9th International Conference on Recent Development in Engineering Science, Humanities and Management
Mahratta Chamber of Commerce, Industries and Agriculture , Pune (India) ESHM-17
23th December 2017, www.conferenceworld.in ISBN: 978-93-86171-88-7

AGRICULTURAL CROP RESIDUE: A POTENT SOURCE FOR FUTURE FUEL

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ABSTRACT

Agriculture brings in large amount of residues which can be used as a feedstock for their conversion into biofuels Biofuel production paves a roadmap towards a sustainable and carbon-neutral bio economy. Due to the rapid growth in population and industrialization, worldwide bioethanol demand is increasing continuously. First generation Bio-fuels are a subject to food Vs fuel debate. Thus, they are being outsmarted by second generation bio-fuels.The agricultural residues being cost effective and abundant, promises to be an attractive feedstock for bioethanol production. In the present study the production of bioethanol was carried out from 8 lignocellulosic crop residues. The lignocellulosic biomass was subjected to compositional analysis for their cellulose, hemicelluloses, lignin and ash contents. It was subjected to pretreatments by acid /alkali, followed by hydrolysis with a cocktail of enzymes and subsequently fermented by an indigenous strain of Saccharomyces cerevisiae to produce bioethanol. The average percentage composition of cellulose, hemicelluloses, and lignin in the samples of 8 types of residues was found to be in the range of 20-36%, 12-22%, and 9-19%, respectively. The total reducing sugar was significantly increased to 50-60% of the substrate. The highest yield of ethanol was obtained from Sorghum straws and Rice straw which is 33% and 30.5% based on sugar to alcohol conversion. It was found to be 64 and 59 % based on theoretical yields.

Keywords: Bioethanol, lignocellulosic biomass, Fermentation, Pretreatment, Saccharomyces cerevisiae

I.INTRODUCTION

Current instability of oil supplies, continuous fluctuation in the prices of crude petroleum, and worldwide increasing demand of energy inputs have paradigmatically aroused the widespread interest in White Biotechnology, a branch which works on the production of fuels from renewable sources [8, 9, 15]. Bio-fuels derived from biomass have the potential of providing clean, carbon neutral and sustainable energy [7, 18]. Fermentation of sugar-based edible counterparts of plants is referred to as “first generation” bioethanol, whereas the use of lignocellulosic biomass or crop residues is commonly called “second generation” bioethanol. The “third generation” [19] of algal bioethanol is at an early stage of investigation and the “fourth generation” refers

ISBN: 978-93-86171-88-7

to engineering the metabolic machinery of plants to produce higher amount of biofuels.

The lignocellulosic material consists of three types of polymers, namely cellulose, hemicelluloses and lignin which are closely associated with each other.

Cellulose: It is the most abundant component present in the cell wall. It is a polysaccharide consisting of glucose subunits, linearly arranged and linked by B-1, 4 glycosidic bonds. They can be broken down into glucose monomers by treating with concentrated mineral acids and alkalis at high temperatures. It has a highly ordered crystalline structure with various forms due to variations in the presence of hydrogen bonds.

Hemicellulose: It is a branched polymer which consists of varied polymers like pentose, and hexose sugars. Xylan and Glucomannan comprise a major part in hardwood and softwood respectively. It strengthens the whole cellulose-hemicellulose-lignin complex by adding to its rigidity. The solubilization of lignocellulosic biomass depends not only on temperature but also on moisture content and pH. Xylans can be easily extracted by acid and alkali pretreatments.

Lignin: It is an amorphous heteropolymer consisting of 3 phenyl propane units namely p-coumaryl, coniferyl and sinapyl alcohol linked by various bonds. The main purpose of lignin is to give plant structural support, impermeability and resistance against microbial attack. It is the basic binding material between cellulose and hemicellulose and keeps the structure of plant components, intact.

In November 2017, at a national conference on “Ethanol as Transport Fuel”, the Petroleum Ministry at Central Road Transport Institute (CIRT), Pune highlighted the advantages producing bioethanol from Bagasse. According to the data provided by the ministry, the ethanol supply for blending with petrol has increased from 154 million liters to 1110 million liters between 2011-2016 [14] .

The present study includes the conversion of agricultural and domestic refuse into second generation Bioethanol. It includes the collection, analysis, pretreatment, hydrolysis and fermentation to recover the produced alcohol in a economically feasible way. The studies indicate that further research in bioethanol is a step towards uplifting Indian economy by decreasing the need to import fuel and providing employment in the fuel industry of India.

II.MATERIALS & METHODS

2.1 Raw Materials and Microbial Strains

A Strain of *Saccharomyces cerevisiae* (DYPBBI- 11) was procured from the institutional culture collection. Commercial grade enzymes were obtained from M/s Maps (India) Ltd., Ahmedabad, viz.: Palkodex (Glucoamylase), Palkosoft super (Cellulase), Palkozyme ultra (Bacterial alpha amylase), Palkobake (Xylanase) and Palkoscour (Pectinase). The fruits and vegetable peels were collected from local food processing units and vendors (Table 2.1).

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SAMPLE	PLACE OF COLLECTION
Rice Husk	Rice fields in Pune
Rice Straw	Fruit seller in Pune
Groundnut Straw	Groundnut fields in Pune
Groundnut Shell	From municipalty as domestic refuge
Tealeaf Residues	From municipalty as domestic refuge
Sorghum Straw	From Sorghum fields in Pune
Sorghum Bran	From Sorghum fields in Pune
Pigeon Pea Straw	From grocery store in Pune

Table 2.1 Samples and their locations for biomass collection

2.2 Processing of sample

The collected samples were dried in the oven for 5 hours at 80 C. After complete loss of moisture, they were grinded and filtered via sieves to get a fine powder of mesh size < 20 µ sized particles. Moisture content was calculated by the following formula [3]

[(Initial weight- Oven dried weight)/ Oven dried weight]* 100

Compositional analysis was done to obtain cellulose, hemicellulose, and lignin content by methods given by National renewable energy laboratory *[2,11]*. Ash content was determined by placing 1gm sample in muffle furnace at 700C for 4 hours [16].

Ash% = (weight of ash/ weight of sample) * 100

2.3 Pre-treatment of the samples

10 g of samples were soaked in 100 ml distilled water, acid (1N HCl) and alkali (1 N NaOH); heated at 90^{o} C in water bath for 3hrs and allowed to be cooled to room temperature. Microscopic analysis was done to check the effects of pretreatment on the structural integrity of the sample. DNSA was performed to check the free sugar content of the sample [1, 6, 17].

2.4 Saccharification

All the above samples were adjusted to pH 4.5 as it was optimum for activity of enzymes as conferred from previous studies [5] with the help of 1N HCl and 1N NaOH. 0.10 ml of crude commercial enzymes containing glucoamylase (1000 I.U/gm), cellulase (10 FPU/gm), and pectinase (75 I.U/gm) were added as a cocktail; followed by incubation in water bath at 55˚C for 24 hrs.

2.5 Clarification of saccharified samples for analysis

After making the final volume of the above mentioned hydrolysates to 100 ml, a 10 ml portion was centrifuged at 10000 rpm at 5˚C for 10 min and the supernatant was collected for further analysis.

2.6 Determination of hexoses in the hydrolysate

Samples were diluted 100 times appropriately with distilled water and analyzed for total sugar contents by DNSA method [16].

2.7 Alcoholic Fermentation of Hydrolysates

0.5% Corn steep liquor was added to the hydrolysates and autoclaved at 121° C, 15 psi for 5 min. Inoculum of the yeast strain was prepared in 50.0 ml of YPD (Yeast Peptone Dextrose) broth at 5.5pH and incubated at 25˚C for 24 hrs. The growth of yeast was observed microscopically as well as by taking optical density at 550 nm. A 5% inoculum was added to all the production flasks containing 100 ml of above mentioned production media, incubated at 25˚C for 72 hrs to allow the complete conversion of sugar into alcohol by batch fermentation.

2.8 Alcohol determination

The fermented samples were harvested and after making up the volume, a 10 ml portion was centrifuged at 10,000 rpm at 5˚C for 10 min to remove the yeast cells and other solids present in the sample. 1ml portion was used for the determination of residual sugars to calculate their conversion rates. The remaining sample was distilled and a fraction was injected in a gas chromatogram to determine the alcohol contents. (SHIMADZU GC2014 Column Rtx 200) . Potassium dichromate test was another method used for obtaining alcohol yield.

Practical yield of alcohol is= (Actual yield *100)/ Theoretical yield

III.RESULTS AND DISCUSSIONS

3.1 Moisture Contents: Moisture contents of the sample were found to be in the range of 7-8%. Highest moisture was observed in case of Sorghum straw (9.5%) and lowest in case of Tea leaf residues (7.8%). The ash contents of the samples were found to be in the range of 1.77-5.33%. Highest ash content (5.33%) was present in Rice husk and lowest (1.77%) was observed in Tea leaf residues (Table 3.1).

ISBN: 978-93-86171-88-7

SAMPLES	% MOISTURE CONTENT	% ASH CONTENT
Rice Husk	9.0	5.33
Rice Straw	8.5	4.5
Groundnut Straw	8.0	4.5
Groundnut Shell	8.5	3.5
Tealeaf Residues	7.8	1.77
Sorghum Straw	9.5	3.5
Sorghum Bran	8.0	4.0
Pigeon Pea Straw	8.7	3.2

Table 3.1Showing the Moisture and Ash contents in the biomass samples

3.2 Chemical Characterization*:* Cellulose, Hemicellulose and Lignin form the basic structural components of any lignocellulosic biomass. Since Cellulose and Hemicellulose refer to the carbohydrate content of samples, their higher percentage would mean higher amount of sugar and alcohol yields. Lignin being a phenolic compound does not contribute in bioethanol production but has great potential to be converted into antibiotics, terpenes etc [12]. Highest Cellulose, Hemicellulose and Lignin were found in Rice straw (36.55%), Rice Straw (21%) and Rice husk (19%) respectively (Table 3.2).

Samples	% Lignin	% Cellulose	% Hemicellulose
Rice Husk	19	31	19
Rice Straw	17	36.55	21
Groundnut Straw	15	27	17
Groundnut Shell	16	30	18
Tealeaf Residues	9	18	11
Sorghum Straws	12	23	18
Sorghum Bran	12.5	35	21
Pigeon Pea Straw	17	26	19.19

Table 3.2 Compositional analysis of lignocellulosic biomass samples.

ISBN: 978-93-86171-88-7

3.3 Pretreatments: The structural components of lignocellulosic biomass are compactly arranged. For efficient action of hydrolyzing enzymes, these counterparts have to be effectively loosened to allow the enzymes to get the maximum active surface area. Pretreatments plays a significant role by separating Cellulose, Hemicellulose and Lignin subunits to allow the enzymes to act upon them and maximize their conversion into their nonnumeric forms. It has been reported earlier by X-Ray crystallography that pretreatments disrupts the crystallinity of solid lignocellulosic biomass [12]. Decrease in the ordered structure means disruption of bonds which allows enzymes to access the components easily. Microscopic analysis showed acid pretreatment worked well for all the samples for alkali pretreated sample, the sample which gave highest yield of sugar was Sorghum bran and the yield was 6.2%. The yield was very less as compared to 18.2%, which was obtained in case of alkali pretreated rice straw samples . (Table 3.3)

Samples	**Sugar Conc (%) Acid PT**	**Sugar Conc (%) Alkali PT**
Rice Straw	18.2	4.4
Rice Bran		6
	15.2	
Groundnut Straw		3.4
	13.8	
Groundnut Shell		2
	12.6	
Tealeaf Residues		2.14
	11.3	
Sorghum Straws		6.2
	10	
Sorghum Bran		5.8
	12.6	
Pigeon Pea Straw	13.8	4.2

Table 3.3 Total sugar contents in acid and alkali pretreated biomass samples

3.4 Enzymatic Hydrolysis of the pretreated samples: After the Lignocellulosic biomass components viz. Cellulose, Hemicellulose and Lignin were loosened during the pretreatments, they were enzymatically hydrolyzed for conversion into hexoses and pentoses. These monomeric subunits were acted upon by the yeast strain and converted into alcohol. In this investigation the enzymes used were Cellulase, Xylanase, Glucoamylase, alpha-amylase and pectinase as an enzyme cocktail, as reported earlier and also observed by us it works better compared to individual enzymes for the hydrolysis of the substrates[20]. Better pretreatment and the mixture of enzymes leads to better enzymatic hydrolysis resulting in to better saccharifation .The maximum hexoses (59%) were obtained from Rice straw and (56.4%) from Sorghum bran in case of acid pretreatment while Sorghum straw (48.2%) and Rice husk (46.4%) were highest in alkali pretreated sample (Table 3.3)

Samples	Sugar Contents of Acid Pretreated (%)	Sugar Contents of Alkali Pretreated (%)
Rice Husk	54.5	46.4
Rice Straw	59.8	41.4
Groundnut Straw	46.4	41.4
Groundnut Shell	52	37.6
Tealeaf Residues	40	29
Sorghum Straws	53	48.2
Sorghum Bran	56.4	38.4
Pigeon Pea Straw	44.2	39.5

Table 3.3 Percent Sugar concentrations after enzymatic hydrolysis of acid and alkali pretreated samples

ISBN: 978-93-86171-88-7

3.5 Alcohol Yields: The fermented samples were centrifuged and analyzed by DNSA method for determining the residual sugars and finding the conversion yields. The distilled samples were used for the determination of the alcohols by the Gas Chromatographic techniques. Same samples were also used for the further determination of alcohol contents by Potassium Dichromate spectrophotometric method to compare the results. Highest amount of alcohol was produced from acid pretreated Sorghum straw, Rice straw and Sorghum bran which was 33%, 30.5% and 30.2% respectively. Minimum alcohol was detected from tea waste which was around 18%. It can again be seen that acid pretreated samples gave better alcohol yield than alkali pretreated samples as has been reported earlier also (Table 3.4).

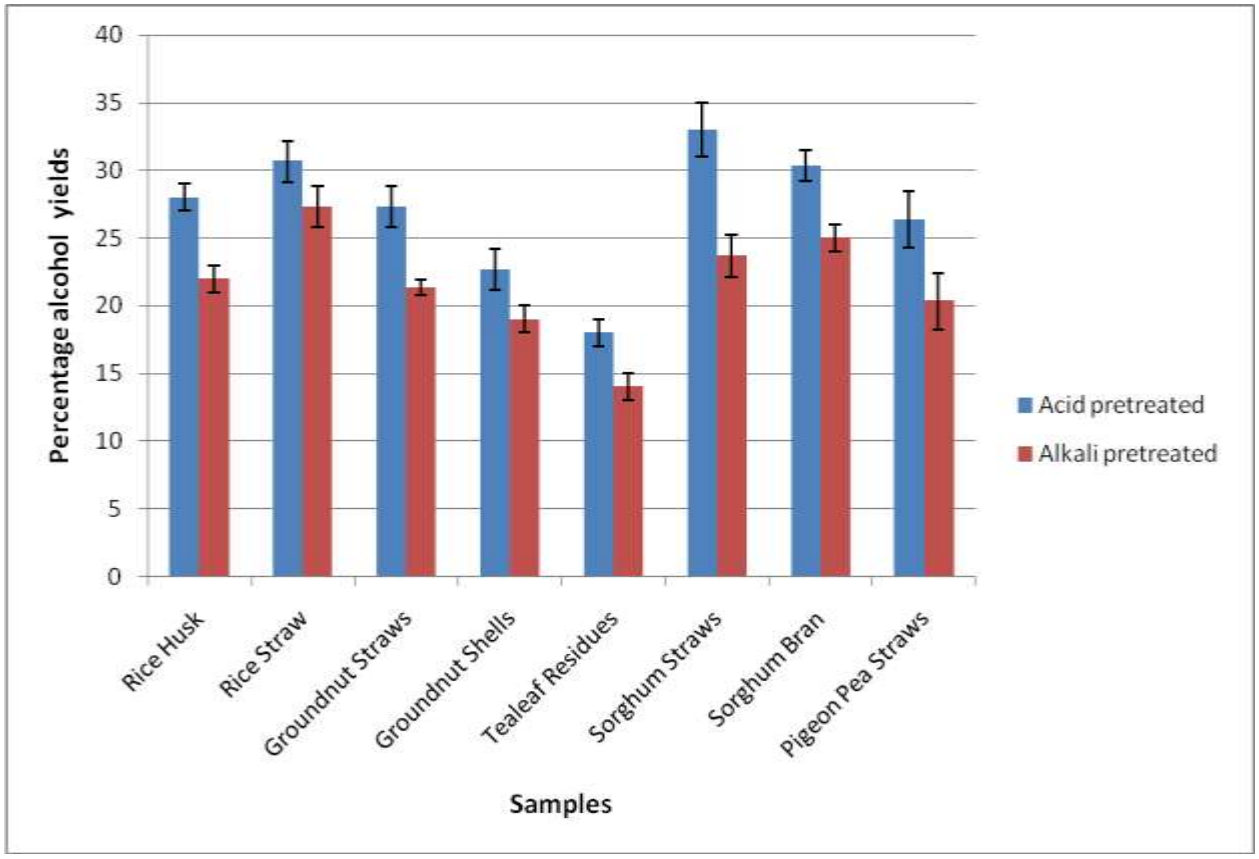

Fig 1. Percent Alcohol yields from the Hydrolysates of biomass samples

IV.CONCLUSION

In the present study, 8 lignocellulosic residual waste samples were collected, pretreated with acid and alkali, hydrolysed with cocktail of enzymes and fermented with a high yielding strain of *Saccharomyces cerevisiae.* The best alcohol yields were obtained with Sorghum straw, pretreated with acid. Tea leaves were found to be

the poorest source among the substrates used. The process can be improved further to give higher alcohol yields and the process can be applied to more domestic crop residues to fulfill the increasing demand of alcohol required for petroleum blending.

VI.ACKNOWLEDGEMENT

1. Authors express their thanks to Dr. D Y Patil Biotechnology and Bioinformatics Institute, Dr. D Y Patil Vidyapeeth for providing research facilities.
2. Sincere thanks to Maps India ltd (Ahmedabad) for providing samples of commercial enzymes.

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