feedstock for ethanol generation are the tedious and time taking hydrolysis step and also costly pretreatment. Usually, lignocellulose raw feedstock has an intractable composition. This complex structure is impervious to biological and chemical attacks. The Pretreatment process is a important with the objective to break the main components (cellulose, hemicellulose and lignin) of the biomass, extract the cellulosic component, eliminate hemicellulose and lignin, and also to make modification in the cellulosic structure to make it be more prone to enzymatic action or biological hydrolysis step, in which enzymes digest and degrade the cellulosic structure to release the cellulose.

One of the other advantage of utilizing lignocellulosic biomass is that in cellulosic ethanol production a large amounts of residual lignin is produced. Hence, a value – added lignin product along with the fermentable sugars, from the same biomass conversion process, will improve the feasibility and economy of the biomass conversion refineries and processes and lessen the disposal rate of a refinery (*Doherty et al., 2011*).



The Figure 1.1:- represents the biorefinery flowchart process showing that the lignocellulosic feedstock is finally converted into fuel

1.1.3 Bioethanol:-

Bioethanol, is a renewable resource of fuel and is a solution various problem related to conventional fossil resources. The largest generated liquid biofuel in the global market is the bioethanol. Bioethanol can used as a transport fuel because of its various advantages such as its lower cost, and green fuel. 35% oxygen content is found in bioethanol which help in its complete combustion and it also reduces the matter emissions that can cause hazard to the human health. Fermentation of bioethanol is one of the most established and mature bioprocesses technique (*Swana et al. 2011*).The octane number of bioethanol is (99) which is quite higher than petrol (80–100). Blending of a small quantity of bioethanol in petrol boost the octane number, it acts as a replacer to the conventional additives (Meta tertiary butyl ether, which can cause bad effects human health).Due to this advantage ethanol is widely used as a fuel (*Chandra et al., 2007*).

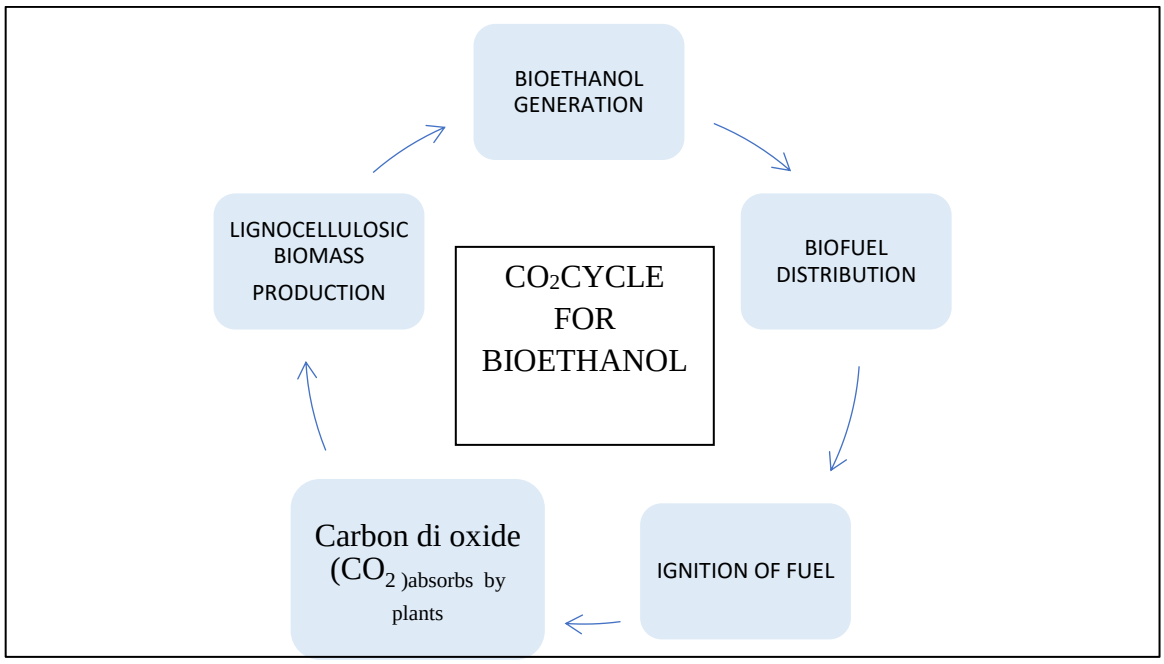


Figure 1.2: CO_2 cycle for bioethanol

This biofuel can also be used as a petroleum replacement to the vehicles for road transport. Ethyl alcohol (C_2H_5OH) or Bioethanol is a colorless clear liquid fuel having very low toxicity and on combustion produces little environmental pollution. Burning of this fuel produces carbon dioxide (CO_2) and water. The combustion of fossil fuel results into the emission of CO_2 which causes the greenhouse effects but by the use of ethanol, this emissions rate is reduced as the plants absorb the CO_2.

Microbial degradation of substrates rich in carbohydrate by yeast, bacteria and fungi results in the production of Bioethanol. *Saccharomyces cerevisiae* is one of the most commonly used yeast strain in industrial as well as pilot scale bioethanol production. The recombinant yeast strain can be operated in a wide range of pH, temperature, and substrate and can also resist high levels of ethanol and other inhibitory substances.

1.1.4 Types of Bioethanol:-

The three kinds of biofuels are: 1st generation, 2nd generation and 3rd generation biofuels based on the biomass source used for the generation of energy.

First generation bioethanol are produced by utilizing food crops such as, sugarcane, groundnut, corn, wheat, barley, rice and sorghum as raw material. Majority of the biofuel production all across the globe was first generation over a few years ago. Major problem regarding the generation of these bioethanol was fuel vs. food competition. As these bioethanol were produced directly from the edible crop these was causing decrease in the volumes of crops from the global food market. Therefore this is one of the major reasons for the increase in world food prices over the last few decades (*Carere et al., 2008*).

The Second Generation biofuel were generated from non-edible crops commonly known as lignocellulosic feedstock. These crops includes agriculture residue, domestic waste, industrial residue, wood, food crop waste, organic waste, and specific biomass crops. The second Generation bioethanol was produced to have overcome the disadvantage of first generation biofuels and also to discard the ‘food vs. fuel’ competition. As the second generation biofuel is considered as green fuel it also helps in decreasing the environment pollution caused by the combustion of domestic, agricultural and industrial residue.

Algae biomass is used for the production of third Generation of biofuels. The algae are sub cultured in special ponds to act as a low-price, high-power and totally renewable raw material in biofuel generation.. The growth of algae is suitable on both the land and water comparable to food production which is only suitable on land, therefore the algae to biofuel generation is decreasing the stress on already depleted water resources (*Chandra et al., 2007; Carere et al., 2008*).

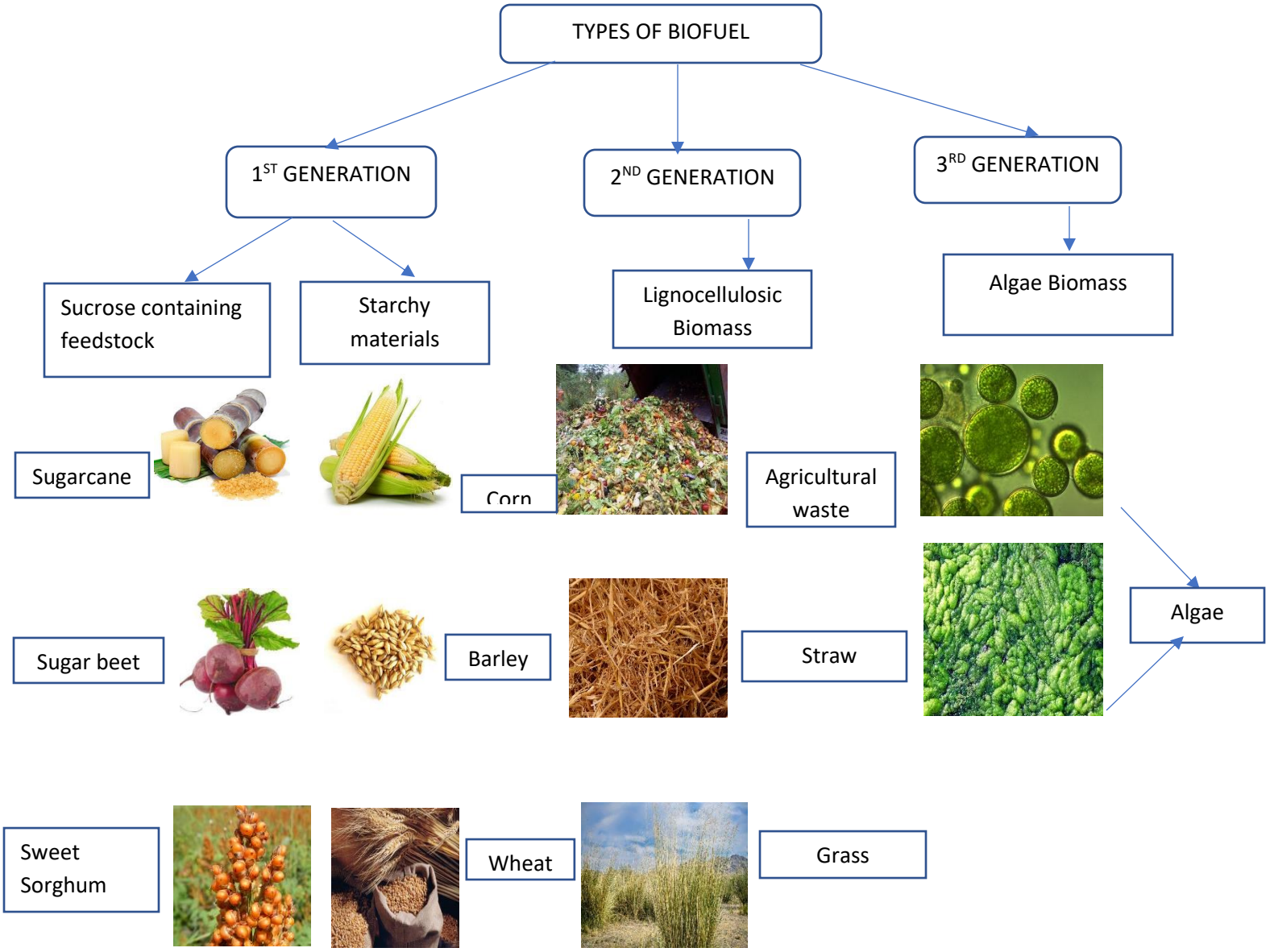


Figure 1.3:-Types Of Bioethanol and there source of production.

1.1.5 Groundnut:-

Groundnut (scientifically known as Arachis hypogaea) is an agricultural crop, whose seeds/fruit are used in the food industry as one of the most necessary raw materials. The annual global production of groundnut (fruit) reported in the year 2009/2010 was estimated to be 33.36 metric million tons (report given by U.S. Department of Agriculture, fas.usda.gov). The peanut seeds are generally used as industrial raw matter or independently as food. Industrial use of peanut includes the production of peanut oil, peanut flour, snacks and peanut butter. The shells or the pods of the groundnut are cellulose rich substrate considered as industrial or agricultural residue.

One of the largest producer of peanut is India after china with the production of about was 33000 tons in 2010 (Department of Agriculture) and with an amount of 60% of this production is done entirely by Gujrat and Andhra Pradesh region. About one third mass of the peanut fruits is the shell, as around 25000 tons of the groundnut shell waste is produced annually which can be processed according to the data represented by the Department of Agriculture.

Groundnut shell waste is a useful industrial and agricultural residue raw material which have not only high cellulose content but also higher content of lignin. Zhang et al in the year 2006 reported that the groundnut shell contains approximately 30% lignin, 19% hemicellulose and 36% cellulose. Therefore if we calculate there is an opportunity of processing about 9000 tons of cellulose, 4800 tons of hemicellulose and 7500 tons of lignin annually.

Figure 1.4:-Groundnut and Groundnut shell

1.2 REVIEW OF LITERATURE

1.2.1 Bioethanol Renewable resource of energy:

Bioethanol is a liquid clear colorless biofuel produced from lignocellulosic feedstock through fermentation procedure. Bioethanol show complete combustion unlike the gasoline fuel and therefore the ethanol is considered as cleaner fuel. Bioethanol can be utilized either as a fuel additive or independent fuel (*Sánchez and Cardona 2008*). Kuhad and Singh 1993 studied that the world wide generation of plant feedstock is about 200 $X10^9$ tons/year, of which the major part was estimated to be lignocellulose approximately over 90 %, 8–20 $X10^9$ tons of the biomass remains accessible potentially for different purposes.

The emissions of co_2, material compatibility and engine performance has been studied for various ethanol-diesel and ethanol-gasoline blends. Bioethanol is an oxygen containing biofuel, meaning it provides better oxidation to the hydrocarbons, resulting into lesser release of aromatics and carbon monoxide (*Sánchez and Cardona 2008*). Though the energy density bioethanol is relatively low, but it has higher octane number (113) than petrol (80-90) and gasoline (87-93) (Renewable Fuels Association 2017). Bioethanol has other various desirable properties, such as, broader flammability range speed of the flame is higher, and heat of vaporization is also higher (*Balat, Balat, and Öz 2008*) when gasoline is compared, therefore can improve the efficiency of the engine (*Balat et al. 2008; Renewable Fuels Association 2017*), and therefore enhance its use as a suitable blend modern engines increases (*Balat et al. 2008; Masum et al. 2013*).

In lesser blended form, bioethanol from lignocellulosic biomass can decrease the GHG release by 4-4% in comparison to the pure gasoline. Ethanol gasoline blend can be prepared by mixing 10-15% of ethanol in gasoline (E10 or E15) (*Moriarty and Yanowitz 2015*) without any modifications to conventional engines. Besides the environmental advantages of the use of bioethanol it has other desirable benefits like low-price octane raising additive for gasoline, bioethanol can also create new jobs, support agriculture based economy. Lignocellulosic biomass represents as a promising alternative in ethanol generation because of its abundant availability, lower cost, high yield of ethanol and output/input energy ratio.

Table1.1:- Global availability of agricultural wastes and their utilization in bioethanol production (*Sarkar et al.,2012*)

Agricultural waste	**Availability (million tons)**	**Estimated bioethanol potential (Gl)**
Corn straw	**128.02**	**58.6**
Sugarcane bagasse	**180.73**	**51.3**
Wheat straw	**354.34**	**104**
Rice straw	**731.3**	**205**

1.2.2 Ethanol production technology

1.2.2.1 Basic Concept

Cellulose, hemicelluloses, and lignin are the major three components of the lignocellulosic raw materials .The conversion of these lignocellulosic feedstock to the biofuel is done majorly by two technologies developed on two platforms. (i) Synthesis gas (or syngas) platform (2) and the sugar platforms.

.2.3 Pretreatment:-

Plant cell wall is a complex structure as mentioned before. The crystallinity of the cellulose, lignin's and hemicelluloses' being physical barriers to the cellulose, and accessible surface area of the cellulose related to the former and latter, makes the cellulosic biomass resistant to hydrolysis (*Mosier et al., 2005*). Therefore pretreatment processes should be performed to remove the lignin, decrease the crystallinity of the cellulose; meanwhile not harming the cellulosic structure; in order to make the biomass susceptible for the further hydrolysis of the cellulose and hemicellulose contents (*Liu et al., 2012; Vancov et al., 2012*). In this framework, pretreatment has come up as the most important step, determining the total cost of the lignocellulose to fuel/chemicals process. Most of the research has been conducted on developing both effective and inexpensive pretreatment methods. Many of the pretreatment

methods focus on disrupting the complex structure of the cellulose-hemicellulose-lignin network, decreasing the crystallinity of the cellulose and removing the lignin and hemicellulose parts in the cell wall (*Wyman et al., 2005; FitzPatrick et al., 2010*).

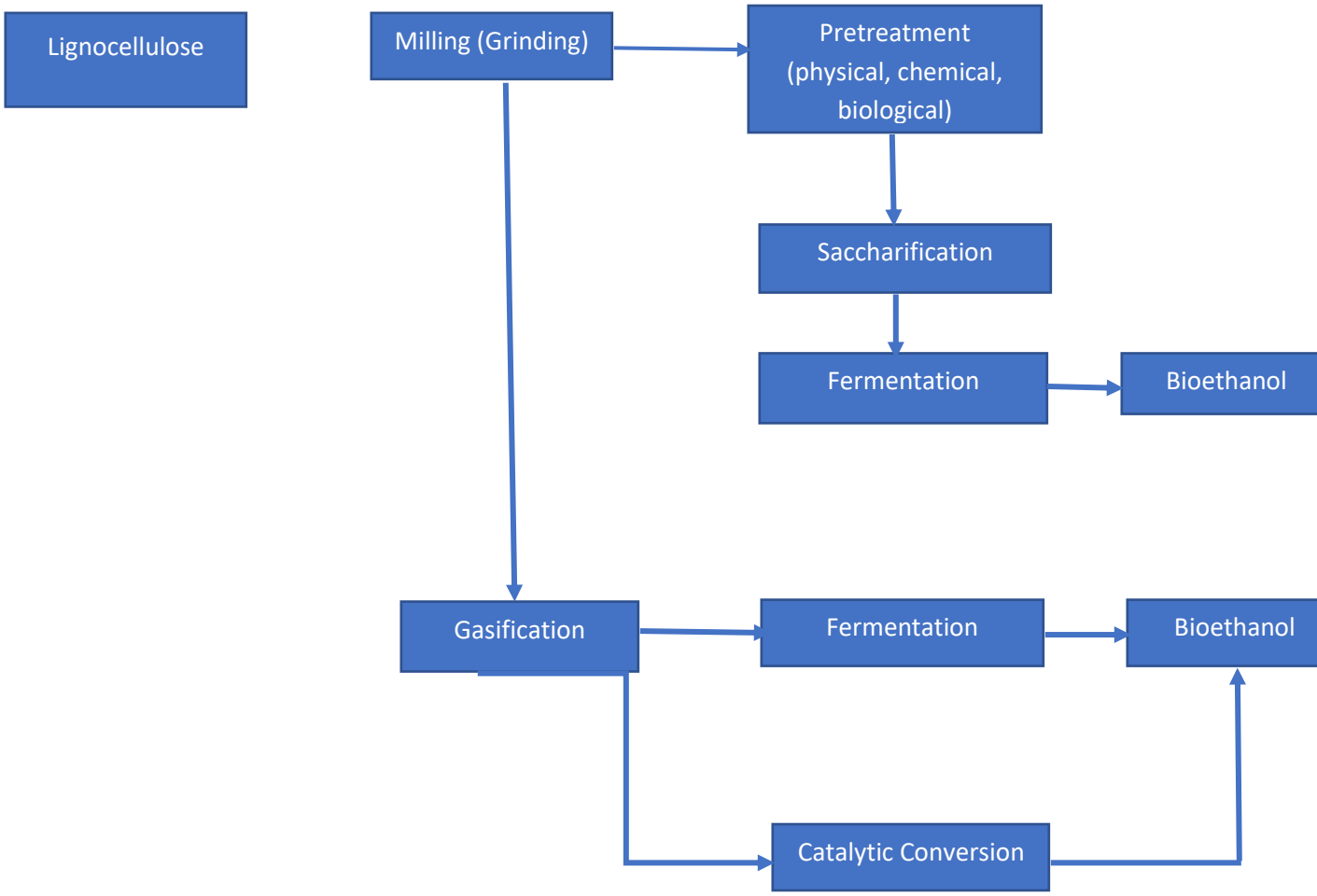


Figure 1.5:-Bioethanol Production Process

1.2.3.1 Physical Pretreatment:

The physical pretreatment of the substrate includes various benefits like it mainly decreases the crystal structure of cellulose, increase the accessible surface area and size of pores, and decrease the crystallinity and degrees of polymerization of cellulose. Commonly used physical treatments are steaming, grinding and milling, irradiation, temperature and pressure.

The particle size of the lignocellulosic feedstock is done by the milling and grinding methods. Thus we can state that the initial steps of the pretreatments are milling and grinding. *Jin et al., 2006* in report stated that the crystallinity of the lignocellulosic feedstock can be reduced with the combination of grinding with other pretreatment process. *Hideno et al., 2009* in his journal reported that ball milling is a better technique then wet disk milling in terms of grinding of the rice straw feedstock. The mall milling process save energy and also increases the glucose recovery. For enzymatic saccharification process different types of grinding techniques have been developed over the last few years like wet disk milling, ball milling and roll milling

1.2.3.2 Chemical Pretreatment:-

The main objective of the pretreatment process applied to the lignocellulosic raw biomass is to reduce the recalcitrance of hemicellulose and lignin parts of the cell wall and also to increase the production of fermentable sugar by the enzymatic saccharification process reported by *Himmel et al., 2007.*

Enzymatic hydrolysis cannot work effectively in the conversion of lignocellulosic raw material to the total reducing sugar without prior chemical treatment process. Ammonia and alkali chemical pretreatment have been proved to be effective in case of rice straw feedstock.

The main aim of the alkali pretreatment is to remove the lignin part of the cell wall and also some part of the hemicellulose which subsequently increases the total accessible surface area of the cellulose. The major types of alkali for the pretreatments available are the NaOH, KOH and Ammonia. There is a Sharpe increase in the yield of reducing sugar by the alkali pretreatment. There are different types in which the alkali pretreatment that can be done firstly at lower temperature with prolong time and very high alkali concentration. In the report submitted by *Gaspar et al., 2007* stated that comparing the acidic or oxidative pretreatment the alkali pretreatment is much more effective one. The alkali pretreatment effectively breaks the ester bonds found between the hemicellulose, cellulose and lignin without fragmenting

the polymers of hemicellulose and also does not distort the cellulosic structure of the lignocellulosic feedstock.

When the acid is used in the diluted form it dominantly effect or solubilize the hemicellulose component present in the lignocellulosic feedstock with a very little or no impact on the lignin component and turn increases the accessibility to the cellulose component. Acidic pretreatment is carried by utilizing different kinds of acids such as H_2SO_4 and HCl . *Jackson et al., 1977* reported that the base pretreatment was mainly done to digest the raw feedstock for animal feeding. The report given Jackson et al., 1977 also stated that the alkali pretreatment solution leads to the distortion of the plant cell wall in turn reducing the hemicellulose, silica and lignin components of the cell wall and also solubilizing the acetic acid esters and uronic substitutes.

Rodríguez-Vázquez et al. reported that the alkali pretreatment done by NaOH is much more effective when compared to other types of alkali pretreatment likes ammonium hydroxide, calcium hydroxide, sodium carbonate and hydrogen peroxide. The sodium hydroxide pretreatment show better degradation of lignocellulosic feedstock and consequently increases the fermentable sugar yield. Rodríguez-Vázquez et al. used alkali (NaOH)solution for the pretreatment of the pith component found in the sugarcane bagasse and concluded that a maximum digestion of 72% at 92C was found. (0.2 g of NaOH per pith gram).

The acidic chemical pretreatment is much more effective and economically feasible as it can increase the anaerobic digestion of the lignocellulosic feedstock reported by *Pandey et al., 2000. Lavarack et al., 2002* reported that solubilization of the hemicellulose is done by diluted acids and this produces a liquid phase which is generally considered as rich in xylose sugar, with very less amounts of lignin derived components therefore this can be considered as an extravagant technique for the recovery of hemicellulose and it can be successfully applied groundnut shell waste. *Geddes et al 2011* in his report suggested that by comparing phosphoric acid and sulphuric acid for the efficiency of hydrolyzing the hemicellulose from lignocellulosic biomass. .

1.2.3.3 Biological pretreatment:

Wyman et al., 1999 in his reported suggested that fungal strain are used to hydrolyze the lignin in the biological pretreatment process. This biological pretreatment technique is simple and economically feasible but the yield is in very low concentration and the rate is also less.

Other advantages of the biological pretreatment process are its energy and chemical input is very low but it require suitable microbial strain for the technique.. *Taniguchi et al., 2005* reported that biological treatment technique is eco-friendly and safe process for the removal or elimination of lignin from the lignocelluloses raw feedstock and it is also cost effective because requires very less investment. The white –rot fungi are found to be the most promising microbial strains for the biological pretreatment technique.

1.2.3.4 Combined pretreatment:

Kumar et al. in his report suggested that single pretreatment singular action on lignocellulosic biomass. They generally reduces degree of polymerization or crystallinity of cellulose. To increase the accessible total surface area there is a need to eliminate the lignin and hemicellulose.. *Rezende et al., 2011* first reported the combination study in which the feedstock was first treated with 1% H_2SO_4 and Then with 1%NaOH(m/v) for higher yield.

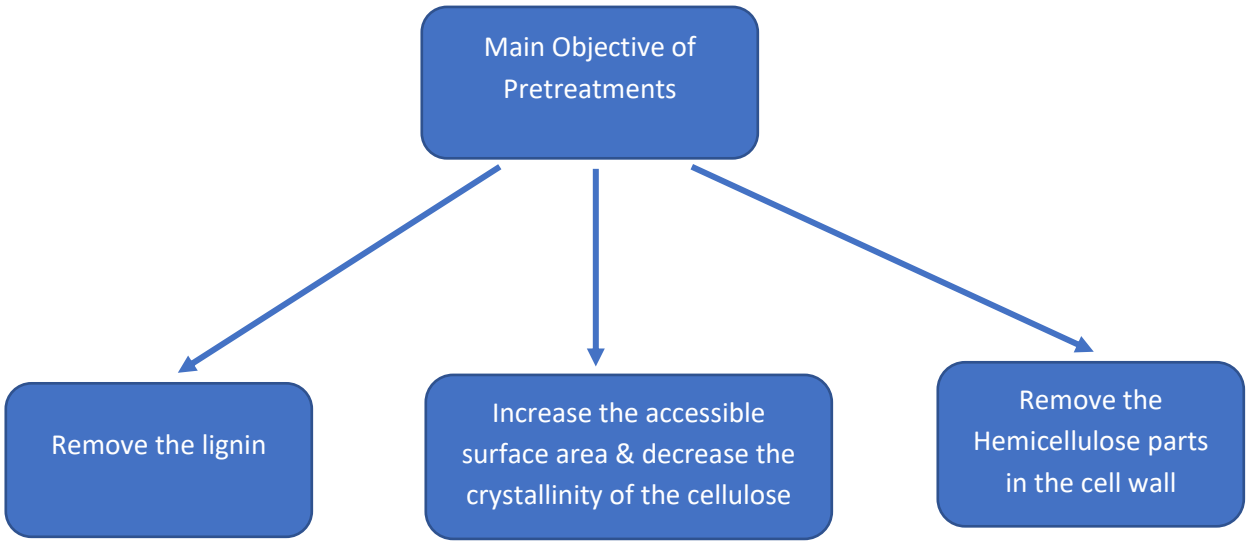


Figure 1.6:- The main objective of pretreatment

1.2.4 Hydrolysis & Fermentation Process:-. The lignocellulosic feedstock is hydrolyzed by the biological enzymes (cellulose and xylase) into fermentable reducing sugars. For the commercialization of the ethanol the following points should be kept into consideration.1) microorganism must utilize a wide range of feedstock,2) reducing sugar yield must be high, 3)microbial strain should be tolerant to high ethanol, pH, temperature and inhibitors present in media. The figure below represent the fermentation pathway by the *Sacchromyces cerevisiae*.

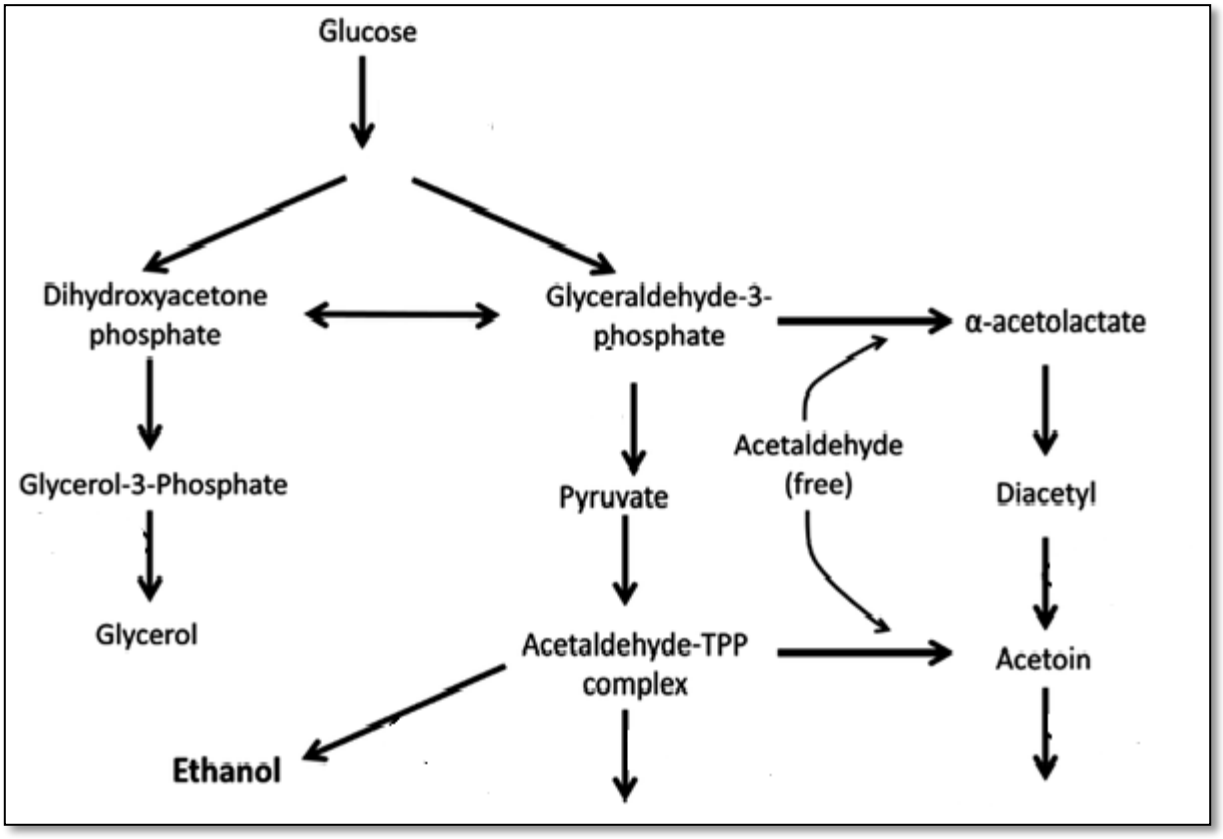


Figure 1.7 Represent the Fermentation process by *Saccromyces cerevisiae*

1.3 AIM & OBJECTIVE

Aim:

Aim of these research is to evaluate the best pretreatment technique for the lignocellulosic raw feedstock to yield higher amount of reducing sugar at the time of biological sccharification process which in turn increases the bioethanol production. And also to find the best optimum way to use this technique at commercial level with economically feasibility.

Objective:

(a) The groundnut shell waste was used as a raw feedstock for bioethanol generation.
(b) Study various pretreatment processes like physical (milling), chemical (alkali and acid) steaming, and biological treatments effect on structure of groundnut shell waste.
(c) Pre-treatment technique is optimized on the basis cellulose content obtained after the process.
(d) Bioethanol generation by *Sacchromyces cerevisiae* & *Zymomonas mobilis*
(e) Compare both aerobic and anaerobic fermentation process.
(f) Extraction of bioethanol by rotary evaporator.
(g) Analyse the bioethanol extracted by both potassium dichromate test and Gas chromatography.
(h) Compare the qualities of the ethanol generated utilizing different pre-treatment techniques on groundnut shell.

1.4 Method and Methodology:-

1.4.1 Collection of raw feedstock:-

The groundnut shell were collected from the local retailer in Behala , Kolkata.

1.4.1.1 Chemical Reagents:-

Sodium hydroxide (Merk, India), Concentrated Sulphuric Acid (Merk, India), Hydrochloric Acid (Merk, India), Agar-Agar (Merk, India), Yeast Extract (Merk, India), Anthrone Reagent (Merk, India), Dinitrosalicylic Acid (Merk, India), Glucose (Merk, India), Magnesium Chloride (Merk, India), Ammonium sulphate (Merk, India), Potassium Dihydrogen Phosphate (Merk, India), Peptone (Merk, India), Dextrose(Merk, India), Czapex dox (Merk, India) , Ethanol , Sodium Nitrate (Merk, India), Magnesium Sulphate Heptahydrate (Merk, India), Ferrous SulpHate (Merk, India) , Potassium Chloride (Merk, India), Dipotassium Hydrogen Phosphate (Merk, India).

1.4.1.2 Microorganism:-

Zymomonas mobilis and *Sacchromyces cerevisiae* strains were obtained from MTCC, pune and Chandigarh respectively. *Aspergillus* strain was isolated from the water sample from sunderban west Bengal.

1.4.2 Pretreatments:-

The plant cell wall is very complicated in the structure. The lignin and hemicellulose crystalline structure is one of the main physical barrier to the accessible total surface area of the cellulose (*Mosier et al., 2005*). Therefore the main objective of pretreatment processes is to remove or reduce the hemicellulose and lignin content in the cell wall , also to reduce the crystalline structure of cellulose ,reduce the degree of polymerization of cellulose ; meanwhile not disrupting the cellulosic structure. (*Liu et al., 2012; Vancov et al., 2012*).

1.4.2.1 Physical treatment Technique:-

The main objective of a effective the physical treatment process is to reduce the size of the feedstock, increase the surface availability and size of the pores of cellulose, reduce the crystallinity of cellulose and polymerization degree of cellulose. The physical pretreatment process is executed prior to other pretreatment processes like chemical and biological pretreatments (*Taherzadeh et al.,2008*).

1.4.2.1.1 Pulverization:-

The physical pretreatment starts with the milling or grinding of the raw feedstock with the main aim of decreasing the size of the lignocellulosic biomass. Crystallinity of Cellulose is also decreased by the Milling process (*Taherzadeh et al., 2008).* The grinding process is done before the other chemical treatment processes. However, with the milling process, only size reduction of the lignocellulosic feedstock takes place without any change or elimination of lignin and hemicellulose. In this process the sun dried groundnut shells were powdered and then sieved with 100 um mesh to get fine powder

1.4.2.2 Chemical treatment technique:-

An effective chemical pretreatment process is done in order to disrupt the lignocellulosic raw material by utilizing a range of different types of chemical agents. This chemical pretreatment technique either decrease or removal the lignin or the hemicellulose.

1.4.2.2.1 Alkali treatment:-

Among the various **p**retreatment techniques the alkali pretreatment is one of the most effective technique. In this process different concentration of alkali (1%-5%)were used to treat 3 gram of shell powder at a constant temperature and pressure .The alkali pretreated feedstock was kept for 30 minutes and then ware further washed with distill water until the pH reached near 7.Then finally the groundnut shell powder was oven dried at 50°C overnight to have a constant weight . The main purpose of alkaline treatment is to eliminate the lignin part of the cell wall and also to remove substitutes of acetyl and uronic acid from the hemicellulose. The alkali treatment can also help in breaking the ester bonds found in hemicellulose and lignin (*Mosier et al., 2005; Taherzadeh et al., 2008*).

1.4.2.2.2 Acid treatment:-

One of the leading effective chemical pretreatment technique are the acid pretreatment (Li et al., 2010). By the Acidic pretreatment process, hemicellulose can be solubilized and furthermore the hydrogen bond found in lignocellulosic biomass is also disrupted. The acidic pretreatment method was approached by 2 different acids (sulphuric acid and hydrochloric acid). The groundnut shell powder was first treated with different concentrations of hydrochloric acid ranging from (0.5% to 2.5%) H_2SO_4 solutions. In the second method the feedstock powder was pretreated with sulphuric acid from (0.5%-2%) range. Both the pre-treated groundnut powder were kept at room temperature for 30 minutes and then washed and dried overnight to have a constant weight. One of the major disadvantage of using of acidic pretreatment can be that lignin part of the cell wall can be condensed which might affect or restrict the further hydrolysis process (*Li et al., 2010*).

1.4.2.2.3Alkali and Acid:-

In this process integration of alkali (NaOH) and Acid (HCl, H_2SO_4) were used in a single pretreatment process and there effect on the raw feedstock was studied. In this method different alkali (1%-5%) pretreated groundnut shell samples were retreated with 1% H_2SO_4 and 1%HCl solutions separately. Both the pre-treated groundnut powder were kept at room temperature for 30 minutes and then washed until the pH reached approximately 7 and then dried overnight at 50°C to have a constant weight and then the pretreated powder was stored in air tight container for further analysis.

1.4.2.2.4 Alkali (steamed) treatment:-

Pretreatment by alkali steamed is one of the most effective pretreatments among all the discussed above ones. In this process different concentration of alkali (1%-5%) were used to treat shell powder at 121°C and 15 psi pressure in the autoclave. The autoclaved feedstock was then cooled ,filtered and washed several times to get the pH near 7.Finally dried overnight at 50°C to have a constant weight and then the pretreated powder was stored in air tight container for further analysis.

1.4.2.2.5 Alkali (steamed) and acidic treatment:-

In this process the alkali (steamed) feedstock were further treated with both sulphuric acid or hydrochloric acid one at a time. Feedstock was then, filtered and washed several times to get the pH near 7.Finally dried overnight at 50°C to have a constant weight and then the pretreated powder was stored in air tight container for further analysis. The results observed after this treatment were also very promising.

1.4.3 Determination of Cellulose & Hemicellulose content in Groundnut shell waste:-

1.4.3.1 Cellulose Estimation:-

Anthrone test is done for the estimation of cellulose content. In this test the carbohydrate gets dehydrated when reacted with concentrated sulphuric acid (H_2SO_4) forms furfural. This furfural which is formed in the previous step reacts with anthrone reagent to give bluish green coloured complex. This coloured complex intensity is measured by spectrophotometer at 630nm (Perkin Elmer, Germany).

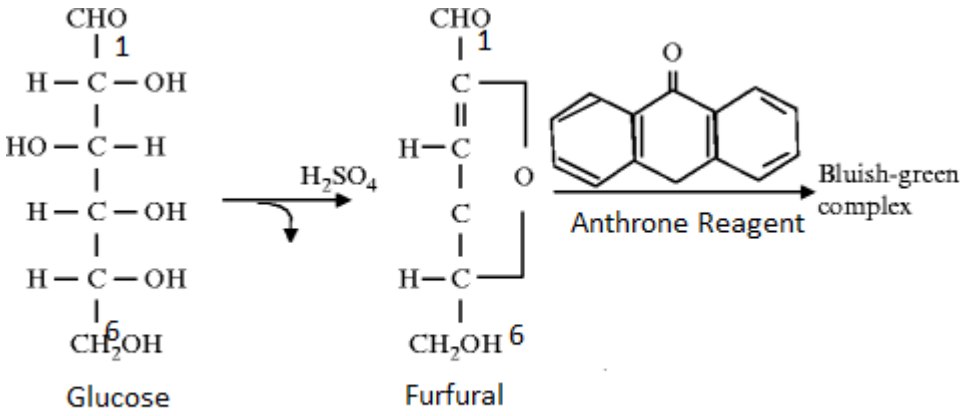


Figure 1.8:- Anthrone test principle

1.4.3.1.1Chemical Reagents:-

1 Pretreated (acid, alkaline, acid+ alkaline) groundnut shell waste

2 Anthrone Reagents: 100ml of ice cold H_2SO_4(95% conc.) was take in which 200mg of anthrone was dissolved to prepare the anthrone reagent solution. The solution was prepared fresh and refrigerated for 2hrs before use.

3. 67% sulphuric acid (H2SO4).

1.4.3.1.2 Standard curve preparation:-

For the standard curve preparation cellulose (10 mg) was dissolved in 100 ml of double distilled water. From the stock solution aliquots of 0.25, 0.5, 0.75 and 1 ml were taken. Further 10 ml of anthrone reagent was added and the solution was heated in water bath for 15 minutes. Finally the absorbance was measured at 630nm and the standard graph was plotted.

4.3.1.3 Experiment:-

- 0.5 gram of each pretreated samples were taken in different test tubes.
- 10ml of 67% H_2SO_4 was added to each test tubes and was kept for 1hr at room temperature.
- 1ml from the previously prepared solution was taken and was further diluted to 100ml by adding distil water.
- 1ml from the above stock solution was taken to which 10ml of Anthrone reagent was added and thoroughly mixed.
- The test tubes were heated in the water bath at boiling for 15 minutes.
- The above solutions in the test tubes were cooled and finally the absorbance was measured at 630nm.

1.4.3.2 Determination of Hemicellulose content:-

For the determination of the hemicellulose content in the lignocellulosic raw material firstly the Acid Detergent Fibre(ADF) and Neutral Detergent Fibre(NDF) are calculated.

Hemicellulose=%NDF-%ADF

Determination of %Neutral Detergent Fibre(NDF):-

Neutral detergent solution preparation:-6.81 gram of sodium borate decahydrate and 18.61 gram of Disodiumethylene diaminetetraacetate were added to 200ml of distill water by continuous stirring and heating. 100ml of sodium lauryl sulphate (30g) solution and ethoxy ethanol (10ml) were added to the above prepared solution. Furthermore 100ml (4.5%)sodium hydrogen phosphate was added and finally the volume was made upto 1 liter and the pH was adjusted to 7.

1.4.3.2.1 Procedure:-

10 ml of neutral reagent solution was added to each pretreated substrate and further the following reagents were added:-

Sodium sulphite (0.5g) and Decahydronapthalene (2ml) and was added to the above solution and then was heated in water bath at boiling 1 hour. The above solution was cooled and further filtered by sintered glass crucible (G-2). The filtrate was washed several times by hot water and then washed with acetone twice to thrice. The residue obtained after the treatment was dried at 100^{o}C until the residue obtain a constant weight .The residue was cooled in the desiccator and the weight of the residue was measured and recorded. The residue obtained was designated as neutral detergent fibre (NDF).

Determine the % Acid Detergent Fibre(ADF):-

Acid Detergent reagent preparation: - The reagent solution was prepared by adding (2%) cetyl trimethyl ammonium bromide in sulphuric acid(1N).

1.4.3.2.2 Procedure:-

100ml of Acid detergent reagent solution was added to 1 gram each of the pretreated substrate. The above solution was heated at 100C for 1 hour. The above solution was then cooled and further filtered by sintered glass cruicible (G-2) and was washed thrice with hot water to remove the reagent and to get a constant pH. The residue was further washed with acetone to make it colourless and finally dried overnight to get a constant weight.

1.4.2.3 Biological Treatment Process:-

Cellulase enzyme Production by simultaneous saccharification and fermentation (SSF):-

1.4.2.3.1 Inoculum preparation:-

Aspergillus species (Genbank: MH119104)was isolated from the soil sample collected from the sunderban area of West Bengal. The identified and isolated fungal strain was further subcultured on the potato dextrose agar media as well as modified czapekdox medium. The Aspergillus strain was streaked on the agar plates and were incubated at 35°C for 7 days. After 7 days fully sporulated plates were obtained which were further treated with 0.1% Tween 80 to collect the spores. Due to the detergent properties of tween 80, spores were dislocated and were gently collected from the plate using pipet. This spore suspension was then centrifuged and washed several times to remove the tween and finally refrigerated to be used as inoculum.

1.4.2.3.2 Media Preparation for Enzyme Production:-

Basal Media was prepared by adding salts of (0.2%) Sodium Nitrate ($NaNO_3$,) , (0.05%) Potassium chloride (KCl), (0.05%) magnesium sulphate ($MgSO_4$), (0.001%) Ferrous sulphate ($FeSO_4$),(0.1%) Dipotassium hydrogen phosphate (K_2HPO_4) and Peptone. The nitrogen source used in this media was the Peptone.

1.4.2.3.3 Experiment:-

Three grams each of the pretreated groundnut shell were taken into 250ml Erlenmeyer flasks and were then humidified with freshly prepared basal media. The media containing the pretreated substrate was sterilized by autoclaving at 121°C and 15psi pressure. The media containing the flask were then inoculated with 3ml of the spore suspension. The flask was incubated at 35°C in the incubator for 6 days.

In the current research the effect of different important factors on the hydrolysis process (amount of reducing sugar produced) has been studied. The *Aspergillus sp*. used in the process breakdown the long chains of polysaccharides into simpler components such as – glucose, fructose, galactose and maltose etc.

1.4.2.3.3.1 Temperature:-The optimum temperature of the *Aspergillus sp*. is 35-37°C. To determine whether *Aspergillus sp*. can yield the reducing sugar better in any other temperature or not, different ranges of temperature are studied (26-46°C).

1.4.2.3.3.2 Substrate:-

The standard substrate concentration used in this experiment was about 3 grams. To find the better sugar yield the substrate concentration was varied from 1gram to 5 gram.

1.4.2.3.3.3 Dose:-

The dose concentration for the groundnut shell powder used in the standard process was 3ml of (1.25x106 spore/ml). So, dose concentration was varied to study more detail effect of dose on the yield of reducing sugar by the *Aspergillus sp*. The dose was varied in a range of 1ml to 5ml.

1.4.2.3.3.4 pH**:-**

The effect of pH on the sugar yield was observed by varying the pH from 4 to 10.

1.4.3 Estimation of the total reducing sugar by DNS:-

3,5-Dinitosalicylic acid(DNSA) is extremely used in biochemistry for the determination of reducing sugar. DNSA can detect the presence of free carbonyl group present in the reducing sugars. In this experiment the oxidation of the ketone functional group (in fructose) and aldehyde functional group (in glucose) involves. During this reaction the DNSA is generally reduced to 3-amino-5-nitroslicylic acid (ANSA) which in turn shows reddish brown colored complex which has maximum absorbance at a wavelength of 540nm.

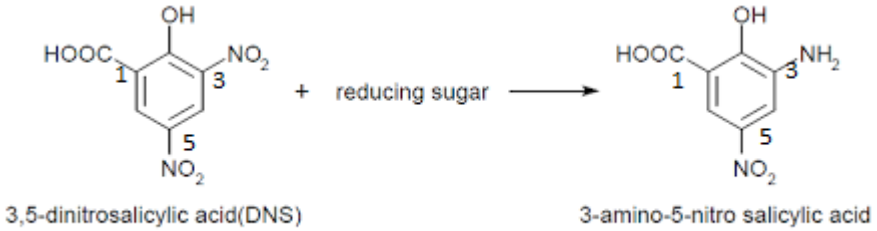


Figure 1.9:-DNS result

1.4.3.1 Dinitrosalicylic acid (DNS) reagent preparation:-

1.4.3.1.1 The DNS reagent was prepared by adding dinitrosalicylic acid (DNS) (1gram), crystalline phenol (200mg), sodium sulphite (50mg) in 100ml of 1%NaOH solution by continuous stirring.

Sodium-potassium tartrate solution was prepared by adding 40 gram of Rochelle salt in 100ml of distill water.

1.4.3.1.2 Standard stock and curve preparation:-

The standard stock solution was prepared in 100ml of distill water by adding 100mg of commercial glucose powder. The working solution was prepared by taking 10 ml aliquot from the stock solution and making the volume to 100 ml by adding distill water. Further different concentration of the working solution were taken (0.2,0.4,0.6,0.8 and1 ml) and the final volume was made to 3 ml by adding water. 3ml of the DNS reagent was added to each test tube and then heated for about 10 minutes. After cooling and appearance of reddish brown colour 1 ml of 40% Rochelle salt was added and thoroughly mixed. Finally the absorbance was recorded at 540nm using Spectrophotometer (Perkin Elmer, Germany).

1.4.3.1.2 Experiment:-

1ml filtrate each of the hydrolysed media(which was previously inoculated by aspergillus sp.) was taken and 2 ml of distil water was added to it to make up the volume to 3ml. 3ml of the DNS reagent was added to each test tube and then heated for about 10 minutes. After cooling and appearance of reddish brown colour 1 ml of 40% Rochelle salt was added and thoroughly mixed. Finally the absorbance was recorded at 540nm using Spectrophotometer (Perkin Elmer, Germany).

1.4.4 Fermentation

1.4.4.1 Microbial Species:-

Saccharomyces cerevisiae (MTCC 170)

Saccharomyces cerevisiae is a very common species of yeast. It is universally used microorganism for the purpose of fermentation. *Saccharomyces cerevisiae* is capable of fermenting polysaccharide majorly the hexose sugars like the glucose.

The growth medium (YEPD) for *Saccharomyces cerevisiae* is

Working Solution:-

Yeast extract – 0.3gm

Agar – 1.5gm

Distilled water – 100ml

Stock solutions :

Peptone - 5.00g / 50mL

Dextrose - 10.00g / 50mL

The stock solution were then sterilized by autoclaving at 121°C and 15psi pressure 10ml of the stock solutions were simultaneously added to the working solution. The solution were poured in the petri plate and allowed to cool until gel formation takes place. The identified and isolated *Saccharomyces cerevisiae* was subcultured on the petri plate by streaking and incubated 37°C. Growth was observed between 2-3 days.

Zymomonas mobilis

Zymomonas mobilis is a gram negative, non sporulating, polarly flagellated, rod shaped ,facultative anaerobic. *Zymomonas mobilis* have notable bioethanol generating properties which can some time surpass the capability of yeast. This microorganism can ferment more sugar than *Saccromyces cerevisiae* such as arabinose and xylose which cannot be fermented by yeast.

1.4.4.2 Growth Media:-

The growth medium for this anaerobic bacteria is :

Working solution:-

Yeast extract – 1.0gm

Agar – 1.5gm

Distilled water – 100 ml

Stock solutions:

Glucose - 2gm / 10mL

Magnesium chloride - 1gm / 10mL

Ammonium sulphate - 1gm / 10mL

Potassium di-hydrogen phosphate - 1gm / 10mL

The stock and the working solutions were autoclaved and then 10mL of glucose solution was added to the working solution and 1ml each of the other stock solutions were simultaneously added to the working solution. The *Zymomonas mobilis* was subcultured on the previously mentioned growth media. The media plates were incubated at 35°C and growth was observed within 3 days.

1.4.4.3 Experimental procedure:-

Two Fermentation condition, two organisms

The media containing the substrates were further treated with both microbial strains, to identify the amount difference in the product by fermentation in both the cases.

1.4.4.3.1 Aerobic Fermentation-

3gm of pretreated groundnut shell powder which was previously pretreated with Aspergillus strain was further autoclaved at 121°C and 15psi pressure to kill the fungal strain. The same media containing substrate was filtered and the filtrate media was divided into 2 equal parts and one was treated with *Saccharomyces cerevisiae* and another was treated with *Zymomonas mobilis*. Fermentation showed by *Zymomonas mobilis* was observed within 3 days at 35.5°C whereas *Saccharomyces cerevisiae* showed it after 4 days at 37°C.

1.4.4.3.2 Anaerobic fermentation –

3gm of pretreated groundnut shell powder which was previously pretreated with Aspergillus strain was further autoclaved at 121°C and 15psi pressure to kill the fungal strain. The same media containing substrate was filtered and the filtrate media was divided into 2 equal parts and one was treated with *Saccharomyces cerevisiae* and another was treated with *Zymomonas mobilis.* Both the media was sparged by nitrogen to remove the oxygen as well as to maintain the anaerobic condition. The flask containing the media were sealed with Para film and were kept in anaerobic bioreactor for 3 days after that bioethanol was recovered and analyzed.

1.4.5 Extraction of bioethanol:-

The fermented samples obtained after 3-4 days, of pretreated groundnut shell both by *Zymomonas mobilis* and *Saccharomyces cerevisiae* and were taken for the extraction of bioethanol by the Rotary Evaporator. The water-ethanol mix was obtained by evaporator was further analyzed for the estimation of ethanol in each pretreated samples..

1.4.5.1 Estimation of ethanol by Dichromate test:-

The ethanol water mix obtained after the fermentation process was further used to estimate the amount of ethanol in it.

1.4.5.1.1 Potassium dichromate reagent preparation:-

In I liter volumetric flask 400 ml of distill water was added, then carefully 325 ml of concentrated sulphuric acid was added to the volumetric flask. After through mixing the solution was allowed to cool down to (70-90°C).Potassium dichromate powder 33.768 gram was added to the previously prepared solution and finally the volume was made upto 1 liter by adding double distill water.

1.4.5.1.2 Preparation of standard solution:

The standard solution of Ethanol was prepared by mixing pure ethanol into water in a range of 0-20% (v/v).

1.4.5.1.3 Standard curve for ethanol preparation:

Standard plot was obtained by taking 1mL aliquot of the standard solutions(0-20%) in a volumetric flask of 50 ml .The beaker was already containing 25 ml of freshly prepared potassium dichromate solution($K_2Cr_2O_{7)}$ .9ml distill water was added to make the volume 35ml each and then this samples were heated in water bath at 65°C for 25 minutes. Finally after cooling of the above solution 15 ml of water is added so that the final volume becomes 50ml. Absorbance was measured at a wavelength of 600nm by UV-VIS spectrophotometer.

1.4.5.1.4 Estimation of Bioethanol in the fermented sample:-

1ml of the extracted ethanol-water mix was taken in 50 ml volumetric flask previously containing 25 ml of freshly prepared potassium dichromate solution($K_2Cr_2O_{7)}$ .9ml distill water was added to make the volume 35ml each and then this samples were heated in water bath at 65°C for 25 minutes. Finally after cooling 15 ml of water is added to the above

solution so that the final volume becomes 50ml. Absorbance was measured at a wavelength of 600nm by uv-vis spectrophotometer.

1.4.6 Characterization:-

1.4.6.1 Fourier Transform Infrared (FTIR) Spectroscopy:

The infrared spectra images of the cellulose and lignin (wave numbers in cm-1) were produced on a Perkin Elmer spectrophotometer (Magma - IR 560 E.S.P) utilizing KBr disk containing the finely grounded samples in 3%.

1.4.6.2 HPLC (high-performance liquid chromatography):-

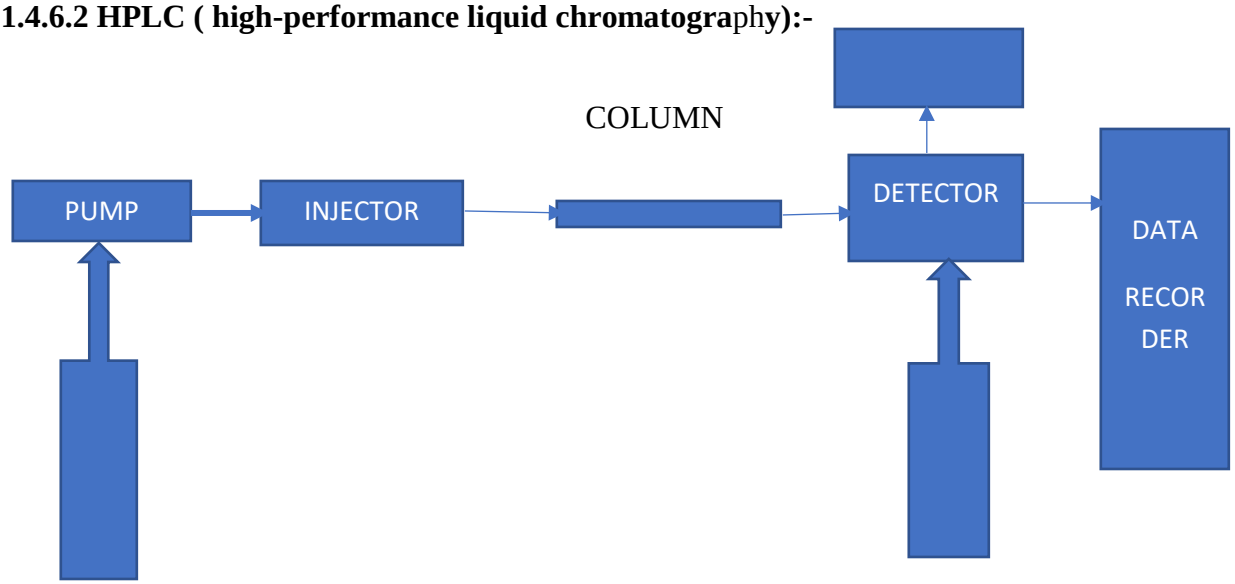


Figure 2.0:-HPLC Principle

High-performance liquid chromatography is a process which is used to separate identify or quantify each component of a mixture. HPLC work on principle that some components/molecules of the mixture takes longer time than other components to pass the chromatography column. This retention time depends upon the affinity of the molecule towards the mobile phase (gas or liquid) and also the stationary phase (solid or liquid). The one which have greater affinity towards the stationary phase have higher retention time to pass through the column and vice versa.

1.5 Result And Discussion:-

1.5.1 Physical Pretreatment:-

1.5.1.1 Milling:-

Figure2.1-(Peanut shell waste) (Groundnut after milling) (Peanut After Pretreatment)

1.5.2 Chemical Pretreatment:-

1.5.2.1 (Alkali and Alkali (steamed))Pretreatment:- treat .

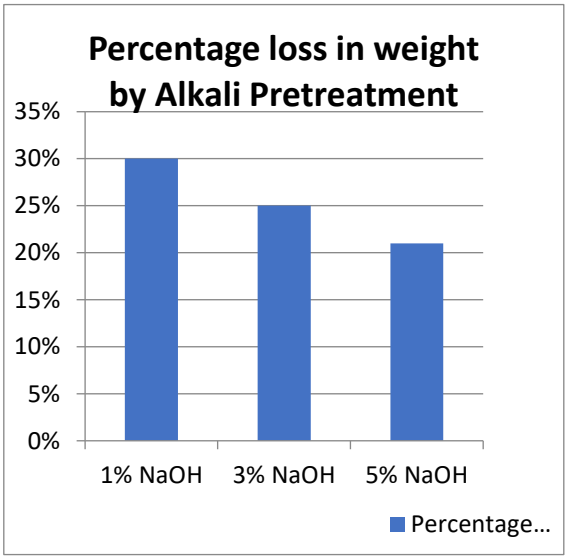


Figure 2.2 (Alkali Pretreatment)

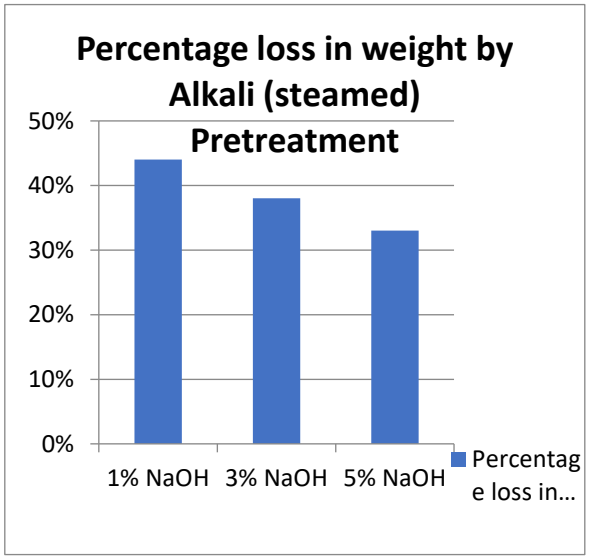
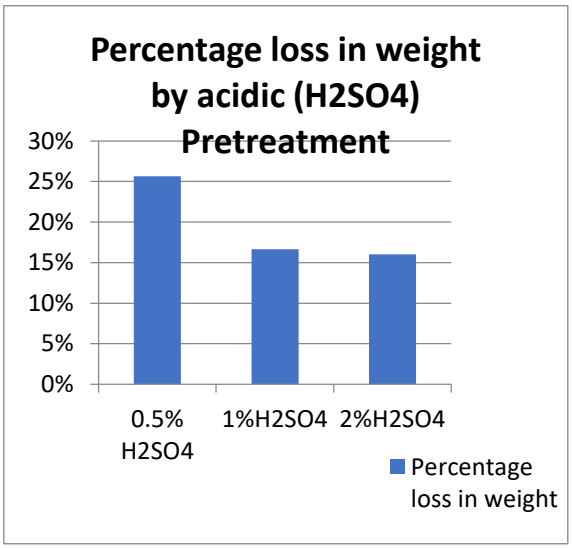


Figure 2.3 (Alkali steamed Pretreatment)

Discussion:- The lignocellulosic raw biomass were treated with different alkaline concentration(NaOH) (1%,3% and 5%) .This method was approched by two method in the first half peanut waste were treated with 1%,3% and 5% and then autoclaved at 121°C and 15 psi pressure. In the next part the shell waste were directly treated with alkali without autoclave kept for 30 minutes and then washed and dried. From the result observed we can concluded that alkali(steam) pretreatment is much better than simple alkali method .The maximum weight loss encountered by steam pretreatment is approximately 44% and by alkali treatment it is 30%.

1.5.2.2 Acidic(H_2SO_4 & HCl) Pretreatment:-



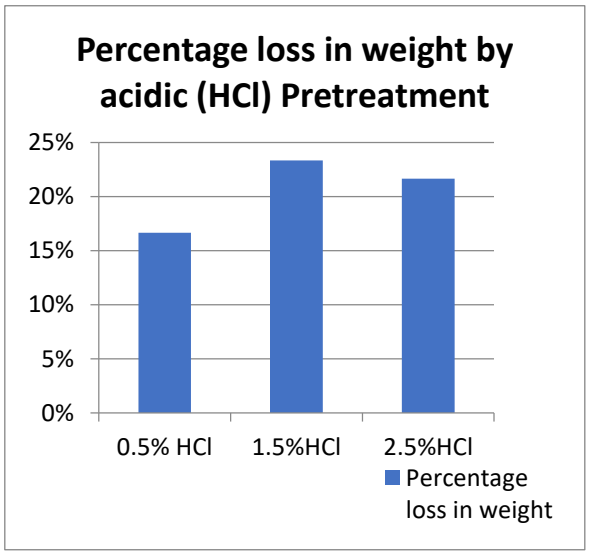
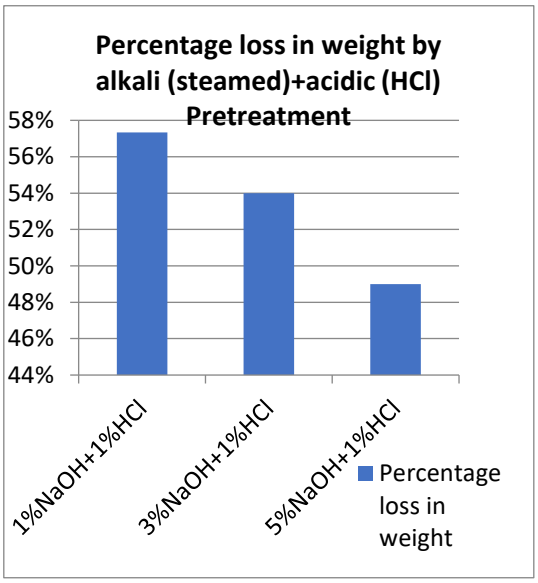


Figure 2.4 (H_2SO_4 Pretreatment) **Figure 2.5 (HCl Pretreatment)**

Discussion:- In this present study two different types acid pretreatment (HCl and H_2SO_4) has been studied on the lignocellulosic biomass. The hydrochloric acid (HCl) was taken in different range of concentration (0.5%,1.5% and 2.5%). And the maximum weight loss encountered was 23.33% by HCl. The sulphuric acid showed maximum weight loss of 25.66% when treated with 0.5% H_2SO_4. The H_2SO_4 were taken in the range of (0.5%,1%,2%)

1.5.2.3 Alkali(Steamed)+Acidic(HCl & H_2SO_4):-

Discussion:-Kumar et al reported that single pretreatment technique have singular effect on the lignocellulosic feedstock. .In this procedure the raw material is first pretreated NaOH at different concentrations(1%,3%and5%).The alkali treated biomass was then autoclaved at 121°C and 15psi pressure ,washed and oven dried to get a constant weight. Ultimately the autoclaved biomass was again pretreated with either 1% HCl or 1%H_2SO_4 respectively. The alkali (steam) +HCl pretreatment showed better results than h2so4 with maximum weight loss of about 57.33%. The alkali (steam) +H_2SO_4 pretreatment showed maximum weight loss of about 49%.



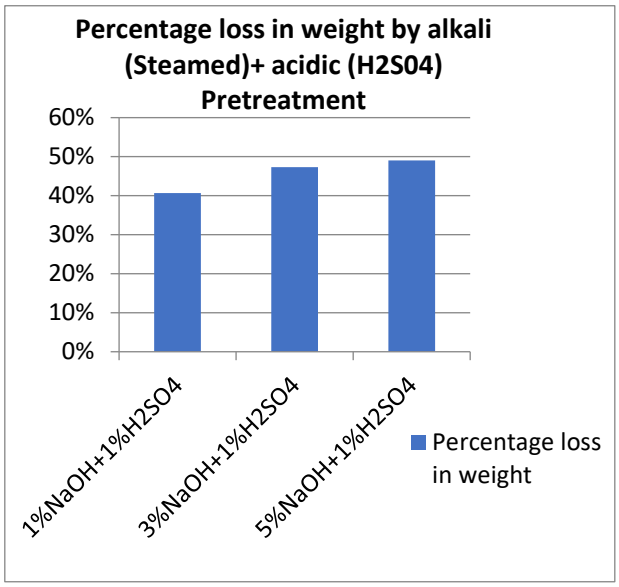
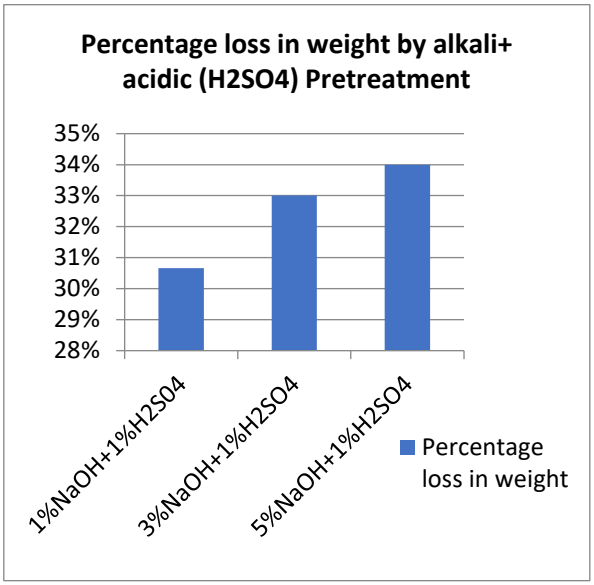


Figure 2.6(Alkaline(S)+ HCl) Pretreatment **Figure 2.7(Alkaline(S)+ H_2SO_4) Pretreatment**

1.5.2.4 Alkali +Acidic(HCl & H_2SO_4):-

Discussion:- In this procedure the raw material is first pretreated NaOH at different concentrations(1%,3%and5%).. Ultimately the biomass was again pretreated with either 1% HCl or 1%H_2SO_4 respectively. The maximum weight loss encounter was 34% by alkali+HCl.



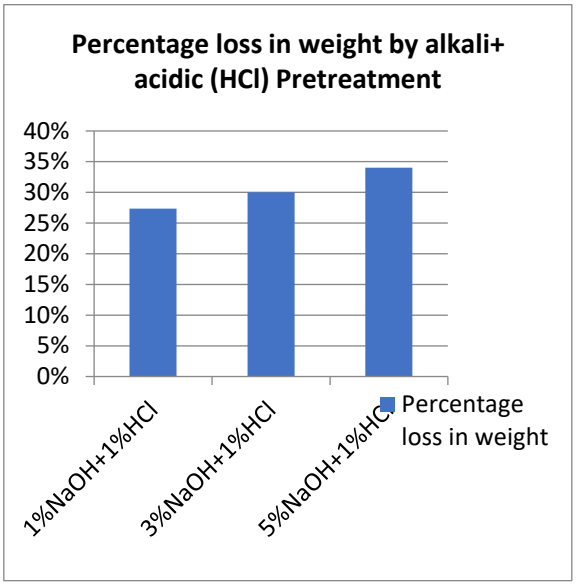


Figure 2.8:-(Alkaline+ HCl) Pretreatment **Figure 2.9:-(Alkaline+ H_2SO_4) Pretreatment**

1.5.3 Cellulose Content of Groundnut shell:-

Table 1.2 :-Anthrone result

Pretreated lignocellulosic raw material	Amount of Cellulose content (mg/ml)
Raw groundnut shell waste	3.78 mg/ml
1% NaOH treated	5.94 mg/ml
3% NaOH	5.10 mg/ml
5% NaOH	4.35 mg/ml
1% NaOH (Autoclave)	7.68 mg/ml
3% NaOH (Autoclave)	8.04 mg/ml

5% NaOH (Autoclave)	10.74 mg/ml
0.5% HCl	8.77 mg/ml
1.5% HCl	9.20 mg/ml
2.5% HCl	6.99 mg/ml
0.5% H_2SO_4	9.17 mg/ml
1% H_2SO_4	8.42 mg/ml
2% H_2SO_4	7.58 mg/ml
1% NaOH +1%HCl	7.22 mg/ml
3% NaOH +1%HCl	5.42 mg/ml
5% NaOH +1%HCl	5.35 mg/ml
1% NaOH +1%HCl(autoclave)	8.36 mg/ml
3% NaOH +1%HCl(Autoclave)	11.36 mg/ml
5% NaOH +1%HCl(Autoclave)	14.94 mg/ml
1% NaOH +1% H_2SO_4	6.47 mg/ml
3% NaOH +1% H_2SO_4	8.64 mg/ml
5% NaOH +1% H_2SO_4	7.23 mg/ml
1% NaOH +1% H_2SO_4 (Autoclave)	10.31 mg/ml
3% NaOH +1% H_2SO_4 (Autoclave)	7.92 mg/ml
5% NaOH +1% H_2SO_4 (Autoclave)	10.99 mg/ml

Discussion:- The cellulose content of the raw groundnut shell waste was estimated to be 3.28mg/ml by the anthrone test. In order to increase the cellulose content of the shell waste different type of the pretreatments are done on the groundnut shell. The main aim of the different types of pretreatments are to increase the cellulose content and decrease the hemicellulose content. In various journal it has been reported that the acid pretreatment breaks the crystallinity of cellulose and release different types of sugar like arabinose, glucose and fructose. Anwarl etal in his journal "Optimization of Dilute Acid Pretreatment Using Response Surface Methodology for Bioethanol Production from Cellulosic Biomass of Rice Polish" reported similar results. The acid pretreatment (HCl, H_2SO_4) increases the cellulose content to 9.20 mg/ml and 9.17mg.ml respectively. The alkali steam pretreatment also increase the cellulose content which was initially found in the shell waste to 10.74mg/ml. and the hemicellulose content was found to be 5.60% The cellulose content obtained after the 5% NaOH +1%HCl(Autoclave) was maximum with 14.94mg/ml and the hemicellulose content decreased to 4% from 15.41% found in the raw groundnut shell.

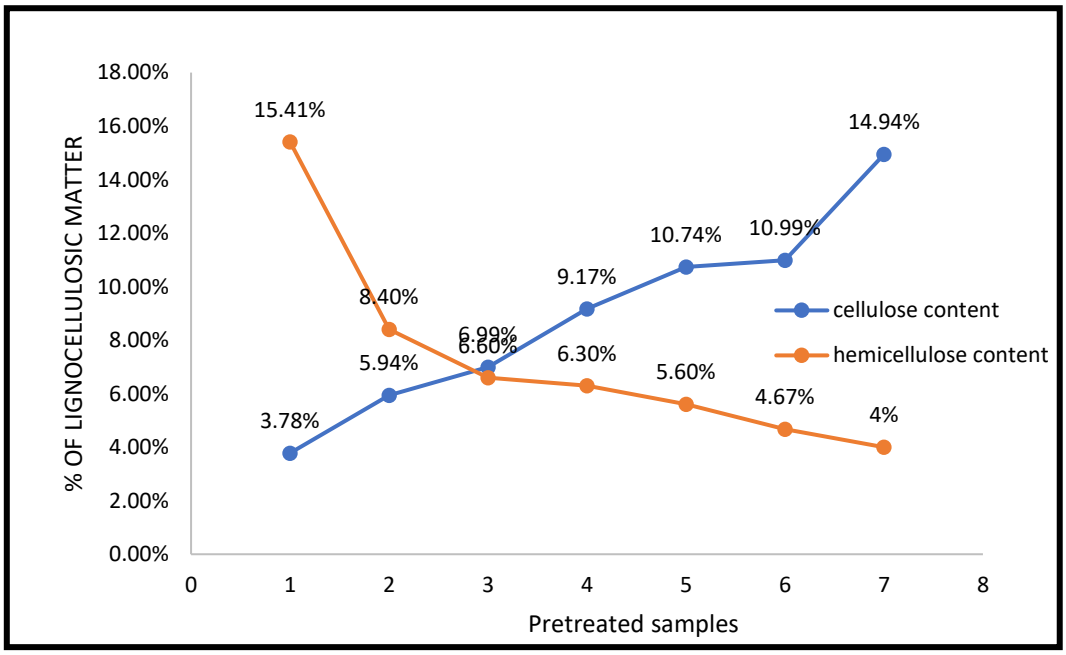


Figure 3.0- GraPH of percentage of cellulose and hemicellulose in groundnut shell

1.5.4 Biological Pretreatment:-

The total amount of reducing sugar produced by hydrolysis process on the lignocellulosic biomass by *Aspergillus sp.* is shown in the grapHs below. Different types of parameters (such as temperature, dose, pH, and substrate) are applied on the lignocellulosic biomass and their effect on the yield of reducing sugar by the *Aspergillus sp.* is observed and reported. Analysis of the reducing sugar is done by using Di-nitrosalacylic acid test. The biological conversion of the polysaccride (cellulose) into its monomeric sugar glucose is done in two step process. In the first part the cellulose is broken down to cellobiose by the action of enzyme beta-1,4 glucanase. This cellobiose is a glucose dimer which is consequently broken to glucose by the beta-glucosidase enzyme

Cellulose ⟶ Cellobiose ⟶ Glucose

b-1,4 glucanase . b-glucosidase

1.5.4.1 Effect of Substrate:-

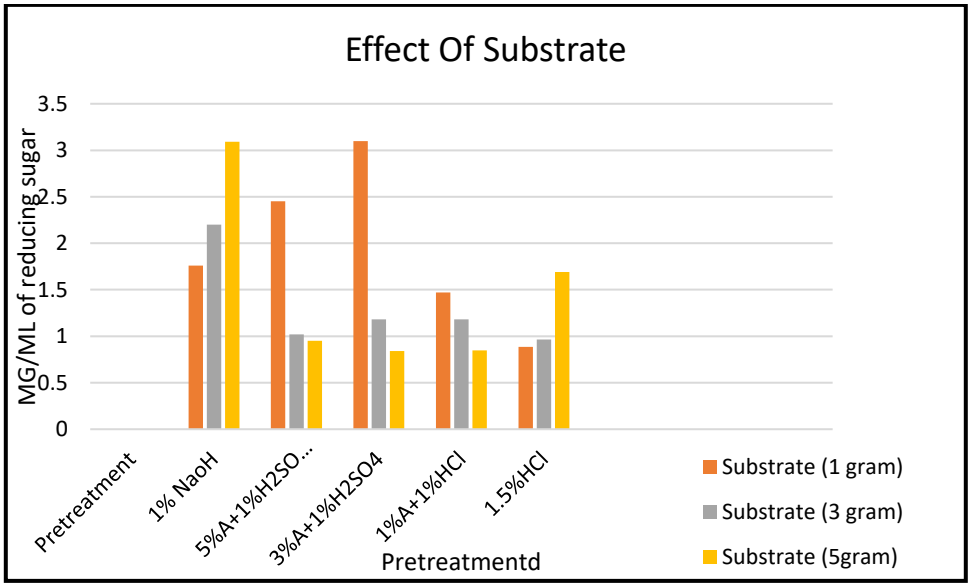


Figure 3.2- Effect of Substrate on Reducing Sugar Production:-

Discussion:- The concentration of substrate have a significant effect on the reducing sugar yield. From the above experiment we can conclude that with the combination pretreatment the amount of substrate can be reduced for the sugar yield that is maximum sugar is produced at 1 gram substrate concentration and minimum at 5 gram. Concentration of substrate at 1% is found to be perfect or ideal condition for Aspergillus sp. to generate the enzyme cellulose because enzyme adsorption on the substrate and oxygen diffusion will be optimally run at this substrate concentration (*Stewart and Parry,1981*). In case of 1%NaOH and 1%HCl the maximum reducing sugar yield is at 5 gram substrate concentration.

1.5.4.2 Effect of Dose:-

Table 1.3 Effect of the Dose:-

Pretreatment	Dose($1.25x10^6$ Spore/ml)	Dose($3.75x10^6$ Spore/ml)	Dose($6.25x10^6$ Spore/ml)
1% NaoH	**2.03**	1.87	1.73
5%A+1%H2SO4(A)	1.75	1.48	1.36
3%A+1%H2SO4	1.95	1.34	1.88
1%A+1%HCl	1.56	2.10	1.36
1.5%HCl	1.74	1.59	1.76

Discussion:- The above grapH represent that effect of dose on the amount of total reducing sugar produced *Aspergillus sp.* The dose are taken in a range of (1.25x106,3.75x106 and 6.25x106). From the above data it can be concluded that in most of the cases that maximum sugar yield was at the minimum dose concentration(1.25x106spore/ml) expect in the case of

1%alkali+1%HCl. But the difference between the sugar yield is not very much .The decrease in the reducing sugar yield with increase in spore volume can be explained as the increase of microorganism there is depletion of the nutrient pool and also there is a decrease in the oxygen availability at the early stage of the experiment. Alternatively we can also state that the reduction in cellulose generation after increasing the inoculum volume is due to the creation of an anaerobic condition and imbalance nutrition as a result of rapid growth of aspergillus sp. at very early stage of the experiment.

1.5.4.3 Effect of Temperature:-

Discussion:- Aspergillus sp. have a optimum temperature of growth at 36°C. Therefore to determine the effect of temperature on the yield of reducing sugar production three different temperatures were varied from 26°C, 36°C and 46°C. And it was observed that in most cases the yield of sugar was maximum at 36°C expect in case of 1%NaoH and 1.5%HCL .They both showed better result at 26°C .But the amount of reducing sugar produced at 26°C was nearly same to that 36°C. In each case the yield of sugar was minimum at the highest temperature that is 46°C. Mrudula etal 2011 reported that 83% of cellulose production took place at 35°C in his journal paper "Production of cellulase by *Aspergillus niger* under submerged and solid state fermentation using coir waste as a substrate".

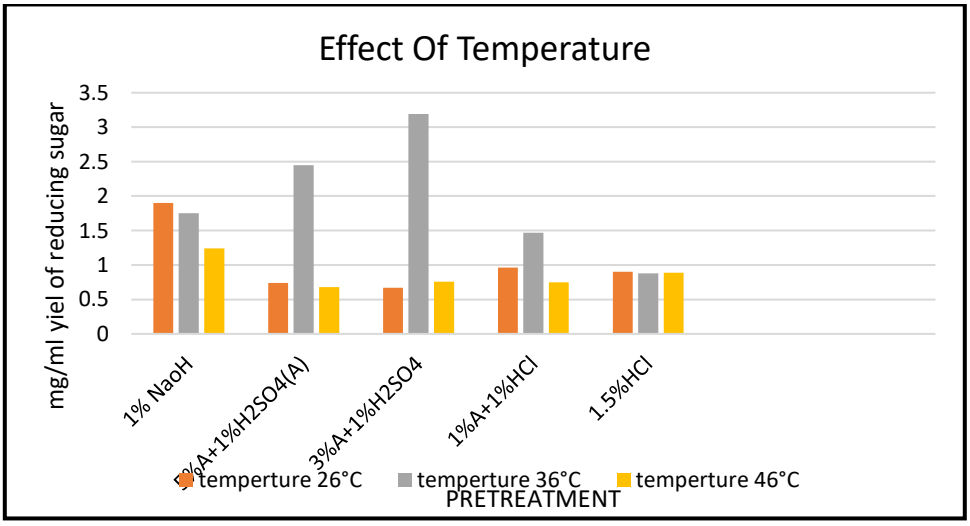


Figure 3.3 Effect Of Temperature:-

1.5.5 Fermentation:-

Fermentation of the lignocellulosic biomass or ethanol production is carried out by two different microbial strain *Sacchromyces cerevisiae* and *Zymomonas mobilies* for about 3 days at a optimum temperature of 34-37C. Both HPLC and dichromate test were performed to determine the amount bioethanol present in the ethanol water mix. The medium which was previously hydrolyzed was further fermented by the two microbial strains of *Zymomonas mobilis (MTCC 92)* and *Sacchromyces cerevisiae (MTCC 170).* Aerobical and anerobical conditions were provided for the fermentation step.

1.5.5.1 Dichromate Result For Aerobic fermentation:-

Discussion:-The maximum amount of Ethanol generated by *Zymomonas mobilis and Sacchromyces cerevisiae aerobically is about* 23.29% (from 1%NaOH treated shell powder) and 15.33% (from 1%NaOH+1%HCl treated shell waste) respectively. **Anaerobically** by ***Sacchromyces cerevisiae*** 27.75% of ethanol is generated from the 1.5%HCl treated peanut shell waste whereas in case of *Zymomonas mobilis* ethanol produced is about 24.72% from 5%NaOH +1%HCL treated shell waste by only by using 1 gram of each treated substrates. In Anaerobic fermentation better results of bioethanol generation can be reported this is due to the fact that both the microbial strains used for this process are facultative .Anaerobic fermentation shows better results when the carbon source used in the media is glucose reported by (*cheng et al 2014)* . (*Rabah et al 2011*) in her journal reported that bioethanol production from groundnut shell waste have shown poor result when *Zymomonas mobilis strain is used.*

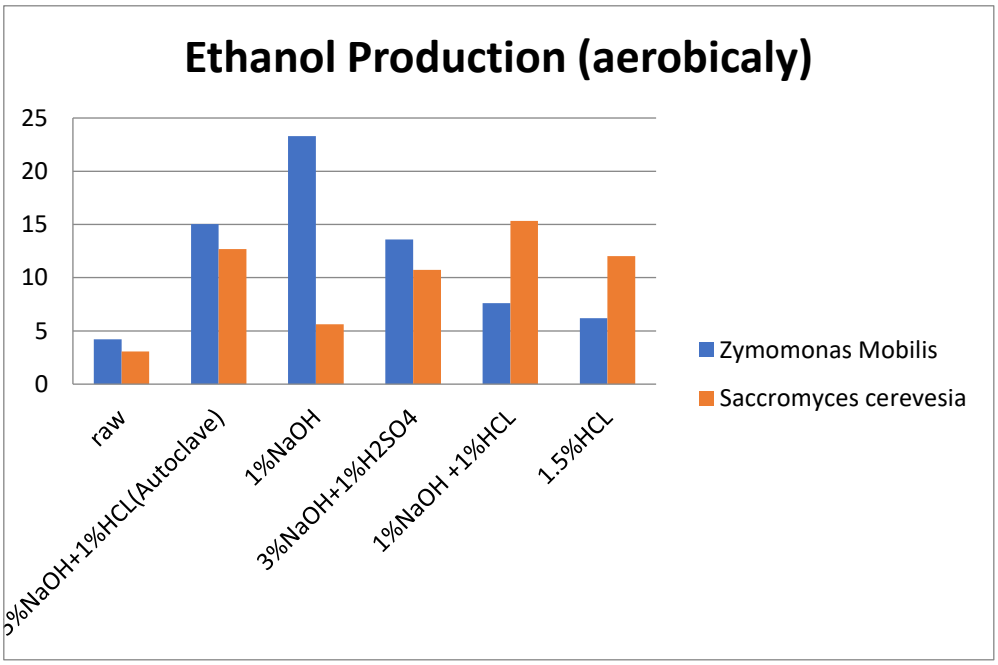


Figure 3.4 Dichromate Result For Aerobic fermentation:-

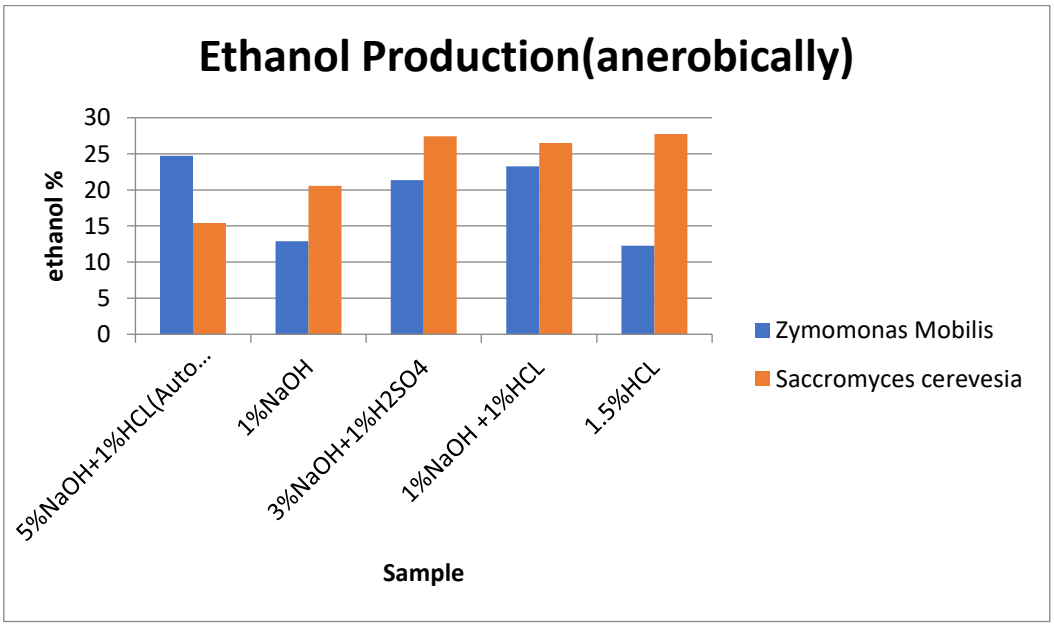


Figure 3.5 Dichromate Result For Anerobic fermentation:-

1.5.5.2 Ethanol Estimation By HPLC:-

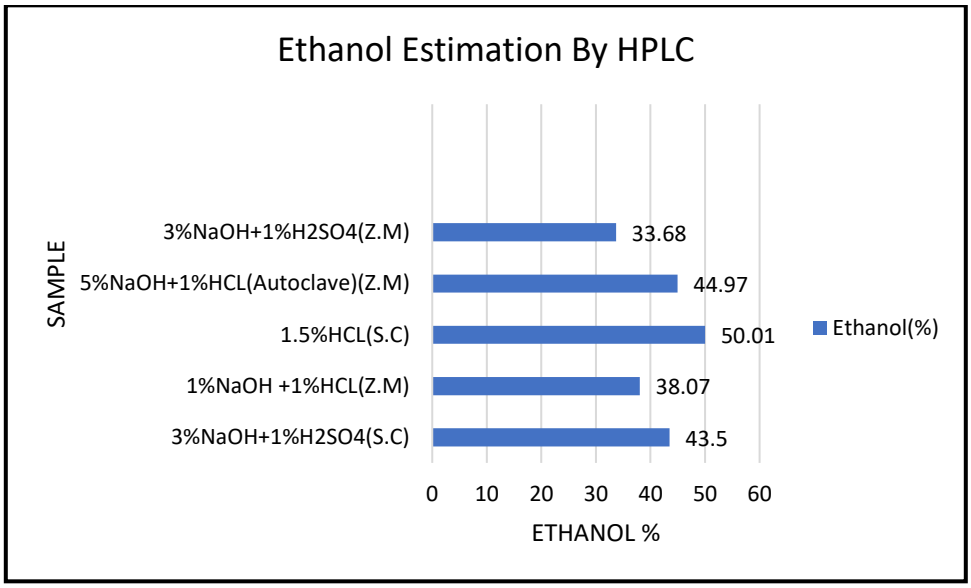


Figure 3.6 :-Ethanol Estimation By HPLC:-

Discussion:- The substrate showing higher bioethanol yield by the potassium dichromate test where further analyzed by the HPLC. HPLC result shows that 1.5% HCl treated shell powder when fermented by *Sacchromyces cerevisiae* under anaerobical condition produce 50% of ethanol. Similarly in case of *Zymomonas mobilis* the highest ethanol yield reported was 44.97% from 5%NaOH +1%HCL(A) treated shell waste.

1.5.6 FTIR of Pretreated Groundnut Samples:-

The FTIR analysis of different pretreated samples of groundnut shell waste. The best pretreated groundnut samples were observed from all the earlier analysis and were chosen for analysis in FTIR .

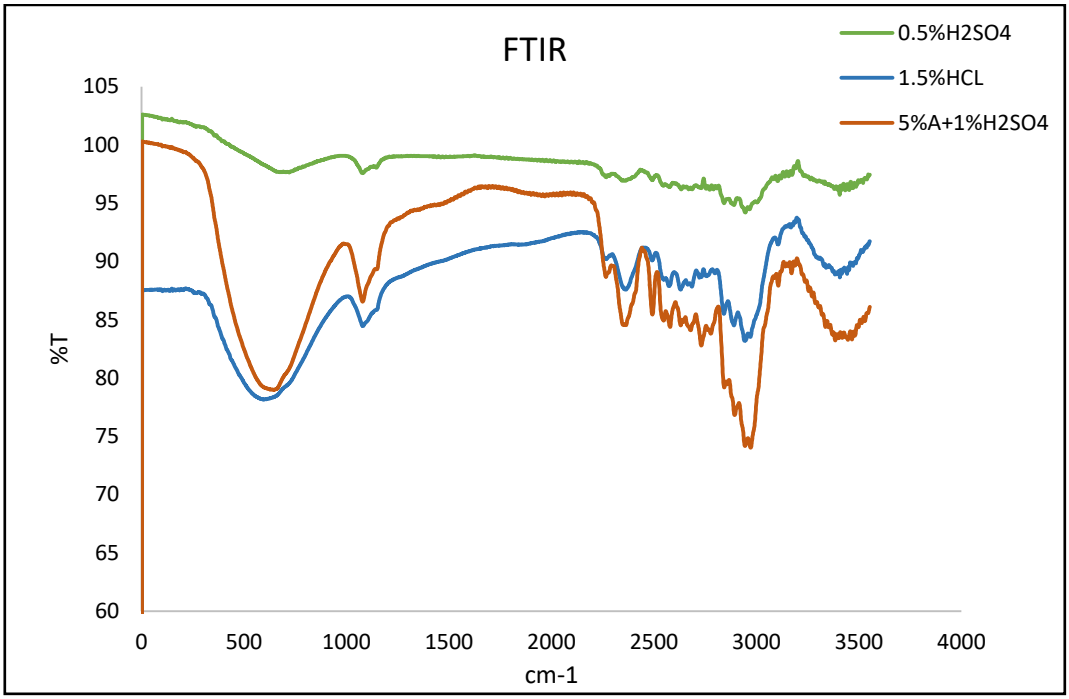


Figure 3.7 FTIR of different pre-treated groundnut shell

The FTIR plots shows the characteristic feature of the lignocellulosic raw biomass(*Balatet al., 2008*). On treatment with different acid,base and combinations of acid and base sharp peaks can be distinguished at 900 cm^{-1} due to the presence of (β,1-4)-glycosidic bond corresponding to the cellulose this was first reported by (*Sekkalet al., 1995*).. A wide range of vibrating bonds can be found near the wavelength of 3323 cm^{-1} which show the presence of a number of different types of hydrogen No significant bond vibration is found in any of the pretreated samples in the range1800-2700cm^{-1}. Reported by *Morrison and Stewart (1992)* The band found at the wavelength of 1214cm^{-1} is the evidence for the C-O-C in esters and ethers bond *(Bhat et al 2009.,)* .At 2889 cm^{-1} the presence of C-H stretching has been reported .

1.6 Conclusion:-

This experiment leads us to the conclusion that there are many pretreatments methods are there to remove hemicellulose fraction and decrease cellulose crystallinity. Therefore from the study we can conclude that:-

- Groundnut shell with milling and acid alkali pretreatment leads to better extraction of cellulose from which higher percentage of reducing sugars could be extracted.
- The reducing sugars extracted from different pretreated groundnut shell samples results production of ethanol.
- The highest ethanol percentage estimated in ethanol water mixture was obtained to about 50 %.

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Chapter 2

Extraction of Cellulose and Lignin in a Single process& Utilization of cellulose in bioethanol production

2 Introduction:-

2.1.1 Agriculture waste as Feedstock for Bioethanol production

Among the various agriculture based nations one of the most important country is India with the cultivation of various types of edible crops such as pulses, fiber crops, cereals, plantation crops, spices and oilseed. The increasing population of India along with high standard of living is resulting into higher production of agriculture which in turn is increasing the volume of the waste generated from this agricultural cultivation. There can be different types of agricultural waste domestic as well as industrial. There is no proper management designed for the full combustion of this agricultural. As a result this wastes are burned by the cultivator in the open field which generates the CO2 and other harmful pollutants in the environment. Therefore the inefficient management of these agricultural waste is contributing to adverse environmental change, contaminating soil and water, and polluting the air. It has been reported by the environmental agencies that by the combustion of lignocellulosic biomass, such as crops, wood, trees, grasses and leaves, which are including the main agricultural waste generates about 32% of carbon monoxide (CO), 50% of polycyclic aromatic hydrocarbons (PAHs) 40% of carbon dioxide (CO_2), and 20% of particulate matter (PM), which are released directly into the environment and causing adverse effect on climate all over the globe. Furthermore on incomplete burning of these generated waste produces dioxins which is a very toxic carcinogenic pollutant for the human health. The proper management, of these agricultural waste lignocellulosic biomass can be converted to various value added products (*Carere et al., 2008*).

2.1.2 Lignocellulosic biomass:-

The lignocellulosic biomass mainly consist of the plant cell wall .The plant cell wall is mainly composed of cellulose (40 – 50%), hemicellulose (25 – 35%) and lignin(15 – 20%) (*Wyman et al., 2005*). On the type of lignocellulosic feedstock the complexity, structure and the amount of various components (cellulose, hemicellulose and lignin) depends. Agricultural waste such as corn stalk, cotton Stover, peanut shell waste, corncob, energy corps and other woody materials are the main sources of lignocellulosic feedstock. (*Liu et al., 2012*).

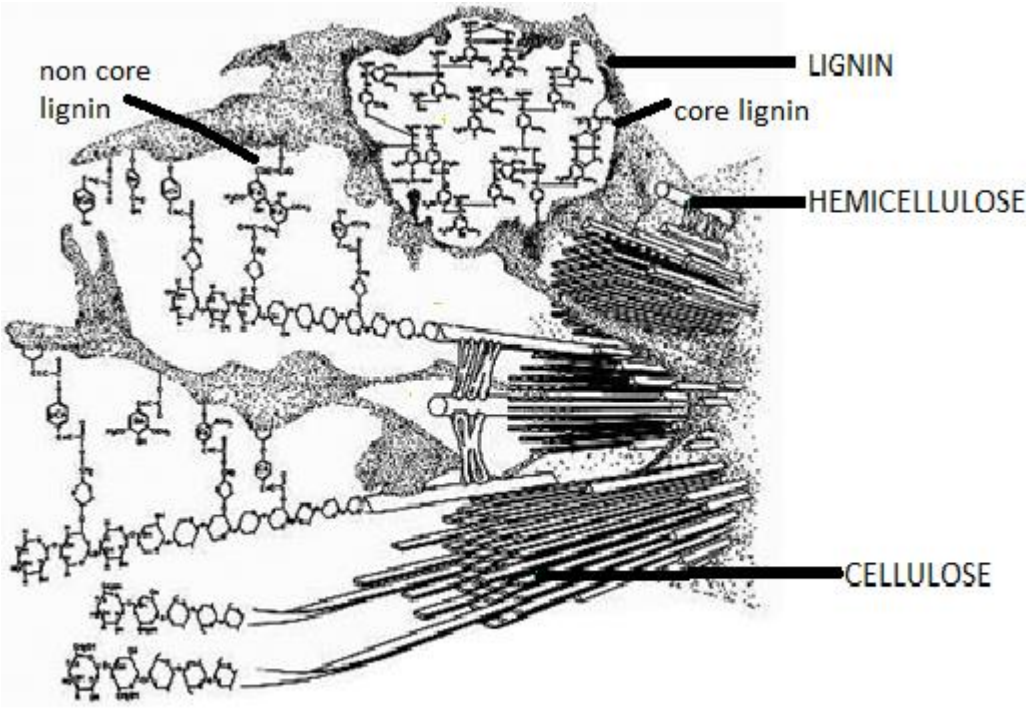


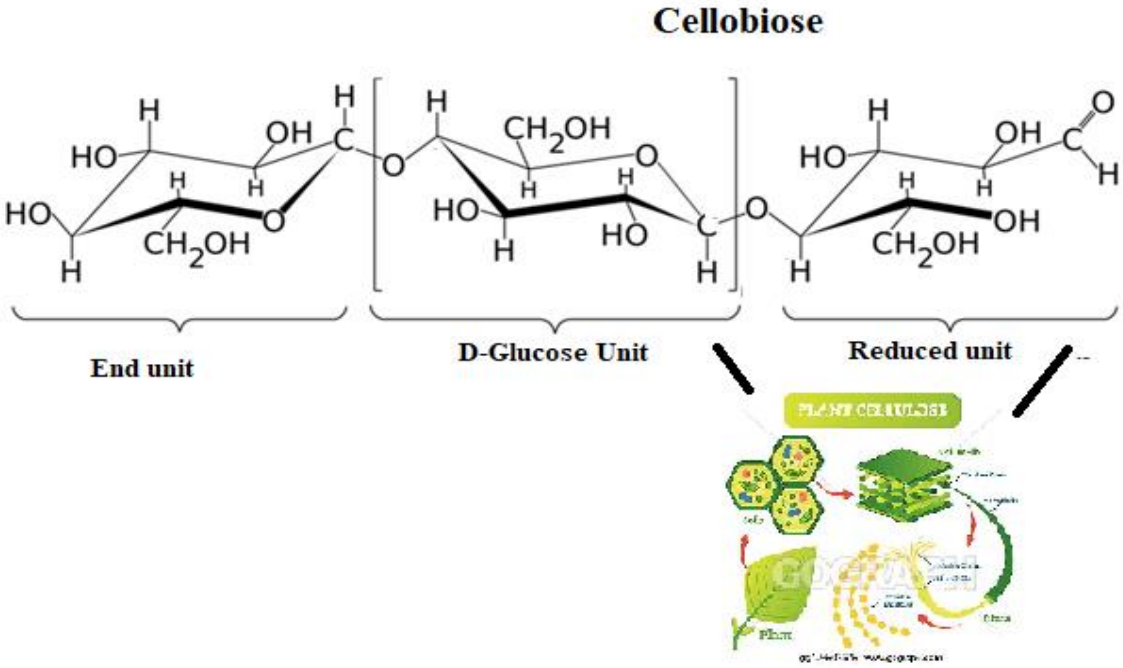
Figure 3.8 Represent the Cell wall model

The biggest renewable bioethanol sources are made up of the Lignocellulosic raw biomass. The lignocellulosic biomass production of U.S. is estimated to be 1.4 billion tons per year. 30% of this lignocellulosic biomass production is from forest biomass, which mainly constitutes of 370 million tons annually. For the first time reported that Woody feedstocks are of two types, commonly known as hardwoods and softwoods. Softwoods may be defined as the biomass which has been originated from gymnosperm and conifers plants. Softwoods

are faster growing and have less density than that of hardwoods plants. Most common examples of softwood trees are Douglas fir, juniper, redwood, pine, cedar and spruce. Hardwood trees on the other hand are originated from angiosperm biomass. Alder, balsa, beech .oak, teak, mahogany, willow, cottonwood and hickory are some common examples of hardwood trees.

2.1.2.1 C**ellulose**:-

Cellulose is a organic compound and the most biopolymer on the earth. The cellulose is one of the most important constitute of the plant cell wall (*Wyman et al., 2005*).cellulose is a polysaccharide consisting of a straight chain of thousands of B-linked D-glucose units . The major characteristic of cellulose is that it is crystalline in nature and insoluble in water (*Wyman et al., 2005; Mosier et al., 2005*). The main application of Cellulose or cellulose derivatives are in textile, paint, polymer, fibre, and paper industry (*Swatloski et. al, 2002*).



. **Figure 3.9:- Molecular Structure of Cellulose.**

The Beta (1-4) glucosidic linkage found in the cellulose biopolymer is entirely different to that are (1-4) glucosidic linkage found in glycogen and starch. Furthermore, branch chain polymers does not exist in cellulose which are present in the starch. Cellulose consist of porous heterogeneous internal and external surfaces. The external surface area of cellulose can be determined by the size and shape of the cellulose particles whereas the internal area can be determined by determines capillary structure of cellulose fibres. Cellulose fibres consist of both amorphous and crystalline regions on it. The part of crystalline structure present in the total surface of cellulose is considered an important factor because it has a severe effect on the hydrolysis of cellulose as it provides resistance to degradation. The commercial name of microcrystalline cellulose is Avicel.

2.1.2.2 Hemicellulose:-

Hemicellulose is a hetropolymer like arabinoxylans found along with cellulose polymers in most of the terrestrial plants cell wall. It is heterogeneous branched polymers of beta – glucan, xylan, xyloglucan, and mannan polysaccharides (Mosier et al., 2005; Maki-Arvela et al., 2010). Hemicellulose is mainly composed of -D-galactose ,-D-xylose, -D-glucose -Larabinose, -D-mannose like hexose and pentose sugar along with some uronic acids substitutes, like -D-4-Omethylgalacturonic ,-D-glucuronic, and -D-galacturonic acids). The most readily available hemicellulose are glucomannans and Xylans (Girio et al., 2010). According to the biomass originated source the hemicellulose structure differs. For example, The main hemicellulose components of the hardwoods are glucuronomannans and glucuronoxylans . About 15 – 30% dry mass of the hardwood is composed of glucuronoxylans. Whereas in the softwoods, galactoglucomannans are dominant than that of glucuronoxylans present in the hardwood,, making 25% of the dry biomass.

The other major components of the hemicellulose structure is Xyloglucans and arabinoxylans. These structures are commonly found in the hardwoods, for example cereal grain and grasses cell walls . D-glucose backbone is the main constituent of the Xyloglucans Xyloglucans have necessary role in hydrogen bonding by interacting with fibrils of cellulose (*Girio et al., 2010*).

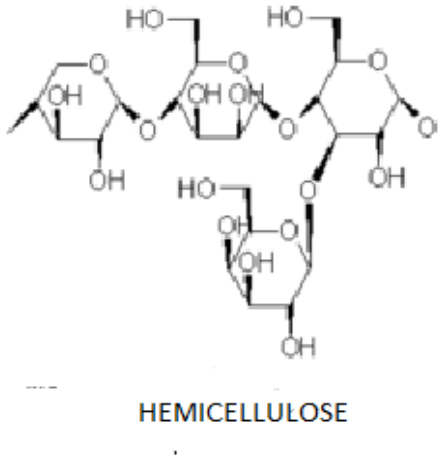


Figure 4.0:-Structure of Hemicellulose

Hemicelluloses are mainly used in the industries like emulsifiers, sweeteners and thickeners (*Maki-Arvela et al., 2010*). D-xylose is an industrial raw material in the production of xylitol. Similarly like glucose, xylose, and fructose other pentose sugars can also be used for the purpose of fermentation. For furfural production Xylose is the main feedstock. Furthermore, from the hemicellulose part the acetic acid can be directly. The hemicellulose have amorphous structure unlike the crystalline structure of cellulose which can be easily hydrolysed by dilute alkali or acid. Hemicellulose is soluble in water.

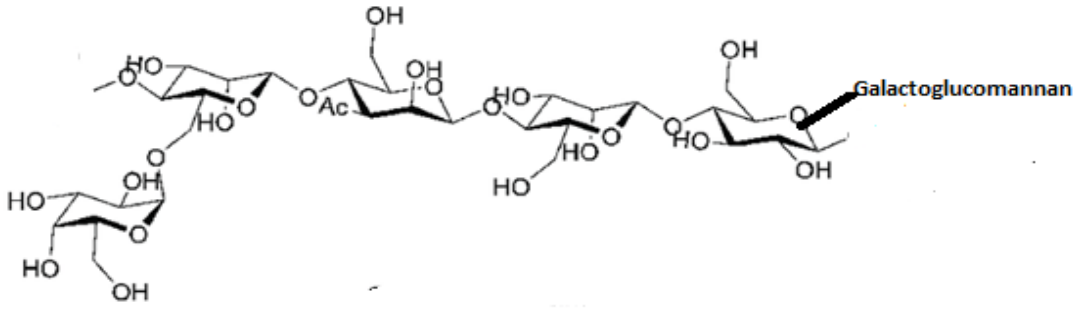


Figure 4.1 :-Represent the molecular structure of Galactoglucomannan

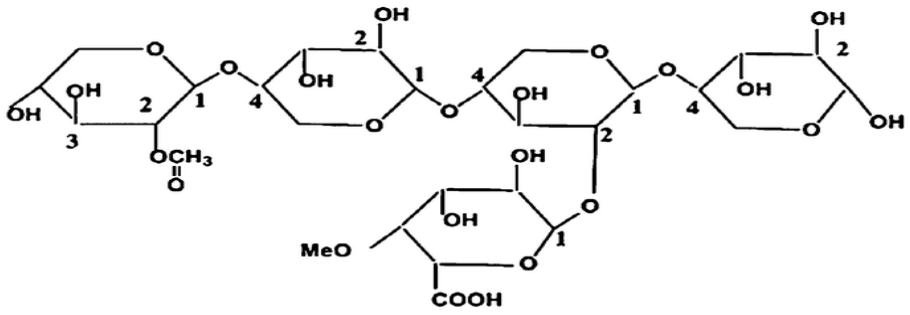


Figure 4.2 :-Represent the molecular structure of Glucuronoxylans

2.1.2.3 Lignin:-

The second most abundant biopolymer on the earth is the (*Kim et al., 2011*). This is one of the major component of the plant cell wall .Lignin component of the cell wall provide the rigidity and strength to the plant cell wall. The lignin constitute around15 wt% - 40 wt% of the total dry mass of the plant cell walls. Lignin are more recalcitrant to chemical, physical, and biological attacks to any other components of the cell wall (*Doherty et al., 2011*). Lignin is an amorphous phenolic, three dimensional, cross – linked polymer mainly composed of p-coumaryl, coniferyl ,monolignols; and sinapyl alcohols. The monomeric units of lignin are connected by heterogeneously interunit linkages (*Kim et al., 2008*). The Molecular weight of the lignin is in the range of 1000 - 20000 g/mol. The lignin polymer's glass transition temperature is dependent on the molecular mass of lignin. The temperature increases with increase in the molecular mass (*Doherty et al., 2011*).

Between the monomers of lignin carbon – oxygen bonds and carbon – carbon bonds can be seen. The lignin structure provide the strength and rigidity to plant cell wall which is dependent on the degree of substitution and crosslinking. The sinapyl alcohol type is predominant in the hardwood tress whereas the conyferil alcohol dominant in softwood

tress.in contrast to the above in grasses p-coumaryl is found dominating (Doherty el al., 2011).

Figure 4.3:-Represent the molecular structure of lignin.

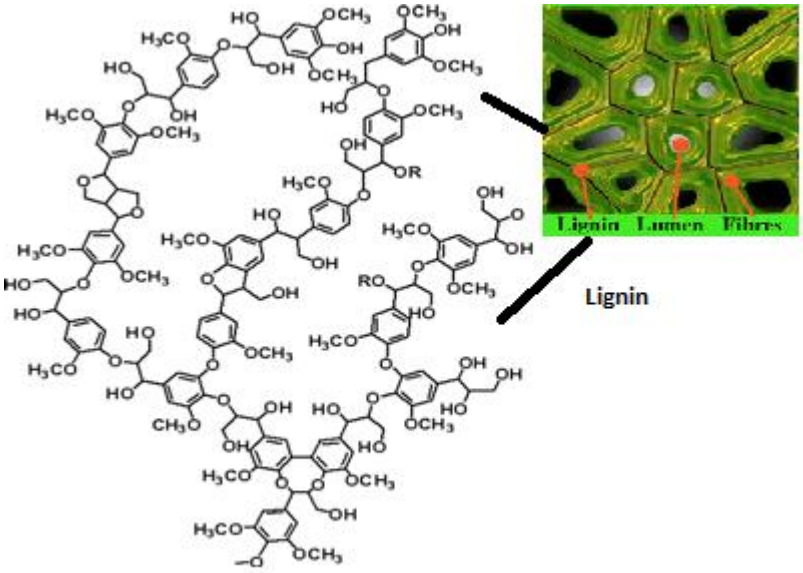
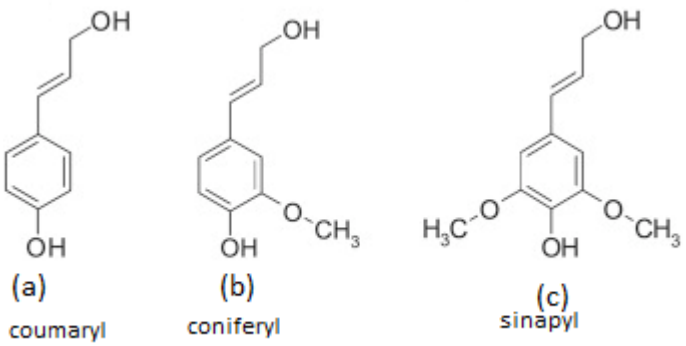


Figure 4.4:- Monolignol monomers: p-Coumaryl alcohol, Coniferyl alcohol, and Sinapyl alcohol



Lignin is a three – dimensional, heterogeneous, complex in structure polymer. Lignin has different types of intermolecular linkages, which are not present in cellulose. The primary type of intermolecular linkage found in lignin is arylgycerol- -O-4-aryl ether bonding. Arylgycerol- -O-4-aryl ether bonding in lignin makes about 50 – 60% of the total

intermolecular linkage (*Braun et al., 2005*). Lignin has been used for industrial purpose. Lignin is derived as a byproduct from newspaper pulping industries. Most significant use of lignin is its lignosulphonate form. Lignosulfonate has been used in oil industry, cement and concrete industry and also used in dye industry for the removal (*Doherty et al., 2011*) .Lignosulfonates can also be used as a feedstock for the production of various value added products like pesticides, carbon black dispersant, dyes and pigments, animal feed pelleting aids, emulsifiers, battery expanders, industrial cleaners and industrial cleaners (*Doherty el al., 2011*).The cold crystallization temperature and modulus can increased if lignin is mixed with synthetic or natural polymer whereas the melting temperature is decreased.

2.1.3 Different feedstocks and their respective conversion technology

Bioethanol generation from sugar derived raw material is done by fermentation process directly. This is a simpler technique in comparison to other raw materials due to the fact that oligosaccharide can be easily broken down by the yeast. Sugar derived feedstock are sugarbeet ,sorgum, sugarcane and molasses

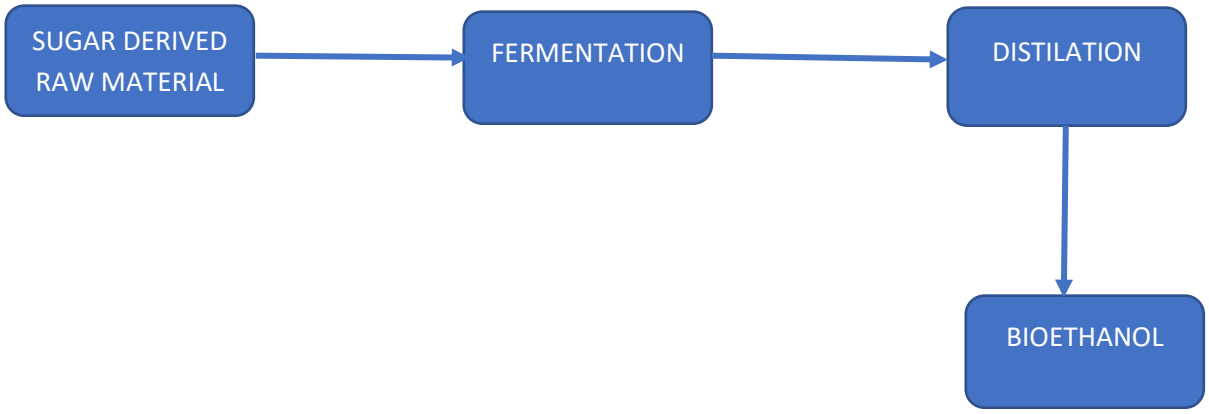


Figure 4.5 :-Bioethanol from Sugar derived compounds

Bioethanol generation from starch based raw material requires an additional step for the purpose. For the starchy raw material extraction of the reducing sugar is done prior to the fermentation process commonly known as hydrolysis step. Starch or amylum is a polymer carbohydrate consisting of large number glucose (monomeric) units joined by glyosidic

bond. Therefore the fermentation process is only possible by breaking down this long chains of polysaccharides. Wheat and corn are main raw feedstocks used for the ethanol production in this purposes (*Knauf et al., 2004*).

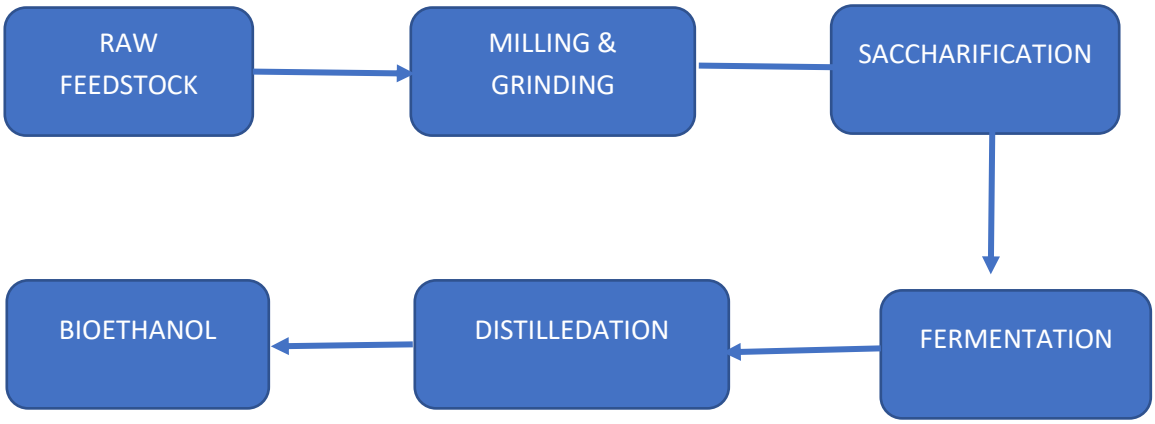


Figure 4.6 : Bioethanol from Starchy biomass

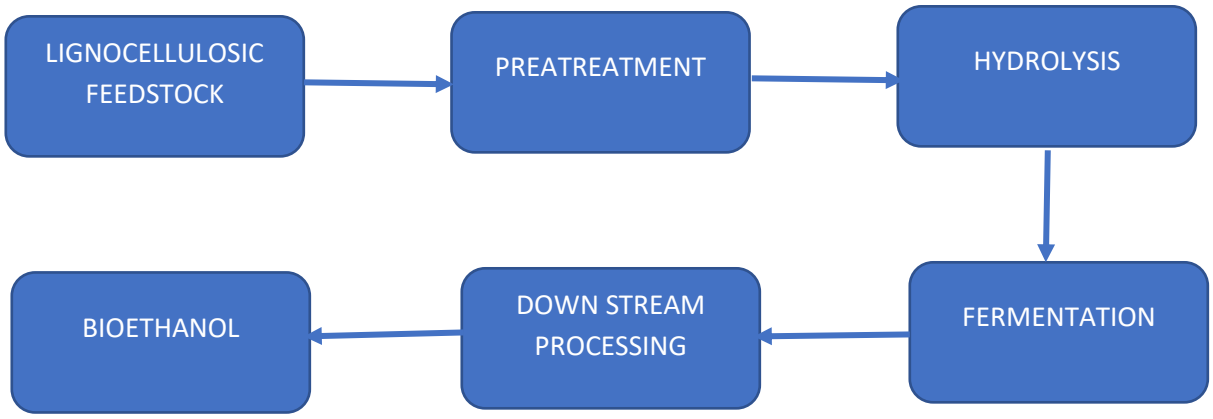


Figure 4.7: Bioethanol from lignocellulosic feedstock

Generation of biofuel from lignocellulosic feedstock is a challenge because of the recalcitrant structure of cellulose, hemicellulose and lignin which act as a barrier in the hydrolysis step. For the ethanol production from the lignocellulosic feedstock first pretreatment is done in order to remove the lignin and solubilize the hemicellulose part.

2.2 Literature Review:-

2.2.1 Lignocellulosic Feedstocks Of Bioethanol Production:-

The sugar based raw materials are the most common type of feedstock used all over the globe for bioethanol production. The sugar based feedstock mostly includes sugarbeet, sweet sorghum and sugarcane which are generally high in production for fermentable sugar or the other type of feedstock which can be used are generally rich in polysaccharides that can be subsequently saccharified to produce sugar which are then fermented to bioethanol.

The second type of feedstock for bioethanol generation is the starch based raw materials, which generally includes the rice, wheat, pulses or corn. The third type or the most economically viable type of bioethanol production is from cellulose rich lignocellulosic biomass. The reaction involved in the conversion of the edible biomass to bioethanol is represented below. (*Cardona and Sánchez 2007; Guo et al. 2015; Sánchez and Cardona 2008*)

The hydrolysis of polysaccharides, cellulose and starch to simpler sugar monomers like fructose, glucose and xylose sugars.

$$(C_6H_{10}O_5)\ n + nH_2O \longrightarrow nC_6H_{12}O_6$$

The hemicellulose part of the plant cell wall is generally hydrolysed to pentose sugar which includes arabinose, mannose and xylose.

$$(C_5H_8O_4)\ n + nH_2O \longrightarrow nC_5H_{10}O_5$$

Enzyme invertase catalyses the hydrolysis process of sucrose sugar to monomeric glucose and fructose units in Saccharomyces cerevisiae

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

Exothermic reaction is found in bioethanol production from the hexose and pentose sugars (enthalpy calculated in ethanol formation is about $\Delta fH^\circ = -278$ kJ/mol)

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

$$C_5H_{10}O_5 \longrightarrow 5C_2H_5OH + 5CO_2$$

More than 90% of theoretical yield was found in the first ethanol generation process (*Gombert and van Maris 2015*).

2.2.1.1 Sucrose or Sugar-based Raw material:-

Global production of bioethanol is done by the edible sugar crops. The conversion of sugar to ethanol is more direct and simple, unlike the starch to bioethanol conversion technique. The polysaccharide sugar is firstly converted to the fermentable sucrose by the invertase enzyme generally secreted by the microbial strain yeast and this entire process is known as hydrolysis. In the juice condition step partially hydrolysis can take place. Industrially or commercially used sugar based raw materials are the sugar beet, sweet sorghum and sugarcane (*Cardona and Sánchez 2007; Zabed et al. 2014*).

The sugarcane is a type of C4 crop (plants which avoid phosphorespiration and are capable of high carbon fixation) and is one of the most important raw feedstock used for the biofuel production in most of the developed and developing countries. Sugarcane (Saccharum officinarum) can be used as sugarcane juice or molasses for the bioethanol production. The fermentable sugar content of sugarcane juice is about 12-18% most of this is composed of sucrose (90%). The sugar cane juice contains mainly vital organic nutrients, minerals are also present in minute quantity which are useful for the growth of microbial strain. 13 industries in Brazil represented a renewable energy model which is based on the sugarcane juice conversion to bioethanol and this model is subsequently achieving the twice of renewable fuel generated by the corn feedstock used in US. (*Cardona and Sánchez 2007; Chum et al. 2014; Ergun and Ferda Mutlu 2000; Ghosh and Ghose 2003; Laluce et al. 2016; Zabed et al. 2014*).

One of the temperate climate growing food crop is Sugarbeet (Beta vulgaris) which are mostly common in the European nations. The farming of sugarbeet is easier than that of sugarcane as it requires 35-40% lesser fertilizer and water than sugarcane. The sucrose contain in the sugarbeet juice is about 16.5% and around 85%-90% of this sugar is the fermentable one. The sugarbeet juice is generally used as a source of sugar for the process of bioethanol generation. This juice can directly be used as substrate after adjusting the pH (*Balat et al. 2008; Dodić et al. 2012; Ergun and Ferda Mutlu 2000; Zabed et al. 2014*) .

Sweet sorghum is another type of the C4 crop able to grown in both tropical and temperate climatic conditions. The juice extracted from Sweet sorghum as well as the crop itself can be used in the bioethanol generation process. Sweet sorghum contains about 13 to 17% of the fermentable sugar out of which about 10-14% is the sucrose content (*Akbulut and Özcan 2008*). Micronutrients are found in the juice of Sweet sorghum which help in growth and metabolism of yeast (*Cao, Gao, and Gu 2006*). There are various advantages of using Sweet sorghum as a raw feedstock which are as follows:-1) higher tolerance for cold temperature and drought 2) lesser amount of fertilizer and nitrogen requirement 3) efficiency of photosynthesis is high 4)the growth cycle is short (3 months)and 5)assimilation of carbon is higher (*Kim and Day 2011*). The juice extractability of sweet sorghum is the highest with 71.9% the report was submitted by *(Kim and Day 2011)* which is the highest among all the other sucrose based crops. The crop when used as whole can be potentially used for about 8000 L ha-1 of bioethanol yield which is about 30% higher than that of sugarcane and twice of corn (*Deesuth et al. 2012*). This advantage of sweet sorghum is making it a very suitable feedstock for bioethanol generation *Andrzejewski et al. 2013; Barcelos et al. 2016; Kumar et al. 2013; Laopaiboon et al. 2009; Yu, Zhang, and Tan 2009; Zabed et al. 2014*).

2.2.1.2 Starchy feedstocks:-

Starch is a homopolymer of long chains of D-glucose unit, which can be hydrolysed to get monomeric glucose unit which are suitable for the bioethanol generation. This type of bioethanol generation is common process in Europe and North America (*Balat et al. 2008*). The principle crops for starch based raw material are the corn and wheat. The other types of starch based feedstock which can be used for bioethanol generation are the cassava, potato, sweet potato, barely and rice.

Corn scientific name Zea mays L. is a type of C4 grass which was native to Mexico. The corn is commonly cultivated in America (*Matsuoka et al. 2002*). At the growing stage the sugar is mainly found in the stalk part of the plant but on maturity the sugar is accumulated in the corn kernel as starch (*Abendroth et al. 2011; Taylor et al. 2010*). The starch containing kernel is either dry milled for processing or is wet milled which is important prior the ethanol production steps reported by (*Bothast and Schlicher 2005*. Amylase enzyme is used after the

milling process to breakdown the complex structure of starch to monomeric glucose unit which is finally used for the bioethanol production process. About 95% of ethanol in US is produced by utilizing corn starch as a feedstock Renewable Fuels Association 2017).

2.2.1.3 Lignocellulose containing feedstocks:-

As the first two generation used edible crops for ethanol generation uses food crops, the functionality of the feedstock is restricted.

Lignocellulosic biomass mainly consist fast growing trees , energy crop ,Agriculture and forest biomass, which together constitutes the world's most available and recyclable resource of energy, this makes the lignocellulosic feedstock available for biofuel generation (*Balat et al. 2008; Guo et al. 2015; Mohr and Raman 2013; Refaat 2012*). Presently available and most common lignocellulosic raw material are the industrial or domestic waste which is used for biofuel generation (*Mortimer and Elsayed 2004*). The fuel generated from this waste reduces the emission of co2 and increases the RER (*Chum et al. 2014; Gallagher, Yee, and Baumes 2016; Mortimer and Elsayed 2004*). Lignocellulosic biomass also reduces the food vs fuel competition. Lignocellulosic feedstock mainly consist of sugarcane bagasse (*Cardona, Quintero, and Paz 2010; Dasgupta et al. 2013; Pandey et al. 2000*), sweet sorghum waste (*Barcelos et al. 2016; Goshadrou, Karimi, and Taherzadeh 2011*), wheat and rice straw (*Kaparaju et al. 2009; Karagöz and Özkan 2014; Mortimer and Elsayed 2004; MurpHy and Power 2008*) and corn (*Gallagher et al. 2016; Humbird et al. 2011; Luo, Van Der Voet, and Huppes 2009*).

Lignocellulosic biomass requires pretreatment process prior to the hydrolysis step, pretreatment is mainly done to reduce the degree of polymerization and to enhance the accessible surface of cellulose. Different types of pretreatment are studied extensively (*Aditiya et al. 2016; Alvira et al. 2010; Refaat 2012*).

2.3 Aim And Objective

Aim:-

This Research aimed at investigating the potential of extracted cellulose in bioethanol generation .The lignin and cellulose was extracted in a single process by utilization of singular action which was economically feasible. High fermentable sugar yield to be converted to ethanol production was seen and also to find best ways to use this technique at commercial level with financial profit.

Objective:-

(a) To utilize waste groundnut shell as feedstock for cellulose production.
(b) .Production of bio ethanol by *Saccromyces.cerevisiae* and *Zymomonas.mobilis* utilizing substrate as extracted cellulose.
(c) Comparative study of ethanol production by two different microbial strains.
(d) Recovery of bioethanol.
(e) To study the quality of bioethanol produce from different sources,.
(f) Extraction of lignin as a value added product.

2.4 Materials And Methodology:-

2.4.1 Materials:-

Groundnut shell waste were procured from the local food venders (Behala, India) and were used as raw feedstock for this research study. The initial all tastings of the raw feedstock were dine in Jadavpur university laboratory based on the standard methods given in the standard handbooks. Analytical reagent grade chemicals were used for the experiment. This chemical reagents were used as they were received without any purification in them.

2.4.1.2 Chemicals:-

The chemical agents used for this research study are as follows:- Sulphuric Acid(Merk, India), Sodium Hydroxide(Merk, India),Sodium Sulfite (Merk, India).Sodium Hypochlorite(Merk, India), Ethanol, Nitric Acid (Merk, India) ..Agar-Agar (Merk, India), Yeast Extract (Merk, India), Anthrone Reagent (Merk, India), Dinitrosalicylic Acid (Merk, India), Glucose (Merk, India), Magnesium Chloride (Merk, India), Ammonium sulphate (Merk, India), Potassium Dihydrogen PHospHate (Merk, India), Peptone (Merk, India), Dextrose(Merk, India), Czapex dox (Merk, India) , Ethanol , Sodium Nitrate (Merk, India), Magnesium Sulphate Heptahydrate (Merk, India), Ferrous Sulphate (Merk, India) , Potassium Chloride (Merk, India), Dipotassium Hydrogen Phosphate (Merk, India).

2.4.1.3Microorganism:-

Zymomonas mobilis and *Sacchromyces cerevisiae* strains were obtained from MTCC, pune and Chandigarh respectively. *Aspergillus* strain was isolated from the water sample from sunderban west Bengal.

2.4.2 Isolation of Microcrystalline Cellulose:-

The groundnut shell waste were collected from local merchant in behala Kolkata. The peanut shell were washed in tap water and then in distilled water for several times in order to remove the unwanted impurities and dust particles. The peanut shell were sundried for 4-5 days and then grinded into fine powder. The powder of the peanut shell waste was sieved through 100um mesh in order to discard the larger particles of groundnut. The sieved groundnut shell

powder was treated with (0.5M) Sodium hydroxide with continuous stirring at 700rpm on (REMI 1MLH) stirrer at about 92°C. The black liquor obtained after the above process was allowed to cool down and then filtered. The filter cake obtained after the process was washed several times to get constant pH whereas the black slurry obtained was kept in a air tight borosil beaker for further extraction of lignin. By the NaOH treatment the lignin was removed. The filter cake was further treated with 20% nitric acid in pure ethanol (22.1 ml of nitric acid in 78.9 ml of ethanol). This nitric acid treatment was carried 2-3 in order change the residue colour from reddish brown to pale yellow respectively. The residue obtained after this treatment was washed till the pH drop downs approximately to 7. Sodium hypochlorite chemical was finally used for the bleaching purpose of the residue from pale yellow to white. The white cellulose obtained after the process was first oven dried and then lyophilized for 4-5 hours, fine powdered cellulose was obtained after this complete procedure *(Punnadiyil et al 2016).*

2.4.3 Isolation of lignin from Black Slurry:-

The black dark slurry obtained during the cellulose extraction was poured down in a thick (500ml) plastic tight lid bottle with a magnetic bead in it in order to enhance through mixing. In order to enhance the ionic strength of the dark liquor Sodium sulphate(1gram in 250 ml liquor) was added .The lid of the plastic container was tightly closed and the bottle was placed in water bath at a temperature of 45ºC. The temperature was allowed to increase upto 75ºC which took approximately 1 hour. At a regular interval of 10 minutes the plastic bottle was taken out from the water bath and was stirred at 600 rpm for 5 minutes and then again placed back to the water bath .After 1 hour the black slurry was allowed to cool down and then finally treated with (6M) H_2SO_4 which was added dropwise. Lignin starts to precipitate. The precipitate obtained from the process is filtered and then oven dried. This precipitation of lignin obtained is used for the dye removal purpose.

2.4.4 Characterizations of Extracted Microcrystalline cellulose and Lignin:-

2.4.4.1 Fourier Transform Infrared (FTIR) Spectroscopy:

Perkin Elmer spectrophotometer (Magma - IR 560 E.S.P) was used to produce infrared spectra images of lignin and microcrystalline cellulose (wave numbers in cm-1) using KBr disk accommodating the finely grinded sample about 3% in quantity.

2.4.4.2 Scanning Electron Microscopy (SEM):

To observe the modification of cellulose after the hydrolysis step done by Aspergillus sp. scanning electron microscopy (SEM – EVO 18 / Carl Zeiss/United Kingdom) was used. The sample was firstly attached to a carbon tape the excess sample were removed by spraying air on the carbon tape further platinum coating was done on the sample to get better resolution (S7620 Quoter Model/Quorum/United Kingdom). To guarantee the authenticity of the result different parts of the samples were focused and imaged by the SEM in order to produce many images.

2.4.4.3 X Ray Diffraction (XRD):-

Shimadzu Diffractometer is used for the purpose of X-Ray diffraction analysis. It is mainly operated at 30mA and 40 Kv . Graphite made filter is used for this experimental purpose The filter used for the process CuK(=1.5432 A°).

2.4.4.4 Gas Chromatography (GC):-

Gas chromatography is an systematic instrumental method that can measure different component of a sample. The analysis performed under the gas chromatograph is called gas chromatography. When the sample is injected into the gas chromatography instrument it directly enters to a gas phase which help in transporting the sample solution into a differentiating tube commonly known as the "column."(nitrogen or Helium are generally used as the carrier gas.) In the column the different component in the mixture is separated. The detector is a device which help to measures the quantity of the components in the sample that exit the column.

2.4.5 Estimation of cellulose content in extracted Sample:-

2.4.5.1 Cellulose Estimation:-

Anthrone test is done for the estimation of cellulose content. In this test the carbohydrate gets dehydrated when reacted with concentrated sulphuric acid (H_2SO_4) forms furfural. This furfural which is formed in the previous step reacts with anthrone reagent to give bluish green coloured complex. This coloured complex intensity is measured by spectrophotometer at 630nm (Perkin Elmer, Germany).

2.4.5.2 Chemical Reagents:-

1 Extracted sample from peanut shell waste.

2Anthrone Reagents: 100ml of ice cold H_2SO_4(95% conc.) was take in which 200mg of anthrone was dissolved to prepare the anthrone reagent solution. The solution was prepared fresh and refrigerated for 2hrs before use.

3. 67% sulphuric acid (H_2SO_4).

2.4.5.3 Standard curve preparation:-

For the standard curve preparation cellulose (10 mg) was dissolved in 100 ml of double distilleded water. From the stock solution aliquots of 0.25, 0.5, 0.75 and 1 ml were taken. Further 10 ml of anthrone reagent was added and the solution was heated in water bath for 15 minutes. Finally the absorbance was measured at 630nm and the standard graph was plotted.

2.4.5.4 Experiment:-

- **0**.5 gram of the extracted sample from peanut shell is taken in a test tubes.
- 10ml of 67% H_2SO_4 was added to the test tube and was kept for 1hr at room temperature.
- 1ml from the previously prepared solution was taken and was further diluted to 100ml by adding distil water.
- 1ml from the above stock solution was taken to which 10ml of Anthrone reagent was added and thoroughly mixed.
- The test tube were heated in the water bath at boiling for 15 minutes.

- The above solutions in the test tube was cooled and finally the absorbance was measured at 630nm.

2.4.6 Biological Treatment Process:-

Cellulase enzyme Production by simultaneous saccharification and fermentation (SSF):-

Inoculum preparation:-

Aspergillus species (Genbank: MH119104)was isolated from the water sample collected from the sunderban area of West Bengal. The identified and isolated fungal strain was further subcultured on the potato dextrose agar media as well as modified czapekdox medium. The Aspergillus strain was streaked on the agar plates and were incubated at 35°C for 7 days. After 7 days fully sporulated plates were obtained which were further treated with 0.1% Tween 80 to collect the spores. Due to the detergent properties of tween 80, spores were dislocated and were gently collected from the plate using pipet. This spore suspension was then centrifuged and washed several times to remove the tween and finally refrigerated to be used as inoculum.

2.4.6.1 Media Preparation for Enzyme Production:-

Basal Media was prepared by adding salts of (0.2%) Sodium Nitrate ($NaNO_3$,) , (0.05%) Potassium chloride (KCl), (0.05%) magnesium sulphate ($MgSO_4$), (0.001%) Ferrous sulphate ($FeSO_4$),(0.1%) Dipotassium hydrogen phosphate (K_2HPO_4) and Peptone. The nitrogen source used in this media was the Peptone.

2.4.6.2 Experiment:-

One grams of the extracted cellulose from the groundnut shell were taken into 250ml Erlenmeyer flasks and were then humidified with freshly prepared basal media. The media containing the cellulose as substrate was sterilized by autoclaving at 121°C and 15psi pressure. The media containing the flask were then inoculated with 3ml of the spore suspension. The flask was incubated at 35°C in the incubator for 6 days.

In the current research the effect of different important factors on the hydrolysis process (amount of reducing sugar produced) has been studied. The *Aspergillus sp*. used in the

process breakdown the long chains of polysaccharides into simpler components such as – glucose, fructose, galactose and maltose etc.

2.4.6.2.1 Temperature:-

The optimum temperature of the *Aspergillus sp*. is 35-37°C. To determine whether *Aspergillus sp*. can yield the reducing sugar better in any other temperature or not, different ranges of temperature are studied (26-46°C).

2.4.6.2.2 Substrate:-

The standard substrate concentration used in this experiment was about 1 grams. To find the better sugar yield the substrate concentration was varied from 1gram to 5 gram.

2.4.6.2.3 Dose:-

The dose concentration for the groundnut shell powder used in the standard process was 3ml of (1.25x106 spore/ml). So, dose concentration was varied to study more detail effect of dose on the yield of reducing sugar by the *Aspergillus sp*. The dose was varied in a range of 1ml to 3ml.

2.4.6.2.4 pH:-

The effect of pH on the sugar yield was observed by varying the pH from 4 to 10.

2.4.7 Estimation of the total reducing sugar by DNS.

3,5-Dinitosalicylic acid(DNSA) is extremely used in biochemistry for the determination of reducing sugar. DNSA can detect the presence of free carbonyl group present in the reducing sugars. In this experiment the oxidation of the ketone functional group (in fructose) and aldehyde functional group (in glucose) involves. During this reaction the DNSA is generally reduced to 3-amino-5-nitroslicylic acid (ANSA) which in turn shows reddish brown coloured complex which has maximum absorbance at a wavelength of 540nm.

2.4.7.1 Dinitrosalicylic acid (DNS) reagent preparation:-

The DNS reagent was prepared by adding dinitrosalicylic acid(DNS) (1gram), crystalline phenol(200mg), sodium sulphite (50mg) in 100ml of 1%NaOH solution by continuous stirring.

Sodium-potassium tartrate solution was prepared by adding 40 gram of Rochelle salt in 100ml of distilled water.

2.4.7.1.2 Standard stock and curve preparation:-

The standard stock solution was prepared in 100ml of distilled water by adding 100mg of commercial glucose powder. The working solution was prepared by taking 10 ml aliquot from the stock solution and making the volume to 100 ml by adding distilled water. Further different concentration of the working solution were taken (0.2,0.4,0.6,0.8 and1 ml) and the final volume was made to 3 ml by adding water. 3ml of the DNS reagent was added to each test tube and then heated for about 10 minutes. After cooling and appearance of reddish brown colore 1 ml of 40% Rochelle salt was added and thoroughly mixed . Finally the absorbance was recorded at 540nm using Spectrophotometer (Perkin Elmer, Germany).

2.4.7.1.3 Experiment:-

1ml filtrate of the hydrolysed media(which was previously inoculated by aspergillus sp.) containing cellulose was taken and 2 ml of distil water was added to it to make up the volume to 3ml. 3ml of the DNS reagent was added to each test tube and then heated for about 10 minutes. After cooling and appearance of reddish brown colour 1 ml of 40% Rochelle salt was added and thoroughly mixed. Finally the absorbance was recorded at 540nm using Spectrophotometer (Perkin Elmer, Germany).

2.4.8 Fermentation:-

2.4.8.1 Microbial Species:-

***Saccharomyces cerevisiae* (MTCC 170)**

Saccharomyces cerevisiae is a very common species of yeast. It is universally used microorganism for the purpose of fermentation. *Saccharomyces cerevisiae* is capable of fermenting polysaccharide majorly the hexose sugars like the glucose.

The growth medium (YEPD) for *Saccharomyces cerevisiae* is

Working Solution:-

Yeast extract – 0.3gm

Agar – 1.5gm

Distilleded water – 100ml

Stock solutions :

Peptone - 5.00g / 50mL

Dextrose - 10.00g / 50mL

The stock solution were then sterilized by autoclaving at 121°C and 15psi pressure 10ml of the stock solutions were simultaneously added to the working solution. The solution were poured in the petri plate and allowed to cool until gel formation takes place. The identified and isolated *Saccharomyces cerevisiae* was subcultured on the petri plate by streaking and incubated 37°C. Growth was observed between 2-3 days.

2.4.8.2 Zymomonas mobilis

Zymomonas mobilis is a gram negative, non sporulating, polarly flagellated, rod shaped, facultative anaerobic. *Zymomonas mobilis* have notable bioethanol generating properties which can some time surpass the capability of yeast. This microorganism can ferment more sugar than *Saccromyces cerevisiae* such as arabinose and xylose which cannot be fermented by yeast.

The growth medium for this anaerobic bacteria is :

Working solution:-

Yeast extract – 1.0gm

Agar – 1.5gm

Distilled water – 100 ml

Stock solutions:

Glucose - 2gm / 10mL

Magnesium chloride - 1gm / 10mL

Ammonium sulphate - 1gm / 10mL

Potassium di-hydrogen phosphate - 1gm / 10mL

The stock and the working solutions were autoclaved and then 10mL of glucose solution was added to the working solution and 1ml each of the other stock solutions were simultaneously added to the working solution. The *Zymomonas mobilis* was subcultured on the previously mentioned growth media. The media plates were incubated at 35°C and growth was observed within 3 days.

2.4.8.3 Experimental procedure:-

Two Fermentation condition, two microorganism

The media containing the substrates were further treated with both microbial strains, to identify the amount difference in the of fermentation in both the cases.

2.4.8.3.1 Aerobic Fermentation-

1gm extracted cellulose containing media which was previously pretreated with Aspergillus strain was further autoclaved at 121°C and 15psi pressure to kill the fungal strain. The same media containing substrate was filtered and the filtrate media was divided into 2 equal parts and one was treated with *Saccharomyces cerevisiae* and another was treated with Zymomonas mobilis. Fermentation showed by *Zymomonas mobilis* was observed within 3 days at 35.5°C whereas *Saccharomyces cerevisiae* showed it after 4 days at 37°C.

2.4.8.3.2 Anaerobic fermentation –

1gm extracted cellulose containing media which was previously pretreated with Aspergillus strain was further autoclaved at 121°C and 15psi pressure to kill the fungal strain. The same media containing substrate was filtered and the filtrate media was divided into 2 equal parts and one was treated with *Saccharomyces cerevisiae* and another was treated with Zymomonas mobilis. Both the media was sparged by nitrogen to remove the oxygen as well as to maintain the anaerobic condition. The flask containing the media were sealed with Para film and were kept in anaerobic bioreactor for 3 days after that bioethanol was recovered and analysed.

2.4.9 Extraction of bioethanol:-

The fermented samples obtained after 3-4 days, of pretreated groundnut shell both by *Zymomonas mobilis* and *Saccharomyces cerevisiae* and were taken for the extraction of bioethanol by the Rotary Evaporator. The water-ethanol mix was obtained from the rotary evaporator was further analysed for the estimation of ethanol from the samples..

2.4.9.1 Estimation of ethanol by Dichromate test:-

The ethanol water mix obtained after the fermentation process was further used to estimate the amount of ethanol in it.

2.4.9.1 .1 Potassium dichromate reagent preparation:-

In I litre volumetric flask 400 ml of distilled water was added, then carefully 325 ml of concentrated sulphuric acid was added to the volumetric flask. After through mixing the solution was allowed to cool down to (70-90°C).Potassium dichromate powder 33.768 gram was added to the previously prepared solution and finally the volume was made upto 1 litre by adding double distilled water.

2.4.9.1 .2 Preparation of standard solution:

The standard solution of Ethanol was prepared by mixing pure ethanol into water in a range of 0-20% (v/v).

2.4.9.1 .3 Standard curve for ethanol preparation:

Standard plot was obtained by taking 1mL aliquot of the standard solutions(0-20%) in a volumetric flask of 50 ml .The beaker was already containing 25 ml of freshly prepared potassium dichromate solution($K_2Cr_2O_7$) .9ml distilled water was added to make the volume 35ml each and then this samples were heated in water bath at 65°C for 25 minutes. Finally after cooling of the above solution 15 ml of water is added so that the final volume becomes 50ml. Absorbance was measured at a wavelength of 600nm by uv-vis spectrophotometer.

2.4.9.1 .4 Estimation of Bioethanol in the fermented sample:-

1ml of the extracted ethanol-water mix was taken in 50 ml volumetric flask previously containing 25 ml of freshly prepared potassium dichromate solution ($K_2Cr_2O_7$) .9ml distilled water was added to make the volume 35ml each and then this samples were heated in water bath at 65°C for 25 minutes. Finally after cooling 15 ml of water is added to the above solution so that the final volume becomes 50ml. Absorbance was measured at a wavelength of 600nm by uv-vis spectrophotometer.

2.5 Results and Discussion:-

2.5.1 Figure Below represents the extracted cellulose and lignin from a single extraction technique.

Figure 5.0 (a) Groundnut powder 1(b)alkali treated 1(c)Sodium hypochlorite treated 1 (d) MC

1(e) Precipitation of lignin 1 (f) Extracted lignin

2.5.2 Fourier-transform infrared spectroscopy (FTIR):-

Microcrystalline extracted cellulose were analysed through the FTIR, which evidently stated the presence of a broad bands of spectrum in the range of 3323 cm^{-1} which indicated the presence of huge number of hydrogen bonds of various types many types. The conformation of certain molecules are stabilized due to the presence of –OH groups. From the range 1800-2700cm^{-1} there is absence of any significant bond vibration .Similar results are stated by *Morrison and Stewar*t *(1992)* in their study which reported that absence of any absorbance band in 1800-2700cm^{-1} . in the microcrystalline cellulose extracted the absorbance band found at 1214cm^{-1} shows the presence of asymmetrical C-O-C linkage in ether and esters*(Bhat et al 2009.,)* .The absorbance band at wavelength 2889 cm^{-1} is representing the C-H bending and stretching bonds indicating towards the CH bond in cellulose microcrystal. A week S=O bond is also found in cellulose .The stretching vibrational bond C-O is found near the wavelength of 1028 cm^{-1}*(Gelbrich et al 2013)* .

A wide range of absorbance bands are seen at wavelength 3448 cm^{-1} in extracted lignin because of the existence of OH bending and stretching vibrations which is mainly caused due to the presence of alcohol or phenol groups which were forming different types of hydrogen bond. Abundant of side chains are found predominantly in lignin which represents bonds at 2935 cm^{-1} whereas at the wavelength 2842 cm^{-1} C-H of methylene units is present (*Yan, et al.,2015*).

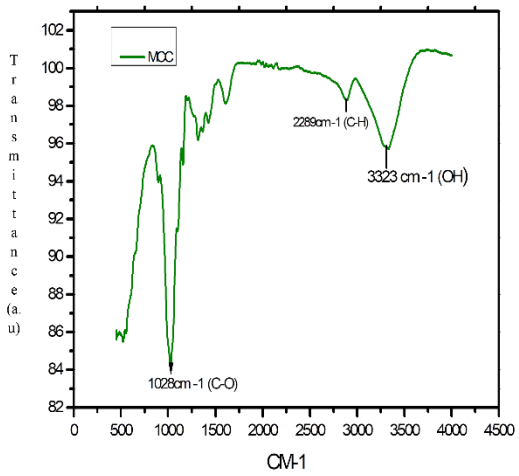


Figure 5.1 :- FTIR of Microcrystalline Cellulose

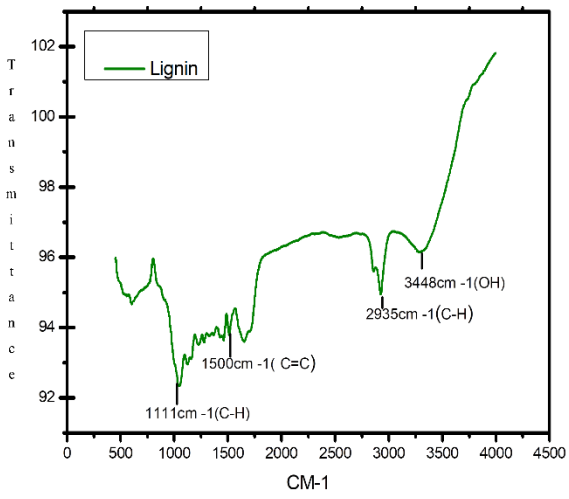
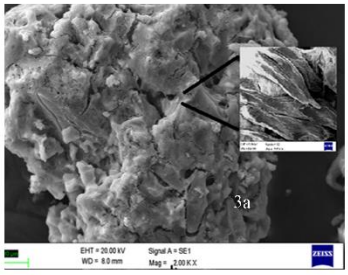


Figure 5.2 :-FTIR Of Lignin

2.5.3 Scanning Electron Microscopy (SEM):-

To investigate the Surface morphology of extracted cellulose crystal sample (MCC) Scanning electronic microscope (SEM) was done. The first figure shows the surface morphology by SEM of the extracted cellulose from groundnut shell waste. The second picture represent the elaborated image of cellulose after the hydrolysis process done by *Aspergillus sp.(*SEM micrographics) .After the saccharification process the surface the surface structure of the extracted cellulose from groundnut waste changed evidently. The surface morphology of the extracted microcrystalline fibrous cellulose changed from smooth surface to rough fibrous surface by the action of *Aspergillus sp.* This change in the surface structure is seen by the different images taken by the Scanning Electron microscope (SEM).



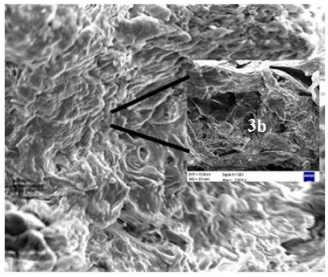


Figure 5.3 (a) SEM of the extracted cellulose fibres

Figure 5.4 (b) SEM of the cellulose after the process of hydrolysis by *Aspergillus sp*.

2.5.4 X –Ray Diffraction:-

The figure shown below is the X ray diffraction (XRD) pattern determined of the extracted microcrystalline cellulose fibre done through various chemical pre-treatments. Two sharp and distinguishable reflection peaks are seen at 2 θ =22.6 and 34.8 in the extracted cellulose. The absence of peak at 2 θ=16 which was predominantly found in the raw peanut shell is due to the surface morphology change in extracted cellulose by the different pretreatments. The shift in the peak at 2 θ=22.6 can also prominently seen. The cellulose I (native type) has been converted to Cellulose II by chemical pretreatment (*Maki-Arvela et al 2010.,Wang et al 2011.,Bhcegul et al., 2012*). The broad and pre-eminent peak at 2 θ=22.6 in the extracted microcrystalline cellulose is because of the hydrolysis step which digest the native structure of cellulose. Similar results were given by *Cheng et al 2012., and Bahcegul et al 2012* in their research. The amorphous nature of the extracted cellulose can be determined by the peak at 2 θ=22.6. (*Punnadiyil etal 2016*) and others already investigated the crystallinity percentage of the extracted cellulose.

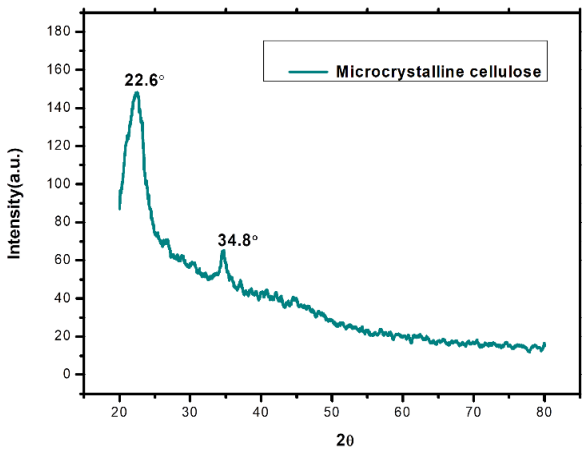


Figure 5.5-shows the X RD of the extracted microcrystalline cellulose

2.5.5 Anthrone test results:

The main component of the lignocellulosic biomass is the cellulose which are organic component with long linear chains of polysaccharides. The anthrone test is done to determine the cellulosic component of the extracted sample done through different chemical pretreatment. In a report submitted by (*Suryawanshi et al 2017*) stated that by 4% hydrolysate pretreatment of the lignocellulosic shell waste 3.9mg/ml was the maximum amount of carbohydrate content estimated. In this study total carbohydrate content was found nearly 11.41mg/ml in the extracted microcrystalline cellulose.

2.5.6 Dinitrosalicyclic Acid Test Result :-

Di-nitrosalacylic acid test was done for the investigation of the amount of total reducing sugar produced by the hydrolysis process on the extracted cellulose by *Aspergillus sp.*Different kinds of parameters were investigated ((such as temperature, dose, pH, substrate).

2.5.6.1 Effect of pH:-

The effect of pH on the amount of total reducing sugar production by the *Aspergillus sp.* Is investigated. The highest amount of total reducing sugar generated by *Aspergillus sp.* was reported to be (1.17 mg/ml) at pH (7) by utilizing only 1 gram of the substrate that is the cellulose. (*Sazci, et al.1986),* observed similar results at pH 7.0 At neutral pH condition the reducing sugar production was highest whereas at acidic and basic pH the reducing sugar production is compatible less . *The pH was taken in between the range of 4,7 ,10respectively . And a bell shaped grapH was seen. Akiba et al 1995.*in his report submitted that pH activity is maximum at 6.0 and 7.0 pH whereas another reported submitted by (*McCleary and Glennie-Holmes 1985)* stated that optimum pH for *Aspergillus sp.*is 4.0-4.5 pH. Due to difference in the species type of Aspergillus a difference in the reducing sugar production yield was observed.

2.5.6.2 Effect of substrate:-

Effect of substrate concentration on the amount of reducing production was shown in Figure below. With the increase of substrate concentration there is an increase of considerable amount of reducing sugar was observed. *The substrate concentration were taken in range between the 1,3 ,5 gram respectively* The maximum amount of reducing sugar production by this experiment was observed to be 3.16 mg/ml by utilizing 5 gram of extracted cellulose. *Acharya*

et al 2008) reported similar results with substrate concentration of 9.6 gram (. Higher cellulose activity was obtained with higher substrate concentration)

2.5.6.3 Effect of Temperature:-

On the reducing sugar production by *Aspergillus sp.* The effect of temperature is investigated. In this experimental research work the temperature was taken in a range from 26-46°C . The maximum reducing sugar generation was observed at 36°C .The total amount of reducing sugar generated was estimated to be 1.27mg/ml using only 1 gram of extracted microcrystalline cellulose. (*Kulkarni et al 2017)* found similar temperature results, whereas (*Gupta et al 2015)* reported maximum sugar production by utilizing *Aspergillus sp with* wheat bran and bagasse pith at a temperature of 32°C using twenty three different fungal strain which was firstly isolated from soil samples from paper mill industry..

2.5.6.4 Effect of dose of *Aspergillus sp*:-

To investigate the effect of reducing sugar production on the inoculum volume of *Aspergillus sp.* this part of the experiment was conducted. The dose were taken in the range of 1,2and 3 ml of $1.25x10^6$ spores/ml respectively .The amount reducing sugar generated maximum is about 0.9 mg/ml with 3 ml of $1.25x10^6$ spores/ml utilizing 1 gram of the extracted cellulose substrate. (Abo-state 2010) found similar result by *Aspergillus flavus* and found that cellulose activity was maximum at 3.0ml of 1x106 spores/ml of spore suspension. Sharma et al 1996 further confirmed this result in his report.

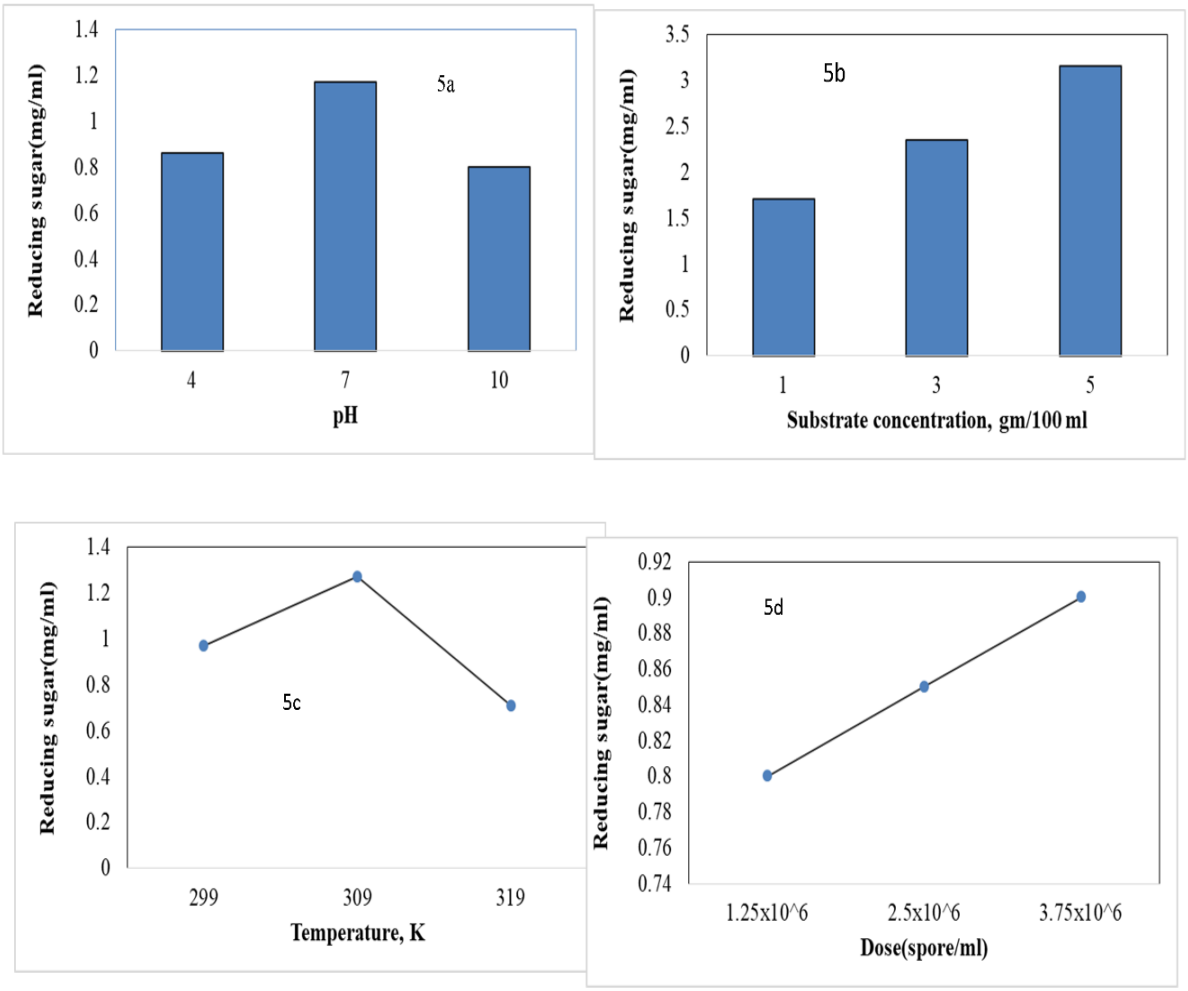
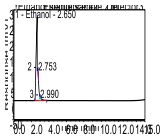


Figure 5.6 :-Represent the effect of different parameters(dose, substrate, temperature and pH) on reducing sugar production by *Aspergillus sp*

2.5.7 Bio-Ethanol Generation:

Determination of the bioethanol was done by both the dichromate test and Gas ChromatograpHy. The saccharified media was further fermented by both *Zymomonas mobilis (MTCC 92)* and *Sacchromyces cerevisiae (MTCC 170)* microbial strains. Aerobic and aerobic fermentation conditions were applied and the results were recorded.

By Dichromate test the amount of bioethanol generated by *Zymomonas mobilis and Sacchromyces cerevisiae* in aerobic environmental condition was 15.14% and 12.986 % respectively., whereas **in absence of oxygen the amount of bioethanol extracted was** it 19.13**%** in case of ***Sacchromyces cerevisiae*** *and Zymomonas mobilis* was able to produce 14.15% by using 1 gram of the extracted microcrystalline cellulose substrate. From the research it can be concluded that Anaerobic fermentation was much more better for bioethanol generation process because both the microbial strains used for this technique are facultative anaerobes which consequently showed better result in absence of oxygen. (*cheng et al 2014)* in his reported submitted the reason for the better results shown in anaerobic fermentation is that glucose solely was used as a source of carbon . (*Rabah et al 2011*) previously reported in his journal that lower amount of bioethanol is extracted from peanut shell waste when *Zymomonas mobilis is used in the experiment.*



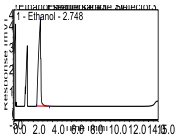


Figure 5.7 :-The above GC plot shows the ethanol estimation when fermented anaerobically

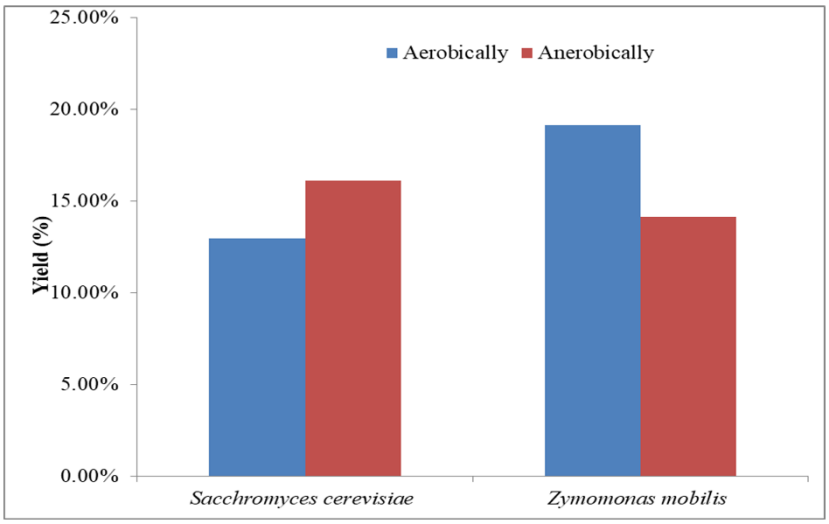


Figure 5.8: Ethanol by fermentation (Aerobic and anaerobic) and its recovery .

2.6 Conclusion:-

From this experiment we can come to this conclusion that bioethanol generation can be done by utilizing the extracted cellulose from the peanut shell waste. Therefore we can come to this conclusion that:-

A. Peanut shell waste have high concentration of cellulose which can be utilizing for biofuel generation if proper pre-treatments are performed.
B. *Sacchromyces cerevisiae* and *Zymomonas mobilis* have potential for ethanol production from peanut shell waste
C. *Sacchromyces cerevisiae* better ethanol production in absence of oxygen whereas good results by *Zymomonas mobilis* is obtained in presence of oxygen.
D. During this experiment a important value added product is also generated which has potential in waste water treatment and other industrial purposes.

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Chapter 3

Cellulose, Lignin And LCS as absorbents for the removal of dye from waste water

3 Introduction:-

Modern day activities and rapid commercialization have led in the production of large amount of lesser value materials. These materials are generally considered as waste and there is a problem with their disposal. Utility of such material is common now a days but their disposal must be highly investigated. This toxic waste includes acid, metal salts and radioactive materials. Besides these toxic waste other industrial, domestic waste also add to many water pollutants.

3.1.1 Problem Associated With Waste water:-

Severer exploitation of natural sources have cause the deterioration of the climate and environment which in turn is causing harm to human health. The most important element of life is water after air. The intense use of fresh and purified water resources is causing an increase in the presence to toxic materials (such as organic and inorganic matters),biological entities as well as heat and radiation on the surface of water bodies. The characteristic of this chemical agents varies from place to place, industry-industry and also due to modern civilization. Therefore on the dependency on various activities the water bodies can contain different types of pollutants.

The removal of these toxic component from water resources is a tedious and difficult task because there are numerous numbers of different components are present in different concentration(high-to-very low).the governmental agencies as well as industries are becoming aware of the fact to clean or reduce the pollutants in rivers. The standards of effluent discharge by the industries is becoming very strict.

3.1.2 Types of pollutants:-

The substances or the matters which can gradually change the natural quality of the environment by any chemical, physical or by biological means is termed as pollutant. The most common types of pollutants found in water bodies includes dyes, phenol, poly-nuclear

hydrocarbon, poly chlorinated biphenyls, detergents heavy metals, insecticides, pesticides etc. If this compounds are present over a certain level they are considered as toxic.

3.1.3 Biological Agents:-

Biologically active microorganism which are common in the sewage effluents are Salmonella, Shigella, E.coli, Streptococcus, Pseudomonas aeroginosa ,Giardia and mycobacterium according to the report submitted by Water quality and health council. Some of these microbial strains caused water born diseases like dysentery, cholera, jaundice and typhoid. Shigella outbreak have resulted into scarcity of freshwater shellfish.

3.1.4 Heavy metals:-

Industrial effluents are mainly predominated by heavy metals. Even at relatively lower concentration these heavy metal pollutants are toxic to aquatic fauna and flora. Chromium is the main metallic discharge from the tannery industries. Metal plating industries mainly discharge cadmium, copper, zinc and chromium. Mercury is resulted by the process of mining, production of electrical equipment, fossil fuel burning and smelting which also contribute towards the pollutant load of water bodies. Lead is produced generally by mining process as well as various industries.

Other heavy metal discharging industries includes rubber industry, oil refining , chemical and paint industry. Toxic symptoms are produced in the human body if the ingestion of zinc is greater than 2 grams. Most of these heavy metals are important to human body but in trace amount if the amount increase the minimum level this can become toxic.

3.1.5 Phenol:-

Besides the heavy metals among the priority level of harmful pollutant the phenol holds the second toxic pollutant. It can impart carbolic odour and bad taste to the fresh water and are toxic at a minimum level to the marine fishes and human being. The phenol are mainly present in the industrial effluent from chemical, paint ,paper and pulp industry ,gas and coke manufacturer, resin, insecticide, pesticide and dye industries. The permissible limit of concentration of phenol in industrial effluent before discharge in water bodies or municipal sevages is 1-5mg/l.

3.1.6 Heat:-

There is a little or negligible variation in the ideal temperature of the water bodies. Water is used as a cooling media due to its heat capacity. Water carrying away heat are generally discharged from industrial effluents. The higher temperature of waste water not only effect the marine life but also causes different types of corrosive activity and chemical- biological reactions such as tri halo methane formation.

Completed Chart

Type of Water Pollution	Cause of Pollution	Symptoms of Pollution	Effect of Pollution	Source of Pollution
Biodegradable waste	Humans and animals	Decreasing numbers of fish and other aquatic life, increasing number of bacteria	Increased number of bacteria, decreased oxygen levels, death of aquatic life	Run-off, improperly treated effluent,
Nutrients	Nitrates and phosphates	Green, cloudy, slimy, stinky water	Algae blooms, eutrophication of water source	Over use of fertilizers, run-off from fields, improper disposal of containers,
Heat	Increased water temperature	Warmer water, less oxygen, fewer aquatic organisms	Decrease in oxygen levels, death of fish and plants	Industrial run-off, wastewater treatment
Sedimentation	Suspended particles settling out of water	Cloudy water, increased amount of bottom	Warms up water, decreases depth of water source,	Construction sites, farming and livestock operations, logging, flooding, city run-off, dams
Chemicals	Toxic and hazardous chemicals	Water colour changes, develops an odour, aquatic life die out	Kills aquatic life, can enter human food chain, leads to birth defects, infertility, cancer and other diseases in humans and animals	Human-made, improper disposal, run-off, dams, landfill leachate, industrial discharge, acid rain
Radioactive pollutants	Radioactive isotopes	Increased rates of birth defects and cancer in human and animal populations.	Kills aquatic species and leads to cancer and death in humans and other animals	Waste water discharges from factories, hospitals and uranium mines
Medical	Medicines, antibiotics	Infertility in aquatic organisms, and other unknown symptoms	Effect the marine species, adverse effect on flora and fauna	Humans dumping medicines into water systems, wastewater treatment
Microbiological	Bacteria, viruses, protozoa	People and animals become ill with gastrointestinal disorders	Undrinkable water	Improper treatment of water/effluent, can occur naturally

Table 1.4:-Represent the different types of pollutants present in industrial effluent

3.1.7 Dye:-

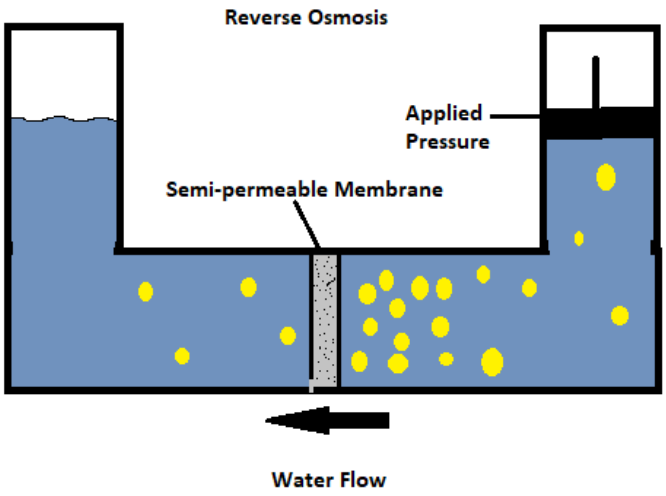
The most frequently occurring organic group of compound in the waste water from industries is the dye. Dye are considered important water pollutant and they have the ability to impart colour to other materials. Economically feasible pre-treatment for colour technique are available in the dyestuff industries effluent. For the purpose of eye appeal, brand identification and colour coding in industries and household reason a wide range of coloured pigments and dye are used which are commonly known as colorants. The used of dye has been started very earlier in the Stone Age even before the signs of civilization started. From plant extracts and animal resources a number of natural dyes are obtained. However petrochemical feedstock is used for the purpose of synthesis of synthetic dyes. There are a wide range of dyes available commercially now a days.

Dyes are mainly used in dyeing, textile, pulp and paper, petroleum refining, tannery, pharmaceuticals, paints, food processing, fertilizer and a numbers of other industries. The effluent discharge from the above industries as well as dye manufacturing companies have appreciable amount of dye in it which is generally considered as unpleasant type of waster pollutants. The dye are considered obnoxious type of pollutant because they can impart undesirable odour, colour, taste to the fresh water which is harmful for the aquatic life and human health because of the carcinogenic and toxic effects. The dyes are resistant to bio-chemical oxidation and remains in water for longer period of time and can reach to a hazardous level with multiple reuse of such effluent. By conventional waste water treatment method it is difficult to remove the dyes in the discharge because they are resistant to aerobic digestion, have oxidizing property and are stable in light. The use of thermally stable dyes now a days are much more difficult to be removed from the waste water. Therefore prior pretreatment of dye containing waste water is need for their removal.

3.1.8 Treatment Method:-

The type of treatment used for the removal of dye depends upon the nature of pollutant present in the waste water. The most commonly used techniques for pollutant removal includes reverse osmosis, dialysis, oxidative destruction by UV/Ozone treatment, biological method, photo catalytic degradation, electrochemical reduction etc. The removal of the pollutants by the above techniques is efficient for the toxic compounds removal but there installation and operational cost is quite high and also they can cause an inhibition to the finishing and dyeing industries.

The impurities in the waste water have different grain size therefore can be removed by the process of sedimentation. The removal of unstable suspended solids can be simply achieved effectively by the gravitational separation by sedimentation technique. Filtration is an effective technique for the removal of solids present in water such as precipitation of manganese and iron present in various well water supplies and also used in precipitating hardness from lime-softened water. Some of the impurities are so small in size that they cannot be removed by gravity separation technique alone. The two most commonly used technique for the treatment coloured waste water are biological method and precipitation. The major drawback of using these techniques are long term biodegradation and disposal of sludge. The frequently used conventional technique to treat textile effluent discharge are activated sludge processes and aerated lagoon technique. The above technique discussed are largely effective for the removal of suspended solids and chemical oxygen demand and have a drawback of not able to remove the colour of the waste water. Therefore new physico-chemical technique has been investigated for the removal of colour from textile effluent discharge. These study include ultra-filtration, membrane filtration, coagulating agents, precipitation, chemical oxidation, adsorption, ion exchange technique and electro chemical method.



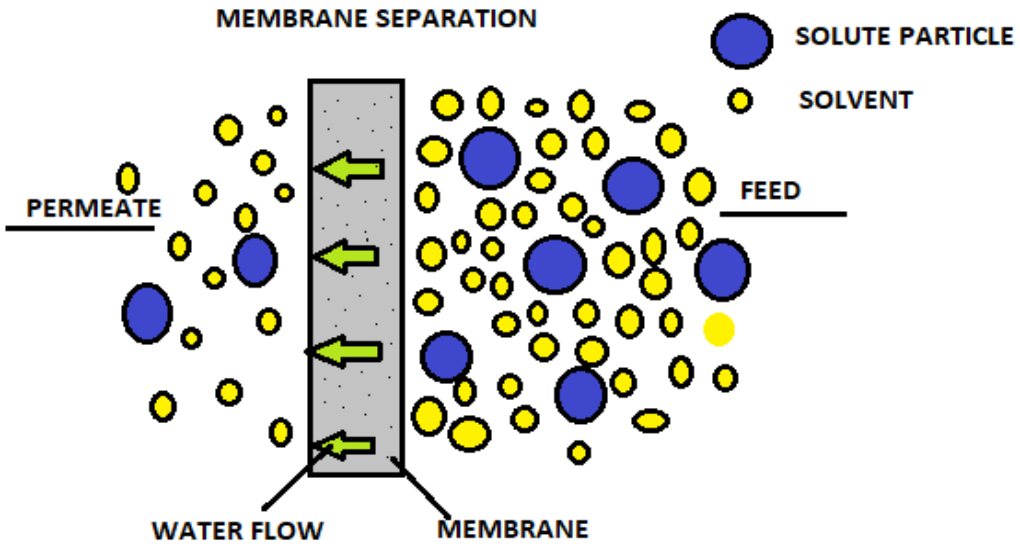


Figure 5.9:- Represent common treatment practices

3.1.9 Adsorption:-

The alternative to different expensive dye removal technique from waste water is the adsorption. A survey submitted in 2008 claimed that among the various methods used for the removal of pollutants from waste water, the adsorption technique for the removal of pollutants on the solid film is broadly used technique because is it economically feasible, efficient, effective and also able to remove diverse verity of pollutants. Adsorption process is superior because water can be reused, low initial and instalment cost, simple in design, low energy requirement and there is a possibility of regeneration of the adsorbent.

It has been proved one of the best methods for removal of waste residues which are not able to be removed from other techniques. Kayser introduced the term adsorption to suggest the condensation of gaseous molecules on the free surface. In gaseous adsorption process the gas molecule penetrate the solid bed. Adsorption process is widely used for the elimination of heavy metals, dyes and other colour pigments, phenolic compounds, non-degradable matters and refractory organics.

A wide range of solid adsorbent beds are developed and are commonly used for adsorbing solutes from gases as well as solutions. In pollution control the wide application and versatility of adsorption has been recognized. The adsorption process have various advantages over other method because it is a clean technique, sludge free and complete removal of dye from the aqueous solution takes place.

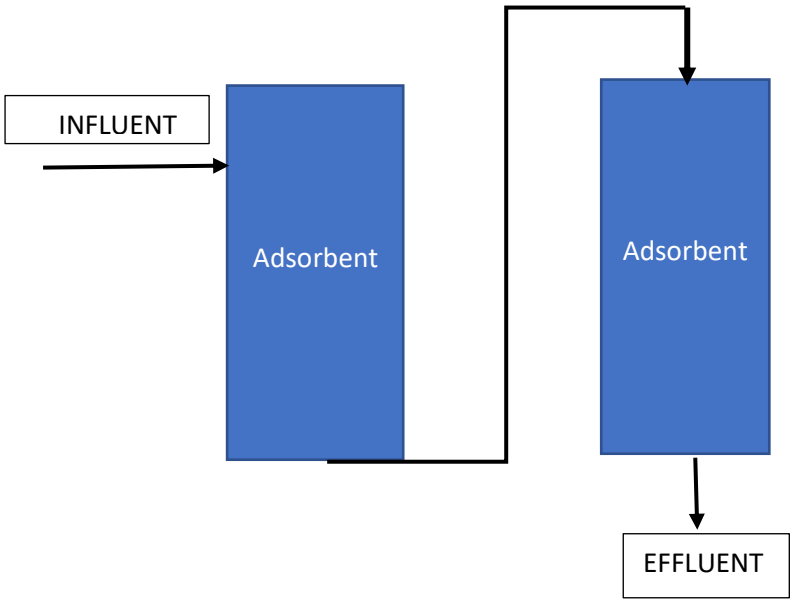


Figure 6.0: Principle of Adsorption

3.1.9.1 Adsorbents:-

The most important characteristic of a good adsorbent is that it should possess higher surface to volume ratio that is it should have porous structure which will result in board surface area. The other advantages of a good adsorbent is that the equilibrium of the adsorption process should reach in minimum time possible, so that the pollutants are removed in low time. Therefore from above we can conclude that higher the surface area of the pollutant faster will be the kinetic and lesser time will be required.

Commonly used Adsorbent:-

3.1.9.2 Cellulose:-

Cellulose is a organic compound and the most biopolymer on the earth. The cellulose is one of the most important constitute of the plant cell wall (*Wyman et al., 2005*).cellulose is a polysaccharide consisting of a straight chain of thousands of B-linked D-glucose units . The major characteristic of cellulose is that it is crystalline in nature and insoluble in water (*Wyman et al., 2005; Mosier et al., 2005*). The main application of Cellulose or cellulose derivatives are in textile, paint, polymer, fibre, and paper industry (*Swatlowski et. al, 2002*).

The Beta(1-4) glucosidic linkage found in the cellulose biopolymer is entirely different to that are (1-4) glucosidic linkage found in glycogen and starch. Furthermore, branch chain polymers does not exist in cellulose which are present in the starch. Cellulose consist of porous

heterogeneous internal and external surfaces. The external surface area of cellulose can be determined by the size and shape of the cellulose particles whereas the internal area can be determined by determines capillary structure of cellulose fibres. Cellulose fibres consist of both amorphous and crystalline regions on it. The part of crystalline structure present in the total surface of cellulose is considered an important factor because it has a severe effect on the hydrolysis of cellulose as it provides resistance to degradation. The commercial name of microcrystalline cellulose is Avicel.

3.1.9.3 Lignin:-

The second most abundant biopolymer on the earth is the *(Kim et al., 2011*). This is one of the major component of the plant cell wall .Lignin component of the cell wall provide the rigidity and strength to the plant cell wall. The lignin constitute around15 wt% - 40 wt% of the total dry mass of the plant cell walls. Lignin are more recalcitrant to chemical, pHysical, and biological attacks to any other components of the cell wall. Lignin is an amorphous phenolic, three dimensional, cross – linked polymer mainly composed of p-coumaryl, coniferyl ,monolignols; and sinapyl alcohols. The monomeric units of lignin are connected by heterogeneously interunit linkages (*Kim et al., 2008*). The Molecular weight of the lignin is in the range of 1000 - 20000 g/mol. The lignin polymer's glass transition temperature is dependent on the molecular mass of lignin. The temperature increases with increase in the molecular mass (*Doherty et al., 2011*).

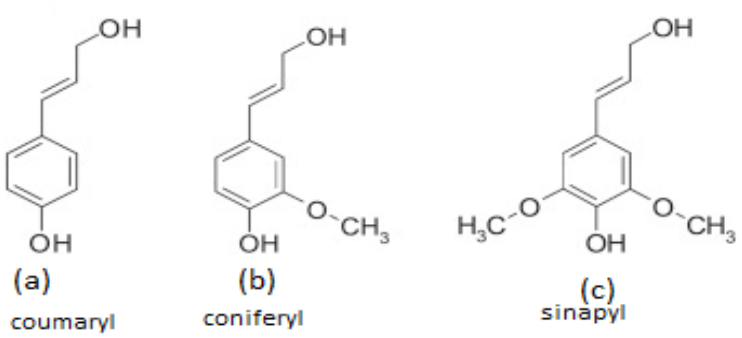


Figure 6.1:- Monolignol monomers: p-Coumaryl alcohol, Coniferyl alcohol, and Sinapyl alcohol

Lignin is a three – dimensional, heterogeneous, complex in structure polymer. Lignin has different types of intermolecular linkages,which are not present in cellulose. The primary

type of intermolecular linkage found in lignin is arylgycerol- -O-4-aryl ether bonding. Arylgycerol- -O-4-aryl ether bonding in lignin makes about 50 – 60% of the total intermolecular linkage (*Braun et al., 2005*). Lignin has been used for industrial purpose. Lignin is derived as a byproduct from newspaper pulping industries. Most significant use of lignin is its lignosulphonate form. Lignosulfonate has been used in oil industry, cement and concrete industry and also used in dye industry for the removal (*Doherty et al., 2011*). Lignosulfonates can also be used as a feedstock for the production of various value added products like pesticides, carbon black dispersant, dyes and pigments, animal feed pelleting aids, emulsifiers, battery expanders, industrial cleaners and industrial cleaners (*Doherty el al., 2011*).The cold crystallization temperature and modulus can increased if lignin is mixed with synthetic or natural polymer whereas the melting temperature is decreased.

3.2 Literature Review:-

3.2.1 The resource of colour in dyes:-

An organic compound that can impart colour to different materials such that plastic materials, food, hair, wax, textile fibre and leather is referred as dye (*Zollinger et al 1991*). In early development of civilization the dye were originally extracted from the natural plant. Now a days all the dyes available commercially even the natural occurring dyes are synthetically synthesized. The dyes mainly contains two types of groups 1) auxochromes 2) chromophore. The colour of the dye is due to the presence of chromophore groups. This chromophore are mainly electron withdrawing groups and also this are the atoms which also control the colour of the dye. –N=N–,–C=C–, –C=N–, –C=O–, –NO2, –NO groups is the most important chromophores groups. –NH2, –NR2, –NHR, – COOH, –SO3H, –OH and –OCH3 groups are the main auxochromes group (Dos Santos 2005). The main electron donating substitute in dye are the auxochromes whose main function is to intensify the colour caused due to the chromophore and also provide the adherence and solubility of the dye towards the fibres. Auxochromes and chromophore together in the dye molecules are referred as chromogen (*Wallace 2001*).

3.2.2 Categorization of Dye:-

The dyes are mainly classified in two groups either due to their application (colouristic classification) or composition of dye (chemical classification) (*Rys and Zollinger 1972; Trotman 1990*). The mainly considered classification is the chemical classification and have various advantages over the colouristic classification. To list the dye and pigments available commercially for the colourization purpose such as paints, printing inks, pigment colouration of plastics, dyeing of textile fibres, and the colouration of liquids (solvents) both type of classification(colouristic classification)(chemical classification) are used by the colour index in 1917. On the basis of the classification based on application the list of commercially available dyes are given below (*Agarwal 2006*).

3.2.2.1 Acidic Dye:-

Acidic dye are water soluble dyes with various groups substituted on the acidic functional groups which includes sulphonic acid, nitro- and carboxyl. The presence of this substitutes on

the functional group of acid are responsible for the solubility of the dye. This acidic dye are mainly used for dying of animal fibres such as wool and silk but are not useful for plant extracted fibres like linen and cotton. This are always useful only in solutions of acid. The most commonly known acidic dyes includes orange II, martius yellow and picric acid. To cellulosic fibres the acidic dye is not substantive (*Agarwal 2006*).

3.2.2.2 Basic Dye:-

Basic dyes are water soluble cationic form of dye. The basic dyes are mainly used in the modified polyesters, polyacrylonitrile, modified nylons, and paper industries. The basic dye are additionally used to impart colour to tannin–mordant cotton, wool and silk where the brightness shade of the material is not as much important than of the fastness to washing. Some of the common examples of the basic dye includes, hemicyanine, diazahemicyanine, cyanine thiazine, acridine, triarylmethane and oxazine (*Agarwal 2006*).

3.2.2.3 Direct Dyes:-

Direct dyes are anionic dyes which are water soluble. This dye shows greater affinity towards the cellulose fibres in the presence of electrolytes. The direct dyes have their applications mainly in the paper, cellulose, leather, cotton and nylon industries. In direct type of dye most of them are polyazo compounds along with phthalocyanines, stilbenes and oxazines (*Agarwal 2006*) are constituted by some.

3.2.2.4 Mordant Dyes:-

The dying properties of this type of dyes are generally considered mordent. the major advantage of this dyes are that certain substitutes present in this type of dye molecule are capable of holding metal residues by forming linkages with covalent and coordinate bonds basically involving a chelate compound. The mordant and their metallic salts which are used commercially includes the salt of copper, nickel, iron, cobalt, aluminium, chromium and tin (*Agarwal 2006*).

3.2.3 Methods Available commercially for removal of dye:-

The literature so far reviewed have shown the different types of dye available commercially in the market. The conventional dye removal techniques available includes the biological, chemical and physico- chemical techniques as well as some of the emerging new techniques. Various conventional dye removal technique are not used widely for removal of dye in the

waste water on the industrial level because of the sludge disposal problem and relatively high operational and installation cost. No single technique is available for the treatment of dye removal in waste water because of the complexity present in the industrial effluent discharge (*Crini 2006*).

3.2.4.1 Biological Technique:-

One of the most economically feasible alternative effective method for the removal of dye from waste water when this technique is compared to other microbial biocatalyst. The other advantages of the biological treatment process for the removal of dye pollutants from textile effluent discharge over the conventional pre-treatment process is that it has minimal adverse effect on the environment and also it is economically feasible. The additional benefits with this process includes that rigorous monitoring is not required and there is a complete mineralization process with nontoxic by products.

The biological process can be anaerobic (absence of oxygen) , aerobic(in presence of oxygen) or combination of both (Stolz 2001). In biological treatment process the algae, bacteria and fungi microorganism are extensively studied for the treatment of dye in waste water. The microbial strain which is majorly used in this technique is the fungi. Fungi can be broadly classified in two groups1) living strain mainly used for the biodegradation and biosorbtion of dye molecules whereas the dead strain (fungal biomass) to absorption of dye (*Fu and Viraraghavan 2001*). The mechanism by which the fungal strain removes the dye is basically done by living fungal cells due to their property of generating the lignin modifying enzyme like manganes peroxidase, laccase and lignin peroxidase which can mineralize the synthetic dye as well as lignin. The physico- chemical interaction which includes deposition, ion exchange and adsorption are basically involved in the mechanism of adsoption by the dead fungal biomass (*wesenberg et al 2003*).

3.2.4.2 Aerobic biodegradation

White Rot fungi (Phanaerochaete Chrysosporium) in anaerobic condition can remove different types of dye such as Direct Red, Direct blue and Direct Yellow. The high fungal capacity to degrade the dyes from the industrial effluent is due to the presence of lignin peroxidase enzyme. *Wong and Jain (1999*) in his report submitted, stated that the commercial application of the fungal strain is not practically possible. The growth of lignin peroxidase is basically inhibited by the carbon and nitrogen presence therefore fungi cannot be used for degradation of different

dye molecules (*Perie and Gold 1991*). From the textile effluent the dye molecules can be removed by using the Trametes versicolor first reported by *Wong and Jain (1999*).

The enzyme Laccase can be used for the biodegradation of different dyes and this enzyme can also be regenerated even in the presence of nitrogen and carbon. Secondary metabolites are not required by the enzyme Laccase to catalyse the oxidation.

Alizarin Red S and Direct blue dye can be decolorized by the use of T.versicolor DSM11269 without any addition of redox mediators. Biodegradation of dye can be successfully done by the use of bacterial strains like, Pseudomonas (*Kulla et al 1983*), Aeromonas hydrophilia (*Jiang and Bishop 1994*) and Pseudomonas luteola (Hu 1994). It has been found that some of the microbial strain requires additional energy and carbon source are required for the formation of micro anaerobic zones in an aerobic system (*Zissi et al 1997*). The anaerobic reduction of azo dyes is facilitated with the creation of anaerobic zones (*Costerton et al 1994*). The ability of microbial strain Kurthia sp to degrade the crystal violet, Pararosaniline, Magenta, Brilliant Green and Malachite Green has been reported (*Sani and Banerjee 1999).*

3.2.4.3 Anaerobic Degradation:-

The azo dye biodegradation in anaerobic condition is studied in many research (*Carliell et al 1998, Banat et al 1996*). The actual anaerobic degradation mechanism of the dye is still known. The oxidation-reduction mechanism is suggested for degradation of the azo dye (*Carliell et al 1998*). The formation of aromatic amines is the major restriction in anaerobic azo dye degradation because further minimization of the reductive cleavage is not possible (*Rafii et al 1990, Chung et al 1992, Van der Zee 2002*). In the above technique discussed the accumulation is the major problem since they can form carcinogenic product such as benzene derivatives and naphthylamine (*Chung et al 1992*). For the complete degradation of dye combination of anaerobic and aerobic process can be utilized. The intermediate products which are produced in the anerobic condition were further degraded in the aerobic condition process (*Forgacs et al 2004, Rai et al 2005*).

3.2.5 Chemical Technique:

Electro kinetic coagulation, oxidation, coagulation/ flocculation and photo catalysis are the major types of chemical treatment methods. Chemical reaction mainly occurs in order to separate the pollutants from the waste water or to neutralize the hazardous components present in the pollutants. In this technique different types of chemical agents such as oxidizing agents

like permanganate, ozone, and hydrogen peroxide are used for the dye pollutant removal. But this type of chemical reagents can produce harmful disinfecting by products.

3.2.5.1 Chemical oxidation:-

One of the most traditional techniques for the dye removal is the oxidation method. It is mainly useful for removing odour, colour and impurities present in the waste water. The oxidising chemical reagent which includes ozone, hydrogen peroxide, chlorine and hypochloride attacks on the chromophore part of the dye which basically impart the colour to the materials (*Letterman 1999*)

a) Ozone:-

The most effective method for the removal of dye from the textile effluent discharge is ozonation due to its fast nature and as well as no toxic or sludge by-product is produced. There are two techniques of ozonation 1) direct application and 2) indirect application. In the ozonation technique the ozone gas is passed which breaks the conjugate double bonds present in the dye which is responsible for colourization in the dye (Srinivasan et al 2009). For the decolourization of the dye continuous discharge of ozone gas is required therefore this technique is higher in cost (*Xu and lebrun 1999*).

b) Sodium hypochlorite:-

The presence of chlorine in the hypochlorite leads to avoidance in the use of sodium hypochlorite. Now a days the use of chlorine gas for waste water treatment has been avoided because of the formation of harmful ,toxic byproduct such as haloacetic acid and trihalomethanes which inhibits the carcinogenetic and mutagenic properties which are harmful for mankind as well as to the aquatic life. When the reaction between the hypochlorite and dye molecules takes place they results in the production of some aromatic amines which are harmful, toxic and carcinogenic (*Banat et al 1996*).

3.2.6 Physical Technique:-

A effective conventional treatment for dye removal cannot consist a single process or technique; it is generally composed of various different processes, such that the output of one process becomes the input for the very next process. In this technique generally the first stage is made up for physical treatment processes. The most commonly used physical techniques are shown as followed.

3.2.6.1 Membrane separation processes

The membrane separation process includes mainly the ultra-filtration, reverse osmosis, microfiltration and Nano filtration for the dye removal technique which is based on the principle of membrane pressure. One of the most recent margining techniques foe the waste water treatment is the membrane separation technique. This technique comes with high separation efficiency, easy installation and operation, low energy cost and no by production formation, no pollution and so on. In this technique a porous membrane is used to filter and separation of the dye pollutant and impurities present in the waste water. Howerver this technique cannot be used on the industrial level because of certain drawbacks which includes membrane fouling, high investment and so on (*Ranganathan et al 2007*).

3.2.6.2 Adsorption:-

The utilization of adsorption technique for dye removal has become popular now a days. It basically refers to the accumulation of particulate matters at the interface of two phases which includes either solid and liquid or solid and gas. The material/bed on which adsorb the particulate matter is known as the adsorbent. Adsorption process can either be chemical or physical but it is found that mostly it involves combination of both (Cheremisinoff and Morresi 1978). On the chemical and physical properties of both adsorbate and adsorbent the adsorption capacity depends. This basically includes concentrate in which adsorbate is present in the liquid phase and the experimental conditions like pH of the solution, temperature, amount of adsorbate and the contact time between the adsorbate and the adsorbent.

3.2.7 Commercially Available Adsorbents:-

Various types of substances has been invigilated as an adsorbent for dye removal in waste water treatment. Some of them are as follows 1) activated alumina, 2) activated carbon, 3) silica gel and 4) Zeolites.

Silica gel is produced by decreasing the pH of the alkali silicate solution to a level less than eleven. The silica gel thus generated are inert, efficient support and nontoxic in nature. The silica gel consist of very large numbers of reactive site and hence the number of immobilization of the organic molecule on the gel is higher which ultimately result in higher adsorption capacity for metallic ions (*Rangsayatorn et al 2004*). Various researches are made to investigate the potential of silica gel for adsorption of the basic dye although the adsorption capacity is quite high there is a major drawback in the system that is silica gel production is a

very costly technique. Therefore the silica gel produced are also expensive adsorbent (*Gupta and Suhas 2009).*

Zeolites are low cost abundant resources. This are mainly crystalline hydrated aluminosilicates with a structure containing pores. This pores are preoccupied with alkali. Alkaline earth cations and water. In a study showed by *Han (2010)* reported that naturally available zeolite can be used for the removal of dye (Malachite Green) from the aqueous phase in a batch technique. And also regeneration of the zeolite can take place by the help of microwave irradiation. The used zeolites were treated with the radiation at 160 W, for about 10-12 minutes, and it was found that 85.8% of zeolite was regenerated. The adsorption capacity of this zeolite was found to be 25.14 ± 0.59 mg/g.

The activated alumina mainly consist of a series of non-equilibrium hydroxylated alumina oxide, Al2O3 which are in partial form. Activated alumina is mainly used to treat the ore of aluminium by making filter media, therefore it becomes highly porous and adsorptive in nature. The major disadvantage of using activated alumina is that only at low pH conditions the adsorption capacity is high and also the pollutants like arsenites firstly must be converted to arsenates by peroxidation prior to adsorption. The use of other pretreatment technique is necessary to decrease the level of pollutants present for human health concern (*Johnson et al 2005*). In order to make the result obtained less complicated in this study pure adsorbate are used.

3.3 Aim and Objective:-

Cellulose, lignin and LCS act as an important bio-adsorbent and show high adsorption capacity. All these bio-adsorbent carries negative charge on their surface therefore attract more strongly the positively charged ions thereby giving a higher adsorption capacity for the cationic dyes. The higher affinity towards the cationic dye is due to columbic attraction found between the anionic charge on the surface of adsorbate and basic charge on the dye molecule. In this study crystal violet dye CVD is used as adsorbate.

Aim:-

In this research study adsorption of CVD on lignin, cellulose and LCS is potentially investigated. In order to obtain information regarding the suitability of cellulose, lignin and LCS as potential adsorbents and also assess the possibility of efficiently treating textile effluent discharge. And also to find best ways to use this technique at commercial level with financial profit.

Objective:-

(a) Extraction of cellulose and lignin in a simple, effective and economically feasible way.
(b) Recovery of the left cellulose after hydrolysis process.
(c) Utilization of the cellulose, LCS and lignin as potential bio-adsorbents.
(d) Study different parameter (pH, contact time and adsorbent dose) for LCS and cellulose.

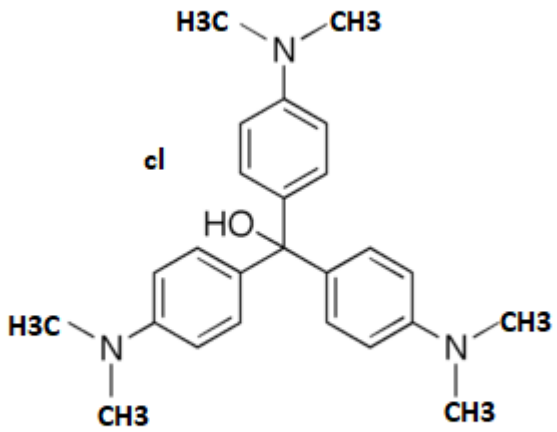
3.4 Method And Methodology:-

3.4.1 Materials:-

Peanut shell waste were obtained from the local food venders (Behala, India) and are used as the feedstock for this research study. The tastings of the raw feedstock were initially done in Jadavpur university laboratory which was based on the standard protocols that were given in the standard handbooks. Analytical grade chemical reagents were used for this experiment. This chemical reagents were used as they were received without any purification in them.

3.4.1.1 Dyes:-

For this experiment crystal violet dye was used which was procured from the Loba chemi Pvt. Ltd. Mumbai. The chemical formula of crystal violet dye is $C_{25}H_{30}CIN_3$ FW=407.98, CI=42,555 and the maximum absorbance is observed at 580-590 nm).



Molecular Structure Of Crystal Violet Dye

Figure 6.2- Structure of Crystal Violet

3.4.1.2 Other chemical used in the experiment:-

For this experiment the chemical reagents used are Sodium Hydroxide (NaOH)(Merk, India), Nitric Acid(HNO_3)(Merk, India), Ethanol(C_2H_5OH), Sodium Hypochlorite(NaOCl)(Merk, India), Sulphuric Acid(H2SO4)(Merk, India), Sodium Sulfite(Na2SO3).

3.4.1.3 Instruments:-

In this experiment for the temperature pH and substrate concentration study incubator was used at 30C for different time span. To check the dye removal percentage the absorbance was checked by spectrophotometer (Perkin Elmer, Germany).

3.4.2 Isolation of Microcrystalline Cellulose :-

The groundnut shell waste were collected from local merchant in behala Kolkata. The peanut shell were washed in tap water and then in distill water for several times in order to remove the unwanted impurities and dust particles. The peanut shell were sundried for 4-5 days and then grinded into fine powder. The powder of the peanut shell waste was sieved through 100um mesh in order to discard the larger particles of groundnut. The sieved groundnut shell powder was treated with (0.5M) Sodium hydroxide with continuous stirring at 700rpm on (REMI 1MLH) stirrer at about 92°C. The black liquor obtained after the above process was allowed to cool down and then filtered. The filter cake obtained after the process was washed several times to get constant pH whereas the black slurry obtained was kept in a air tight borosil beaker for further extraction of lignin. By the NaOH treatment the lignin was removed. The filter cake was further treated with 20% nitric acid in pure ethanol (22.1 ml of nitric acid in 78.9 ml of ethanol). This nitric acid treatment was carried 2-3 in order change the residue colour from reddish brown to pale yellow respectively. The residue obtained after this treatment was washed till the pH drop downs approximately to 7. Sodium hypochlorite chemical was finally used for the bleaching purpose of the residue from pale yellow to white. The white cellulose obtained after the process was first oven dried and then lyophilized for 4-5 hours, fine powdered cellulose was obtained after this complete procedure *(Punnadiyil et al 2016).*

3.4.3 Isolation of lignin from Black Slurry:-

The black dark slurry obtained during the cellulose extraction was poured down in a thick (500ml) plastic tight lid bottle with a magnetic bead in it in order to enhance through mixing. In order to enhance the ionic strength of the dark liquor Sodium sulphate (1gram in 250 ml liquor) was added .The lid of the plastic container was tightly closed and the bottle was placed in water bath at a temperature of 45ºC. The temperature was allowed to increase upto 75ºC which took approximately 1 hour. At a regular interval of 10 minutes the plastic bottle was taken out from the water bath and was stirred at 600 rpm for 5 minutes and then again placed back to the water bath .After 1 hour the black slurry was allowed to cool down and then finally treated with (6M) H_2SO_4 which was added dropwise. Lignin starts to precipitate. The

precipitate obtained from the process is filtered and then oven dried. This precipitation of lignin obtained is used for the dye removal purpose.

3.4.4 Extraction of Left Cellulose After Saccharification (LCS):-

The isolated crystalline cellulose were further hydrolysed by Aspergillus microbial strain to produce D-glucose monomeric units which in turn is fermented to bioethanol. The cellulose left after the process of hydrolysis was further washed with distill water ,dried and utilized for the purpose of dye removal. In this experiment the left cellulose after hydrolysis is abbreviated as LCS.

3.4.5 Stock preparation of Dye:-

The stock preparation was done dissolving 100 mg of dye in 1 litre of the distill water. The working solutions were prepared of 5ppm, 10ppm, 15ppm and 20 ppm respectively. The 5 ppm working solution was prepared by taking 95 ml of double distill water and then 5 ml of previously prepared stock solution. In the similar way the 10 ppm solution was prepared by adding 10 ml stock solution in 90 ml distill water . 15 ppm and 20 ppm were prepared by adding 20 ml and 15 ml of the stock solution in 85ml and 80 ml respectively.

3.4.5.1 Estimation of Dye:-

The crystal violet dye was estimated by using spectrophotometer. The absorbance of dye was measured at different wavelength and was concluded that maximum absorption of the dye was at 585.8 nm wavelength. λmax is defined as the wavelength at which the crystal violet dye showed the maximum absorbance of light rays at constant pH and temperature. The crystal violet dye was generally determined at a pH of 7 and temperature is about 30C

3.4.6 Adsorption Studies:-

3.4.6.1 Batch Method:-

The batch technique is used commonly because it is relatively simpler and economically feasible. The other major advantages of using batch technique includes the ease of invigilation at different types of conditions, this technique is free from complexities of hydraulic parameters native to flow kinetics of the system, its adaptability to smaller work volume and have general facility of operation.

For the adsorption studies a series 100 ml Erlenmeyer flask are used in this experiment. The different ppm aqueous solutions were taken and the final volume was made to 100 ml and a desired temperature and pH is maintained. Equal amount of lignin, cellulose and (LCS) was added to each conical flask. The flask were shaken for 2-3 minutes and then incubated at a fixed temperature and 2 ml of the solutions were taken at regular interval of 15, 30minutes 1,2 and 24 hours respectively from different ppm flasks. The vial containing 2ml of each different solution were then centrifuged the supernatant was analysed for the estimation of the dye concentration residue. For the calibration curve to be drawn the concentration of the residue dye in the diluted dye solution is to be estimated. The amount absorbed by the cellulose, lignin and LCS residue are estimated by the difference between final and initial concentration expression as percentage of adsorbent.

The concentration of the residue dye in the solution which is not diluted was estimated, by knowing the dilution factor. If in any case the absorbance of the residue dye stand out the absorbance range then further dilution should be made in the dye solution with distill water at a fixed pH concentration.

3.4.6.1.1 Effect of Adsorbate concentration:-

To observe the effect of concentration of adsorbent on the rate of adsorption was estimated in this experiment. Different concentrations of substrates were taken for different ppm of the dye. The adsorbate concentration was taken in the range of 50-200 mg/ml for crystal violet dye. It is very important to estimate the dose concentration of adsorbent required to achieve a desired level of dye removal from the waste water. Swamy etal showed in his report that with increase of the dose concentration the dye removal per unit volume is also increasing.

3.4.6.1.2 Contact time of the adsorbate with dye molecule:-

From the design and monitoring point of view the contact time is an important factor to be investigated. Adsorption by the adsorbate molecule of the dye solution is faster during the initial stages of the contact time but after some time it reaches equilibrium and then desorption is also observed at certain cases. The contact time was found to be dependent on certain factors such as rate of agitation, temperature, particle size etc. If any pretreatment conditions are applied on the adsorbate than also the contact time differs. The efficiency decreases when the contact time is less. The contact time was taken in range of 15 minutes, 30 minutes, 1 hour , 2 hour and -24 hours. In case of cellulose and LCS the adsorption was observed till 24 hour,

whereas in the case of lignin desorption was seen at 24 hour at all the different ppm (5,10,15 and 20 ppm)

3. 4.6.1.3 Effect of pH:-

In order to determine the influence of pH on the adsorption kinetics, three different substrate concentrations were taken for crystal violet dye at different pH (range between 2-10). Conventional and non-conventional adsorbent used of the removal of crystal violet dye from waste water is highly sensitive to the Ph. The pH may also have an effect on the charges present on the adsorbents surface, rate of adsorption and on the degree of ionization. The dissociation of the functional groups present on the adsorbate and adsorbent is affect by the change in the pH.

3.4.7 Characterization of Lignin, cellulose and LCS:-

3.4.7.1 Scanning Electron Microscopy (SEM): To observe the modification of cellulose after the hydrolysis step done by Aspergillus sp. scanning electron microscopy (SEM – EVO 18 / Carl Zeiss/United Kingdom) was used. The sample was firstly attached to a carbon tape the excess sample were removed by spraying air on the carbon tape further platinum coating was done on the sample to get better resolution (S7620 Quoter Model/Quorum/United Kingdom). To guarantee the authenticity of the result different parts of the samples were focused and imaged by the SEM in order to produce many images.

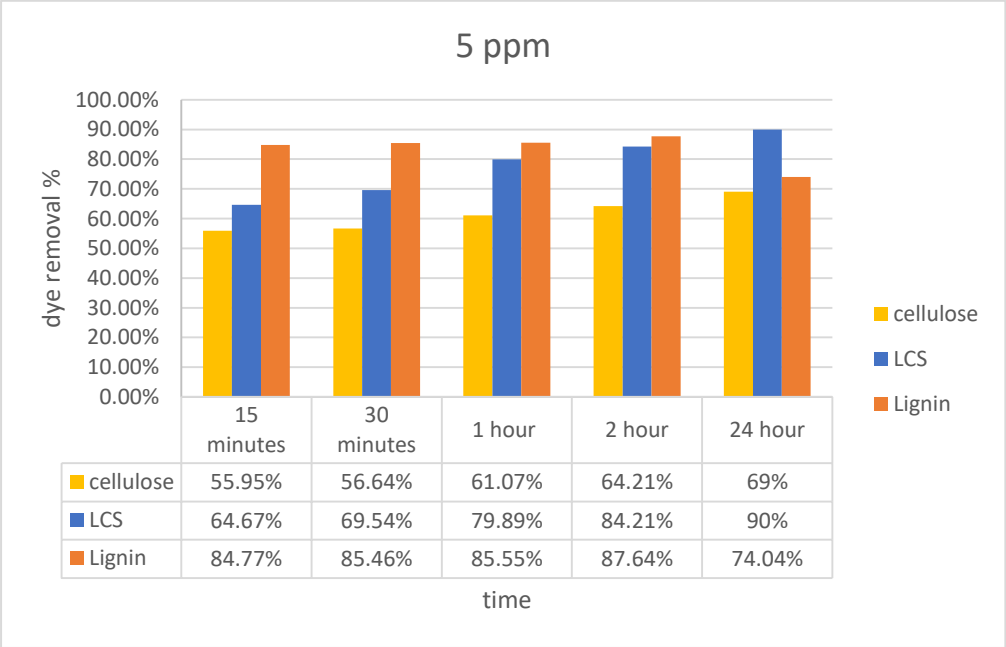
3.5 Result and Discussion:-

3.5.1 Result:-

In order to estimate the absorbance with the different types of substrate (cellulose, LCS, Lignin) it was important to assure thatno chemical reaction between the substrate and the dyes is taking place. The probability of any physico-chemical reaction taking place between the dyes and substrates was ruled out in this research study (Namasivayam et al., 1993). The substrates were extracted at different pH similarly the dye solutions was also prepared at various pH values. The absorbance of the dye solution was measured prior to mixing as well as after mixing. There was minute change in absorbance, so it can be neglected clarifying that there was no chemical reaction between the adsorbents and the dye.

3.5.2 Contact Time:-

The potential investigation of the effect of contact time on dye removal percentage by cellulose, LCS and lignin were studied and presented in the figures below. In the initial part of the experiment the Removal of dye increased rapidly by the different adsorbents. But after sometime slowed down as it approached towards the equilibrium time. The initial dye concentration were studied over the range of 5ppm,10ppm ,15ppm and 20 ppm respectively. It is shown from the table and fiqure below that with increase in the contact time the amount of dye removed also increases. From above we can concluded that removal of dye is dependent on the contact time.



	15 minutes	30 minutes	1 hour	2 hour	24 hour
cellulose	55.95%	56.64%	61.07%	64.21%	69%
LCS	64.67%	69.54%	79.89%	84.21%	90%
Lignin	84.77%	85.46%	85.55%	87.64%	74.04%

Figure 6.2:- Represent the dye removal percentage by different Adsorbents at 5 ppm initial dye concentration.

Discussion:-

The dye solution prepared in this case was of 5 ppm. From the above graph we can conclude that in case of lignin the maximum adsorption took place with 15 minutes after that equilibrium is reached and after 24 hours deadsorption can be observed. Whereas in Cellulose and LCS adsorbent case the maximum adsorption by the adsorbent was observed after 24 hours. The LCS showed maximum dye removal percentage (90%) after 24 hours .The initial rate of crystal bio sorption is fast because higher concentration gradient and the high availability of large surface area for absorption.

TIME	Dye removal percentage (%)	**(10 ppm)**	
	Cellulose	LCS	Lignin
15 minutes	43.33%	38.76%	75.67%
30 minutes	47.15%	44.55%	76.56%
1 hour	48.75%	51.68%	78.57%
2 hour	49.73%	66.75%	80.96%
24 hour	72%	93.34%	65.38%

Table1.5:- Represent the dye removal percentage by different Adsorbents at 10 ppm initial dye concentration.

Discussion:-

In the present experiment above, the initial dye solution was prepared for 10 ppm and the effect of different absorbent with the respect to time is evaluated. For cellulose the maximum dye

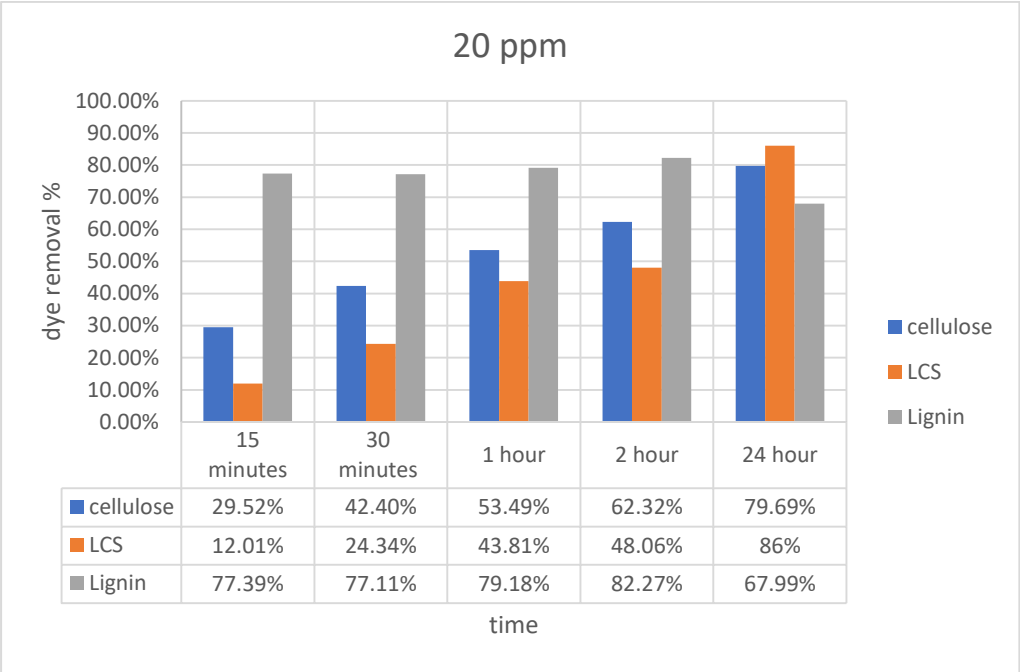
removal percentage achieved was 72% whereas after 15 minutes 43.33% of dye removal was already observed. In case of lignin the maximum adsorption seen is after 2 hours (80.96%) and at 24 hour deadsorption kinetics can be studied. At 10 ppm dye concentration the maximum amount of dye removal is achieved by LCS after 24 hour about 93.34%. Form this we can conclude that LCS has better removal properties than the cellulose and lignin adsorbent.

Time	Dye removal percentage (%)	(15 ppm)	
	Cellulose	LCS	Lignin
15 minutes	38.72%	53.90%	74.00%
30 minutes	49.66%	57.18%	74.31%
1 hour	53.12%	60.07%	77.86%
2 hour	58.55%	66.50%	78.74%
24 hour	74.79%	90.13%	69.07%

Table 1.6:- Represent the dye removal percentage by different Adsorbents at 10 ppm initial dye concentration.

Discussion:-

In the above table , the initial dye solution was prepared for 15 ppm and the effect of different absorbent with the respect to time is evaluated. For cellulose the maximum dye removal percentage achieved was 74.79% whereas after 15 minutes only 38.72% of dye removal was already observed. In case of lignin the maximum adsorption seen is after 2 hours (78.74%) and at 24 hour deadsorption kinetics can be studied with 69.07%. At 10 ppm dye concentration the maximum amount of dye removal is achieved by LCS after 24 hour about 90.13%. Form this study also we can conclude that LCS obtained after hydrolysis has better removal properties than the cellulose and lignin adsorbent.



	15 minutes	30 minutes	1 hour	2 hour	24 hour
cellulose	29.52%	42.40%	53.49%	62.32%	79.69%
LCS	12.01%	24.34%	43.81%	48.06%	86%
Lignin	77.39%	77.11%	79.18%	82.27%	67.99%

Figure 6.3:- Represent the dye removal percentage by different Adsorbents at 20 ppm initial dye concentration.

Discussion:-

From the above graph we can state that at 20 ppm the best result again is shown by the LCS adsorbent with dye removal percentage of 86%. Whereas the lignin also showed 82.27% of dye removal after 2 hour and deadsorption is found at 24 hour. The Lignin at the beginning showed Adsorptive nature with time but after 2 hour desorption of lignin was reported this is because the surface of the lignin are already occupied with dye particles within 2 hour. The lignin particles are negatively charged therefore can able to adsorbed more strongly towards the positively charged dyes (Crystal Violet). For cellulose the maximum dye removal percentage achieved was 79.69%

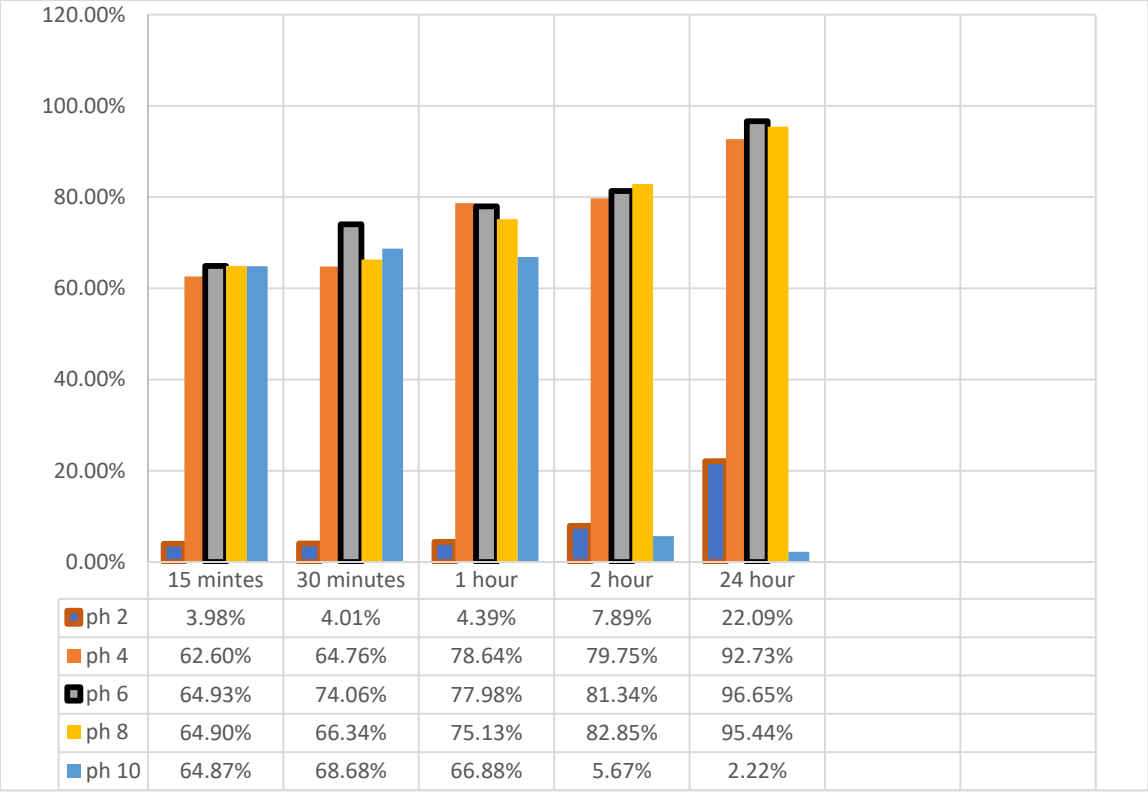
In case of LCS the maximum dye removal percentage was achieved after 24 hours in all the cases (5.10,15,20 ppm)respectively. The maximum percentage of dye removed was obtained at 10 ppm (93.34%), but at different concentrations of dye the removal rate is not very different therefore we can conclude that the concentration of dye from 5-20 ppm does not effect much

the removal rate in case of LCS. Therefore in case of LCS we will further study the different parameters by taking 5 and 10 ppm because of maximum dye removal rate (93.34% and 90.%) at this concentration.

Lignin is showing high dye removal percentage after 15 minutes and then on further increase of time there is a very little increase in the dye removal percentage as well as in all the case deadsorption is observed at 24 hours. Finally with cellulose the dye removal percentage is the least one from lignin and LCS, the maximum dye removal is observed after 24 hours at 15 and 20 ppm. At lower concentration of dyes the dye removal percentage is very low. Therefore for cellulose study we will be taking 15 and 20 ppm to estimate the different parameters.

3.5.3 pH:-

3.5.3.1 Effect of pH on LCS :-



	15 mintes	30 minutes	1 hour	2 hour	24 hour
ph 2	3.98%	4.01%	4.39%	7.89%	22.09%
ph 4	62.60%	64.76%	78.64%	79.75%	92.73%
ph 6	64.93%	74.06%	77.98%	81.34%	96.65%
ph 8	64.90%	66.34%	75.13%	82.85%	95.44%
ph 10	64.87%	68.68%	66.88%	5.67%	2.22%

Figure 6.4- The effect of pH on the dye removal percentage by LCS adsorbent

Discussion:-

In the above graph the influence of different pH ranges from (2-10) on dye removal rate by LCS adsorbent is estimated. The maximum dye removal percentage is observed at pH 6 towards the neutrality. In both extreme acidic and basic condition poor dye removal percentage is achieved. At pH 4,6 and 8 similar type of results are obtained but at 6 the maximum amount of dye removed encountered was about 96.65%. From above we can conclude that the pH have a significant influence on the dye removal rate. From above we can conclude that the pH have a significant influence on the dye removal rate and for LCS the best pH is 6. A similar trend of pH was observed for the adsorption of dye with biogas waste slurry (Yamuna, 1990) that is maximum dye removed at 5 and the decrease in removal at pH 12.

3.5.3.2 Effect of pH on cellulose :-

Time	pH 2	pH 4	pH 6	pH 8	pH 10
15 minutes	50.74%	59.42%	63.68%	62.20%	33.33%
30 minutes	58.14%	66.52%	66.59%	65.14%	30.98%
1 hour	62.95%	69.97%	70.71%	68.57%	24.76%
2 hour	67.51%	74.95%	75.40%	70.08%	12.14%
24 hour	75.28%	82.85%	85.19%	83.32%	2.34%

Table 1.7:- The effect of pH on the dye removal percentage by cellulose adsorbent

DISCUSSION:-

In the above graph the influence of different pH ranges from (2-10) on dye removal rate by Cellulose adsorbent is estimated. The maximum dye removal percentage is observed at pH 6 towards the neutrality. In both extreme acidic and basic condition poor dye removal percentage is achieved. At pH 4,6 and 8 similar type of results are obtained but at 6 the maximum amount

of dye removed encountered was about 85.19%. From above we can conclude that the pH have a significant influence on the dye removal rate.

3.5.4 Substrate Concentration:-

3.5.4.1 Effect of adsorbent LCS on dye removal percentage-

Time	Substrate 50 mg/ml	Substrate 150 mg/ml	Substrate 200 mg/ml
15 minutes	71.48%	83.35%	72.11%
30 minutes	75.43%	87.46%	75.67%
1 hour	87.91%	92.97%	81.70%
2 hour	92.12%	95.57%	84.76%
24 hour	93.84%	98.26%	88.12%

Table 1.8 :- The effect of substrate concentration on the dye removal percentage by LCS adsorbent

Discussion:-

The effect of adsorbate concentration on dye removal rate is estimated in the above graph. From the graph it was observed that by increase in dosage of adsorbent from 50 to 150 mg/ml, the percentage removal was subsequently increased from 93.84% to 98.26% , due to increase in the surface area of adsorbent. But on continuously increasing the substrate concentration will result in decrease in the dye removal rate this decrease in the adsorption capacity at higher adsorbent concentration was due to the fact that low surface area may be contributed that result in the saturation of adsorption sites due to interaction of particulate such as aggregation.

3.5.4.2 Effect of Cellulose adsorbent on dye removal percentage:-

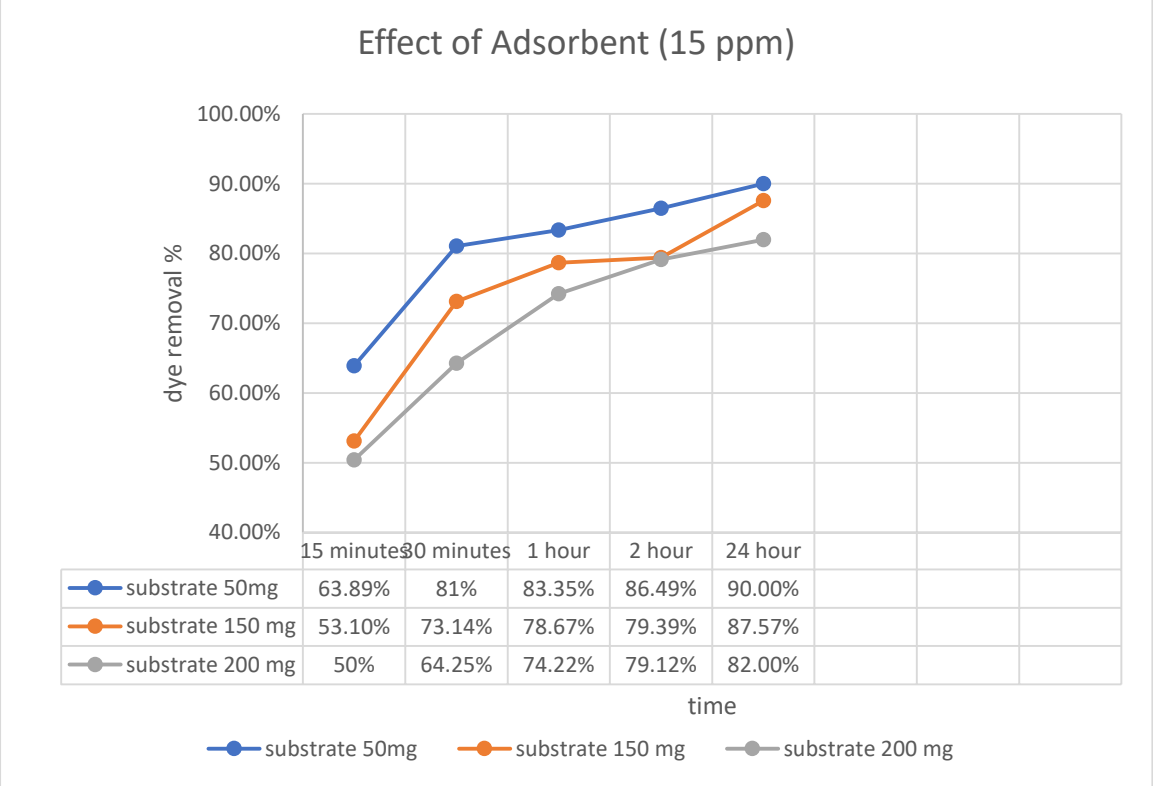


Figure 6.5-The effect of substrate concentration on the dye removal percentage by Cellulose adsorbent

Discussion:-

The effect of adsorbate concentration on dye removal rate is estimated in the above graph. From the graph it was observed that by increase in dosage of adsorbent from 50 to 200 mg/ml, the percentage removal was subsequently decreased from 90.00% to 82.00%. But on continuously increasing the substrate concentration will result in decrease in the dye removal rate this decrease in the adsorption capacity at higher adsorbent concentration was due to the fact that low surface area may be contributed that result in the saturation of adsorption sites due to interaction of particulate such as aggregation.

3.5.3 Characterization of Cellulose, LCS and lignin:-

3.5.3.1 SEM Analysis of Lignin:-

To analysis the surface morphology of the adsorbent the SEM imaging were used. The SEM image of the lignin from peanut shell waste is observed to be rough and raged prior to adsorption of dye molecule. Prominent interspatial pores in the lignin was also observed within the lignin matrix. This pores are the index for good adsorption capacity. The second image of SEM indicates the lignin after the adsorption process which showed deposition of particles on the adsorbent surface. The pores found in the first image were completely absent in the second image this is due to the penetration of crystal violet dye (CVD) in the interspatial pore network. This result can conclude that the surface morphology of the lignin adsorbate completely changed after the adsorption of CVD

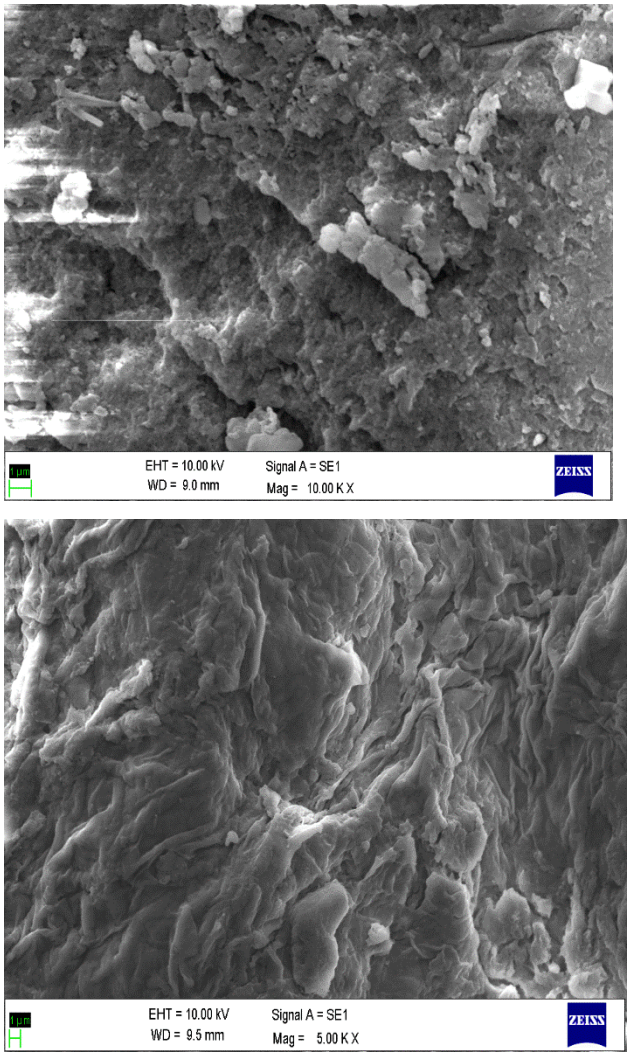
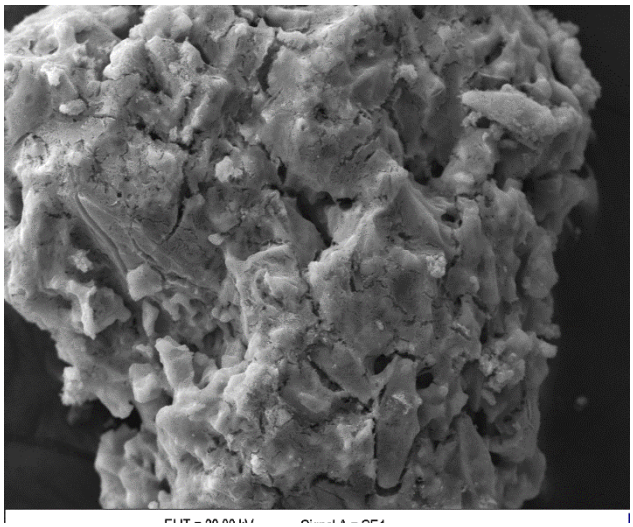


Figure 6.6.1(a) :- Indicates the lignin adsorbate prior to adsorption

Figure 6.6.1(b) :- Indicates the lignin adsorbate after adsorption

3.5.3.2 SEM Analysis of Cellulose:-

SEM imagining of the surface of adsorbate was carried out prior and after adsorption process of crystal violet dye using scanning electron microscopy (SEM – EVO 18 / Carl Zeiss/United Kingdom) 20 kV and at 20,000 times of resolution. The images of the adsorbate are shown in the figure below. In the first figure the surface of the adsorbate is relatively free and porous in structure of any type of aggregations. The second image shows the surface morphology after the penetration of CVD there are absence of porous walls in the figure. The surface of CVD loaded cellulose adsorbent is wrinkled due to the dye molecules which indicates the involvement of ion exchange in the dye molecule while removal.



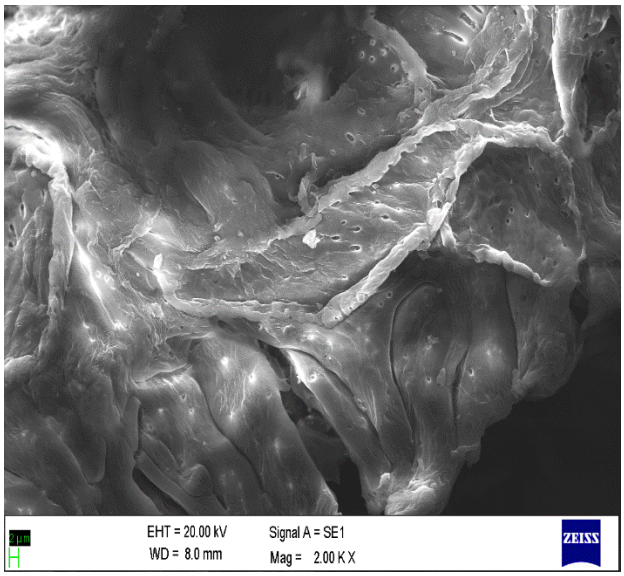


Figure 6.7.1(a) :- Indicates the cellulose adsorbate prior to adsorption

Figure 6.7.2(b) :- Indicates the cellulose adsorbate after adsorption

3.5.3.3 SEM Analysis of LCS:-

SEM imagining of the surface of LCS adsorbate was done by (SEM – EVO 18 / Carl Zeiss/United Kingdom) scanning electron microscopy at 20 kV and at 20,000 times of resolution. The images of the adsorbate are shown in the figure below. In the first figure the surface of the adsorbate porous in structure of any type of aggregations. The second image shows the surface morphology after the penetration of CVD there are absence of porous walls.

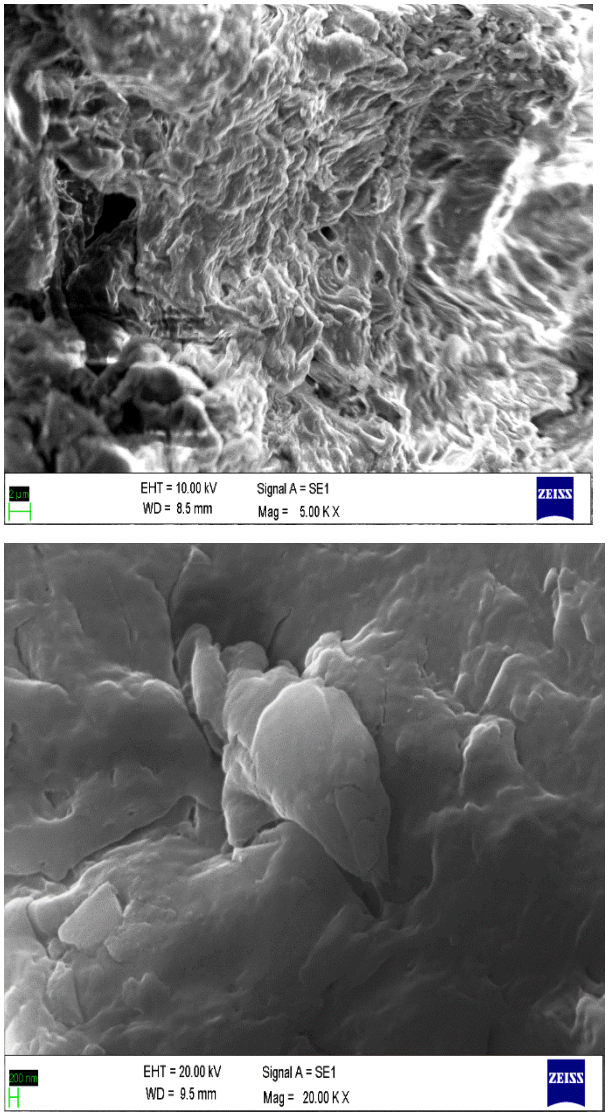


Figure 6.8.1(a) :- Indicates the LCS adsorbate prior to adsorption

Figure 6.8.2(b) :- Indicates the LCS adsorbate after adsorption

3.6 Conclusion:-

Large quantities of low cost bio-adsorbent are needed for the removal of dye from the waste water. Lignin, cellulose and LCS are the nontoxic, economically feasible and available in abundant quantities in waste lignocellulosic biomass. The present study demonstrate the use of cellulose, left cellulose after saccharification and lignin all extracted in a single method in dye removal waste water treatment. The raw lignocellulosic material (peanut shell waste) is abundantly available and are inexpensive. The extraction of these materials are simple and cost efficient. The basic dye binding property of all the absorbent used above is high.

Following conclusion are drawn from the above study:-

1. Results indicates that lignin can be used as a good adsorbent for the removal of CVD. It can remove about 87.64% of dye at pH-6 and temperature-30C after 2 hours of contact. Deadsorption ability of lignin is also observed in this study.
2. The best dye removal property is shown by the LCS which are obtained after the hydrolysis step. The crystal violet dye removed by LCS is about 99% when the adsorbate is used at a concentration of 2mg/ml pH is maintained at 6 and the temperature at 30C. The dye removal percentage of 99% was achieved after 24 hours of contact time.
3. The dye removal on average by the cellulose is the least. The CVD removal by cellulose is obtained about 85% at 50 mg/100ml of substrate pH is maintained at 6 and the temperature at 30C after 24 hours.

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