EXTRACTION OF ALCOHOLIC FROM AGRICULTURE BIOMASS

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ABSTRACT

Bio fuels appeared to be more climate-friendly than burning fossil fuels. There are different types of alcoholic fuels that can be produced easily and may used as alternative source of existing fuels. Butanol may be utilized as a fuel in an internal combustion engine and shares more similarities with gasoline. Butanol is a compatible fuel that may be used in gasoline-powered cars without any alterations. Both n-butanol and isobutanol have been researched as potential fuels. Butanol is derived from starch- or sugar-based materials like maize grain, sugar cane, or cellulosic feedstocks. Biofuel can have a significant impact on Pakistan due to the country's reliance on oil. Second-generation cellulosic biofuels provide a solution to decrease carbon emissions from transportation and to generate electricity for residential and commercial use. A study was done to establish a strategy for managing agriculture and other organic wastes for producing alcoholic fuels. Cellulosic materials such as wheat and rice straws, together with fruit wastes, were utilized in this study. Samples were examined for several criteria. Biological and chemical pretreatments were evaluated for each substrate. An assessment was conducted on the efficacy of microbial enzymes in breaking down agricultural substrates into sugars. The study is anticipated to boost biofuel output and decrease reliance on imported fossil fuels.

Key words; Fossil fuels, Bio butanol, Biomass, Greenhouse gases, Climatic changes

1. INTRODUCTION

Exploration of alternative energy sources has been heightened due to rising issues about energy security and climate change. The transportation industry significantly contributes to greenhouse gas emissions through the use of fossil fuels. Replacing oil-derived fuels with alternatives like ethanol or butanol might decrease environmental consequences and provide social and economic benefits.Several options for producing environmentally friendly biofuels are now

under investigation. Biological energy resources include bioelectricity, biogases, biodiesel, and bio alcohols. Bioalcohol has significant potential to lower greenhouse gas emissions, reduce reliance on fossil fuels, and serve as a chemical feedstock and transportation fuel (Dhamole et al., 2015).Alcoholic fuel generation has significantly advanced as many countries aim to decrease oil imports, enhance air quality, and boost rural economies. Global ethanol output is 51 billion liters according to the Renewable Fuels Association in 2007. Ethyl alcohol is advantageous as a fuel due to its increased oxygen concentration. Increased oxygen levels allow for better oxidation of hydrocarbons, resulting in reduced aromatic compounds and carbon monoxide emissions. Ethanol exhibits superior octane rating characteristics (Thomas and Wong, 2001).

Biomass is a crucial energy source in Pakistan due to its agricultural nature. The biomass generated in the livestock and agriculture sectors includes animal waste, sugarcane bagasse, and rice husk (Amiri et al., 2014). Second-generation biomass consists primarily of lignocellulosic material. Lignocellulosic biomass is a widely available organic material on Earth, including cellulose (35-50%), hemicellulose (20-35%), and lignin (5-30%) (Huber et al., 2006).Renewable energy resources consist of various agricultural materials such as green leaves, fruit shells, straws, nut shells, and fruit seeds. The primary feedstocks utilized are wheat straw, wheat bran, maize stover, corn steep liquor, and apple pomace (Ejezi et al., 2006).Currently, agricultural waste is utilized for producing biofuels such as biodiesel, bioethanol, biohydrogen, and methane instead of energy crops due to competition with food crops. Due to the abundance of agricultural waste and the issue of disposal, using lignocellulosic biomass is proposed as an alternative to alleviate the conflict between fuel and food sources (Mahro and Timm, 2007). Grasses are regarded a dependable source for extracting ethanol. Using perennial grasses can be beneficial and may reduce the cost of producing ethanol and using it as fuel (Gomez et al., 2008).

Grasses may be cultivated year-round globally, especially in subtropical and tropical regions. Cogon grass has been utilized to improve soil stability and as fodder. It is considered a severe weed and classified as a pest in around 73 nations affecting over 35 different crops. Cogon grass roots contain secondary metabolites that has medicinal significance. It is a perennial grass that may be grown on soil often deemed unsuitable for crop production.

Cellulose, a primary sugar found in wood, is decomposed by bacteria present in the termite's stomach and transformed into other substances such as fatty acids and ethanol.Clostridium is a genus of rod-shaped, gram-positive bacteria commonly found in soil, water, and the digestive tract.

Clostridium acetobutylicum metabolizes sugar into a blend of organic solvents such as acetone, butanol, and ethanol. *Saccharomyces cerevisiae*, also known as baker's yeast, are single-celled eukaryotes often utilized in the fermentation process to produce ethanol and other alcoholic products. Hence, the present work aimed to conduct chemical and biological investigation of cellulosic biomass to determine the necessary parameters for biobutanol synthesis, a type of alcohol fuel.

2 MATERIAL AND METHODS

2.1. Collection of Agricultural Substrates

Assorted samples of wheat and rice straw, together with peel debris, were gathered from different locations. Approximately 1 kg samples of each sample were collected in high-quality plastic bags. The samples were initially shaded and then dried under the sun before being oven-dried overnight at 55 °C. The materials were pulverized into a fine powder using an electric grinder and then filtered through a 40-mesh standard size sieve. The powdered samples were stored in labeled plastic bags and kept in a refrigerator at 4°C until needed.

2.2. Proximate Analysis of Samples

All samples underwent analysis for ash content, volatile matter, crude protein, crude fiber, crude fat, and both wet and dry weights. The total solids and moisture contents were estimated using established procedures involving drying at 105 ºC to eliminate moisture from the samples (AOAC, 1990).

2.3. Chemical analysis of raw biomass

The cellulose content of the sample was determined using a previously established technique. The hemicellulose content was calculated by comparing the values of Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF).The lignin contents were determined using the standard technique described in AOAC (1990).

2.4. Chemical Pretreatment

Two chemicals, acid (H2SO4), were utilized for chemical treatments. An experiment was conducted with pretreatment utilizing H2SO4 solutions of varying concentrations (1.0%, 1.5%,

and 2%) at varied temperatures (100°C, 110°C, and 120°C) for various periods (15, 30, and 45 minutes). A solid sample of 10% concentration by weight in a reagent bottle was used in the experiment. The vacuum filtration assembly was utilized to filter the sample in each bottle after pretreatment, and the contents were then discharged onto filter paper. After filtering, the solid was rinsed away with 300 cc of distilled water to neutralize the pH. The filter paper was dried at 105 °C and then weighed.

Figure 1. Biomass

2.5. Enzymatic Hydrolysis

The biomass, pretreated at 5% (w/v), underwent hydrolysis using cellulose and β-glucosidases at 50 °C and 160 rpm for 72 hours in a water bath shaker containing a 0.05 M sodium citrate buffer at pH 4.8. Cellulases have an activity of 30FPU g-1. Samples were extracted from the reagent container every 12 hours to measure the sugar content. H2SO4 (µl) was added after enzymatic hydrolysis. The unhydrolyzed material was separated by centrifugation for 10 minutes at 13,500g. The supernatant was obtained using syringe filters for sugar determination by the dinitrosalicylic acid (DNS) technique.The sugar content was determined using the p-hydroxybenzoic acid hydrazide (PAHBAH) technique. A standard curve was created using xylose concentrations ranging from 1mM to 25mM. The sugar content in the pretreatment sample was evaluated by comparing it to the reference sugar concentration. The optimal pretreatment condition was chosen following the enzymatic hydrolysis procedure. The sample with the highest amount of sugar released was chosen for the fermentation procedure. The solid biomass was held at 4 °C and

thereafter utilized for the fermentation process as referenced by Demirbas (2001), Iram et al. (2021), and Maria et al. (2021).

2.6. *Clostridium acetobutylicum* function for butanol

The clostridium specie *Clostridium acetobutylicum*was maintained at at -20^{0}C. Enzymatically hydrolysed sample was then used for fermentation. The pH was maintained at 6.5 with NaOH. 1ml *C. acetobutylicum*spores were added in 100ml enzymatically hydrolysed solution in reaction bottle for separate hydrolysis and fermentation. These reaction bottles were placed in shaking incubator having 120 rpm at 37^{0}C for 72 hours. The butanol concentration was determined by alcohol meter after 72 hours, and values were expressed as % butanol obtained from wheat straw, from rice straw and from corn stover. Among these three substrates wheat straw produced higher yield of butanol (Xue et al., 2012).

2.7. Analysis of sugar and alcohol by using HPLC

Prior to analysis, all glucose samples and standard solutions were filtered using a 0.22 μm filter. Approximately 20 microliters of the sample were injected into the HPLC apparatus using the injection loop. Glucose samples were enzymatically hydrolyzed and analyzed using gradient mode for 10 minutes, as described by Shields and Cathcart (2010), Sluiter et al. (2008), Tao et al. (2014), and Zhao et al. (2012).

3. RESULTS AND DISCUSSION

Results regarding chemical analysis of biomass samples as well as fermentation of sugars into acetone-butanol- ethanol are given in the following sections. Therefore, in current study acetone-butanol-ethanol (ABE) were produced from organic wastes material of agriculture and municipal sources by using bacterial fermentation.

3.1. Biomass analysis

Data in table 1 represents various parameter found in biomass samples. Whereas ligno-cellulosic contents of the samples are given in table 2. It was observed that wheat straw has higher cellulosic contents as compared to other substrates used for analysis.

Table 1. Proximate analysis (%) of biomass samples

Substrate	Dry Matter	Moisture	Crude Protein	Crude Ash	Crude Fiber	Ash
Wheat straw	91.12±0.48	7.83±0.25	8.15 ±0.24	8.17±0.33	34.45±0.43	5.36±0.2
Rice straw	89.15±0.26	7.19±0.27	7.16 ±0.24	9.15±0.33	36.45±0.45	6.25±0.3
Peel(wastes)	92.43±0.47	8.56±0.35	5.95 ±0.23	5.92± 0.43	34.86±0.36	4.76±0.4

Analysis of organic wastes samples

Table 2. Chemical analysis (%)of biomass samples

Substrate	NDF	ADF	Hemicellulose	Cellulose	Lignin
Wheat straw	83.4±0.58	57.85±0.26	28.14 ±0.25	28.16±0.36	24.46±0.46
Rice straw	84.16±0.27	54.13±0.26	26.15 ±0.23	27.16±0.43	26.48±0.46
Peel(wastes)	79.5±0.56	51.1±0.35	25.2±0.35	26.6±0.65	23.4±0.46

Mean ± standard deviation (n=3) NDF = Neutral Detergent Fiber and ADF=Acid Detergent Fiber

3.2. Dilute H_2SO_4 pretreatment

The biomass samples underwent pretreatment with dilute acid at concentrations of 1%, 1.5%, and 2% in an autoclave at temperatures of 105°C, 120°C, and 135°C for durations of 15 minutes, 30 minutes, and 45 minutes. An optimal temperature of 120 °C was used for both samples, with a retention time of 15 minutes for peel wastes and 30 minutes for cogon grass at concentrations of 1.5% and 1%, respectively. These conditions were optimized for the enzymatic experiment (Figures 2-5).

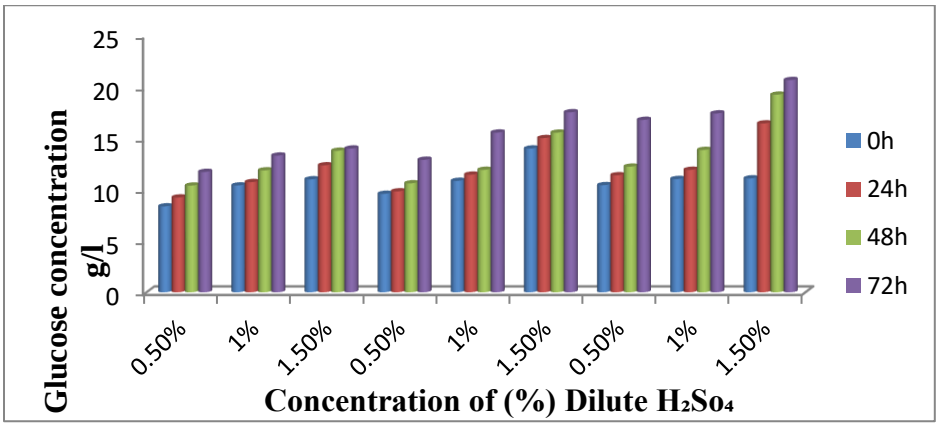

Figure 2. Glucose yield obtained from Wheat straw at H_2SO_4 pretreatment conditions at 120^{0}C

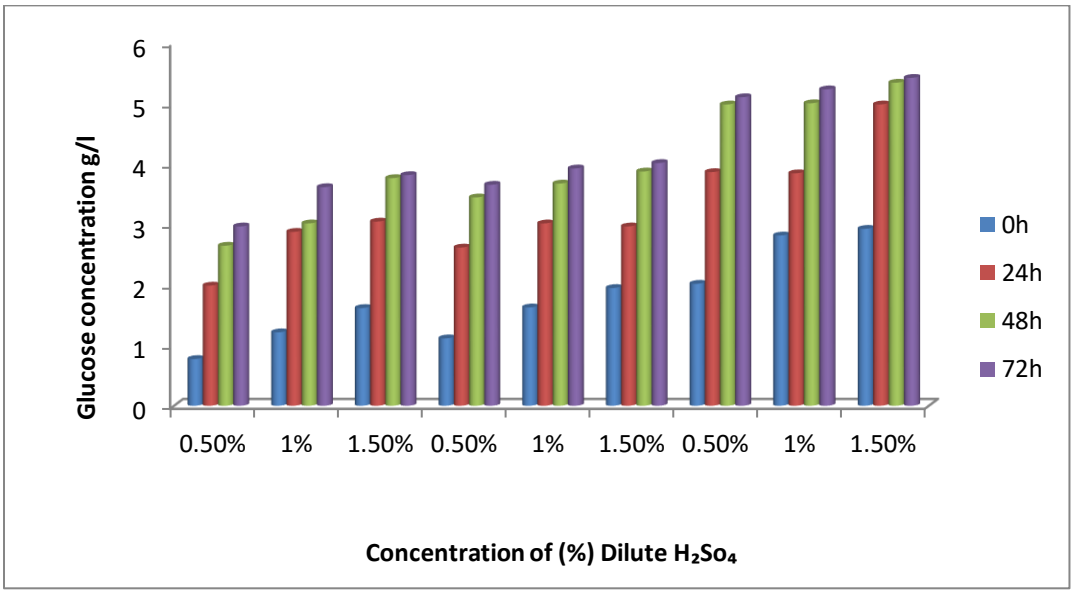

Figure 3. High glucose yield obtained from Rice straw at H_2SO_4 pretreatment conditions at 110 ^{o}C.

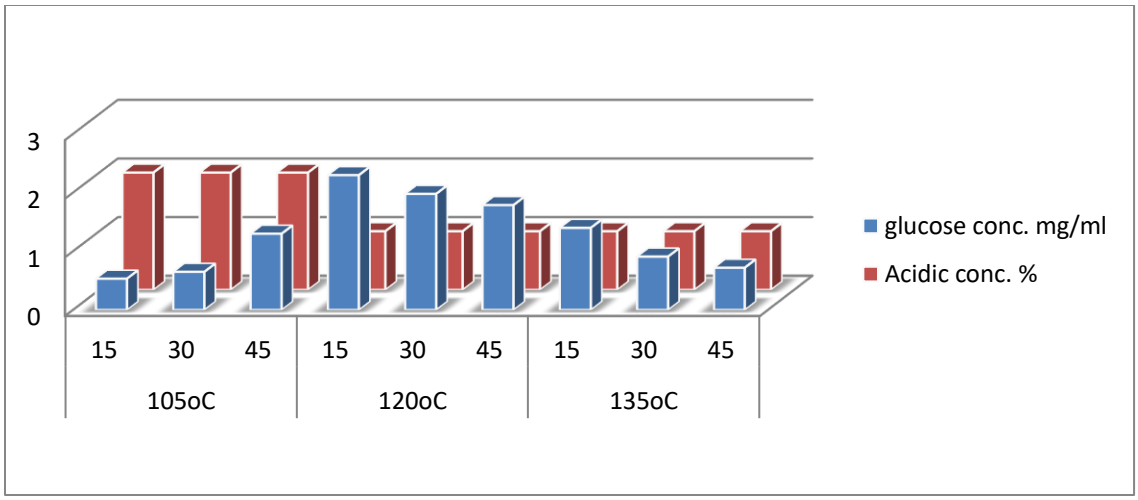

Figure 4. Comparison of glucose concentration at various temperatures after dilute acid pretreatment.

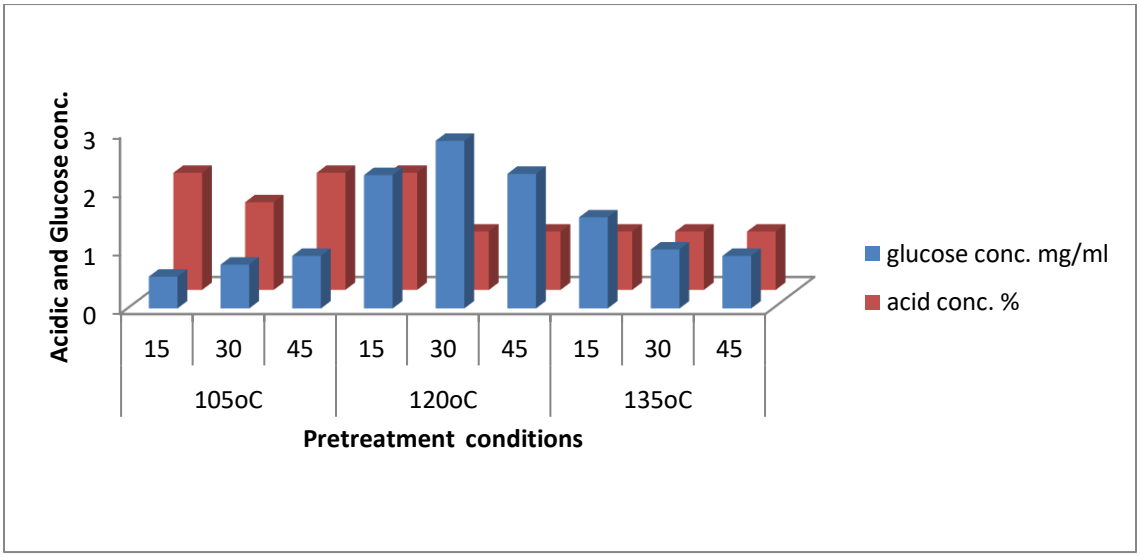

Figure 5. Comparison of glucose concentration at various temperature

3.3. Saccharification of biomass samples with enzymes

More glucose was released from the agricultural waste after 36 hours, eliminating the need to extend the experiment (Fig. 6). The sugar that has been released can be utilized for fermentation studies as documented by (Becerra et al. 2015), (Dheeran et al. 2012), and (Garcia et al. 2011). Glucose was released at a concentration of 11.55 mg/ml during the initial 48 hours after the enzyme

was added. After that, the release of sugar stabilized and remained constant, as indicated by the linear trend exhibited in Figure 6.

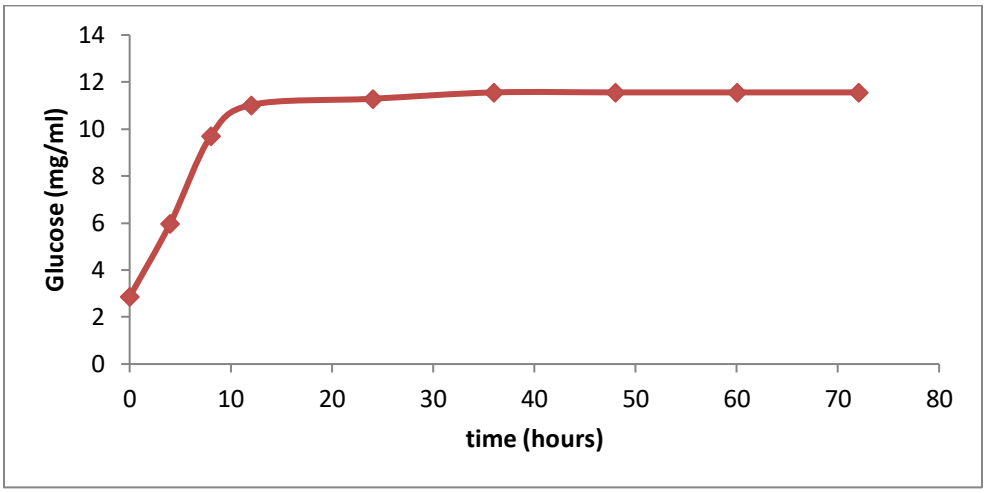

Figure 6. Enzymatic Saacharification of sugar from biomass

3.4. Fermentation

Tables 3 to 5 display the notably increased levels of butanol produced from various substrates. As time elapsed, glucose concentration decreased and ethanol concentration increased, but only up to a specific point. After 72 hours, the glucose concentration was insufficient to sustain ethanol synthesis. Agrowaste with higher cellulosic and lower lignin contents is more suitable for alcohol fuel generation, as shown by Gregg and Saddler (1996), Hanifeng et al. (2015), and Jiang et al. (2015).

3.5. Fermentation with *Clostridium acetobutylicum*

The primary outcome of this fermentation process is referred to as ABE (acetone, butanol, and ethanol) fermentation. The ratio of acetone, butanol, and ethanol in the fermentation process is commonly stated as 3:6:1 by various writers. It was estimated that *Clostridium acetobutylicum* yields higher butanol quantity at acidic pretreatment Among three substrates the yield of butanol was high in wheat straw because of number of factors i.e. the amount of carbohydrate was high in wheat straw as compared to rice straw and peel waste . Low lignin content in wheat straw is responsible for high glucose yield as well as high yield of butanol than other two substrates. Wheat straw contains low lignin contents as compared to rice straw. The higher butanol production from

wheat straw may be due to its low lignin contents. Among three substrates wheat straw yields highest quantity of butanol at acidic pretreatment conditions. It was estimated from previous studies that wheat straw hydrolysate contain furfural and hydoxymethyl furfural that supported the production of biobutanol by fermentation (Kathleen et al., 2015; eMoretti and Thorson, 2008; Quershi and Blaschek, 2000).

Table 3. Acetone, Butanol and Ethanol production (%) from various agrowaste by *Clostridium acetobutylicum*

Samples	Acetone%	Butanol%	Ethanol%
Wheat straw	1.5	6.3	1.8
Rice straw	1.2	6.2	1.3
Peel wastes	1.0	5.2	1.1

ABE production from Biomass samples

Table 4. Acetone, Butanol and Ethanol production from agrowaste by *Clostridium acetobutylicum* at H_2SO_4 pretreated samples

Samples	Acetone %	Butanol%	Ethanol%
Wheat straw	2.5	7.2	2.1
Rice straw	1.7	4.9	2.2
Peel waste	1.1	4.5	2.3

ABE production from Biomass sample

Table 5. Acetone, Butanol and Ethanol production from agrowaste by *Clostridium acetobutylicum* at NaOH pretreated samples

Samples	Acetone %	Butanol%	Ethanol%

Wheat straw	1.9	4.9	2.8
Rice straw	1.5	4.6	2.4
Peel waste	1.3	4.2	2.2

ABE production from Biomass samples

3.6. HPLC Analysis of reaction mixtures

The enzymatically hydrolyzed samples of wheat and rice straws treated with acid were evaluated using HPLC. The samples that exhibited elevated levels of glucose under optimum circumstances were selected for examination. The samples were collected at various time intervals during enzymatic hydrolysis, then centrifuged at 14,000 rpm at 4 ºC for 15 minutes. The supernatant was separated and filtered using a 0.22 µm syringe filter. A portion of the sample (500 µl) was mixed with 1ml of methanol to adjust the concentrations of the samples to fit within the calibration curve range. Methanol was utilized because of the sugars' solubility. Peaks are identified depending on their retention time (tR). Glucose was identified in three samples (wheat straw, rice straw, and maize stover) by injecting a recognized standard by HPLC. A single conspicuous peak was seen with a retention time of 3.255 minutes (Table 6 and Fig. 7).

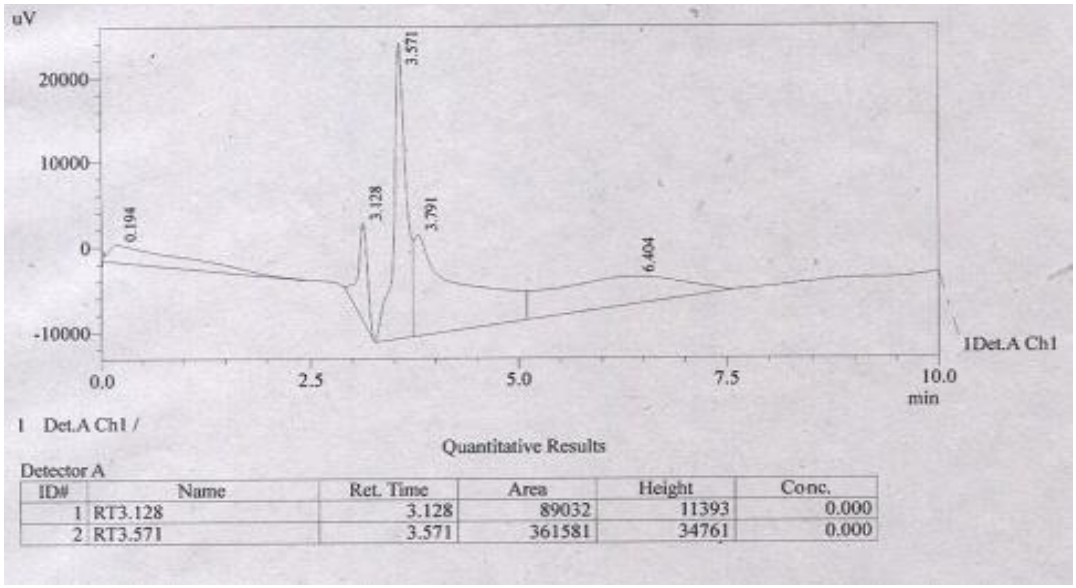

Figure 7. Chromatogram of Wheat straw hydrolysate sugar at acidic pretreatment. Peak of glucose (retention time, 3.128).

Table 6. Analysis of wheat and rice straws samples for sugars with HPLC

Components	Retention time (min)	Concentration (mg/ml) Rice straw	Concentration (mg/ml) Wheat straw
Glucose	8.6	22.52	28.3
Cellobiose	7.1	1.02	1.05
Xylose	11.6	4.3	5.6
Arabinose	12.0	1.4	1.8
Mannose	13.2	1.5	2.8
Galactose	15.5	1.2	1.5
Furfural	42.5	1.4	2.65
HMF	28	1.2	2.84

Analysis of sugar with HPLC

4. DISCUSSION

The generation of alcoholic fuels (Butanol and Ethanol) from lignocellulosic feedstock involves many technical procedures such as acid or alkali pretreatment, saccharification, and fermentation. Properly modifying all units of the system is crucial for achieving cost-effective generation of biofuels. Various nations have enhanced the production of alcoholic fuels by advancements in processes such as pretreatment, enzymatic hydrolysis, fermentation, and increased ethanol recovery (Zhao, 2012). Developing countries can benefit from studying the prevalent instances of biomass-based fuel generation in affluent nations. Novel approaches like biorefinery and directed conversion of categorized composition have been explored for ethanol generation. Similar approach may also be used for butanol synthesis from lignocellulosic biomass (García et al., 2011; Demirbas, 2001). Cost of fuels may reduce further when produced at an industrial scale. Efficient integration of these techniques might lead to competitive biofuel production from plant biomass, which is currently underutilized (Talo et al., 2014).

The fermentation of sugars in cellulosic biomass has the potential to produce key products such as acetone, butanol, ethanol, and other related alcohols, which may be utilized as liquid fuels.

The primary sources of biomass rich in carbs are wood wastes, agricultural crops such as wheat, rice, and cotton straws, maize covers, sorghum straws, fruit and vegetable wastes, and other comparable substrates (Iram et al., 2021). Cellulose is a primary sugar used in alcohol (fuel) manufacturing and is a complex molecule found in plant materials. The intricate cellulose material is broken down into smaller pieces by acid treatment, enzymatic hydrolysis, and bacterial/fungal fermentation. These types of alcohols are significant as they may be utilized as fuels. Thus, biofuels might offer a solution for addressing climate change by helping to decrease the amount of carbon emissions released via transportation, among other sources. Various methods of producing alcoholic fuels from cellulosic substrates were achieved. Straws have shown superior yields of alcoholic fuels compared to other biomass substrates.The quantity of acetone, butanol, and ethanol generated is influenced by the kind of cellulosic biomass employed and the distillation processes carried out post-fermentation for purifying these alcohols (Maria et al., 2021).

5. CONCLUSION

Renewable and sustainable energy resources are superior alternatives to traditional fuels and energy sources. Converting lignocellulosic biomass into alcoholic fuels like butanol and ethanol offers a sustainable and cost-effective method. Continual efforts are required to enhance the efficiency and cost-effectiveness of fermentation processes via a thorough comprehension of diverse pretreatment methods. Additionally, the advancement of affordable detoxification methods necessitates the use of increasingly effective microbial strains. The integration and optimization process aims to decrease energy use and boost yields to replace fossil fuels that are depleting. Scientists worldwide are researching cost-effective solutions for alternate energy sources, particularly by using cellulosic biomass. These sorts of research projects are anticipated to be crucial for the country's growth by utilizing local resources in the future.

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