PRODUCTION OF BUTANOL (C_4H_9OH) FROM SWITCHGRASS

Submitted by

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Under the guidance of

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CERTIFICATE

The thesis entitled, ***"PRODUCTION OF BUTANOL (C_4H_9OH) FROM SWITCHGRASS***" submitted by **"SARBESWAR SOREN"** bearing roll no. **110CH0110** bonafide project work and is worthy in partial fulfillments for the requirements for the award of Bachelors of Technology Degree in Chemical Engineering at National Institute of Technology, Rourkela (Deemed University) is certified to be an authentic work carried out by him under my supervision and guidance.

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

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ABSTRACT

Switchgrass being lignocellulosic biomass is most abundant renewable resource suitable for the unlimited supply of biofuel. Ethanol (C_2H_6O) and butanol (C_4H_9OH) procured a prevailing place in the incomplete supplanting or mixing with gas. Butanol (C_4H_9OH) created through successful treatment that makes a desire for future energy. Acid hydrolysis above room temperature with proper mass transfer can help in dissociation of lignocellulosic fibres and enhances sugar yield. This work carried out explained the pre-treatment effects of acid hydrolysis on switchgrass using different concentrations and acid types and further fermentation of butanol (C_4H_9OH). The maximum amount of sugar (33.15 mg/ml) produced after the hydrolysis by HNO_3 with 0.5 mol/ltr concentration. The maximum amount of butanol (2.32mg/ml) was found by hydrolyzing with 0.5 mol/ltr of HNO_3 after the fermentation of hydrozylate.

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CHAPTER-1

INTRODUCTION

INTRODUCTION

The increment in worldwide population and general financial yield has prompted an increment for transportation of fuels in the course of recent decades and fuel utilization is required to increment more or less 60% in the following twenty years. To the direction of this vitality challenge, there have been increments in innovative work of biofuels [1]. Lignocellulosic biomass can be a compel-ling prospect pertaining to substitute energy resource generation because doing so will be readily accessible, prevents problems encompassing 'food as well as fuel' and possesses the actual potential of experiencing a little environmentally friendly effect. Using forage plants as a supply of lignocellulosic about biofuel generation has attracted restored curiosity throughout the last few years [2].

The sort of plants containing proved to be beneficial will be switchgrass. This agriculture asset was the warm season perpetual C4 grass that is discovered comprehensively all through the North America. Switchgrass is exceptionally versatile, and it can develop in a wide range of districts of the nation incorporating areas with not as much as perfect soil quality [3]. This type of plant is known to have a great resilience to cold, insects and disease. As the biofuel asset, it is a profitable harvest with a few studies indicating yields of 15Mg ha^{-1} or more, and can be promptly coordinated to existing cultivating practices [4]. These advantages happen, partially because it is modest to seed and makes itself decently fast. It can likewise be developed with customary cultivating hardware and built reaping methods.

Another purpose of enthusiasm of using switchgrass is the common favorable circumstances as highlighted by Keshwani. The use of switchgrass, in admiration to other yearly line yields, prompts a 95% lessening in the soil breaking down and a 90% decreasing in pesticide utilization. It has been represented that the switchgrass improves soil quality and carbon sequestration due to its extensive root structure that extends carbon stockpiling in soil [5]. For example, Gebhart exhibited that grasses, for instance, switchgrass can be store 1.1 Mg of carbon each hectare in the upper 1 m of soil consistently. The investigation has also seen that switchgrass was more suitable at ousting nitrogen and phosphorous from flood appeared differently about other cool season grasses. These type of results exhibit that switchgrass can be used to upgrade the surface water quality.

So we can turn biomass so as to invigorates, there is ordinarily a couple of essential procedures that may be used, these being a bio- logical stage and thermo-chemical advances. The main technique comprises of exchanging biomass keeping in mind the end goal to ethanol and also related to liquid fuels by a fermentation and saccharification process [6]. This obliges deconstructing the polysaccharides keeping in mind the end goal to monosaccharides and after that fermentation to ensuing and also second or third innovation biofuels. Taking this strategy, a standout amongst essential criteria inside of general biofuel creation is the data in regards to hemicellulose and cellulose inside the cell surfaces of the biomass. It is noted of which inside herbaceous energy crops, the aggregate cell holding divider bit is pretty nearly 80% of the plant dried abundance weight and is formed essentially in regards to hemicellulose, cellulose and lignin. Lignin is the 3rd critical part found in the cell surfaces with respect to biomass, which is in regards to higher quality. Not at all like cellulose and hemicellulose, lignin is not changed over to ethanol utilizing ordinary maturation advancements. The estimation of the lignin inside of biomass could be caught through methods like pyrolysis containing another bio-oil. This needs hoisted temperatures in a free oxygen environment for brief times to volatilize diminished sub-atomic weight mixes that are then consolidated quickly to a fluid bio-crude [7].

Basically, switchgrass contains a mixed bag of inorganic components which may be doubtlessly not helpful from the change on this bio asset keeping in mind the end goal to biofuels. These sorts of components ought to be managed similar to an aspect stream amid the handling and change with respect to biomass keeping in mind the end goal to bio-fuels, and in order to decrease and comprehend their specific impact, it is critical to focus the measure of these sorts of mixtures from the switchgrass tests. Accordingly it may be seen the assembling with respect to powers from biomass depends upon around the substance material and structure with the basic variables from the versatile or convenient surfaces and the inorganic constituents [8]. The objective of this sort of work ought to be to survey the genuine plant science with respect to switchgrass in light of the fact that it identifies with it is change so as to ethanol or bio- crude. Furthermore, the real change science with respect to switchgrass will probably be sketched out taking a shot at the genuine pretreatment methods and pyrolysis to bio-oil [9, 10].

1.1 PRETREATMENT OF BIOMASS:

1.1. (A) PRETREATMENT OF LIGNOCELLULOSE:

We know lignocellulosic biomass consist of hemicellulose, cellulose and lignin. Lignin, is the significantly cross-associated polymer complex including phenolic liquor monomers, gives assistant support for the plant cell divider. Lignin associations and structures a firm physical seal around cellulose and hemicellulose to maintain a strategic distance from dissolvable penetrability and microbial invasion. Hemicellulose is made out of the hetero-polysaccharide spine (formed by arabinose, xylose, mannose and galactose) with little branches joined similarly by β-(1-4)-glycosidic bonds. Hemicellulose behaves like filler amidst cellulose and lignin microfibrils [11]. Here cellulose is the major assistant parts in the plant cell divider, and it is for the most part squeezed into tight littler scale fibrils on account of the hydrogen security linkage of long cellulose chain. In plant cellulose, biomass is for the most part in the crystalline structure with a little separation in ill-defined structure, which chooses the hard-to-breakdown the nature of cellulose by both destructive and impetus hydrolysis. To viably change over cellulose to fermentable sugars, hemicellulose and lignin must be emptied. The target of the pre-treatment is to evacuate hemicellulose and lignin, diminish the crystallinity of cellulose, and grow the porosity of the lignocellulosic biomass.

1.1.1 PHYSICAL OR MECHANICAL PRETREATMENTS:

The other name of Physical pretreatment is mechanical pretreatment, where grinding, hardware chipping or possibly processing to alleviate the measurements of biomass alongside the cellulose crystallinity enhancing simple acid or chemical access. Concerning the determinations, biomass can without much of a stretch starting wind up being guided through a chipping machine to get contaminants on estimations in regards to 10-30 mm; on the off chance that alright powder is favored, this biomass is normally further guided with respect to cultivating or possibly processing to reduce the measurements so as to 0.2-2 mm [12]. Typically, little this molecule measurements, the simpler for that microorganism or enzyme to digest. Little measurements besides helps you to intrude on this crystalline structure in regards to cellulose unrivalled. Indeed, even along these lines, higher expense is every now and again with respect to better molecule measurements.

1.1.2 THERMAL PRETREATMENT:

Steam blast uses high-temperature steam (160-270^{o}C) at high weight (0.69-4.83MPa) to treat the lignocellulosic biomass for a few seconds to minutes before the biomass is out of the blue introduced to climatic weight, amid which the biomass encounters an unsafe decompression in light of the sudden weight drop [13]. It was accounted for that steam blast can altogether form the enzymatic hydrolysis profitability and reducing sugar yield from an extensive variety of lignocellulosic biomass for instance wheat straw, switchgrass, corn stover and wheat fibre.

1.1.3 STEAM EXPLOSION:

The steam blast has been associated with and is seen as a champion amongst the best pre-treatment schedules for lignocellulosic materials, particularly horticultural build-ups and hardwood. Preferences of the steam blast in a far-reaching way join diminishing the biomass size, convincing departure of hemicellulose and lignin without weakening of the resulting sugars and lower vitality cost diverged from mechanical processing [14].

1.1.4 CHEMICAL PRETREATMENTS:

1.1.4. (A) ACID PRETREATMENT:

According to acid pre-treatment it can be apportioned into weaken acid and concentrated acid pre-treatment. The goal of acid pre-treatment is to almost or thoroughly hydrolyze hemicellulose, separate the lignin structure and bother the cellulose crystallinity for further enzymatic preparing to release fermentable sugars. By and large, focused acid (H_2SO_4 and HCl) pre-treatment is thought to be unnecessarily dangerous and unsafe to work. Besides, a ton of base is required for equalization, achieving high salt concentration in the hydrolyzate significantly inhibitory to the aging [15]. In this way, dilute acid pretreatment is generously all the more by and large used diverged from the concentrated acid pretreatment.

Dilute H_2SO_4 and HCl are commonly used as a piece of dilute acid pre-treatment of biomass with center reaching out from 0.5% to 5 %(w/v) or 0.05 to 1N depending upon the biomass sort or procedure time. Dilute acid treatment is effect in evacuating hemicellulose, with all the hemicellulose hydrolyzed and recouped as the disintegrated sugars, for instance galactose,

arabinose, xylose thus on in the hydrolysis [16]. The clearing of hemicellulose opens the cellulose to enzyme attack, extending the enzymatic absorbability and sugar yield in the development strong left after the acid pretreatment. Diverse agro- mechanical deposits, including corn fiber, switchgrass, corn stover, corn cob, whey straw, barley, sugarcane bagasse, whey grain and cassava bagasse has been considered under particular acid obsessions and residence times in journey for a perfect condition. A mixture of debasement items (furan subordinates, phenolic blends, and so forth) as a rule accompany acid pretreatment. Conforming the sugar yield, acid center and pretreatment can control the inhibitors present in the hydrolyzate, assuaging the weight on the accompanying aging procedure [17].

1.1.4. (B) ALKALINE PRETREATMENT:

Alkaline pre-treatment alongside solid bases like sodium hydroxide, potassium hydroxide, calcium hydroxide, alongside alkali hydroxide is additionally far reaching. Contrasted with acid pretreatment, basic pretreatment makes utilization of genuinely direct conditions, including space or possibly a bit expanded temp alongside environmental weight. Due to this sort of moderate circumstance, this length connected with basic pretreatment commonly takes hours to help days to weeks as opposed to a couple of minutes. Raised temp can unquestionably significantly decrease the reaction time accordingly, 80-120°C is typically used in alkaline pretreatment. Amongst each one of the far reaching strong aspects, lime is unquestionably brought about by picked a consequence of the sensibly focused diminished cost alongside renewability. A few feedstock's happen to be dealt with alongside alkaline, including bagasse, Corn Stover, wheat straw, change turf, wood chips and a great deal more. The principle objective with the alkaline pretreatment ought to be to kill the lignin through biomass, while hemicellulose is likewise in some measure broke up leaving cellulose accessible to help digestive catalysts. It had been moreover recorded that will inside of the notoriety of an oxidizing real estate broker including oxygen, uprooting lignin is really definitely supported while cellulose inside of the biomass is just not beset [18].

1.1 (B) DETOXIFICATION OF LIGNOCELLULOSIC HYDROLYZATE:

Diverse side effects, likewise called inhibitors in the last bioconversion methodology, are made amid the pretreatment process. The genuine reactions fuse furan backups (furfural and 5-hydroxymethylfurfural (HMF), sugar corruption), phenolic blends (syringaldehyde, vanillin, vanillic acid, syringic acid, p-coumaric acid, ferulic acid, lignin debasement), and weak acid (acidic acid, lignocellulose structure corruption).

Hexose and pentose are released at the time hydrolysis of lignocellulosic biomass, and after that further corrupted into furfural and HMF, exclusively. Furfural and HMF are all things considered as the critical inhibitors to the microorganisms. Aldehydes, fragrant mixes and phenolic are corruption things delivered from lignin. These blends, especially the low sub-nuclear weight ones, are uncommonly unsafe to maturing microorganisms, notwithstanding when their fixations are low. Acetic acid ($C_2H_4O_2$) is gotten from the acetyl side-gatherings of hemicellulose, and it is considered as an aftereffect of lignocellulosic structure debasement. The inhibitory effect of acetic acid ($C_2H_4O_2$) is normally not as great as furan auxiliaries or phenolic mixes. At low focuses, a few reports exhibited that acidic acid truly redesigned the dissolvable era and kept the method for culture degeneration [19].

Right when we using lignocellulosic hydrolyzate most of the previously stated substances can be achieve a couple of degrees of restraint in the maturation process. So that the presence of various inhibitors, the slack stage is postponed, sugar utilization is decreased, and the thing advancement (efficiency, fixation, and yield) is basically irritated. The inhibitory centralization of each compound can't be completely chosen on account of the different characteristics of microorganism. Likewise, it has been represented that while an individual compound may not achieve restraint, when in the vicinity with diverse strengthens a noteworthy "synergistic effect" may demonstrate. Detoxification is by and large anticipated that would re-condition the lignocellulosic hydrolzsates to a suitable substrate for microorganisms to process. Physical detoxification for the most part uses vacuum dissemination methodology to clear the unstable lethal substances, for instance, furfural and acetic acid ($C_2H_4O_2$). Commonly the furfural can be viably evacuated by this technique, and the sugar is concentrated after water vanishes. The downside of this pretreatment is that non- unstable substances total and stay in the concentrated

hydrolyzate. With everything taken into account, manufactured detoxification joins using pH adjustment to empower and evacuate toxic substances, and adsorption with enacted charcoal or particle trade tars. Since a couple of inhibitors are shaky at a certain pH, pH modification with $Ca(OH)_2$ (lime) is the most frequently used detoxification procedure for a blended sack of lignocellulose hydrolyzates. Generally, lime is added to change the pH to 9 to 10, and after that acid (H_2SO_4 or HCl) is added to straighten out pH to 5.5 to 6.5. It was represented that over lime detoxification decreased more than 41% of phenolic compounds, 51% of furans and just 8.7% of sugars [20].

1.2 HYDROLYSIS OF LIGNOCELLULOSIC MATERIALS:

Basically cellulose particles are made out of long chains of glucose atoms. In the hydrolysis prepare, these type of chains are broken to "free" the sugar, before it was fermented for alcohol generation. There are two major hydrolysis forms in the chemical reaction utilizing acids or an enzymatic reaction.

1.2.1 ACID HYDROLYSIS:

Acid hydrolysis have been analyzed as a conceivable procedure for regarding lignocellulosic materials, for example, wood chips, the mineral acids act essentially and quickly as response catalyzers of polysaccharide fractions. The sugarcane bagasse can be hydrolyzed utilizing dilute acid to get a mixture of sugars with xylose just as the key segment. Then again, in the hydrolyzate some by-items are produced in the hydrolysis, for example, acetic acid ($C_2H_4O_2$), furfural, phenolic compounds or lignin debasement items. These are potential inhibitors of a microbiological usage of this hydrolyzate. Procedures for example two-stage acid hydrolysis can be utilized to deliver glucose and xylose. Treatment with weaken hydrochloric acid (HCl) at moderate temperatures has ended up being a proficient method for delivering xylose from the hemicellulose. At the second stage more intense response conditions are utilized and glucose may be delivered from the cellulose hydrolysis.

Generally, acid treatment is most capable throughout solubilizing the particular hemicellulosic

element of the biomass. Appropriate mixtures associated with pH, heat range, as well as impulse time can result in substantial assure associated with sugar, primarily xylose through hemicellulose. Hydrochloric acid is usually a switch because of this impulse as well as, in this particular operate; it's utilized to analyze the particular hydrolysis associated with sugarcane bagasse hemicellulose. The results associated with heat range, acid concentration as well as impulse time are researched, as well as the potency of the particular hydrolysis seemed to be considered when it comes to hemicellulose solubilization. Enzymatic hydrolysis associated with cellulose can be performed simply by digestive enzymes that are highly distinct. The products on the hydrolysis usually are lowering sugar such as glucose. Electric cost associated with enzymatic hydrolysis can be reduced when compared to acid as well as alkaline hydrolysis since enzyme hydrolysis is generally done with minor circumstances (pH 4. 8 as well as heat range 45–50°C) as well as doesn't need the deterioration problem. Each bacteria as well as fungi can easily develop cellulases to the hydrolysis associated with lignocellulosic resources. Although some cellulolytic bacteria, particularly the cellulolytic anaerobes in the same manner with Clostridium thermocellum along with substantial distinct activity, they cannot develop substantial enzyme titers. Because the anaerobes possess a reduced growth fee as well as require anaerobic growth circumstances, many investigations intended for cellulase professional creation has focused on fungi [21]. Cellulases usually are mixtures associated with many digestive enzymes. The components in which have impacts on the particular enzymatic hydrolysis associated with cellulose contain substrate, cellulose activity, as well as impulse situation (temperature, pH, as well as other parameters). To further improve the particular produce as well as a fee on the enzymatic hydrolysis, the investigation has dedicated with optimizing the particular hydrolysis procedure as well as bettering cellulase activity.

1.3 FERMENTATION PROCESS:

Different type of microorganisms, general yeast, bacteria or fungi, ferment carbohydrates to butanol (C_4H_9OH) under free oxygen conditions. They do as such to obtain energy and then develop. Systems for C6 sugar were fermentation at that point known (at least) 6000 years back when Babylonians, Sumerians and Egyptians started to perfect and describe the procedure of making lager from grain (starch). After it had got to be possible to free the C6 sugars in lignocellulosic yields (end 19th century), conversion of the C5 sugars got to be interesting. They

speak to a high percentage of the accessible sugars, the capacity to recuperate and ferment them into ethanol is important for the efficiency and financial matters of the procedure. Just in the 1980s research on xylose fermentation started to bear fruit when various wild-type yeast was recognized that could change over xylose to ethanol. Bacteria have drawn exceptional consideration from scientists in light of their speed of fermentation, In general; bacteria can ferment in minutes when contrasted with hours for yeast [22].

CHAPTER – 2

LITERATURE REVIEW

2 LITERATURE REVIEW

2.1 LIGNOCELLULOSIC BIOMASS:

Lignocellulosic biomass, particularly its synthetic structure, it is fundamental for making successful pretreatment advances to deconstruct its unbending structure, outlining chemicals to free sugars, particularly cellulose to release glucose, from headstrong cellulose furthermore assembling microorganisms to change over sugars into ethanol and other bio-based chemicals.

Lignocellulosic biomass is fundamentally made out of plant cell dividers, with the basic carbohydrates cellulose and hemicellulose and heterogeneous phenolic polymer lignin as its key parts. On the other hand, their substance contrasts considerably, depend upon the species, mixture, atmosphere, soil fertility and fertilization practice, however by and large, for farming build-ups, for example, corn stover, wheat and rice straw, the cell dividers contain 40% cellulose, 30% hemicellulose and 15% lignin on a dry weight premise [22].

The specific segment of plant cell dividers is their two-area structure, as demonstrated in Fig. 1. A vital cell divider is delivered with cell division and amplified amid cell improvement to a fiberglass-like structure; with crystalline cellulose microfibrils introduced in a network of polysaccharides for instance hemicelluloses. The essential mass of nearby cells is held together by a sticky layer, called the centre lamella, made out of pectin's, to shape the directing tissue framework masterminded in various vascular groups. Then again, when cells stop to grow, an optional cell divider is regulated spared between the plasma film and the essential cell divider for better mechanical quality and fundamental backing through the solidification of lignin into xylem filaments, which speaks to the main part of lignocellulosic biomass that can be changed over to fills and chemicals.

The change of the main tissue framework with the unbending auxiliary cell divider is a basic versatile occasion in the advancement of area plants, which not just encourages the transport of water and supplements and in addition broad upright improvement, furthermore raises its obstinacy to defilement in light of the communication and cross-connecting of cellulose, hemicellulose and lignin.

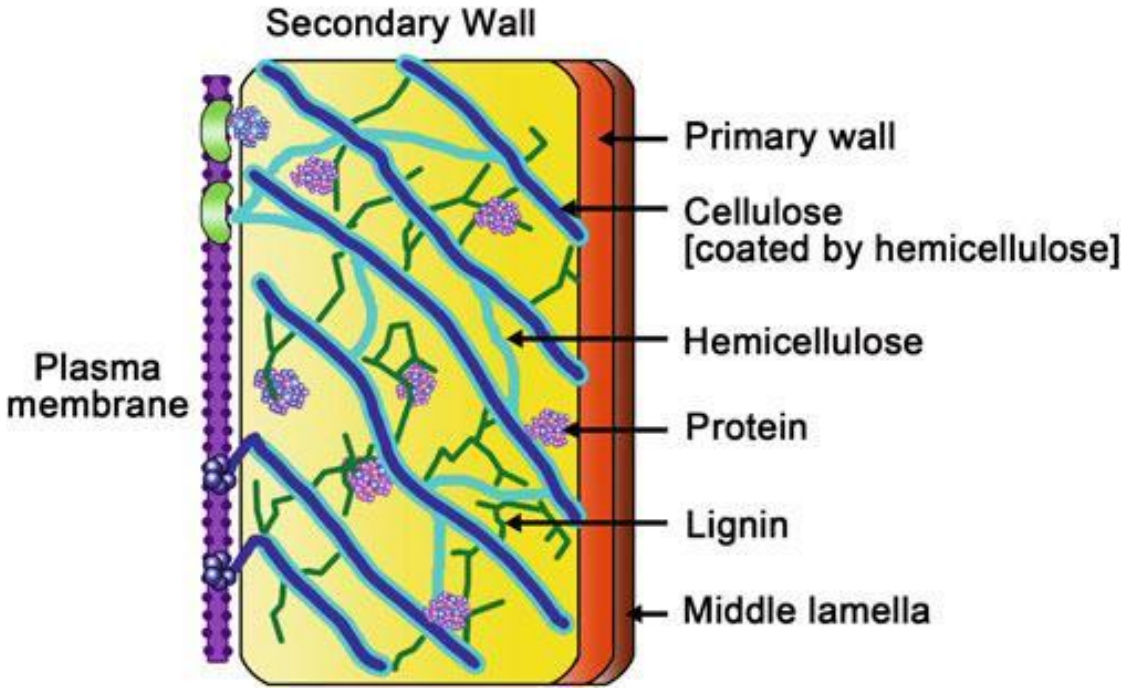

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Figure 1 Lignocellulose structure showing cellulose, hemicellulose and lignin.

2.1.1 CELLULOSE:

Cellulose is a polysaccharide made out of straight glycan chains that are joined together by β-1,4-glycosidic bonds with cellobiose build-ups as the rehashing unit at diverse degrees of polymerization relying upon assets, and pressed into microfibrils which are held together by intermolecular hydrogen bonds and intermolecular Vander Waals forces. In spite of the fact that polymorphy has been archived for cellulose, nearest cellulose happens as cellulose I, which is a blend of two polymorphs I_α and I_β. Cellulose I_α is combined at the same time with the expansion of the microfibril framework, and accordingly is overpowering in lower plants to shape the essential divider, furthermore in some microscopic organisms. While, cellulose I_β is stored inside of the auxiliary mass of higher plants for quality. The decipherment of crystalline structure demonstrates that cellulose I_α is described by the triclinic unit containing one chain, while there are two chains in the monoclinic unit of cellulose I_β giving more intermolecular hydrogen bonds, making it all the more consistent. Cruel conditions are thus anticipated that would change cellulose I_β of plant biomass into undefined polymorphs that can be assaulted all the more effectively by cellulases.

2.1.2 HEMICELLULOSES:

Hemicelluloses are a heterogeneous group of polysaccharides with the β-(1→4)-joined spine structure of pentose (C5) sugars, for instance, xylose and arabinose, and hexose (C6) sugars, including mannose, galactose and glucose as the rehashing units, which have the same tropical setup at C1 and C4, as outlined in Fig.2. The auxiliary likeness of hemicelluloses to the β-1→4 glycosidic obligations of the cellulose atom benefits by a conformational homology, which can prompt an in number non-covalent relationship with cellulose microfibrils [23].

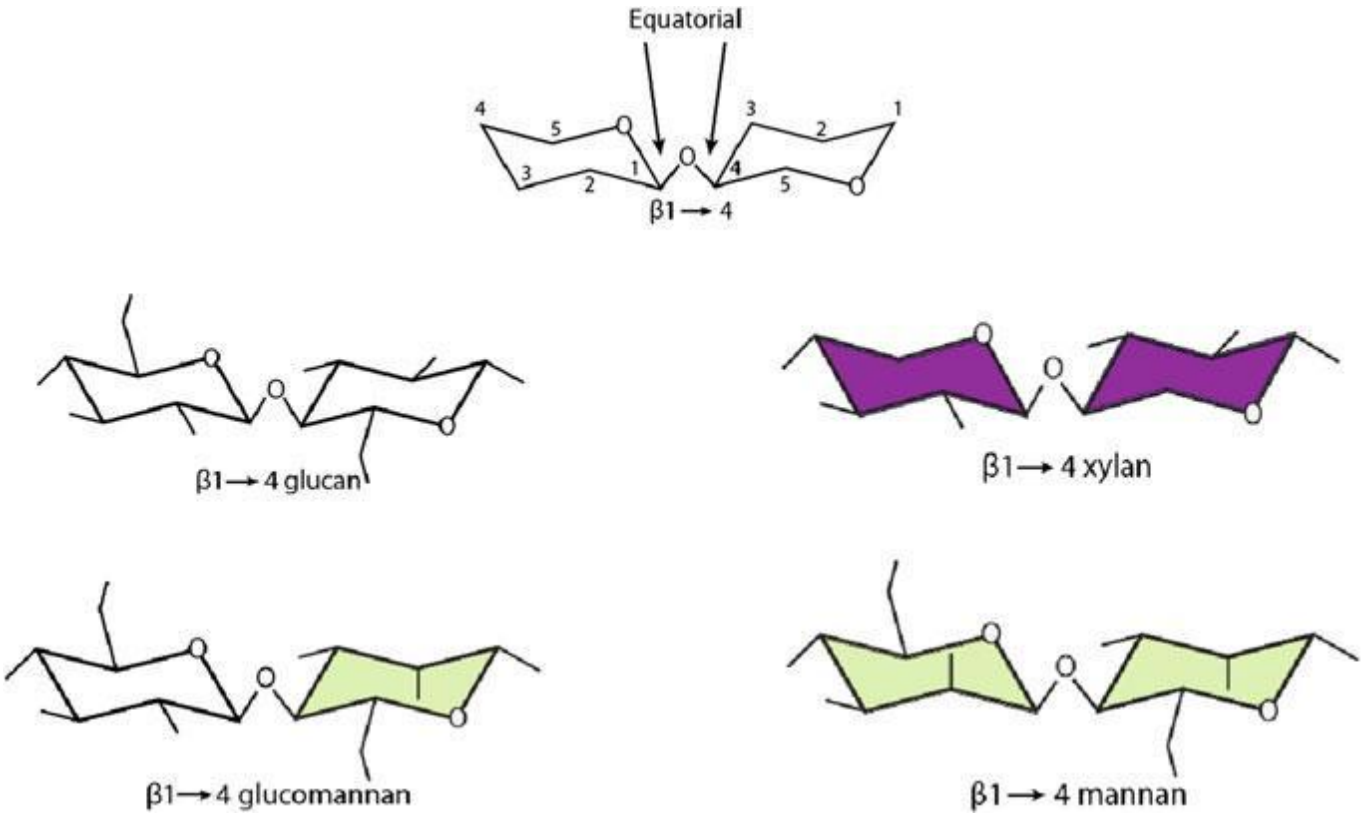

Figure 2 Hemicelluloses

Dissimilar to cellulose that is crystalline and impervious to debasement, hemicelluloses are arbitrary and undefined, and henceforth effortlessly hydrolyzed to monomer sugars. Nonetheless, hemicelluloses are embedded and implanted with cellulose and lignin, which essentially build the quality and durability of plant cell dividers.

2.1.3 LIGNIN:

In spite of the way that lignin is a non-sugar-based polymer and can't be used as feedstock for ethanol creation by method for microbial fermentation, it applies a significant impact on the monetary execution of the relating bioconversion shapes, ensuing to most inhibitors of microbial improvement and maturation begin from this compound in the midst of the pretreatment that is relied upon to render cellulose amiable to enzymatic strike. Mean-while, as the second most abundant segment in biomass after cellulose, lignin yields more vitality when smoldered, and hence is a decent decision for combined heat and power (CHP) era in an eco- and environment-accommodating system for the bio refinery. Furthermore, lignin is an astounding starting material for distinctive items including transportation fills, and quality included chemicals, which may add credits to bioconversion techniques and make bioethanol more monetarily competitive.

It is evident that understanding the fundamentals of lignin biosynthesis is the crucial for becoming more compelling pretreatment and molding strategies and ensuing enzymatic hydrolysis of cellulose and additionally designing small scale creatures with upgraded resilience to inhibitors so they can mature the hydrolysate more rapidly with extraordinary yields. As demonstrated in Fig. 4, lignin bio-combination starts with the deamination of phenylalanine to cinnamic acid, trailed by the change of the sweet-smelling ring by hydroxylation and O-methylation and decrease of the side bind to a liquor moiety, bringing about the three noteworthy monolignols: p-coumaryl, coniferyl and sinapyl alcohols, which are exchanged over the plasma layer into the apoplast [23].

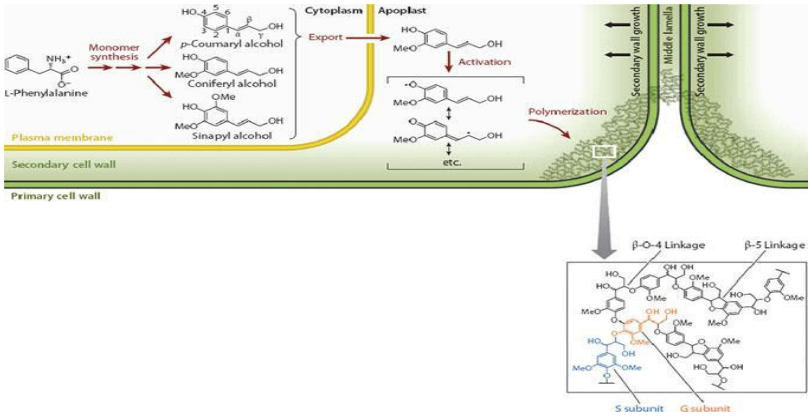

Figure 3 Lignin

Table 1 Contents of Cellulose, Hemicellulose and Lignin in the lignocellulose material.

S. No.	Lignocellulosic Material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
1	Softwood stems	45-49	24-34	26-34
2	Hardwood steams	41-54	24-40	19-24
3	Corn cobs	44	34	14
4	Nut shells	26-29	24-28	28-38
5	Grasses	26-39	36-48	9-29
6	Paper	86-98	0	0-14
7	Wheat straw	29	49	14
8	Sorted refuse	58	18	18
9	Leaves	16-18	80-84	0
10	Cotton seed hairs	82-94	6-19	0
11	Newspaper	42-53	26-38	18-28
12	Waste papers from chemical pulps	59-68	8-18	6-10
13	Primary wastewater solids	7-13	NA	25-28
14	Solid cattle manure	1.7-4.6	1.5-3.2	2.6-5.6
15	Coastal Bermudagrass	26	35.6	6.3
16	Switchgrass	45	31.4	12
17	Swine waste	5.8	27	NA

2.2 TYPES AND YIELD OF SWITCHGRASS:

After some time, switchgrass advanced specifically into extraordinary ecotypes having particular genetic, morphological qualities that have been deserving of particular areas. These sorts of various sorts are for the most part ordered as lowland and upland verities. The specific lowland mixed bags are portrayed by tall, thick stems and are by, and largely found in the heavier soil alongside wetter areas. The specific upland cultivars lean toward drier soils and development better inside of semi-parched areas. Luckily they are shorter alongside flimsy stemmed. Cassida et al. uncovered that will hereditarily, the real lowland forms are equipped for the add to a considerable measure more dry subject rather than upland sorts. The specific upland sorts of incorporate switchgrass Trailblazer, Blackwell, Cave in Rock, Pathfinder alongside Caddo. Normal lowland assortments are Alamo and Kanlow. A record of distinctive characteristics found in different cultivars can be shown in Table 2 [14, 15]. Yields of switchgrass inside a break down for every framed inside Iowa uncovered that they can different through 6.9 to 13.1 Mg ha^{1} through a typical yield of 9 Mg ha^{1}. Studies have demonstrated that these lowland sorts of switchgrass created a standout amongst the most biomass contrasted with alternate cultivars [23].

Table 2 Switchgrass cultivars and characteristics

S. NO.	VARIETY	CHARACTERISTICS
1	Blackwell	Adapted to Kansas, Oklahoma, southern Nebraska, and northern Texas. Regions with 20 inches or a greater amount of yearly precipitation discharged in 1944.
2	Caddo	Good recovery after mowing, Good forage yield under irrigation, released in 1955.
3	Kanlow	Developed for soil conservation in poorly drained or flooded sites, released in 1963.
4	Pathfinder	Winter tough, develops late discharged in 1967
5	Cave-in-Rock	Tolerant to flooding, adjusted to Midwest, discharged in 1973
6	Alamo	Heavy yields particularly in south, discharged in 1978
7	Trailblazer	Adapted to Midwest states and Central Great Plains, discharged in 1984

CHAPTER - 3

MATERIALS & INSTRUMENTS

3 MATERIALS & INSTRUMENTS

3.1 CHEMICALS REQUIRED:

Chemicals used are obtained from Merck. Hydrochloric Acid (HCl), Sulfuric Acid (H_2SO_4), Nitric Acid (HNO_3), Ortho-Phosphoric Acid (H_3PO_4),Concentrated and diluted NaOH for pH adjustments, Phenol, DNS, Sodium hydroxide (NaOH),Sodium metabisulphite ($Na_2S_2O_5$), Potassium Sodium Tartrate (40%)

3.2 INSTRUMENTS USED:

3.2.1 SEIVEING USING MESH:

Three different mesh sizes 14, 16, 18 are used for obtaining particle size of 1mm.

3.2.2 VERTICAL AUTOCLAVE:

Vertical autoclave was primarily utilized for disinfection reason. It is basically an encased space which is used to give water bath to any equipment putting it inside it. Electric coils present at bottom heats the water. Vent at the top discharge the steam buy which the desired pressure is maintained. For each filter sterilization is done to maintain the pH.

3.2.3 LAMINAR FLOW CHAMBER:

The samples are placed in the laminar flow chamber which maintains disturbance free conditions and prevents the contamination of the given sample by undesired chemicals and microbes. It is also used for the purpose of sterilization.

3.2.4 UV-SPECTROPHOTOMETER:

UV-Spectrophotometer is utilized to examine the examples. For our analysis, we utilize the specific wavelength which is 540 nm for estimation. In the equipment the sample is placed at one cuvette whereas the other cuvette acts as reference. At a particular wavelength it gives the absorbance of a specific sample.

3.2.5 SHAKING INCUBATOR:

This type of instrument is utilized for fermentation and acid hydrolysis where at a particular rpm is maintained at necessary conditions. As the name suggests there are shaking platform where Flasks are kept which moves at required speed.

CHAPTER - 4

EXPERIMENTAL METHOD

4 EXPERIMENTAL METHOD

4.1 RAW MATERIALS:

Switchgrass was obtained from agriculture field in Rourkela area. The material is sun dried. After that it is allowed to milling and sieving to make the particle size less than 1mm. The material is stored in polythene bags in absence of light until it use for the experiment. H_2SO_4, HNO_3, H_3PO_4 and HCl concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 mol/ltr are used in the next step. Acid treatment is done by biomass loading of 5% that is 2g of the biomass with 38ml of diluted acid in a conical flask

4.2 PREPARATION OF SWITCHGRASS HYDROLYSIS (SH):

4.2.1 STEAM EXPLOSION:

The conical flask with acidified biomass was autoclaved at 121°C with 15 psi for 1 hour.

4.2.2 ACID HYDROLYSIS:

At 50°c and 140rpm the acid mixture was kept inside the shaking incubator and allowed to remain in that condition for 24 hours. The switch grass hydrolyzate was taken out and brought to room temperature. The switch grass hydrolyzate was neutralized to pH utilizing sodium hydroxide (NaOH). Miller assay used for evaluating reducing sugar.

4.3 ADJUSTMENT of pH:

Sodium hydroxide (NaOH) solution of 1M and 10M is added to make that pretreated switchgrass solution to neutral.

Switchgrass hydrolysate were sterilized in the vertical autoclave for 20 minutes at 121°C and 1atm pressure and then cooled.

4.4 PREPARATION OF SODIUM CITRATE BUFFER:

50mM Sodium citrate buffer used in saccharification is prepared by dissolving sodium hydroxide and citric acid and after that it is diluted to 1 L and is adjusted to the value 4.8.

4.5 PREPARATION OF INOCULUM:

Lyophilized Clostridium acetobutylicum (MTCC 481) was procured from microbial type culture collection, IMTECH, which is situated at Chandigarh. It was maintained as spore suspension in sterile water. This culture restored in Reinforced Clostridial Agar and Reinforced Clostridium Medium (Broth) culture media and maintained at 37°C. The inoculum prepared in Reinforced Clostridium Medium containing (g/L): beef extract, 10.0; glucose, 5.0; yeast extract,3.0; peptone, 10.0; starch, 1.0; sodium chloride, 5.0; sodium acetate, 3.0; Agar,0.5 and cysteine hydrochloride, 0.5; pH 6.5 ± 0.1. 100 mL medium autoclaved at 121°C and inoculated in 250 mL screw capped Erlenmeyer flasks. It is incubated for 72 hours at 37 ± 0.5°C at 120 rpm in shaking anaerobic incubator. Actively growing cultures (after lag phase, 18–20 hours) of the Clostridia were added subsequently to experimental flasks.

4.6 FERMENTATION:

Batch fermentation done by inoculating organism with Switchgrass hydrolyzate in 250 mL of screw-capped Erlenmeyer flasks under anaerobic conditions. The anaerobic condition in the flask was generated by addition of 0.5% cysteine hydrochloride to the switchgrass hydrolysis. After filtering the fibrous remains was collected and then dried in a hot air oven 70°C is maintained and then was weighed. After pretreatment the reduced weight of switchgrass was noted. After the fermentation samples were taken for the estimation of C_4H_9OH production and sugar utilization.

4.7 ANALYSIS FOR BUTANOL (C_4H_9OH):

UV-spectrophotometer was used for Butanol (C_4H_9OH) and sugar analysis of the samples. In analysis of C_4H_9OH, the SH filtrate is directly placed in ultraviolet spectrophotometer and its absorbance is noted down at 197 nm wavelength. However for sugar analysis, DNS assay is followed [18].

4.8 REDUCING SUGARS BY DETERMINATION:

100 µL of the neutralized SH was pipetted into the test tube followed by 300 µL of DNS reagent (The procedure for the preparation of DNS reagent preparation is given below). The most important part involves the preparation of Blank solution, which was prepared by mixing 100 µL

of distill water and 300 µL of DNS reagent. The samples were allowed to be kept in hot water bath at 90°C for 5 min. which was then followed by cooling in running water. After that they were diluted 7.5 times i.e. 2.6 ml of distilled water was added to test tubes. After that at 540nm the absorbance of the samples and blank were taken.

4.9 DNS REAGENT:

Distilled Water	30ml
3, 5 dinitro salicylic acid	225mg
Sodium hydroxide (NaOH)	420mg

Dissolve above and then add,

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

CHAPTER - 5

RESULTS, DISCUSSION AND CONCLUSION

5 RESULTS, DISCUSSION AND CONCLUSION

5.1 COMPOSITION OF SWITCHGRASS:

The composition of switchgrass: Glucan 39.2%, Xylan 22.1%, Arabian 6.7%, Lignin 18.2% and others 13.8 (by difference) for this kind of materials [7].

5.2 REDUCING SUGAR ANALYSIS:

Table 3 5.2.1 Standard Dextrose Curve from DNS Assay:

Dextrose conc. (mg/ml)	Volume of distill water (µl)	Volume of DNS Reagent (µl)	Volume of Dextrose (10 mg/ml) (µl)	Absorbance at 540nm
0	100	300	0	Blank
1	90	300	10	0.117
2	80	300	20	0.237
3	70	300	30	0.346
4	60	300	40	0.441
5	50	300	50	0.562
6	40	300	60	0.664
7	30	300	70	0.763
8	20	300	80	0.90
9	10	300	90	0.94
10	0	300	100	1.021

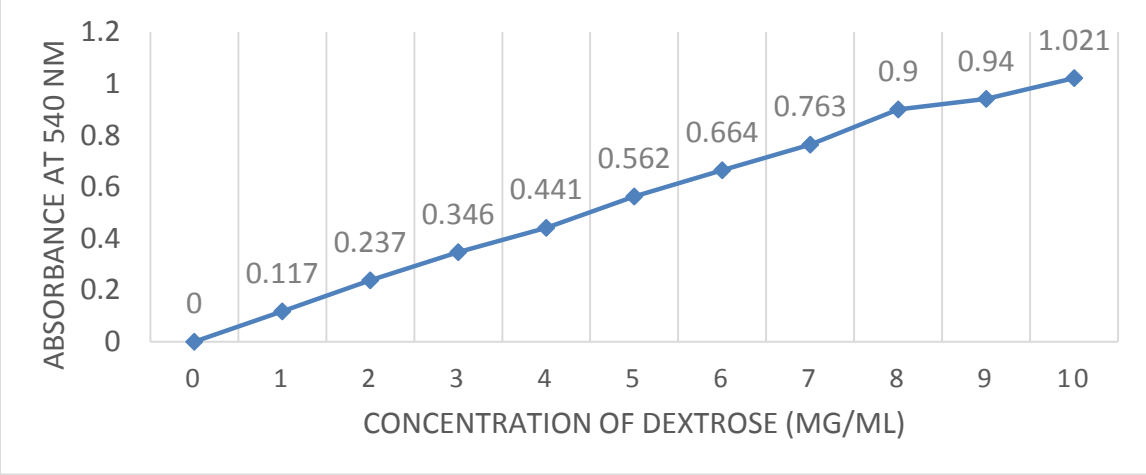

Figure 4 Standard Dextrose Curve from DNS Assay:

5.2.2 SUGARS OBTAINED AFTER STEAM EXPLOSION PRETREATMENT:

0.08mg/ml of reducing sugar was observed for hydrolyzate sample.

5.2.3 AFTER ACID HYDROLYSIS SUGARS PRODUCED:

Reducing sugars (mg/ml of hydrolyzate) which is obtained after the acid hydrolysis at different concentration is given in the below table 5.

Table 4 Reducing Sugars obtained after the acid hydrolysis of switchgrass.

CONC. (mol/Ltr.)	HCl	H_2SO_4	H_3PO_4	HNO_3
0.1	12.4	18.025	7.575	17
0.2	16.25	24.025	9.25	24.75
0.3	21.55	27.325	13.45	29.75
0.4	26.35	28.35	13.6	31.625
0.5	31.15	31.375	13.7	33.15

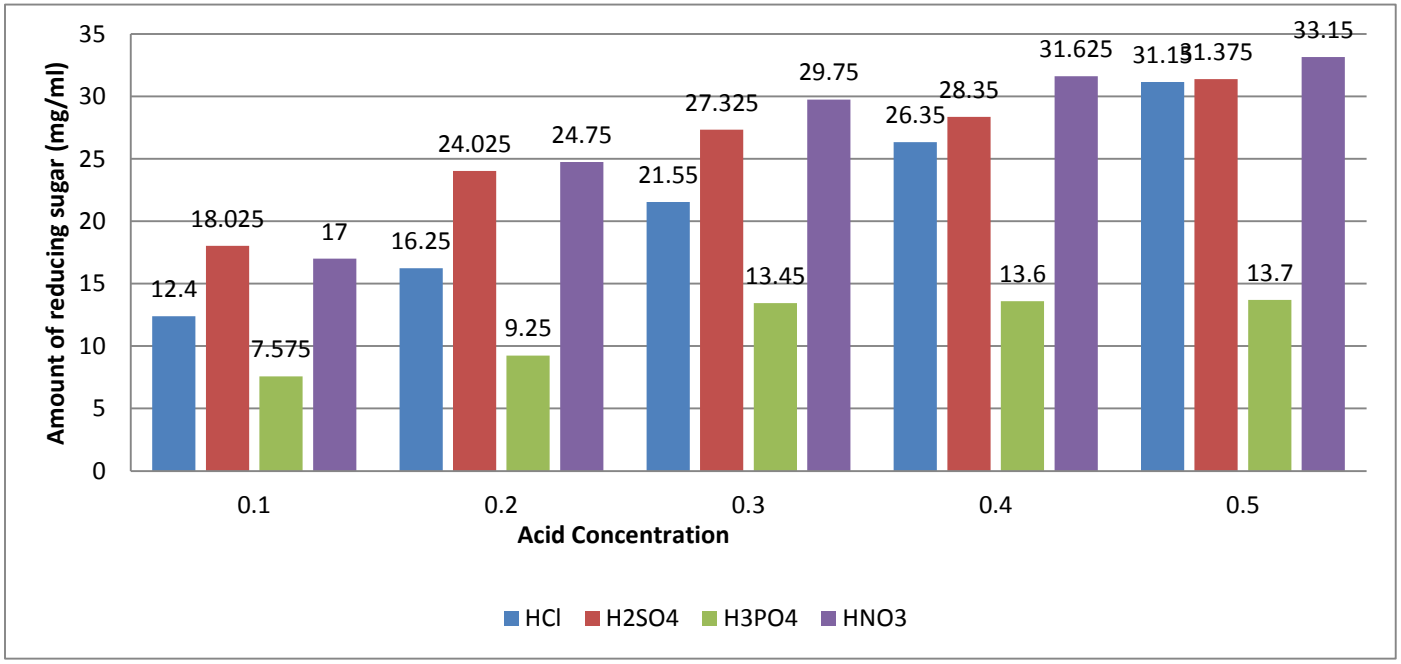

Figure 5 Reducing Sugars obtained after the acid hydrolysis of switchgrass.

5.3 BUTANOL PRODUCED AFTER FERMENTATION:

The amount of butanol (C_4H_9OH) obtained (mg/ml) after the fermentation of acid hydrozylates at different concentration of acid are given in table 6.

Table 5 Butanol (C_4H_9OH) produced (mg/ml) after fermentation.

CONC. (mol/Ltr.)	HCl	H_2SO_4	H_3PO_4	HNO_3
0.1	0.868	1.26175	0.53025	1.19
0.2	1.1375	1.68175	0.6475	1.7325
0.3	1.5085	1.91275	0.9415	2.0825
0.4	1.8445	1.9845	0.952	2.21375
0.5	2.1805	2.19625	0.959	2.32

The butanol (C_4H_9OH) produced from steam explosion hydrozylate after fermentation was 1.36 mg/ml of hydrozylate sample.

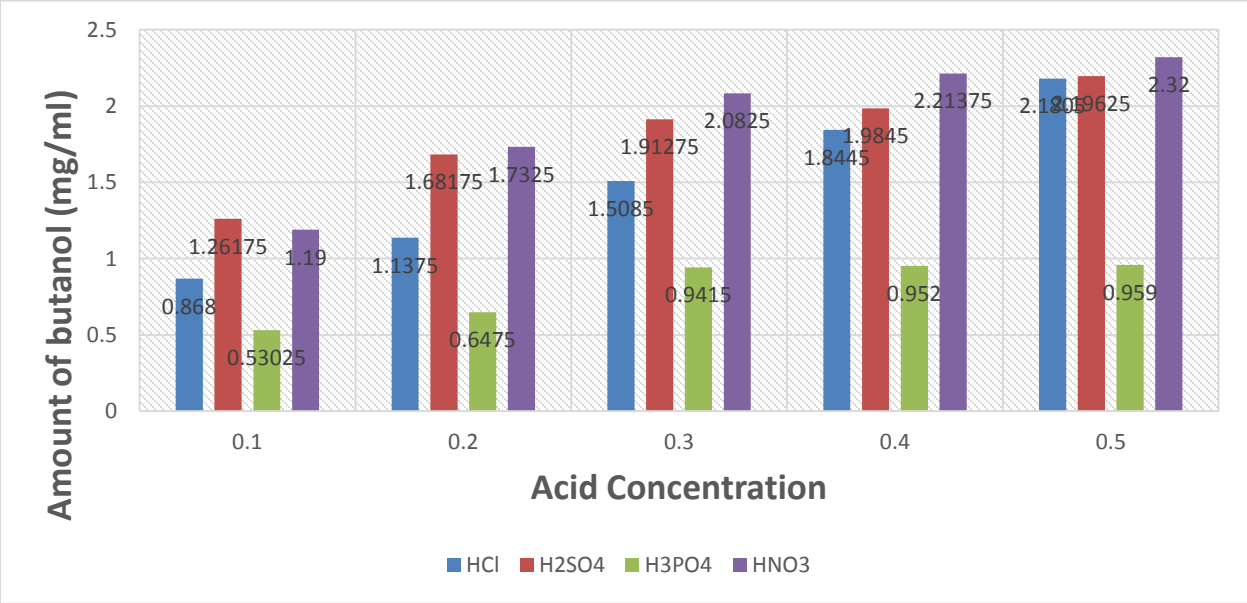

Figure 6 Butanol (C_4H_9OH) produced (mg/ml) after fermentation.

The production of sugar and butanol (C_4H_9OH) increased with the concentration of acid. For sugar the maximum conc. that is 33.15 mg/ml of hydrozylate solution) was produced after the hydrolysis by HNO_3 with 0.5 mol/ltr concentration. However for butanol (C_4H_9OH) the maximum conc. is obtained for 0.5 mol/ltr HNO_3.

5.4 DISCUSSION:

After acid hydrolysis sugar produced at different concentration. When the concentration is at 0.1 mol/litre then sugar produced for Sulphuric acid (18.04 mg/ml), Nitric acid (17 mg/ml), hydrochloric acid (12.4 mg/ml) and Phosphoric acid (7.57 mg/ml). For the concentration 0.2 mol/litre the sugar produced in Nitric acid (24.74 mg/ml), Sulphuric acid (24.025 mg/ml), hydrochloric acid (16.25 mg/ml) and Phosphoric acid (9.24 mg/ml). For the concentration 0.3 mol/litre the sugar produced for Nitric acid (29.75 mg/ml), Sulphuric acid (27.328 mg/ml), hydrochloric acid (21.55 mg/ml) and Phosphoric acid (13.45 mg/ml). For the concentration 0.4 mol/litre the sugar produced in Nitric acid (31.625 mg/ml), Sulphuric acid (28.35 mg/ml), hydrochloric acid (26.35 mg/ml) and Phosphoric acid (13.6 mg/ml). For the concentration 0.5 mol/litre the sugar produced in Nitric acid (33.15 mg/ml), Sulphuric acid (31.375 mg/ml), hydrochloric acid (31.15 mg/ml) and Phosphoric acid (13.7 mg/ml). It was observed that for 1st case when concentration for sugar was 0.1 mol/litre highest sugar produce was in Sulphuric acid which is (18.025 mg/ml). But for rest 4 cases the concentration of Nitric acid was more. Comparing all the concentration it was observed that overall sugars production was high with Nitric acid for Reducing Sugars obtained after the acid hydrolysis of switchgrass pretreatment and afforded an amount of 33.15 mg/ml with Nitric acid where the acid concentration of 0.5 mol/ltr. Amount of butanol produced in (mg/ml) after the fermentation of acid hydrozylates at different concentration of acid are with the concentration 0.1 mol/ litre of concentration Sulphuric acid (1.26175 mg/ml), Nitric acid (1.19 mg/ml), hydrochloric acid (0.868 mg/ml) and Phosphoric acid (0.53025 mg/ml). For the concentration 0.2 mol/litre the sugar produced in Nitric acid (1.7325 mg/ml), Sulphuric acid (1.68175 mg/ml), hydrochloric acid (1.1375 mg/ml) and Phosphoric acid (0.6475 mg/ml). For the concentration 0.3 mol/litre the sugar produced in Nitric acid (2.0825 mg/ml), Sulphuric acid (1.91275 mg/ml), hydrochloric acid (1.5085 mg/ml) and Phosphoric acid (0.9415 mg/ml). For the concentration 0.4 mol/litre the sugar produced in Nitric acid (2.21375 mg/ml), Sulphuric acid (1.9845 mg/ml), hydrochloric acid (1.8445 mg/ml)

and Phosphoric acid (0.952 mg/ml). For the concentration 0.5 mol/litre the sugar produced in Nitric acid (2.32 mg/ml), Sulphuric acid (2.19625 mg/ml), hydrochloric acid (2.1805 mg/ml) and Phosphoric acid (0.959 mg/ml). Maximum amount of butanol in case of all acids i.e. H_2SO_4 (2.19625mg/ml), HCl (2.1805mg/ml), HNO_3 (2.32mg/ml) and H_3PO_4 (0.959 mg/ml) as compare to 0.1, 0.2 0.3 and 0.4 mol/ltr of concentrations. The maximum butanol was found with Nitric acid, the acid concentration of 0.5 mol/litre and was found to be 2.32 mg/ml for the butanol produced from steam explosion hydrozylate after fermentation.

5.5 CONCLUSION:

The sugars production was high with Nitric acid treatment and concentration of 33.15mg/ml sugars produced with the acid concentration of 0.5 mol/litre. 2.32 mg/ml of butanol produced with same acid concentration of 0.5 mol/litre. The acid treatment step is efficient at low temperature which minimizes energy changes for the downstream steps of fermentation.

5.6 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.

REFERENCES:

1. Jang, Y. S., Malaviya, A., Cho, C., Lee, J., & Lee, S. Y. (2012). Butanol production from renewable biomass by clostridia. *Bioresource technology*, *123*, 653-663.

2. Linggang, S., Phang, L. Y., Wasoh, H., & Abd-Aziz, S. (2013). Acetone–butanol–ethanol production by Clostridium acetobutylicum ATCC 824 using sago pith residues hydrolysate. *BioEnergy Research*, *6*(1), 321-328.

3. Bouton, J. H. (2007). Molecular breeding of switchgrass for use as a biofuel crop. *Current opinion in genetics & development*, *17*(6), 553-558.

4. Jain, A., Walker, T., & Kelly, K. (2014). Biological and Biosystems Engineering for Processing of Switchgrass Feedstocks and Biofuel Production.*Compendium of Bioenergy Plants: Switchgrass*, 315.

5. Demain, A. L. (2009). Biosolutions to the energy problem. *Journal of industrial microbiology & biotechnology*, *36*(3), 319-332.

6. Yao, W., & Nokes, S. E. (2014). Phanerochaete chrysosporium pretreatment of biomass to enhance solvent production in subsequent bacterial solid-substrate cultivation. *Biomass and Bioenergy*, *62*, 100-107.

7. Dien, Bruce S., Hans-Joachim G. Jung, Kenneth P. Vogel, Michael D. Casler, JoAnn FS Lamb, Loren Iten, Robert B. Mitchell, and Gautum Sarath. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass and Bioenergy 30, no. 10 (2006): 880-891.

8. Lemus, R., Brummer, E. C., Moore, K. J., Molstad, N. E., Burras, C. L., & Barker, M. F. (2002). Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. *Biomass and Bioenergy*, *23*(6), 433-442.

9. Adler, P. R., Sanderson, M. A., Boateng, A. A., Weimer, P. J., & Jung, H. J. G. (2006). Biomass yield and biofuel quality of switchgrass harvested in fall or spring. *Agronomy Journal*, *98*(6), 1518-1525.

10. Grabber, J. H., Jung, G. A., Abrams, S. M., & Howard, D. B. (1992). Digestion kinetics of parenchyma and sclerenchyma cell walls isolated from orchardgrass and switchgrass. *Crop science*, *32*(3), 806-810.

11. Laser, M., Schulman, D., Allen, S. G., Lichwa, J., Antal, M. J., & Lynd, L. R. (2002). A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. *Bioresource technology*, *81*(1), 33-44.

12. Tucker, M. P., Farmer, J. D., Keller, F. A., Schell, D. J., & Nguyen, Q. A. (1998).

Comparison of yellow poplar pretreatment between NREL digester and sunds hydrolyzer. In *Biotechnology for Fuels and Chemicals* (pp. 25-35). Humana Press.

13. Ma, H., Liu, W. W., Chen, X., Wu, Y. J., & Yu, Z. L. (2009). Enhanced enzymatic saccharification of rice straw by microwave pretreatment.*Bioresource Technology*, *100*(3), 1279-1284.

14. Jurgens, G., Survase, S., Berezina, O., Sklavounos, E., Linnekoski, J., Kurkijärvi, A., & Granström, T. (2012). Butanol production from lignocellulosics. *Biotechnology letters*, *34*(8), 1415-1434.

15. Waldron, K. W. (Ed.). (2010). *Bioalcohol production: biochemical conversion of lignocellulosic biomass*. Elsevier.

16. Laxman, R. S., & Lachke, A. H. (2009). Bioethanol from lignocellulosic biomass. In *Handbook of plant-based biofuels*. Taylor & Francis Group Boca Raton.

17. Guo, Ting, Ai-yong He, Teng-fei Du, Da-wei Zhu, Da-feng Liang, Min Jiang, Ping Wei, and Ping-kai Ouyang. Butanol production from hemicellulosic hydrolysate of corn fiber by a Clostridium beijerinckii mutant with high inhibitor-tolerance. Bioresource technology 135 (2013): 379-385.

18. Zhang, Z., & Zhao, Z. K. (2010). Microwave-assisted conversion of lignocellulosic biomass into furans in ionic liquid. *Bioresource technology*,*101*(3), 1111-1114.

19. Qureshi, N. (2010). Agricultural residues and energy crops as potentially economical and novel substrates for microbial production of butanol (a biofuel).*Plant Sciences Reviews*, 249-256.

20. Zaldivar, J., Nielsen, J., & Olsson, L. (2001). Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration.*Applied microbiology and biotechnology*, *56*(1-2), 17-34.

21. Mes-Hartree, M., & Saddler, J. N. (1982). Butanol production of Clostridium acetobutylicum grown on sugars found in hemicellulose hydrolysates.*Biotechnology Letters*, *4*(4), 247-252.

22. Ni, Y., & Sun, Z. (2009). Recent progress on industrial fermentative production of acetone–butanol–ethanol by Clostridium acetobutylicum in China. *Applied microbiology and biotechnology*, *83*(3), 415-423.

23. Zhang, W. L., Liu, Z. Y., Liu, Z., & Li, F. L. (2012). Butanol production from corncob residue using Clostridium beijerinckii NCIMB 8052. *Letters in applied microbiology*, *55*(3), 240-246.