PRODUCTION OF BUTANOL (C_4H_9OH) FROM BARLEY STRAW

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Submitted by
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CERTIFICATE

This is to certify that thesis entitled **"PRODUCTION OF BUTANOL (C_4H_9OH) FROM BARLEY STRAW"** by **AVANISH KUMAR MAURYA (111CH0077)** submitted to the National Institute of Technology, Rourkela for the Degree of Bachelor of Technology is a record of bonafide research work, carried out by him in the Department of Chemical Engineering under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

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Place:

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Terminology

BS	Barley Straw
BSH	Barley Straw Hydolyzate
$Ca(OH)_2$	Calcium Hydroxide
Conc.	Concentration
C_2H_5OH	Ethanol
C_4H_9OH	Butanol
$(CH_3)_2CO$	Acetone
CO_2	Carbon dioxide
DNS	3,5dinitro salicylic acid
DW	Distilled Water
DI	De-ionized
D-	Dextro-
H_2SO_4	Sulfuric Acid
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
H_3PO_4	Phosphoric Acid
NaOH	Sodium Hydroxcide
psi	pounds per square inch
p	Para-
UV	Ultra violet

ABSTRACT

The energy requirements of the world has accelerated search for new fuels or new sources of fuel. Ethanol or butanol production from lignocellulosic biomass is one example of this. As lignin constrains the access of cellulose or hemicellulose to enzymes for their hydrolysis, it is necessary to remove the lignin. Various pretreatment methods are used before going for the hydrolysis of cellulose present in biomass followed by hydrolysis. In this work, barley straw is chosen as the lignocellulosic biomass and production of butanol has been carried out using steam explosion and acid hydrolysis followed by fermentation. The maximum amount of sugar (37.51 mg/ml of hydrozylate solution) was produced after the hydrolysis by HNO_3 with 0.5 mol/ltr concentration. The maximum amount of butanol (3.046mg/ml) was also found by hydrolyzing with 0.5 mol/ltr of HNO_3 after the fermentation of hydrozylate.

CHAPTER-1

INTRODUCTION

1.1 INTRODUCTION:

Butanol (C_4H_9OH) generation by means of fermentationwas initially reported in 1861 by Louis Pasteur, who initially found and isolated a butyric acid creating strain and later on watched C_4H_9OH production along with the butyric acid. Numerous researchers including Albert Fitz and MartinusBeijerinck proceeded with the work of C_4H_9OH-delivering microorganisms and confined a few extra strains, for example, Bacillus butylicus, and Granulobactersaccharobutyricum. In 1926, McCoy initially utilized the name of Clostridium acetobutylicum in their paper [1], and this name was formally perceived and acknowledged as the C_4H_9OH creating microorganism. Weizmann, alongside a British organization Strange & Graham Ltd. Later on isolated a strain that indicated more $(CH_3)_2CO$ and C_4H_9OH delivering capacity, and created and licensed a methodology taking into account this strain to deliver C_4H_9OH. This procedure played a vital part in World War I, and since 1920 $(CH_3)_2CO$ and C_4H_9OH have gotten to be significant fermentation for their extraordinary properties as solvents[2]. Numerous nations, including USA, England, China, Australia, and Canada, established organic C_4H_9OH plants utilizing ABE aging somewhere around 1920 and 19. Yet they all at long last went to a suspension because of the inexpensive petrochemical production of C4H9OH from crude oils and the high cost of fermentation of biomasses.

A rethink on ABE fermentation in the course of recent decades has made significant advances and leaps forward in the production of C_4H_9OH from variousfeedstocks. As of late, with the exhausting fossil fuel saves and surging raw petroleum cost, biological production of C_4H_9OH as an unrivaled biofuel has turned into an advantageous exploration subject. Contrasted with C_2H_5OH, C_4H_9OH is a prevalent fuel source, and the attributes of C_4H_9OH and gas are comparative. C_4H_9OH has a higher energy content and lower volatility than C_2H_5OH and mC_2H_5OH [3]. In particular, C_4H_9OH can be straightforwardly utilized as a distinct option for fuel or fuel additives in the current IC engines with no alteration. Accordingly, C_4H_9OH could turn into the next generation biofuel later. In table 1 some properties of N-C_4H_9OH, C_2H_5OH, diesel and gasoline are listed. Lignocellulose is the essential structural component of cell walls of the plants[4]. Agriculture biomass is mostly contains cellulose, hemicellulose, and lignin, alongside littler measures of protein,pectin, extractives (dissolvable nonstructural materials. For example, nonstructural sugar, nitrogenous material, and waxes), and fiery debris[5].

Table 1 Properties of N-C_4H_9OH, C_2H_5OH, Diesel and Gasoline.

FUEL PROPERTIES	N-C_4H_9OH	C_2H_5OH	DIESEL	GASOLINE
Energy Density (MJ/L)	29.9	19.6	16	32
Heat of Vaporization (MJ/KG)	0.43	0.97	1.2	36
Research octane number	96	107	106	91-99
Motor octane number	78	89	92	81-89
Air to fuel ratio	11.2	9.0	6.4	14.6
Specific energy (MJ/ Kg air)	3.2	3.0	3.1	2.9

The structure of these constituents can differ starting with one plant types then onto the next. For instance, hardwood has more prominent measures of cellulose though straw and leaves have more hemicellulose (Table 2). Also, the proportions of different constituents inside a solitary plant fluctuate with its age, phase of development, and different situations [6].

Lignocellulose is the fundamental auxiliary component in plant cell dividers and found to be in a sorted out stringy structure. The structure of lignocellulose was indicated in Figure 1. This direct polymer comprises of D-glucose subunits connected to one another by (1,4)-glycosidic bonds. Cellobiose is the rehash unit secured through this bond, and it contains cellulose chains[7]. The long-chain polymers of cellulose connected by van der waalsand hydrogen bonds, which cause the cellulose stuffed into fibrils. The fibrils are covered by Lignin and hemicelluloses[8]. Fermentable glucose can be deliveredthrough the activity of either acid or enzyme from cellulose, breaking the (1,4)- glycosidic bonds. Cellulose present in biomass is exhibited in both crystalline and indistinct structures[9]. Crystalline cellulose includes the real extent of cellulose though a little rate of chaotic cellulose chains structures undefined cellulose. Cellulose is helpless to degration by enzyme in its formless structure.

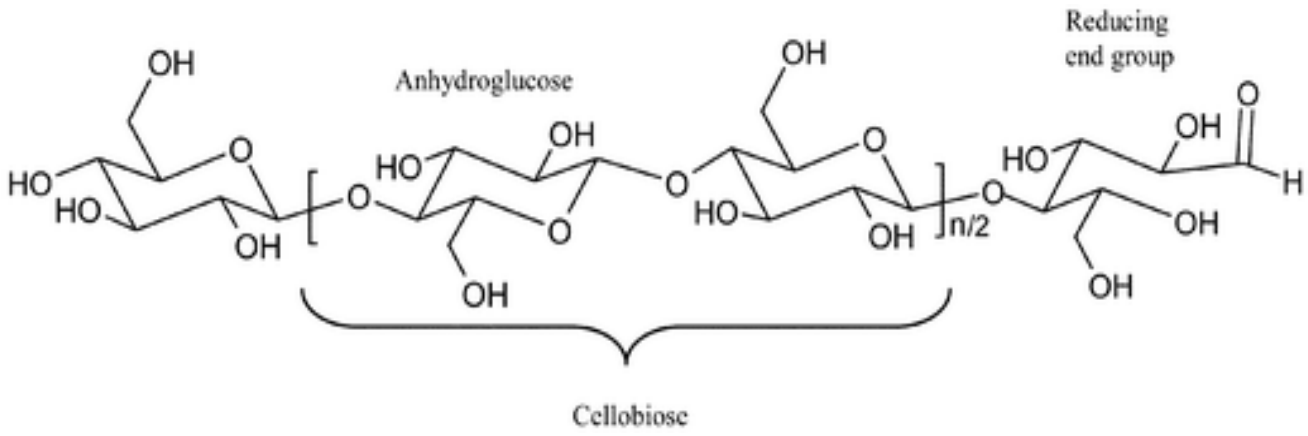

Figure 1. Structure of Cellobiose Chain

The principle includes that separates hemicellulose from cellulose is that hemicellulose has branches with small parallel chains comprising of diverse sugar. These monosaccharides incorporate pentoses, hexoses and uronic acids. The foundation of hemicellulose is either a homopolymer or a heteropolymer with short branches connected by (1,4)-glycosidic bonds and at times (1,3)-glycosidic bonds. Likewise, hemicelluloses can have some level of acetylation, for instance, in heteroxylan. Rather than cellulose, the polymers introduce in hemicelluloses are effortlessly hydrolysable [10]. These polymers don't total, notwithstanding when they co-crystallize with cellulose chains. Lignin is a mind boggling, anextensive atomic structure having cross-connected polymers of phenolic monomers. It is exhibit in the essential cell divider, giving basic bolster, impermeability, and resistance to microbial assault[11]. Three monomers of lignin are: coniferyl liquor, coumaryl liquor, and sinapyl liquor. Alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds connect these phenolic monomers together. As a rule, herbaceous plants, for example, grasses have the least substance of lignin while softwoods contains the most noteworthy lignin substance[12]. Lignin offers resistance to enzymatic activities which are necessary to produce sugar or alcohols from lignocellulose. So it becomes more important to separates lignin from celluloses and hemicelluloses.

Table 2 Cellulose, Hemicellulose, and Lignin Contents in CommonAgricultural Residues and Wastes.

Lignocellulosic Biomass	**Cellulose %**	**Hemicellulose %**	**Lignin %**
Hardwood	40-45	24-40	18-25
Softwood	45-50	25-35	25-35
Nutshell	25-30	25-30	30-45
Rice Straw	28-36	23-28	14-25
Wheat Straw	35-40	20-30	17-20
Corn Cobs	45	35	15
Leaves	15-20	80-85	0
Paper	85-99	0	0-15
Switch Grass	45-50	31-32	12-14
Barley Straw	30-48	20-45	10-20
Cotton Seed Hairs	80-95	5-20	0
Sugarcane Bagasse	40-50	24-28	20-25
News Paper	40-55	25-50	18-30
Corn Stover	38-40	25-28	7-21

1.2 OBJECTIVES:

The objective of this work is the conversion of lignocellulose present in barley straw to butanol (C_4H_9OH). In order to do so two major steps are taken:

1. Pretreatment of biomass with acids (H_2SO_4, HCl, HNO_3 and H_3PO_4) and steam explosion.

2. Fermentation of sugar produced after hydrolysis.

CHAPTER-2

LITERATURE REVIEW

Biofuels obtained from different lignocellulosic biomass, for example, such as wood, farm wastes, or forest residues, can possibly be a profitable replacement for, or supplement to, gasoline. Numerous physicochemical basic and compositional components impede the saccharification of cellulose present in biomass to sugar and other natural compound that can later be converted to fuels [13]. The objective of pretreatment is to make the cellulose open to hydrolysis for production of sugar which are converted to fuels. It has been accounted for that cellulosic C_2H_5OH and C_2H_5OH delivered from different biomass assets can possibly cut greenhouse gas emanations by 86%. Lignocellulosic biomass, for example, agriculture wastes (e.g., wheat straw, sugarcane bagasse, barley straw,cornstover), hardwood, softwood, and devoted yields (switch grass, Salix) are renewable sources of enegy[14]. These materials are sufficiently plentiful and create low net greenhouse gas releases. About 90% of the dry weight of plants containshemicellulose,cellulose, lignin and pectin[14]. The vicinity of lignin in lignocelluloses prompts a defensive layer that protects plant cell decimation by growths and microbial transformations to fuel. For the production of fuel from biomass, the cellulose and hemicellulose must be converted to their relating monomers i.e. sugar, so microbes can use them. So the removal of lignin becomes more important for above purpose.

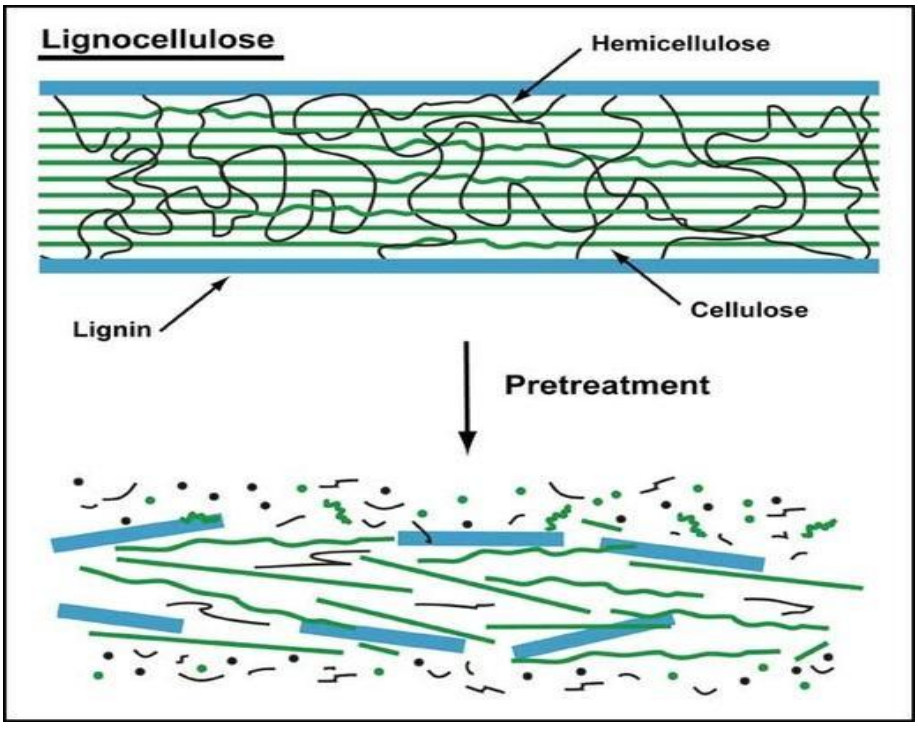

Figure 2. Sructure of lignocellulosic materials and effect of pretreatment on it.

The beneficial impacts of pretreatment of biomass have been perceived long time ago [15]. The objective of pretreatment methodology is to separate lignin and hemicellulose, decrease the crystallinity of cellulose, and expand the porosity of the biomass. Pretreatment must meet the following prerequisites: (1) enhance the formation of sugar or the ability to subsequently form sugar by hydrolysis, (2) reduce the breaking or loss of carbohydrate, (3) reduce the formation of inhibitor to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective[16].

Pretreatment methodology can be divided into different categories: physical (milling and grinding), physicochemical (steam explosion/autohydrolysis, hydro-thermolysis, wet oxidation, CO_2 explosion etc.), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these[17].

n-C4H9OH is a four-carbon essential liquor, and is right now utilized as synthetic intermediate, dissolvable, andextractant cosmetics and pharmaceutical commercial ventures. C_4H_9OH has been pulling in research consideration as an option biofuel to C_2H_5OH in the recent years[18]. Contrasted with C_2H_5OH, C_4H_9OH is considered as the next generation biofuel because of loads of points of interest, for example, higher energy content and lower volatility. C4H9OH can be utilized straightforwardly or mixed with gasoline and diesel as fuel additives in the current vehicle engines with no change or substitution and C_4H_9OH is likewise perfect with the current transportation pipeline for gasoline[19].

2.1 PHYSICAL PRETEATMENT:

2.1.1 MECHANICAL COMMINUTION:

Comminution of cellulosic biomass through a blend of chipping, granulating, and/or processing can be used to decrease cellulose crystallinity. The extent of the biomass materials is generally 10-30 mm after chipping and 0.2-2 mm after grinding or crushing [8]. Vibratory ball grinding was discovered better than conventional ball grinding in diminishing cellulose crystallinity of spruce and chips and in enhancing their absorbability. The last molecule size and biomass attributes focus the force prerequisite for mechanical comminution of agrarian materials. This technique is extremely lavish, in any case, to be utilized as a part of a full-scale process.

2.1.2 PYROLYSIS:

Pyrolysis has likewise been utilized for the pretreatment of lignocellulosic materials. Cellulose quickly breaks down to vaporous items and leftover burn when biomass is dealt with at temperatures more noteworthy than 300°C [25].At lower temperatures, the decay is much slower, and the items shaped are less unpredictable. Chipping, pelletization, torrefecation, and pyrolysis have been mulled over as pretreatment methods for biomass-to-fuel transformation[17]. Pretreatment by torrefecation was discovered to be significantly more alluring than pyrolysis.

2.2 PHYSICOCHEMICAL PRETREATMENT:

2.2.1 STEAM EXPLOSION:

Steam explosion pretreatment is commonly utilized system for the pretreatment of lignocellulosic materials. In this technique, biomass material is treated with high-pressure steam, and afterward the pressure is all of a sudden diminished, which makes the materials experience an explosion decompression. Steam explosion is normally started at a temperature of 160-260°C (relating weight, 0.69-4.83 MPa) for a few seconds to a couple of minutes before the material is presented to atmospheric pressure [25]. The biomass/steam mixture is treated for a period of time to advance hemicellulose hydrolysis, and the methodology is ended by an explosion decompression. The procedure causes hemicellulose corruption and lignin change because of elevated temperature, subsequently expanding the capability of cellulose hydrolysis. Hemicellulose is considered to be hydrolyzed by acidic and different acids discharged amid steam-explosion method. Expulsion of hemicelluloses from the microfibrils is accepted to uncover the cellulose surface and build chemical openness to the cellulose microfibrils. Lignin is removed just to a restricted degree amid the pretreatment however is redistributed on the fiber surfaces as an aftereffect of liquefying and depolymerization\ repolymerization responses. The evacuation and redistribution of hemicellulose and lignin expand the volume of the pretreated specimen[18]. The variables that influence steam-explosion pretreatment are living arrangement time, temperature, chip size, and dampness content. Confinements of steam explosion incorporate pulverization of a part of the xylan portion, fragmented disturbance of the lignin-carbohydrate network, and generation of compounds that may be inhibitory to microorganisms utilized as a part of downstream procedures. Pretreatment effectiveness are assessed by the compositions of the solid and liquid parts produced after filtration

of pretreated material and the exposure of the solid part to enzymatic hydrolysis using commercial grade cellulases.

2.2.2 CARBON DIOXIDE EXPLOSION:

In endeavors to create enhanced pretreatment methods of lignocellulose, the thought of utilizing supercritical CO_2 explosion, which would have a lower temperature than steam explosion and perhaps a lessened cost contrasted with ammonia explosion, was created. Supercritical liquid alludes to a fluid that is in a gaseous form however is packed down at temperatures over its critical point to a liquidlike density. It is speculated that, in light of the fact that CO_2 forms carbonic acid when disintegrated in water, the acid rises the hydrolysis rate. Carbon dioxide atoms are tantamount in size to water and ammonia and ought to have the capacity to enter small pores available to water and ammonia moleclules. Carbon dioxide is proposed to be useful in hydrolyzing hemicellulose and cellulose. Besides, the low temperature keeps any considerable decay of monosaccharides by the acid. CO_2 explosion is worked at low temperatures, it doesn't bring about corruption of sugar, and for example, the deprivation of sugar observed with steam explosion because of the high temperature included[19]. The hemicellulose is debased to oligomericsugar and deacetylated, which is no doubt the reason that the hemicellulose is not solvent. The structure of the material is changed, bringing about expanded water holding limit and higher edibility. AFEX is not an extremely productive innovation for lignocellulosic biomass with moderately high lignin substance, for example, woods and nutshells.

2.3 CHEMICAL PRETREATMENT:

2.3.1 OZONOLYSIS:

Treatment with ozone is one method for diminishing the lignin substance of lignocellulosic biomass. This outcomes in an increment of the enzyme or chemical accessibility of the treated material, and not at all like other compound medications, it doesn't create lethal deposits. Ozone can be utilized to debase lignin and hemicellulose in numerous lignocellulosic materials, for example, wheat straw, bagasse, green feed, shelled nut, pine, cotton straw, and poplar sawdust. The corruption is predominantly restricted to lignin. Hemicellulose is marginally influenced, yet cellulose is most certainly not.

Pretreatment with ozone has favorable feature that the responses are done at room temperature and typical pressure. Moreover, the way that ozone can be effortlessly deteriorated by utilizing a reactant bed or increasing the temperature implies that methods can be intended to minimize ecological pollution[20]. A difficulty of ozonolysis is that a lot of ozone is required, which can make the procedure non-economical.

2.3.2 ACID PRETREATMENT:

Concentrated acids, for example, H_2SO_4 and HCl have additionally been utilized to treat lignocellulosic materials. Pretreatment with acid can bring about change of enzymatic hydrolysis of lignocellulosic biomasses to discharge fermentable sugar. In spite of the fact that they are effective method for cellulose hydrolysis, concentrated acids are dangerous, destructive, perilous, and in this way requires reactors that are impervious to corrosion, which makes the pretreatment handle exceptionally expensive. The concentrated acid must be recovered after hydrolysis to make the procedure economical [25].

Dilute acid hydrolysis has been effectively developed for pretreatment of lignocellulosic materials. Sulfuric acid at concentrations underneath 4 wt %, has been of the most interesting for such studies as it is cheaper and effective. Dilute H_2SO_4 is mixed with biomass to hydrolyze hemicellulose to xylose and different sugar and afterward keeps on breaking xylose down to furfural. The diluteH_2SO_4 pretreatment can give high response rates and altogether enhance cellulose hydrolysis. Dilute acid effectively eliminates and recuperates the greater part of the hemicellulose as disintegrated sugar, and glucose yields from cellulose increase with hemicellulose elimination to very nearly 100% for complete hemicellulose hydrolysis [21]. Recently, acid pretreatment has been utilized on variety of feedstocks running from woods to grasses and agriculture wastes.

Two sorts of dilute acid pretreatment methodologies are regularly utilized: a high-temperature (T > 160 °C), continuous flow for low solids loadings (weight of substrate/weight of response mixture) 5-10%) and a low-temperature (T < 160 °C), batch process for high solids loadings (10-40%) [25].The most generally utilized and tried methodologies are in view of dilutesulfuric acid. Xylose degradation varies directly as a function of acid concentration.

It has been found that the biomasses that have been exposed to acid hydrolysis can be tougher to ferment because of the vicinity of toxic materials. Further, acid pretreatment brings about expensive materials of development, high pressure, neutralization and recovery of hydrolysate before biological treatment, slow cellulose digestion by enzymes, and non-effective attachment of enzyme to lignin.

2.3.3 ALKALINE PRETREATMENT:

A few alkali can be utilized for the pretreatment of biomasses, and the impact of alkaline pretreatment relies on upon the lignin content of the biomasses. Alkaline pretreatment techniques use lower temperatures and pressurethan other pretreatment methods. Alkaline pretreatment can be done at room conditions, however pretreatment duration are on the request of hours or days instead of minutes or seconds. Contrasted and acid pretreatment methodologies, alkaline methods cause less sugar deprivation, and a considerable lot of the alkaline can be recouped and/or redeveloped. Sodium, potassium, calcium, and ammonium hydroxides are appropriate alkaline pretreatment methods. Of these four, sodium hydroxide has been widely utilized.

Chang etal. reported the connections between enzymatic absorbability and three basic elements: lignin substance, crystallinity, and acetyl content. They inferred that (1) greater degradation of lignin is sufficient to acquire high absorbability regardless of acetyl content and crystallinity, (2) removal of lignin and deacetylation remove parallel boundaries to enzymatic hydrolysis; and (3) crystallinity significantly influences earlier hydrolysis rates however,has less impact on extreme sugar yields. These outcomes show that an effective lignocellulose treatment procedure ought to remove the most of the acetyl groups and decrease the lignin content to around 10% in the treated biomass. Therefore, alkaline pretreatment can assume a huge part in the treatment of cellulose to enzyme hydrolysis [22]. Lignin removal increases catalytic process by decreasing the non-productive adsorption destinations and by expanding access to cellulose and hemicellulose.

DiluteNaOH treatment of lignocellulosic biomasses has been found to bring about distension, prompting an increase in interior surface territory, a reduction in the level of polymerization, an abatement in crystallinity, partition of basic linkages in the middle of lignin and starches, and disturbance of the lignin structure. With salt loadings of 0.05-0.3 g of soluble base/g of biomass, microwave pretreatment brought about higher sugar yields than ordinary warming, with the most

astounding yield (90% of greatest potential sugar) being accomplished at an alkali loading of 0.1 g/g [25].

2.3.4 ORGANOSOLV PRETREATMENT:

The organosolvation strategy is an auspicious pretreatment method, and it has pulled in much consideration and exhibited the potential for use in lignocellulosic pretreatment. In the organosolvation process, a natural or fluid natural dissolvable mixture with inorganic acid impetuses (HCl or H_2SO_4) is utilized to break the inward hemicellulose and ligninlinkages. The solvents regularly utilized as a part of the process are C_2H_5OH, C_4H_9OH, ethylene glycol, $(CH_3)_2CO$,tetrahydrofurfurylliquor, and triethylene glycol. Natural acids, for example, oxalic, acetylsalicylic, and salicylic acids can likewise be utilized as impetuses as a part of the organosolvation process [25]. Basically, the organosolv pretreatment includes synchronous prehydrolysis and removal of lignin of lignocellulosic biomass bolstered by natural solvents and `normally, dilute acid arrangements. A high return of xylose can typically be gotten with the expansion of acid.

The biggest part, cellulose, is mostly hydrolyzed into littler parts that still stay insoluble in the alcohol. The second biggest part, hemicellulose, is hydrolyzed generally into solvent constituents, such as oligosaccharides, monosaccharides, and acidic acid. Acidic acid brings down the alcohol pH, fortifying acid-catalyzed hydrolysis of alternate parts[23]. A percentage of the pentose sugar are thusly dried out under the working conditions to shape furfural. The third real polymer part, lignin, is hydrolyzed under the conditions utilized in the process basically into lower-atomic weight sections that break up in the watery C_2H_5OH alcohol.

2.4. DETOXIFICATION OF LINGNOCELLULOSIC HYDROZYLATE:

Numerous byproducts, also known as inhibitors in the ending of bioconversion process, are generated during the pretreatment of biomass. The major byproducts include furan derivatives (sugar degradation, furfural and 5-hydroxymethylfurfural (HMF)), phenolic compounds (syringaldehyde, vanillin, vanillic acid, p-coumaric acid, ferulic acid,syringic acid, lignin degradation), and weaker acids (acetic acid, lignocellulose structure degradation).

Pentose and hexose are produced during the hydrolysis of lignocellulosic biomass, and then further degraded into furfural and HMF, respectively. Furfural and HMF are generally recognized as the inhibitors to the microorganisms. Phenolic, aromatic compounds and aldehydes are produced from the degradation of lignin. These compounds, especially the lighter molecular weight ones, are very toxic to the fermentation microbes, even when their concentrations are extremely low. Acetic acid is generated from the acetyl groups of hemicellulose, and is considered as a product of lignocellulosic degradation. At low concentrations, several researchers found that acetic acid actually enhanced the solvent production and prevented the culture degeneration.

Because of the presence of various inhibitors, the lag phase is increased, sugar utilization is reduced, and the product formation (concentration, yield, productivity) gets significantly hindered. The inhibitory concentration of each compound cannot be strictly determined due to the wide range of microorganism. Physical detoxification usually uses vacuum evaporation technology to remove the volatile toxic compunds, such as furfural and acetic acid. Usually the furfural can be efficiently removed by this method, and the sugar is concentrated after water evaporates. The main drawback of this pretreatment is that non-volatile compounds accumulate and stay in the concentrated hydrolyzate. The chemical detoxification includes using pH adjustment to precipitate and remove toxic compounds, and adsorption with activated charcoal or ion-exchange resins. Since some inhibitors are unstable at a certain pH, pH adjustment with $Ca(OH)_2$ is widely used detoxification method for a huge range of lignocellulose hydrolyzates[17, 23]. Most often, lime is added to adjust the pH to 9-10, and then acid (H_2SO_4 or HCl) is added to readjust pH to 5.5-6.5. It was found that over lime detoxification reduced over nearly 51% of furans, 41% of phenolic compounds, and only 8.7% of sugar.

2.5 HYDROLYSIS OF LIGNOCELLULOSIC BIOMASS:

The cellulose molecules are polymer of glucose molecules. In the hydrolysis process, these polymer chains are broken down to "free" the sugar (monomers), before it is fermented for alcohol production. There are two major hydrolysis processes: (1)chemical reaction using acids or acid hydrolysis, (2) enzymatic reaction or enzymatic hydrolysis.

2.5.1 ACID HYDROLYSIS:

The inorganic acids act primarily and swiftly as reaction catalyzers of polysaccharide sections. Lignocellulosic materials can be hydrolyzed with the help of dilute acid to obtain a combination of sugars with xylose as the main product or sugar. However, during hydrolysis some by-products are created in hydrolyzate, such as acetic acid, furfural, phenolic compounds, or lignin degradation compounds. These are possible inhibitors of a microbiological usages of this hydrolyzate. Processes such as two-stage acid hydrolysis can be used to produce xylose and glucose. Treatment with dilute acid at moderate temperatures has appeared an effective way of producing xylose from hemicellulose. In the second stage more extreme conditions are used and glucose can be produced from cellulose hydrolysis, such as higher concentration of acid and higher temperature. In general, acid treatment is much effective in degrading the hemicellulosic component of biomass. Proper arrangements of pH, temperature, and reaction duration can result in high yields of sugar, primarily xylose from hemicellulose.

2.5.2 ENZYME ENHANCED HYDROLYSIS:

In order to break the cellulose into their respective monomeric form, enzyme enhanced hydrolysis is another method which is widely used. The most commonly used enzyme is cellulose which is typically a combination of 3 enzymes (1) Endocellulases: cuts randomly the cellulose chain, (2) Exocellulases: cuts 2-4 units at the ends to give easy access to endocellulases and (3) Cellobiases: it breaks the dimers of sugar into their respective sugar.

2.6 FERMENTATION:

Wide range of microorganisms, usually either fungi, yeast, or bacteria, are found who can ferment carbohydrates (sugar) to C_4H_9OH under anaerobic situations. Approaches for C_6 sugars fermentation were discovered at least 6000 years ago, when Babylonians, Sumerians and Egyptians began to well establish. At that time the beer was produced from starch present in grains. After it became possible to produce the C_6 sugars in lignocellulosic agriculture products and at the end of 19^{th} century conversion of the C_5 sugars became more interesting. C_5 represent a higher amount of the available sugar. The ability to recover and ferment sugars into alcohol is important for the efficiency and economics of the production. Only in the 1980s exploration on xylose

fermentation started to produce beer from fruits. A large amount of yeast were found that could convert xylose to C_2H_5OH. Bacteria have also drawn more attention from researchers because of their quick movement on sugars for fermentation. In general, bacteria can ferment sugar in minutes as compared to hours for yeast which are found more economical and efficient.

CHAPTER-3

MATERIALS & INSTRUMENTS

3.1 MATERIALS USED:

3.1.1CHEMICALS REQUIRED:

(1) Sulfuric Acid = 0.1, 02, 0.3, 0.4, 0.5 mol/ltr.

(2) Hydrochloric Acid= 0.1, 02, 0.3, 0.4, 0.5 mol/ltr.

(3) Nitric Acid= 0.1, 02, 0.3, 0.4, 0.5 mol/ltr.

(4) Ortho-Phosphoric Acid= 0.1, 02, 0.3, 0.4, 0.5 mol/ltr.

(5) Concentrated and diluted NaOH for pH adjustments.

(6) DNS = 10 gm.

(7) Phenol = 2 gm.

(8) Sodium metabisulphite = 0.5 gm.

(9) Sodium hydroxide = 10 gm.

(10) Potassium Sodium Tartrate (40%).

3.2: INSTRUMENTS USED:

3.2.1 MESH (SCALE):

Mesh analysis is required to determine the particle size of granular material.

3.2.2 VERTICAL AUTOCLAVE:

Vertical autoclave is primarily used for sterilization purpose. It is an enclosed space where steam bath is given to any equipment placed inside it. Water filled in it is heated by electric coils present at bottom. It has a vent at top, from where steam can be released to maintain the desired pressure. For our case, sterilization is required after maintaining the pH and also for each filter.

3.2.3 LEMINAR FLOW CHAMBER:

The laminar flow chamber is used to keep the samples in disturbance free conditions which prevents the contamination of the sample by undesired chemicals and microbes. This instrument can also be used for sterilization purposes.

3.2.4 UV-SPECTOPHOTOMETER:

UV-Spectrophotometer is used to analyze the samples. For our analysis, we use the particular wavelength i.e. 540 nm for measurement. The equipment consists of two cuvettes, one of which acts as a reference while in the other cuvette the sample is placed. Once we kept the samples inside, a UV-light gives the absorbance of a particular sample at a particular wavelength.

3.2.5 SHAKING INCUBATOR:

This instrument is used for acid hydrolysis and for fermentation. This equipment is an enclosed chamber where given conditions are maintained at a particular rpm. Flasks are kept on a shaking platform moving at a desired speed.

3.2.6 PH-METER:

The pH meter is used for measuring the pH of a given solution.

CHAPTER-4

EXPERIMENTAL METHOD

4.1 RAW MATERIALS:

Barley Straw (BS) was obtained from local farmers (Azamgarh, U.P.). The material is well sun dried, milled and sieved to less than 1mm size and stored in plastic bags in dark place until use. H_2SO_4, HCl, HNO_3, and H_3PO_4 of concentrations 0.1, 0.2, 0.3, 0.4 and 0.5 mol/ltr are used. Biomass loading of 5% which means 2g of biomass and 38ml of dilute solvent.

4.2 PREPARATION OF BARLEY STRAW HYDROLYZATE (BSH):

Hydrozylate preparation is by two methods:

4.2.1 STEAM EXPLOSION

5% biomass loading (2g biomass in 38ml of DW) in conical flask is taken. The solution then autoclaved at 121°C and 15 psi for 60 min.

4.2.2 ACID HYDROLYSIS

The biomass loading was 5% w/v i.e. 2g of biomass in 38ml of acid solution. The only was chosen in pretreatment i.e. concentration of acids. The BS and acid mixture was then put in shaking incubator at 50°c and 140rpm for 24 hours. After that, the BSH was taken out and allowed to cool down to room temperature. The BSH was adjusted to neutral pH using sodium hydroxide. Reducing sugar are estimated using Miller assay also called, DNS assay.

4.3 pH ADJUSTMENT:

1. The pH of BSH is checked using pH meter.

2. As the samples are acidic, so NaOH solution of 1M and 10M is added to make that neutral.

After maintaining the pH, the BSH samples are kept in a Vertical autoclave for ½ hr at 120°C (around 2.02 kg/cm2 pressure) and allowed to cool.

4.4 SODIUM CITRATE BUFFER:

Citric acid monohydrate	210 g
DI water	750 ml
NaOH – add until pH equals 4.3	50 to 60 g

Dissolve above and dilute to 1 L, then check pH. If necessary, NaOH needs to be added until the pH becomes is 4.5. If 1 M stock citrate buffer stock is diluted with water to 50 mM the pH must be 4.8. After diluting the citrate buffer check and adjust the pH if requires to pH 4.8.

4.5 INOCULUM PREPATATION:

Lyophilized Clostridium acetobutylicum MTCC 481 has been procured from microbial type culture collection, IMTECH, Chandigarh, India. It was preserved as spore suspension in sterile water. This culture has been invigorated in RCA (Reinforced Clostridial Agar) and RCM (Broth) culture media at 37°C. The inoculum was prepared in RCM containing (g/L): glucose, 5.0; yeast extract, 3.0; starch, 1.0; beef extract, 10.0; peptone, 10.0; sodium chloride, 5.0; sodium acetate, 3.0; Agar,0.5 and cysteine hydrochloride, 0.5; pH 6.5 ± 0.1. 100 mL medium was autoclaved at 121°C and inoculated in 250 mL screw capped Erlenmeyer flasks, and then incubated for 72 h at 37 ± 0.5°C at 120 rpm in shaking anaerobic incubator. Cooked Meat Medium (CMM) is also used for the growth and preservation of clostridia. Incubation was done anaerobically inside an anaerobic culture bag system till active growth was seen (72 h). Actively growing cultures (after lag phase, 18–20 h) of the Clostridia was added subsequently to experimental flasks [26].

4.5 FERMENTATION:

After the hydrolysis, BSH was filtered through cotton cloth and the fibrous leftovers was dried at 70°C in a hot air oven, and then was weighed. The reduced weight of BS after pretreatment was noted and was considered for the final yield calculation. Batch fermentation of BSH samples were carried out in 250 mL of screw-capped Erlenmeyer flasks under anaerobic conditions. Anaerobic condition in the flask was created by addition of 0.5% cysteine hydrochloride to the BSH. The period of fermentation was nearly 120 h (5 days). During complete process pH was maintained at

4.8 using sodium citrate buffer. At the end of fermentation, final samples were taken for estimation of C_4H_9OH production and sugar utilization.

4.6BUTANOL ANALYSIS:

The samples were taken for C_4H_9OH and sugar analysis with the help of UV-spectrophotometer. In case of C_4H_9OH analysis, the BSH filtrate is directly placed in UV-spectrophotometer and its absorbance is noted down at 197 nm wavelength. But in case of sugar analysis, DNS assay is followed.

4.7 DETERMINATION OF REDUCING SUGAR:

100 µL of the nutrilized BSH was pipetted into the test tube followed by 300 µL of DNS reagent (Method for DNS reagent preparation is mentioned in below). Blank solution was also prepared simultaneously by adding 100 µL of DW and 300 µL of DNS reagent. Then samples were kept in hot water bath at 90°C for 5 min. Test tube along with blank were then taken out followed by cooling under running water. They were diluted 7.5 times i.e. 2.6 ml of DW was added to test tubes and then absorbance of the samples and blank were taken at 540nm.

4.8 PREPARATION OF DNS REAGENT:

Distilled Water	30ml
3, 5 dinitro salicylic acid	225mg
NaOH	420mg

dissolve above and then add,

Potassium Sodium Tartrate	6.482 grams
Phenol (melt at 50°c)	0.162 ml
Sodium Metabisulphite	175 mg

Titrate 3ml of sample with 0.1 N HCl using phenolphthalein as indicator. It should take nearly 5-6 ml of HCl. If necessary, add NaOH (2mg=1ml of 0.1 N HCl).

CHAPTER-5

RESULT, DISCUSSION AND CONCLUSION

5.1 COMPOSITIONAL ANAYSIS:

The composition obtained for barley straw was Glucan 39.9%, Xylan 25.2%, Arabian 10.3%, Lignin 13.3% and others 11.3 (by difference) for this kind of materials.

5.2 REDUCING SUGAR ANALYSIS:

5.2.1 STANDARD DEXTROSE CURVE FROM DNS ASSAY

Dextrose conc. (mg/ml)	Volume of Dextrose (10 mg/ml) (µl)	Volume of DW(µl)	Volume of DNS Reagent (µl)	Absorbance at 540nm
0	0	100	300	Blank
1	10	90	300	0.112
2	20	80	300	0.232
3	30	70	300	0.341
4	40	60	300	0.436
5	50	50	300	0.557
6	60	40	300	0.659
7	70	30	300	0.758
8	80	20	300	0.85
9	90	10	300	0.935
10	100	0	300	1.016

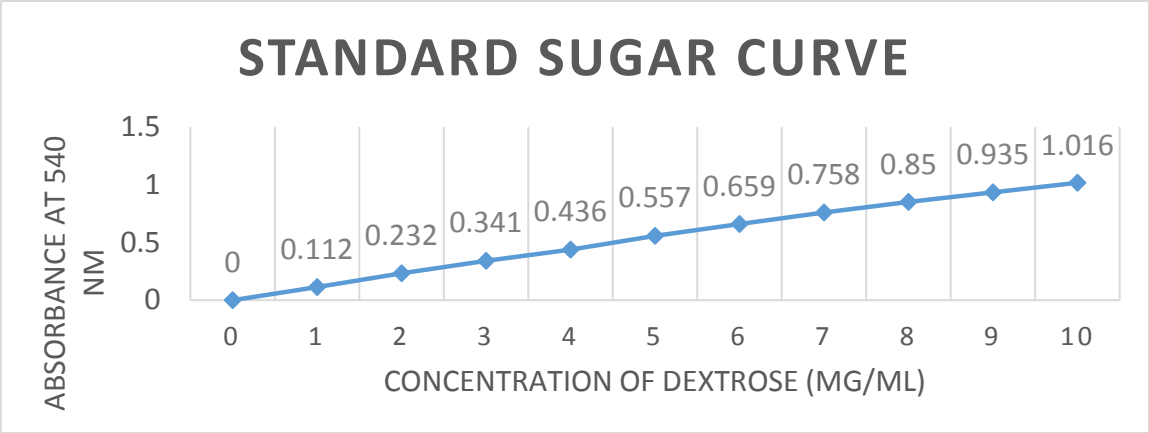

Figure 3 Glucose concentration with respect to absorbance taken at 540nm

5.2.2 SUGAR PRODUCED AFTER STEAM EXPLOSION PRETREATMENT:

The reducing sugar content observed was 0.08mg/ml of hydrozylate sample.

5.2.3 SUGAR PRODUCED AFTER ACID HYDROLYSIS:

The amount of reducing sugar (mg/ml of hydrolyzate) produced after acid hydrolysis for different acids is mentioned in Table 3:

Table 3 Reducing Sugar obtained after the acid hydrolysis of barley straw.

CONC. (mol/Ltr.)	H_2SO_4	HCl	HNO_3	H_3PO_4
0.1	14.40	11.06	17.37	5.94
0.2	29.25	23.09	27.77	13.96
0.3	31.48	29.33	34.67	14.48
0.4	35.94	34.67	36.2	15.22
0.5	36.12	37.0	37.51	14.33

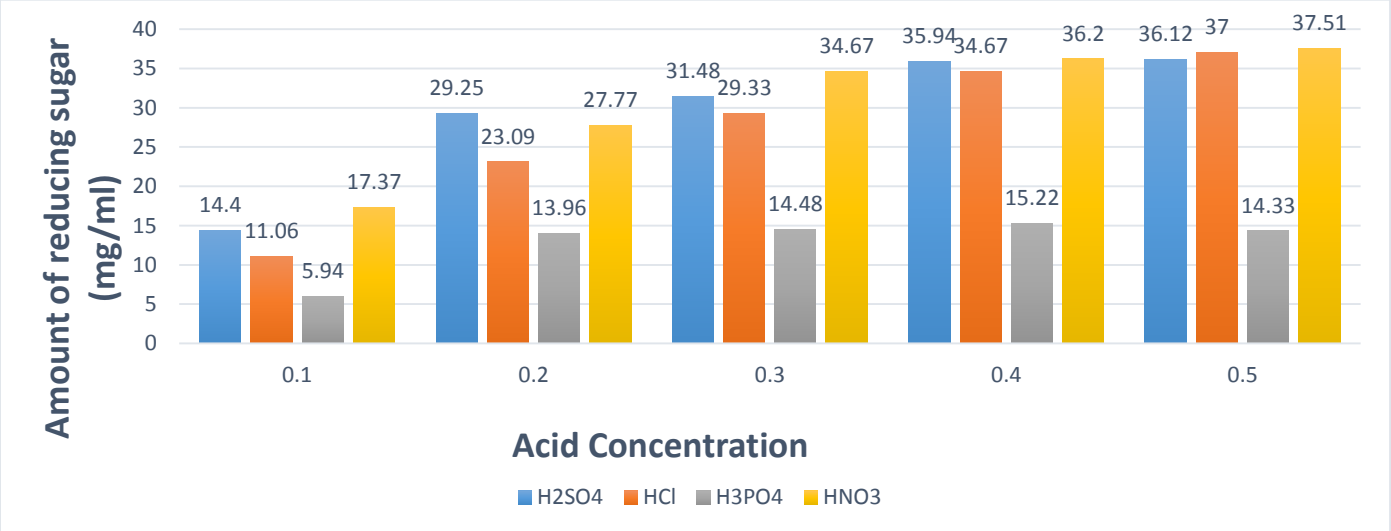

Figure 4 Acid concentration vs. reducing sugar concentration after pretreatment

5.3 BUTANOL ANALYSIS:

The amount of butanol produced (mg/ml) after the fermentation of acid hydrozylates are mentioned in table 4

Table 4 Amount of butanol produced (mg/ml)

CONC. (mol/Ltr.)	H_2SO_4	HCl	HNO_3	H_3PO_4
0.1	1.00	0.77	1.21	0.41
0.2	2.04	1.61	1.94	0.97
0.3	2.20	2.05	2.42	1.01
0.4	2.51	2.42	2.86	1.06
0.5	2.98	2.96	3.046	1.00

The butanol produced from steam explosion hydrozylate after fermentation was 1.36 mg/ml of hydrozylate sample.

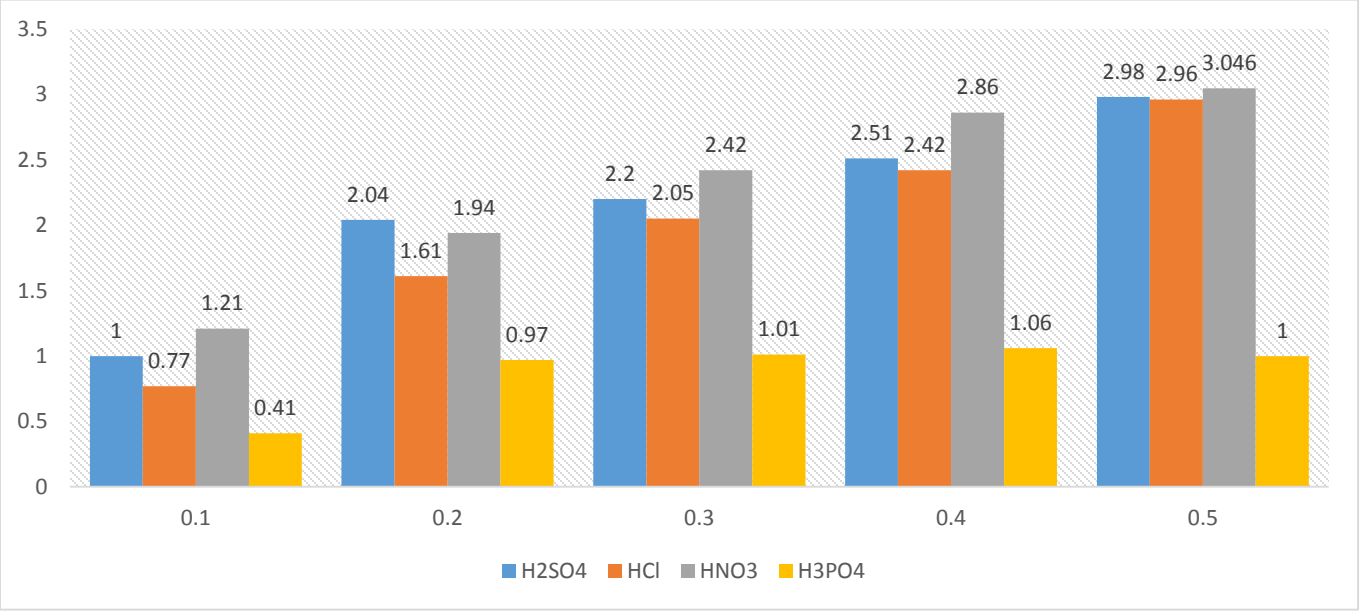

Figure 5 Production of butanol in mg/ml vs. pretreatment acid concentrations

The sugar content as well as the butanol production is increasing as we increase the acid concentrations. In case of steam explosion treatment the amount of sugar produced (0.08mg/ml) was found very less as compared to acid treatment method. If we consider acid treatment with lesser concentration the butanol produced from steam explosion hydrolysate found nearly equal because of production of inhibitors in case of acid treatment. After certain concentration of acids the sugar and butanol production decreases due generation of inhibitors. In case of, $H_3PO_4$the amount of sugar and butanol produced started decreasing after 0.4 mol/ltr concentration.Acid Hydrolysis can be preferred over steam hydrolysis for sugar production but due generation inhibitors at high acid concentrations acid hydrolysis may lag behind the steam explosion method.The maximum amount of sugar (37.51 mg/ml of hydrozylate solution) was produced after the hydrolysis by HNO_3 with 0.5 mol/ltr concentration. The maximum amount of butanol was also found by hydrolyzing with 0.5 mol/ltr HNO_3.

5.4 CONCLUSION:

The sugars production was high with HNO_3 pretreatment and afforded an amount of 37.51mg/ml. With $HNO_3$the acid concentration 0.5 mol/ltr we get maximum amount of butanol in case of all acids i.e. H_2SO_4 (2.98mg/ml), HCl (2.96mg/ml), HNO_3 (3.046mg/ml) and H_3PO_4 (1 mg/ml) as compare to 0.1, 0.2 0.3 and 0.4 mol/ltr of concentrations. The maximum sugar was found 37.51 mg/ml and butanol was found 3.046 mg/ml.

5.5 FUTURE WORK:

1. Detoxification after pretreatment can done to reduce the toxic compounds produced after pretreatment in order to enhance the sugar and butanol yield.

2. The effect of temperature and duration of hydrolysis can be varied in order to get an optimized condition for hydrolysis.

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