BIOMASS AS SOURCE OF BIO PRODUCTS ESPECIALLY ALCOHOLIC BIOFUEL

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ABSTRACT

Compared to burning fossil fuels, biofuels seemed to have less of an impact on the environment. It is possible to make many kinds of alcoholic fuels, which might replace some of the current fuels on the market. Since butanol is chemically more comparable to gasoline, it may be used as a fuel in internal combustion engines. Because it is a drop-in fuel, butanol may be used in gasoline-only automobiles. Potential fuels such as n-butanol and isobutanol have also been investigated. Cellulosic feed stocks, sugar cane, maize grain, and other starch- or sugar-based materials are used to make butanol. Pakistan, a nation heavily reliant on oil, has enormous potential for biofuel. One way to lessen the environmental impact of transportation is to switch to second-generation cellulosic biofuels, which can power both homes and businesses. Researchers set out to find a way to manage agricultural and other organic wastes via their potential use in making alcoholic fuels. Thus, this research made use of cellulosic materials such as straws from wheat and rice, together with fruit waste. The samples were examined for several criteria. Every substrate was subjected to a comparison of chemical and biological pretreatments. It was determined how well microbial enzymes saccharified agricultural substrates. Increased biofuel production and less reliance on imported fossil fuels are two goals that this project aims to achieve.

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

INTRODUCTION

1 Worries about energy security and global warming have prompted a surge in research into potential alternative energy sources. The transportation industry is a major contributor to greenhouse gas emissions because of its reliance on fossil fuels. However, by switching to

fuels made from renewable sources like ethanol or butanol, we may lessen our negative effects on the environment and reap social and economic benefits as well.Diverse options for producing environmentally friendly biofuels are now under study. Energy resources derived from biological sources include bioelectricity, biogas, biodiesel, and bioethanol. According to Dhamole et al. (2015), bioalcohol has the ability to lessen our reliance on fossil fuels, lower emissions of greenhouse gases, and serve as both a chemical feedstock and a transportation fuel.Alcoholic fuel generation has seen tremendous improvement as a result of several nations' efforts to decrease oil imports, enhance air quality, and boost rural economies. According to the Renewable Fuels Association (2007), the world's ethanol output stands at 51,000 million liters. The greater oxygen concentration of ethanol gives it certain fuel benefits. With more oxygen in the air, hydrocarbons may be oxidized more efficiently, leading to a decrease in aromatic compounds and emissions of carbon monoxide. One advantage of ethanol is its higher octane rating (Thomas and wong, 2001).

2 Pakistan is an agriculturally based economy, hence biomass is an essential energy supply for the country. According to Amari et al. (2014), the biomass that is generated in the livestock and agricultural sectors consists of various byproducts such as rice husks and sugarcane bagasse. Lignocellulosic materials make up the bulk of second generation biomass. According to Huber et al. (2006), lignin makes up 5-30% of lignocellulosic biomass, which is composed of cellulose (35-50%), hemicellulose (20-35%), and other organic substances.Green leaves, fruit pits, straws, nut hulls, and fruit seeds are just a few examples of the many agricultural materials that may be transformed into renewable energy sources. Ejezi et al. (2006) lists maize stover, apple pomace, wheat straw, bran, and corn steep liquor as the most popular feedstocks.Instead of growing energy crops, which compete with food crops, agricultural waste is used to produce biofuels such as biodiesel, bioethanol, biohydrogen, and methane. One potential solution to the disposal issue of the vast amounts of agricultural waste is to make use of lignocellulosic biomass, which may alleviate the conflict between food production and fuel use (Mahro and Timm, 2007). The grasses are regarded as a dependable material for ethanol extraction. The usage of long-lived grasses has several benefits, one of which is the potential reduction in production and fuel costs associated with ethanol (Gomez et al., 2008).

3 Grasslands are perennial plants that thrive in both subtropical and tropical climates. Although it has its uses (such as fodder and improving soil stability), cogon grass is considered a pest in 35 different crops in over seventy-three nations and the worst weed in the world. There are medicinally significant secondary metabolites in the roots of the cogon grass plant. It is possible to grow this kind of grass, which is known as perennial grass, on soil that is otherwise unsuitable for farming.

Termites consume wood for its sugar, cellulose, which they then ferment using bacteria found in their digestive tract to produce ethanol and other alcohols and fatty acids (Kim and Dale, 2005).Soil, water, and the intestines are all home to the rod-shaped gram-positive bacteria that make up the genus Clostridium. The fermentation process of sugar by Clostridium acetobutylicum produces a blend of organic solvents, including ethanol, butanol, and acetone. One common eukaryotic organism used in fermentation to make ethanol and other alcoholic beverages is Saccharomyces cerevisiae, more often known as baker's yeast. Consequently, the present investigation aimed to conduct chemical and biological analyses of cellulosic biomass to determine a number of parameters necessary for the manufacture of alcohol fuels, such as biobutanol.

4 MATERIAL AND METHODS

Collection of Agricultural Substrates

Straws from wheat and rice, together with peel trash, were gathered from different locations. We used fine plastic bags to gather about 1 kilogram of each sample. Before being oven-dried for the night at 55 °C, the samples were sun-dried first. We used an electric grinder to finely powder the materials, and then we ran them through a normal size 40 grit sieve. The samples were kept in fine-mesh plastic bags that were clearly labeled with their names. They were then placed in a refrigerator set at 4°C until they were needed again.

The Proximate Analysis of Samples

Wet and dry weight, ash content, volatile matter, crude protein, crude fiber, and crude fat were all measured in each sample. After drying the samples at 105 ºC to eliminate moisture, the conventional procedures for estimating total solids and moisture contents were used (AOAC, 1990).

Chemical analysis of raw biomass

We used the previously described approach to determine the sample's cellulose content. To find the hemicellulose, the differences between acid detergent fiber (ADF) and neutral detergent fiber (NDF) were computed.The AOAC-reported standard technique was used to determine the lignin contents (1990).

Using Chemical Pretreatment

Two chemicals, including acid (H2SO4), were used for chemical treatments. Various concentrations of H2SO4 (1.0, 1.5, and 2%), heated to 100 °C, 110 °C, and 120 °C for varying periods of time (15, 30, and 45 minutes), were used in a pretreatment experiment. For this experiment, we used a solid sample (10% w/v) in a reagent bottle. Following pretreatment, the sample was filtered in each container using the vacuum filtration assembly, and the contents were poured onto filter paper. After filtering, 300 milliliters of distilled water were used to remove the solid and bring the pH level back to neutral. After being dried at 105 °C, the filter paper was weighed.

Figure 1. Biomass

Enzymatic Hydrolysis

In a water bath shaker with a 0.05 M buffer (sodium citrate) at 4.8 pH, the biomass was hydrolyzed with cellulose and β-glucosidases at 50 °C and 160 rpm for 72 hours after pretreatment at a concentration of 5% (w/v). Cellulases with activity of (30FPU g-1). In order to find the sugar content, samples were taken from the reagent bottle every 12 hours. I added µl of H2SO4 after the enzymatic hydrolysis had taken place. For 10 minutes at 13,500g, the unhydrolyzed material was spun in a centrifuge to separate it. The sugar analysis was done using the dinitrosalicylic acid (DNS) technique, and the supernatant was collected using syringe filters.The PAHBAH (p-hydroxybenzoic acid hydrazide) technique was used to determine the sugar content. The standard curve was produced using xylose concentrations ranging from 1Mm to 25mM. To find out how much sugar was in the pretreatment sample, we compared it to the standard sugar concentration. The enzymatic hydrolysis procedure was used to identify the optimal pretreatment conditions. To proceed with the fermentation process, only the samples with the highest amounts of released sugar were chosen. Before being used in the fermentation process, the solid biomass was kept at 4 °C (Demirbas, 2001; Iram et al., 2021; Maria et al., 2021)

***Clostridium acetobutylicum* function for butanol**

The species of Clostridium -20ºC was the temperature maintained for Clostridium acetobutylicum. After the material was hydrolyzed by enzymes, it was fermented. Sodium hydroxide was used to keep the pH at 6.5. To separate the hydrolysis and fermentation processes, 1 milliliter of C. acetobutylicum spores were introduced to 100 milliliters of an enzymatically hydrolyzed solution in a reaction bottle. After 72 hours, the reaction bottles were set in a shaking incubator set at 37 degrees Celsius with 120 revolutions per minute. After 72 hours, the concentration of butanol was measured using an alcohol meter. The results were presented as percentages of butanol extracted from wheat straw, rice straw, and corn stover. Wheat straw outperformed the other two substrates in terms of butanol production (Xue et al., 2012).

Analysis of sugar and alcohol by using HPLC

Before analysis, the samples and glucose standard solution were all passed through a 0.22 µm filter. The HPLC system was supplied with about 20 µl of the sample via the injection loop. The

glucose was determined by subjecting samples that had been hydrolyzed by enzymes to a 10-minute gradient run (Shields and Cathcart.2010; Sluiter et al., 2008; Tao et al., 2014; Zhao et al., 2012).

RESULTS AND DISCUSSION

Subsequent sections include results pertaining to chemical examination of biomass samples and fermentation of sugars into acetone-butanol-ethanol. Consequently, the present investigation used bacterial fermentation to create acetone-butanol-ethanol (ABE) from organic waste materials originating from agricultural and urban sources.

Biomass analysis

Table 1 displays data for many parameters extracted from biomass samples. Table 2 provides the lignocellulosic composition of the samples. Among the several substrates tested, wheat straw was found to have the highest concentration of cellulosic materials.

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

Dilute H_2SO_4 pretreatment

Before being autoclaved at temperatures of 105, 120, and 135 degrees Celsius for 15, 30, and 45 minutes, the biomass samples were pretreated with diluted acids at concentrations of 1, 1.5, and 2%. Figures 2–5 show the optimized conditions for the enzymatic experiment, which included a temperature of 120 °C, a retention time of 15 minutes for peel wastes and 30 minutes for cogon grass at concentrations of 1.5% and 1%, respectively.

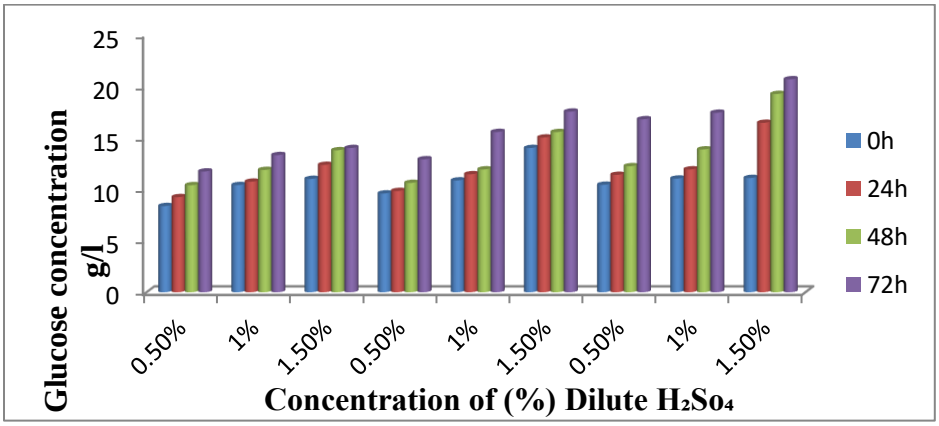

Figure 2. Glucose yield obtained from Wheat straw at H_2SO_4 pretreatment conditions at 120^0C

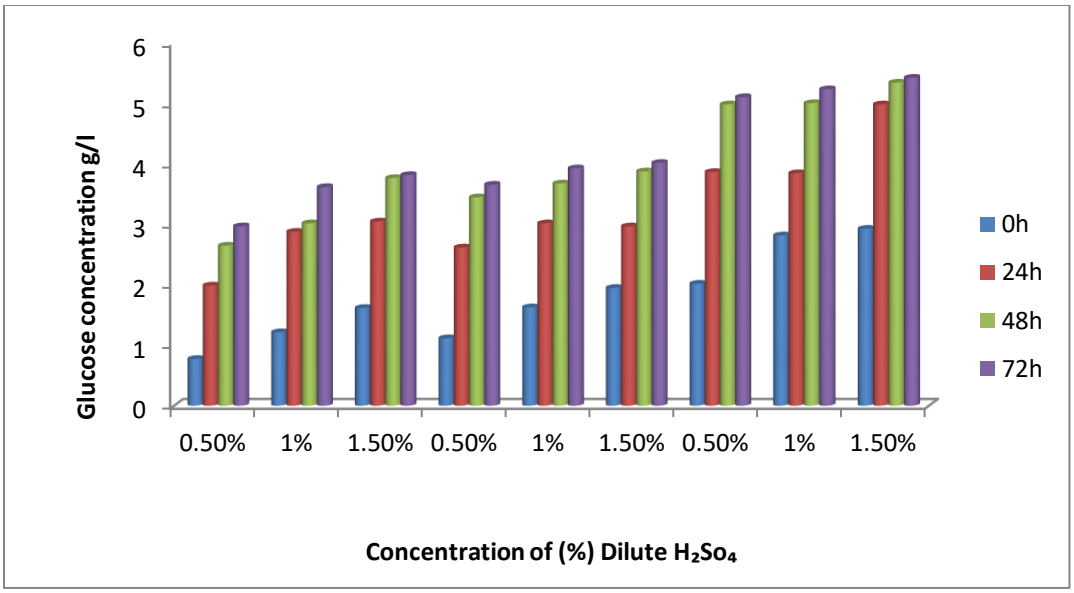

Figure 3. High glucose yield obtained from Rice straw at H_2SO_4 pretreatment conditions at 110 °C.

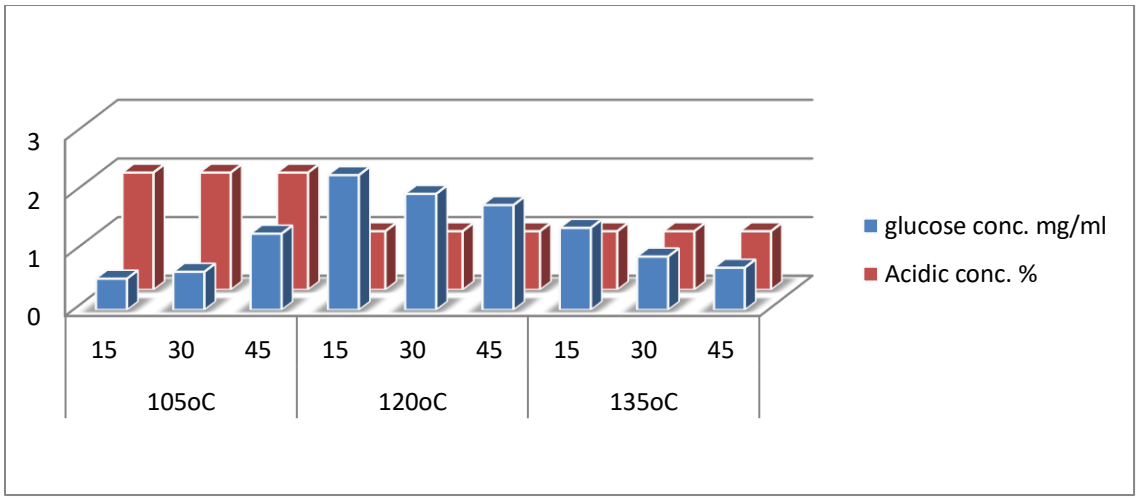

Figure 4. Comparison of glucose concentration at various temperatures after dilute acid pretreatment.

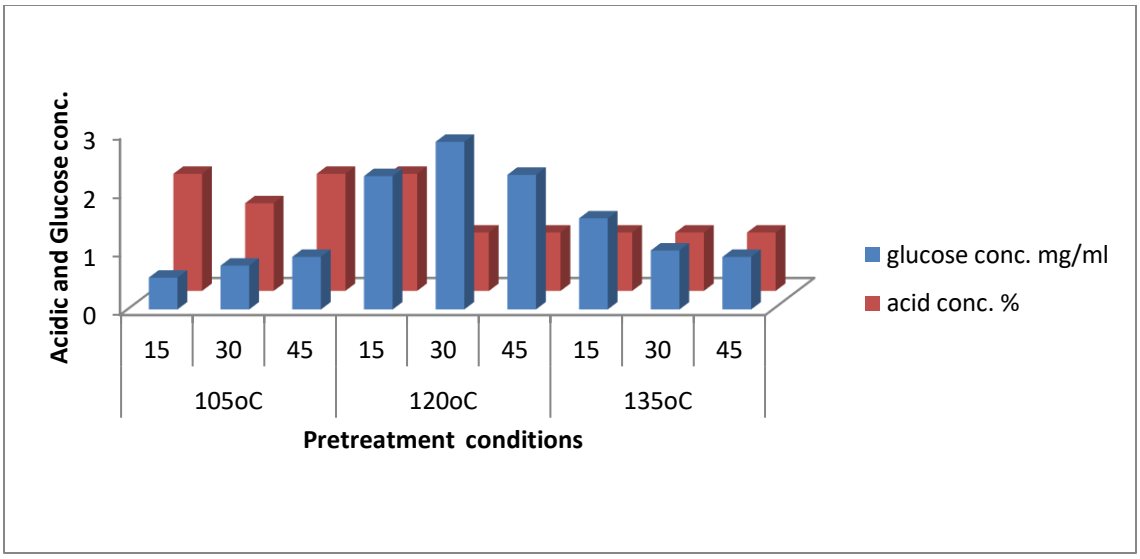

Figure 5. Comparison of glucose concentration at various temperature

Saccharification of biomass samples with enzymes

There was no need to conduct the experiment for longer hours since the highest quantity of glucose could be released from agricultural waste up to 36 hours (Fig. 6). The sugar that is released may subsequently be used in fermentation studies (Garcia et al., 2011; Becerra et al., 2015). According to the straight line in Figure 6, glucose released at a concentration of 11.55 mg/ml for the first 48 hours after enzyme addition, and thereafter, sugar released at a constant concentration.

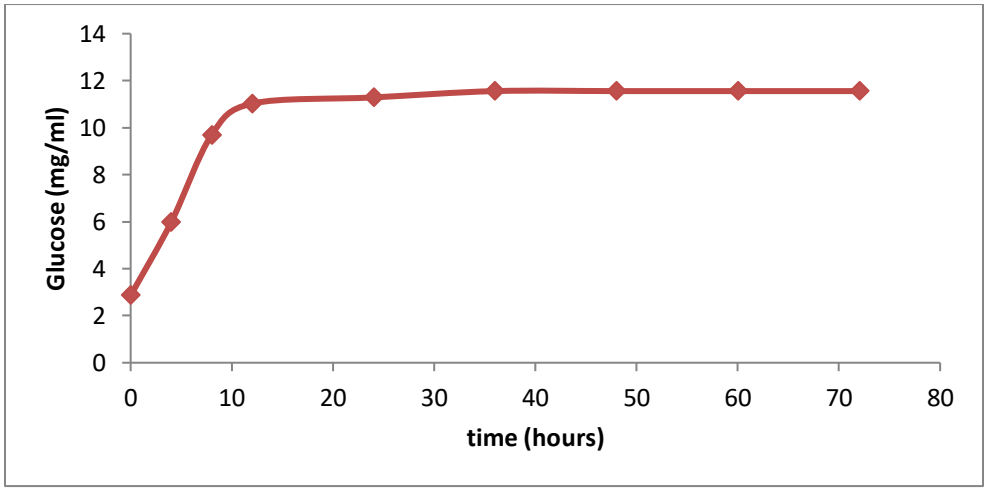

Figure 6. Enzymatic Saacharification of sugar from biomass

Fermentation

As can be seen from Tables 3–5, several substrates yielded butanol concentrations that were noticeably greater. The concentration of glucose decreased while the concentration of ethanol increased with increasing time, up to a specific point. But even with a 72-hour glucose concentration, ethanol synthesis stalled. Agrowaste is a more promising material for producing alcohol fuels because to its higher cellulosic content and lower lignin concentration (Gregg and saddler, 1996; Hanifeng et al., 2015; Jiang et al., 2015).

Fermentation with *Clostridium acetobutylicum*

The major product of this type of fermentation is known as ABE (acetone, butanol and ethanol) fermentation. The ratio of the acetone, butanol and ethanol in the fermentation process ismostly 3:6:1 as reported earlier by many authors. 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

HPLC Analysis of reaction mixtures

Hydrolyzed samples of acidic pretreatment of wheat and rice straws, among other materials, were further examined using high-performance liquid chromatography (HPLC). To achieve this goal, we only analyzed samples that have previously shown a greater concentration of glucose under these ideal circumstances. The samples were centrifuged at 14,000 rpm, 4 ºC for 15 minutes after being drawn at various times throughout the enzymatic hydrolysis process. A 0.22 µm syringe filter was used to separate the supernatant. The sample concentrations were adjusted to fall within the calibration curve range by diluting 500 µl of the sample with 1 milliliter of methanol. The sugars' solubility necessitated the use of methanol. Using the retention time, tR, to determine the peak. Glucose was validated in three samples—wheat straw, rice straw, and corn stover—by injecting the recognized standard by HPLC. One peak, with a retention time of 3.255 minutes, was found (Table 6 and Fig. 7).

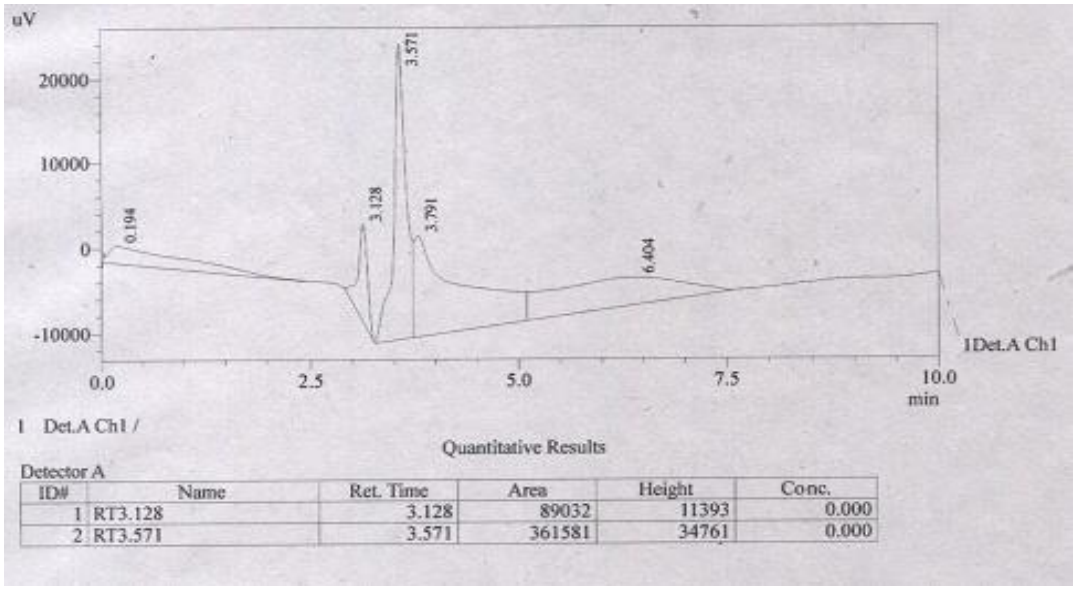

Figure 7. Chromatogram of Wheat straw hydrolysate sugar at acidic pretreatment. Peak of glucose (retention time, 3.128).

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

DISCUSSION

It took a number of technical processes, including saccharification, fermentation, and acid or alkali pretreatment, to convert lignocellulosic feedstock into alcoholic fuels like butanol and ethanol. It is crucial to regulate all system components correctly in order to achieve cost-effective biofuel production. By honing various processes such as pretreatment, enzymatic hydrolysis, fermentation, and greater levels of ethanol recovery, several nations have historically enhanced the production of alcoholic fuels (Zhao, 2012). Developed nations' well-known examples of biomass fuel generation might serve as useful pointers for less developed nations. Biorefinery and the notion of orientated conversion of categorized composition are only two of the numerous innovative approaches to ethanol production that have been studied. The lignocellulosic biomass may also be used to produce butanol using comparable technologies (García et al., 2011; Demirbas, 2001). Producing fuels on an industrial scale and combining these processes efficiently will lead to competitive biofuel production from plant biomass, which is presently underutilized (Talo et al., 2014).

By using the sugars found in cellulosic biomass, valuable byproducts such as acetone, butanol, ethanol, and other comparable alcohols may be produced, which hold great promise as liquid fuels. According to Iram et al. (2021), the most common sources of carbohydrate-containing biomass are agricultural byproducts (such as straws from wheat, rice, cotton, and maize), as well as corn cobs, sorghum stalks, and other comparable substrates. One of the most important sugars found in plant materials, cellulose is also a key ingredient in making alcohol, a fuel. Acid treatment, enzymatic

hydrolysis, and bacterial/fungal fermentation all play a role in breaking down this complex cellulose substance into smaller pieces. The potential use of these alcohols as fuels makes them significant. Biofuels have the potential to address climate change by reducing carbon emissions from sources such as transportation. The results showed a variety of orders for the synthesis of alcoholic fuels from cellulosic substrates. When compared to other biomass substrates, straws have produced the highest yields of alcoholic fuels.Maria et al. (2021) notes that the quantity of acetone, butanol, and ethanol generated is dependent on the kind of cellulosic biomass utilized and the distillation processes that are used after fermentation to purify these alcohols.

CONCLUSION

As an alternative to traditional fuels and power sources, renewable and sustainable energy resources are superior. A sustainable and cost-effective method is the bioconversion of lignocellulosic biomass into ethanol and butanol, two alcoholic fuels. However, there has to be persistent effort to fully comprehend the basics of different pretreatment procedures and to create fermentation systems that are both more efficient and less expensive. Additionally, more efficient microbial strains need to be developed for detoxification that is both cost-effective and environmentally friendly. We can replace the presently available fossil fuels, which are already depleting, with a process of integration and optimization that reduces energy use and increases yields. As a result, researchers throughout the globe are keeping an eye out for new, less expensive ways to generate energy, particularly from cellulosic biomass. These kinds of studies have the potential to become a major trend in the future of national development via the use of indigenous resources.

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