

Bioethanol From Bagasse Pith A lignocellulosic Waste Biomass From Paper / Sugar Industry

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ABSTRACT

Bio fuels produced from various lignocellulosic biomass such as agro forest residues have been recognized to have potential, to be available substitute for or compliment to gasolines. These Biofuels derived from plant materials have the potential to address the two main issues carbon neutral and renewable. In addition they are an integral part of the emerging 'bio-economy' where plant material is used to produce specific chemicals and bulk industrial products.

Central Pulp & Paper Research Institute in association with Indian Institute of Petroleum has been working & exploring the possibility of utilization of pith, available as a soiled waste biomass in paper and sugar industry, as a source of Bio-Ethanol. Efforts were made to achieve maximum prehydrolysis and saccharification efficiency to obtain higher yields of fermentable sugars and ethanol.

The present paper describes the preliminary findings of the study for exploring the possibility of conversion of bagasse pith into Bio-Ethanol. Thereby, helping in meeting out the ever increasing demand of much needed ethanol needed for blending the gasoline. This should also help in addressing the problem of global warming and concern from GHG emissions there by improving the energy & environment status of the country.

Introduction

Ethanol has attracted worldwide attention because of its potential use as an alternative automotive fuel. It has immense importance for countries such as India which depends heavily on import of crude oil, spending a huge sum of its annual budget on import of crude oil. There are numerous advantages of blending ethanol with gasoline that emits lower quantities of carbon mono oxide (CO), nitrogen oxides (No_x), and hydrocarbon after combustion compared to that of gasoline alone because ethanol acts as oxidizing agent(1-3).

Long-term economic and environmental concerns have resulted in a great amount of research in the past couple of decades on renewable sources of liquid fuels to replace fossil fuels. The traditional feed stocks like molasses, sugarcane juice, corn etc. are used for ethanol production but have social and economical barriers. Due to their primary utility as food and feed,

these crops cannot meet the global demand for ethanol production as an alternative transportation fuel.

Apart from these feed stocks, lignocellulosic biomass, which are the most abundant on earth, may prove to be a potential alternative feed stock for bio ethanol production. These biomass including forest residues such as wood; agricultural residues sugarcane bagasse, corn cob, corn stover, wheat and rice straw; industrial residues such as pulp and paper processing waste and municipal solid wastes; energy crops such as switch grass are the most potential feed stocks for fuel ethanol (3-7). The main bottleneck so far has been the technology concerns, which do not support cost-effective and competitive production of lignocellulosic bio ethanol.

Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse emissions (8). Unlike fossil fuels, which come from plants that grew millions of years ago, biofuels are produced from plants grown today. They are cleaner-burning than fossil fuels, and the short cycle of growing plants and burning fuel made

from them does not add CO₂ to the atmosphere. It has been reported that cellulosic ethanol and ethanol produced from other biomass resources have the potential to cut greenhouse gas emissions by 86% (9). Lignocellulosic materials such as agricultural residues (e.g., wheat straw, sugarcane bagasse, corn stover), forest products (hardwood and softwood), and dedicated crops (switchgrass, sllix) are renewable source of energy.

Despite possessing several advantages over conventional ethanol, lignocellulosic bioethanol has not yet been produced on a commercial scale because of the low ethanol yields and high production costs. Economic analyses (US scenario) indicate that the process of sugar release from lignocellulosic biomass, which includes pre-treatment, enzyme production, and enzymatic hydrolysis, contributes to as much as 40-45% of the projected total cost(10-13).

India is the world's largest producer of sugar cane producing nearly 357 million tonnes per annum and nearly 60% of the cane is utilized for the production of sugar of which about 1/3rd of the total sugar cane is obtained as residue so called bagasse containing

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appreciable quantity (up to 35%w/w) of the pith. Presently the pith which is considered to be the undesirable entity from view point of papermaking is separated and pith used as a source of fuel of biomass. Nearly 160t/day of pith is generated in a 300 tpd of Pulp & Paper mill. Due to its inherent chemical composition, pith contains up to 70% of the carbohydrates contents and may become a potential source for Bio-ethanol and other value added products.

Pretreatment of lignocellulosic biomass (Pith) - Technology bottlenecks and possible remedies

With diminishing oil supplies and growing political instability in oil-producing nations, the world is facing a major energy threat which needs to be solved by virtue of alternative energy source. Bioethanol has received considerable attention in the transportation sector because of its utility as an octane booster, fuel additive, and even as neat fuel. Brazil and the USA have been producing ethanol on a large scale from sugarcane and corn, respectively. However, due to their primary utility as food and feed, these crops cannot meet the global demand for ethanol production as an alternative transportation fuel. Lignocellulosic biomass is projected as a virtually eternal raw material for fuel ethanol production. The main bottleneck so far has been the technology concerns, which do not support cost-effective and competitive production of lignocellulosic bio ethanol.

Pretreatment of biomass is requisite to the biological conversion of lignocellulosic biomass to ethanol is the release of the cellulose portion (and subsequently glucose) from the tightly woven lignocellulosic structure. For this, the biomass needs to be pre-treated, either physically, chemically, and/or biologically, to make pre-treated biomass more amenable to subsequent cellulose hydrolysis. Pretreatment is required to alter the size and structure of the biomass, as well as its chemical composition, so that the hydrolysis process can be significantly improved by removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity through pretreatment processes.

About 18-20% of the total projected cost for biological production of cellulosic ethanol can be attributed to pre-treatment, more than for any other

single step. Reducing ethanol cost requires optimizing pre-treatment strategies and condition to the most economical possible. However, not every kind of lignocellulosic biomass can be pre-treated in the same way.

The recovery of fuels from the fermentation broth is achieved by distillation or a combination of distillation and adsorption. The other components, including residual lignin, unreacted cellulose and hemicellulose, and enzymes, accumulate at the bottom of the distillation column.

The economical production of any microbial metabolite is governed by three main factors: (1) the initial cost of raw materials, transportation, and processing; (2) the availability of suitable production and recovery procedures; and (3) the final product yield. The lignocellulosic bioethanol process can be made cost-competitive by: (1) minimizing handling and transportation costs, and incorporating the most efficient and cost-effective pre-treatment and hydrolysis strategy; (2) developing efficient bioprocesses by optimally integrating the preceding and subsequent process steps; and (3) applying overproducing and recombinant strains for enhanced ethanol productivity.

The Central Pulp & Paper Research Institute, as a part of its activity to promote clean and green process technologies in Indian Paper Industry, has been engaged in exploring the potential of utilizing bagasse pith a waste Lignocellulosic biomass rich in carbohydrate as a source of bioethanol adopting a bio refinery approach.

The present paper describes the studies on optimization of the process conditions for pretreatment of biomass (prehydrolysis) and saccharification of the prehydrolysed pith through

chemical and enzymatic route to produce maximum quantity of fermentable sugars to convert into the bio ethanol helping in making the process, techno economically viable.

2. Material & Methods

Materials

Raw material :

Fresh Bagasse was collected from sugar mill, Deoband and depithed using CPPRI patented depithing method. Pith was collected, analysed for chemical composition and used for the present study.

Microorganism :

Ethanol producing Thermophilic yeast

Enzymes :

Enzymes used in the present study were procured from national commercial enzyme supplier (Enzyme 1) and R & D Institute (Enzyme 2).

Methods :

Chemical composition (Lignin, holocellulose, pentosan, ash) of the pith samples after pre hydrolysis and saccharification were analysed by standard TAPPI methods. Total reducing sugars and total sugars was analysed by Miller's (1959) method (16) and Dubois (1956) method (17). Sugar analysis of hydrolysates was done by high performance liquid chromatography (HPLC). Estimation of ethanol was done by both chromic acid method Caputi et.al, 1968 (18) and HPLC.

3. Results & Discussion

3.1 Structure & Composition of Various Lignocellulose Biomass (Agro Residues and Waste)

Table 1. Chemical composition of Common Agricultural Residues and Wastes

Lignocellulosic material	Cellulose, (%)	Hemicellulose, (%)	Lignin, (%)
Hardwood stems	40-45	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	50	15
News paper	40-55	25-40	18-30
Waste paper from chemical pulps	60-70	10-20	5-15
Bagasse	36.4	18.2	22.8
Bagasse Pith	46.0	22.0	21.8
Rice straw	26.0	13.0	4.1

Lignocellulose is the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicellulose, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash (14). The composition of these constituents can vary from one plant species to another.

Bagasse pith was collected and subjected to physico chemical characterization like lignin, holocellulose etc. Table 1 shows the chemical composition of bagasse pith and other various lignocellulosic biomass indicating the potential for use as a source of bio ethanol.

3.2 Conversion of biomass (Bagasse Pith) to bio fuel (Ethanol)

Action of microorganisms and enzymes on biological sources can lead to the production of mostly ethanol and, less commonly, propanol and butanol. These agents carry out the fermentation of sugar, starch, hemicellulose, or cellulose, with cellulose fermentation being the most difficult.

A schematic for the conversion of biomass to fuel is shown in figure 1. The conversion includes the hydrolysis of various components in the lignocellulosic materials to fermentable reducing sugar and the fermentation of the sugars to fuels such as ethanol and butanol. The pretreatment step is mainly required for efficient hydrolysis of cellulose to its constituent sugar. The hydrolysis is usually catalyzed by acids or cellulose enzymes, and the fermentation is carried out by yeasts or bacteria. The factors affecting the hydrolysis of cellulose include porosity (accessible surface area) of the biomass materials, cellulose fiber crystallinity, and content of both lignin and hemicellulose (15). The presence of lignin and

hemicellulose makes the accessibility of cellulase enzymes and acids to cellulose more difficult, thus reducing the efficiency of the hydrolysis process.

Pre-hydrolysis of bagasse pith

Sugarcane bagasse was depithed by CPPRI method. Pith was collected and analysed for its chemical composition and it was subjected to prehydrolysis.

Pre-hydrolysis of bagasse pith

Bagasse pith was subjected to prehydrolysis with an aim to maximum degradation of hemicellulose (xylan) to sugars (xylose) with less or no saccharification of cellulose. Optimisation of process parameters like acid concentration, temperature and retention time were studied for the maximum production of reducing sugars in terms of xylose by hydrolysing the pith in series digester. After treatment, pith was filtered, hydrolysates were collected and the solid residue was subjected to yield analysis. The pre hydrolysates were analysed for estimation of total

reducing sugars (TRS), total solids, Phenolics and lignin content.

Bagasse pith was hydrolysed by dilute acid concentration of 2,4,6 and 8% at 120C for 90 min. Effect of temperature on reducing sugar production from pith was determined by hydrolysing pith 2,4,6 and 8% acid conc. With varying temperatures at 80C,100C,120C and ,140C. Treatment time was optimized so that maximum sugar production was achieved with out loosing much lignin. Pith was hydrolysed with 8% acid concentration for different time periods 60 min, 75 min, 90 min and 120 min. Based on the results, process parameters for prehydrolysis of pith for maximum sugar production (TRS) with out loosing much lignin were optimized i.e acid concentration, 8%, temperature 120°C and retention time 90 min. data is shown in table 2.

Saccharification of Bagasse pith Chemical & enzymatic routes

Pre-hydrolysed solid residue pith subjected to saccharification with an aim to maximum degradation of

Table 2 Prehydrolysis of bagasse pith

Conditions :	
Acid conc. – 8%	
Treatment time – 90 min.	
Temperature , C – 120	
Material to Liquor ratio : 1:8	
Parameters	Percent
Residual Biomass	
Biomass	68.29
Klason Lignin	51.96
Ash	4.96
Unhydrolysed biomass	11.8
Hydrolysate	
Total reducing sugars	28.6
Total sugars	38.3
Soluble lignin	3.33

cellulose to sugars (glucose). Saccharification of prehydrolysed pith was done through both chemical and enzymatic routes.

Chemical Saccharification

Chemical saccharification of prehydrolysed Bagasse pith was done using the optimised acid concentration and process conditions (table -3).

Enzymatic sachharification :

Enzymatic hydrolysis of lignocellulosics is achieved by employing hydrolytic enzymes like

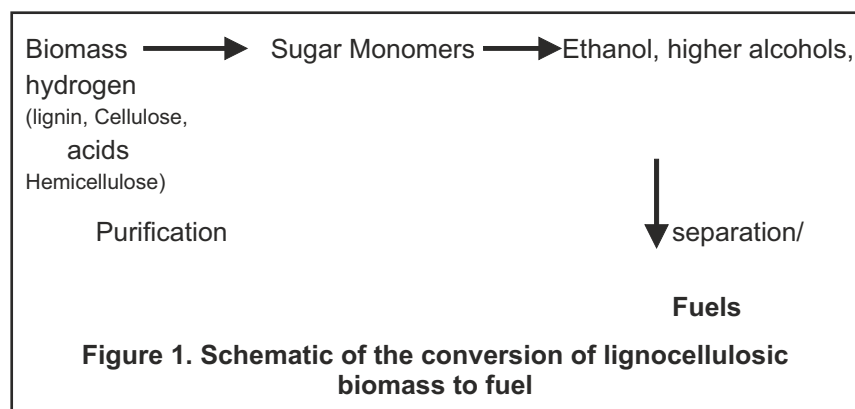


Table 3 Chemical sachharification of prehydrolysed bagasse pith

Conditions : Acid conc. – 72% for 2hrs (Maceration at room temperature) 15% for 4hrs (Digestion at 120 C)	
Parameters	Percent
Residual Biomass	
Biomass	27.13
Klason Lignin	22.29
Ash	4.2
Unhydrolysed biomass	0.64
Hydrolysate	
pH	Below 1.0
Total reducing sugars	42.1
Total sugars	55.2
Soluble lignin	4.88

Table 4 Enzymatic sachharification of prehydrolysed bagasse pith

Conditions : Enzymes dose : Enzyme 1 : 4% , Enzyme 2 : 2% Material to Liquor ratio : 1: 10 Treatment time : 72hr. Temperature , C : 50 pH – 5-6	
Parameters	Percent
Residual Biomass	
Biomass	46.7
Klason Lignin	35.5
Ash	5.19
Unhydrolysed biomass	5.64
Hydrolysate	
pH	6 -7
Total reducing sugars	42.7
Total sugars	53.2
Soluble lignin	4.0

cellulases, laccases and xylanase. Enzymatic hydrolysis is usually done after a pretreatment step. This step is required to open up the bundles of cellulose and hemicellulose fibers and hence to facilitate and increase the efficiency of enzymatic hydrolysis. Enzymatic saccharification was done using two enzymes , enzyme -1 & enzyme 2 following the process conditions given in table - 4. The samples were filtered and filtrates were subjected to analysis. Analysis of prehydrolysates showed that both acid and enzymatic hydrolysates yielded same amount of reducing sugars. Data is shown in tables 3 and 4. As the pH of the acid hydrolysate is acidic it is to be neutralised and detoxified before subjecting it to fermentation which may affect the efficiency and economics of the process. Enzymatic hydrolysate having ph of 6-7 which is suitable for fermentation looks more attractive for

further fermentation process.

Fermentation of bagasse pith hydrolysates :

Acid pretreated and enzymatic

sachharified pith hydrolysed samples was fermented in 500 ml bottles using thermophilic ethanologen , yeast strain at 50C.

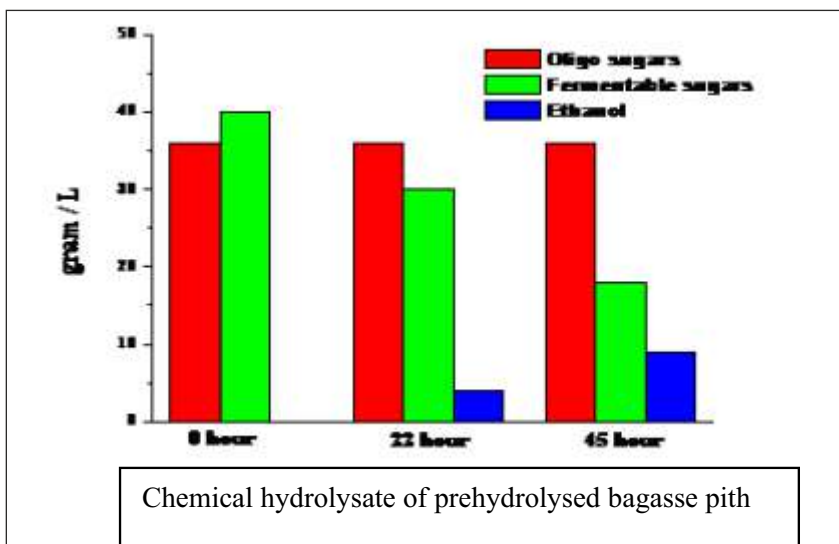
Acid pretreated and enzymatic sachharified pith hydrolysate samples with initial total sugar conc. 36.64 g/l was fermented in 500 ml bottles using thermophilic yeast strain. (Fig -2). Almost 80 % glucose (30g/l) of enzyme hydrolysate was fermented to ethanol (9.5gm/l) with in 40 hr and there after some of the oligosaccharides also converted to ethanol brining the final ethanol concentration 11.5 gm/l in the broth.

Possible utilisation of lignin rich residual biomass

Based on the nature of the residual biomass shown in table 5 which is rich in lignin it was found that it has reasonably high calorific value both obtained from the chemical and enzymatic sachharification. This will provide an opportunity to use it as a source of fuel for sustaining the energy requirement of the process. Further the purity of lignin after chemical sachharification was found to be more than 83% which may find application in rubber and plywood industry.

Conclusion

1. The studies indicated that the bagasse pith available as a waste biomass may proved to be a potential substrate alternate to the conventionally used raw materials viz. molasses ,feedstock like corn, maize starch etc. for production of bioethanol.
2. It is important to prehydrolysed biomass before sachharification to convert he cellulose ion to fermentable sugars under given



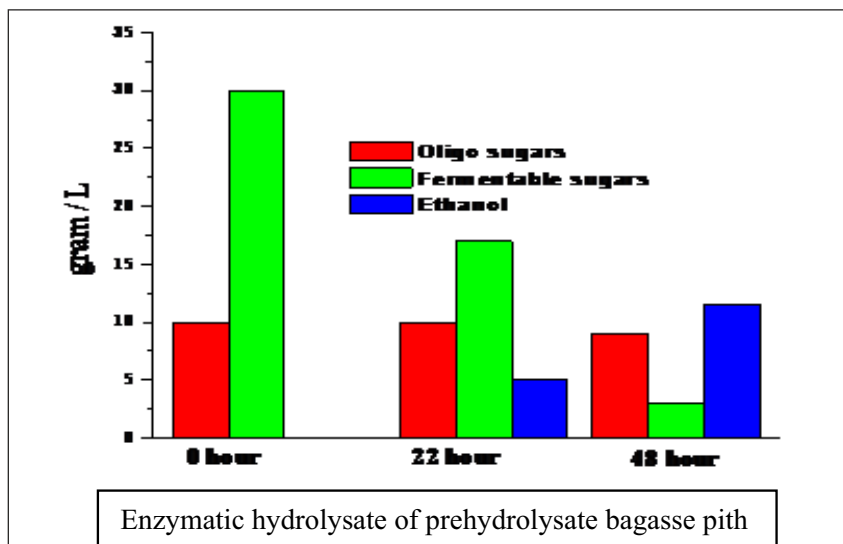


Table 5 Chemical analysis of lignin rich residual biomass after sachharification

Parameters	Residual biomass after sachharification	
	Chemical	Enzymatic
Residual Biomass, %	27.1	46.7
Lignin purity %, w/w	83.28	50.23
Ash, % w/w	4.2	5.2
Silica, % w/w	13.5	6.9
Gross Calorific value, cal/gm	4650	4260
Chlorides, % w/w as Cl ⁻	0.41	0.49
Carbon, % w/w	54.7	47.9
Hydrogen, % w/w	3.34	3.89
Nitrogen, % w/w	0.13	0.54
Sulphur, % w/w	0	0.04

3. Enzymatic sachharification route results it to higher yield of fermentable sugars thereby providing opportunity for higher bioethanol yield.
4. High ethanol conversion was found with enzyme sachharified pith hydrolysate when compared with the acid hydrolysate.
5. Lignin obtained from the above process could be of high purity and better quality finding various industrial applications like fine chemicals including vanillin, paint and varnishes, rubber industry, in batteries and for lignosuphonates for oil industry.

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