PRODUCTION OF BIO PRODUCTS FROM AGRICULTURE BIOMASS

Aafia Islam[1] , Hania Naheem[2]

1.UIBB-Arid Agriculture Rawalpindi

2. COMSATS University Islamabad

Corresponding author; aafiaislam37@gmail.com

ABSTRACT

Biofuels 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. Due to its greater similarity to gasoline, butanol is suitable for use 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. Butanol is made from maize grain, sugar cane, or other starch- or sugar-based materials, as well as cellulosic feed stocks. Pakistan is highly reliant on oil, thus biofuels have the potential to be a game-changer there. An answer to the problem of transportation-related carbon emissions and a source of energy for both business and residential usage are second-generation cellulosic biofuels. 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. The study's results should pave the way for more biofuel production and less reliance on imported fossil fuels.

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

1. INTRODUCTION

As worries about energy security and global warming grow, research into potential alternative energy sources has accelerated. Using ethanol or butanol instead of fossil fuels in vehicles might lessen their influence on the environment and have social and economic benefits. This is because vehicles in this sector rely heavily on fossil fuels, which contribute to greenhouse gas emissions.Many different ways to produce environmentally friendly biofuels are being studied. Bioelectricity, biogas, biodiesel, and bioethanol are all examples of biological energy resources. Bioalcohol has the most promise among these sources for lowering carbon emissions, weaning society off fossil fuels, and serving as both a chemical feedstock and a transportation fuel (Dhamole et al., 2015).The generation of alcoholic fuels has seen significant improvement as a result of several nations' efforts to decrease their reliance on 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. Due to its increased oxygen concentration, ethanol has some benefits as a fuel. An increase in oxygen levels allows for more efficient hydrocarbon oxidation, which in turn reduces aromatic compounds and carbon monoxide emissions. A higher octane rating is one of ethanol's qualities (Thomas and wong, 2001).

Being an agriculturally oriented nation, biomass is an essential energy resource for Pakistan. Biomass from ruminants and other agricultural byproducts, such as rice husks and sugarcane bagasse (Amiri et al. 2014). Lignocellulosic materials make up the bulk of second generation biomass. Cellulose (35–50%), hemicellulose (20–35%), and lignin (5–30%) make up lignocellulosic biomass, the most abundant organic material on Earth (Huber et al., 2006).Agricultural materials such as straws, green leaves, fruit shells, nutshells, and fruit seeds are among the renewable energy resources. According to Ejezi et al. (2006), the most popular feedstocks are maize stover, apple pomace, wheat straw, and corn steep liquor.Biodiesel, bioethanol, biohydrogen, and methane are some of the biofuels made from agricultural waste instead of energy crops, which compete with food crops. To alleviate the conflict between food production and fuel use, one alternative is to make use of lignocellulosic biomass, which is

abundant in agricultural byproducts and poses no disposal problems (Mahro and Timm, 2007). When it comes to extracting ethanol, grasses are a dependable material. 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).

Globally, the grasses may be cultivated year-round, with a special emphasis on tropical and subtropical regions. Cogon grass is a notorious plant that has been used for fodder and to improve soil stability. It is also considered a nuisance in over 35 crops in over 73 nations. 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 often not suitable for growing crops.

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. A combination of organic solvents such as acetone, butanol, and ethanol is produced when Clostridium acetobutylicum ferments sugar. 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 analyze cellulosic biomass chemically and biologically for a number of parameters necessary for the manufacture of alcohol fuels, such as biobutanol.

2. MATERIAL AND METHODS

2.1. Collection of Agricultural Substrates

From several places, we gathered wheat and rice straws, together with peel trash. We used fine plastic bags to gather about 1 kilogram of each sample. After being shad-dried, the samples were sun-dried and then oven-dried for one night at 55 °C. Using an electric grinder, the materials were ground into a fine powder and then passed through a standard size 40 mesh sieve. We put the powdered samples in little plastic bags and tagged them with the name. We kept them in the fridge at 4°C until we needed them again.

2.2. Proximate Analysis of Samples

The ash content, volatile matter, crude protein, crude fiber, crude fat, and wet and dry weight of each sample were examined. The samples were dried at 105 ºC to eliminate any remaining moisture, and then the total solids and moisture contents were estimated according to the standard techniques (AOAC, 1990).

2.3. Chemical analysis of raw biomass

The stated technique was used to estimate the cellulose content of the sample. Determining the hemicellulose was done by estimating the differences between acid detergent fiber (ADF) and neutral detergent fiber (NDF).The AOAC-reported standard technique was used to determine the lignin contents (1990).

2.4. Chemical Pretreatment

There were two substances employed for chemical pretreatment: acid (H2SO4) A pretreatment experiment was conducted utilizing varying concentrations of H2SO4 (1.0, 1.5, and 2%), subjected to varying temperatures (100, 110, and 120 °C) and durations (15, 30, and 45 minutes), among other variables. In the course of the experiment, a solid sample containing 10% (w/v) of the reagent was used. Following pretreatment, the sample was filtered in each container using the vacuum filtration assembly, and the contents were poured onto filter paper. To get the pH level back to neutral after filtering, 300 ml of distilled water was used to wash away the solid. After that, it was weighed and the filter paper was dried at 105 °C.

Figure 1. Biomass

2.5. 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). Every 12 hours, the samples were taken from the reagent bottle in order to measure the sugar content. I added µl of H2SO4 after the enzymatic hydrolysis had taken place. Centrifugation was used for 10 minutes at 13,500g to separate the unhydrolyzed material. Syringe filters were used to collect the supernatant, which was then analyzed for sugar using the dinitrosalicylic acid (DNS) technique.One way to measure sugar content is the p-hydroxybenzoic acid hydrazide (PAHBAH) technique. The concentration range of xylose used to generate the standard curve was 1Mm-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. The fermentation process proceeded using the sample that had the highest quantity of liberated sugar. The fermentation process made use of the solid biomass that had been held at 4 °C (Demirbas, 2001; Iram et al., 2021; 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

Before analysis, the samples and glucose standard solution were all passed through a 0.22 µm filter. The injection loop was used to inject about 20 µl of sample into the HPLC apparatus. Shields and Cathcart (2010), Sluiter et al. (2008), Tao et al. (2014), and Zhao et al. (2012) all state that

enzymatically hydrolyzed samples were run in gradient mode for 10 minutes in order to evaluate the glucose.

3. RESULTS AND DISCUSSION

The next sections provide the results of the chemical analysis of the biomass samples and the fermentation of the sugars into acetone, butanol, and ethanol. Bacterial fermentation was used to create acetone-butanol-ethanol (ABE) from organic waste materials originating from agricultural and municipal sources in the present research.

3.1. Biomass analysis

Table 1 displays data for a number of parameters extracted from biomass samples. Table 2 provides the lignocellulosic composition of the samples. When compared to other analytical substrates, wheat straw was found to have a greater cellulose content.

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

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.

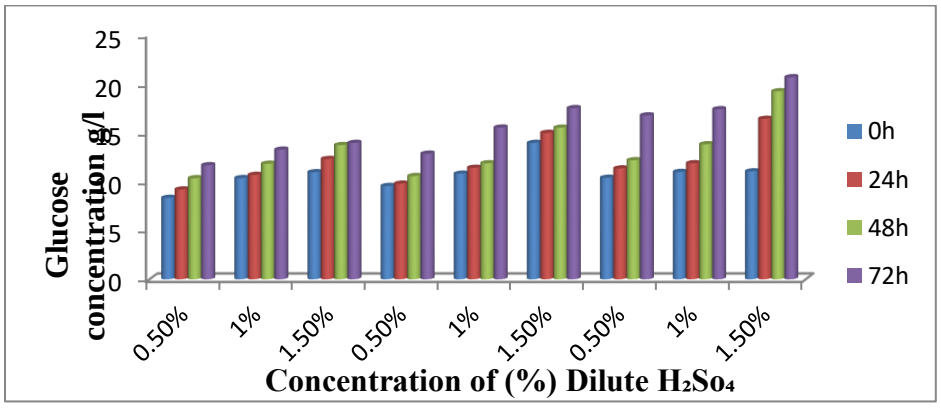

Figure 2. Glucose yield obtained from Wheat straw at H_2SO_4 pretreatment conditions at 120^0C

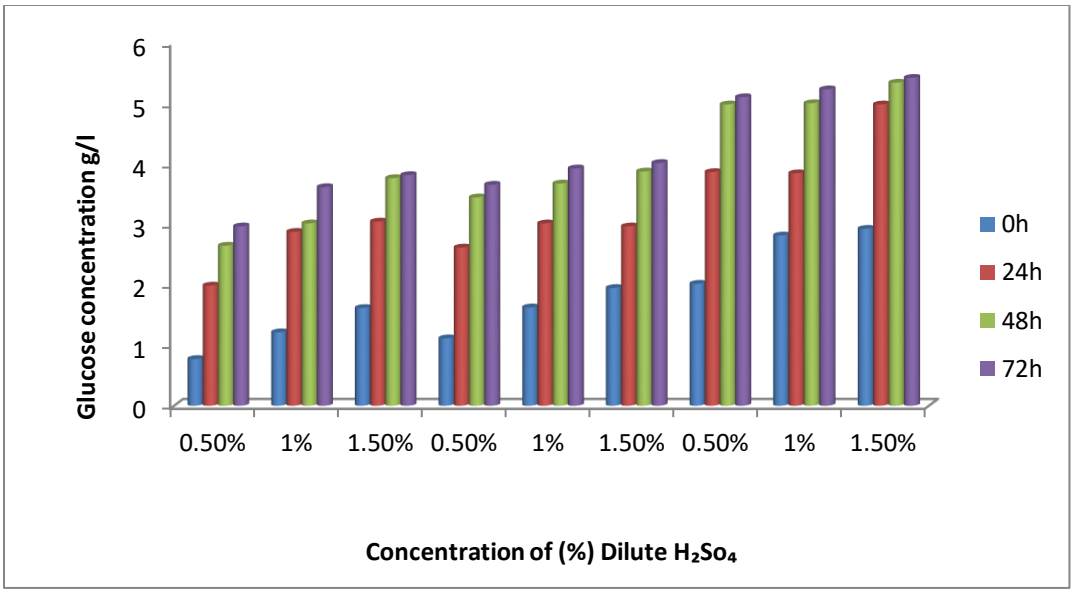

Figure 3. High glucose yield obtained from Rice straw at H_2SO_4 pretreatment conditions at 110 °C.

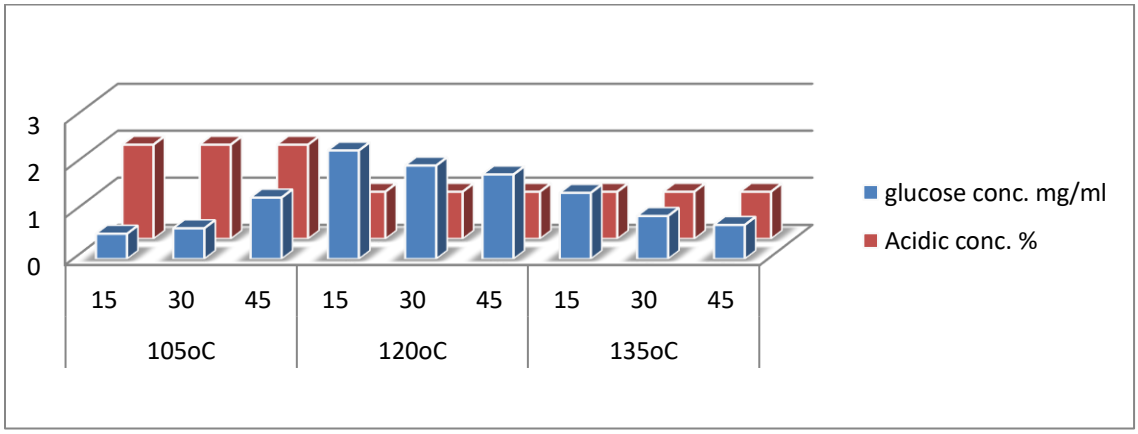

Figure 4. Comparison of glucose concentration at various temperatures after dilute acid pretreatment.

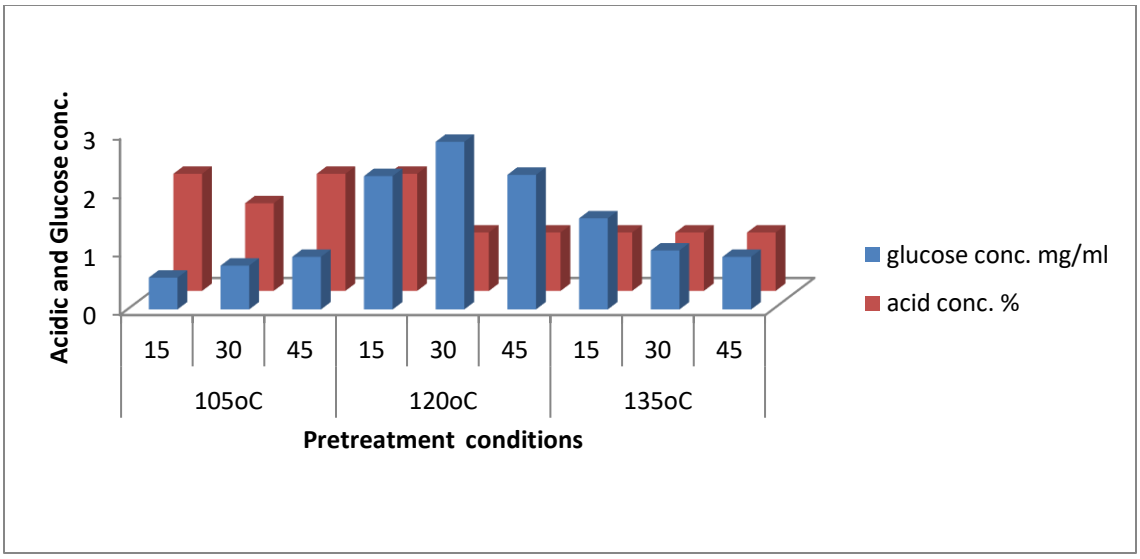

Figure 5. Comparison of glucose concentration at various temperature

3.3. 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 throughout the first 48 hours after the injection of the enzyme. after this, sugar released at a constant concentration.

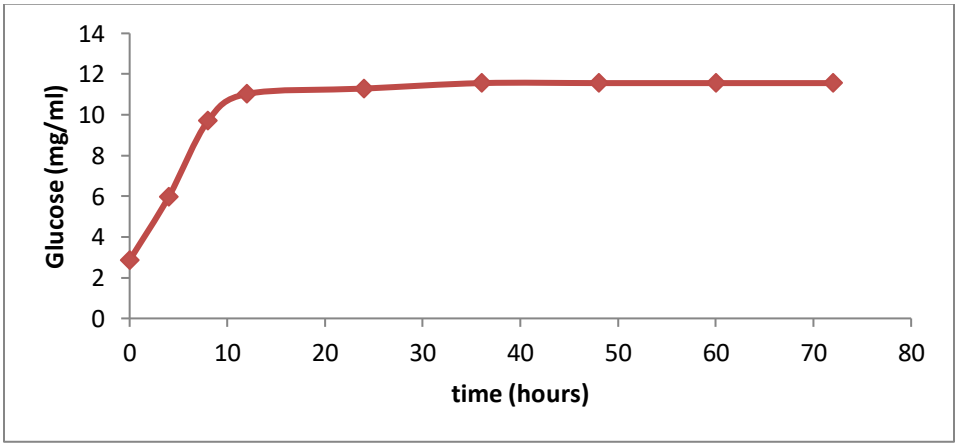

Figure 6. Enzymatic Saacharification of sugar from biomass

3.4. Fermentation

As can be seen from Tables 3–5, several substrates yielded butanol concentrations that were noticeably greater. There was a time limit beyond which the concentration of ethanol increased and the concentration of glucose decreased. After 72 hours, however, the concentration of glucose was too low to keep the ethanol synthesis going. Agrowaste is a more promising material for alcohol fuel generation because to its higher cellulosic content and lower lignin concentration (Gregg and saddler, 1996; Hanifeng et al., 2015; Jiang et al., 2015).

3.5. Fermentation with *Clostridium acetobutylicum*

Acetone, butanol, and ethanol (ABE) are the three main byproducts of this fermentation process. According to previous reports from many writers, the fermentation process typically uses a ratio of 3:6:1. 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

Following enzymatic hydrolysis, acidic pretreatment samples (such as rice straws and wheat straws) were subjected to additional analysis by HPLC. The samples that demonstrated a larger quantity of glucose under optimal circumstances were chosen for this reason. 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. In order to get the sample concentrations within the range of the calibration curve, 500 µl of the sample was diluted with 1 ml of methanol. The sugars' solubility necessitated the use of methanol. Using the retention time tR, the peak may be identified. Table 6 and Figure 7 show that the recognized standard, when injected using HPLC, validated the identification of glucose in three samples: wheat straw, rice straw, and maize stover. The standard showed a single conspicuous peak with a retention time of 3.255 minutes.

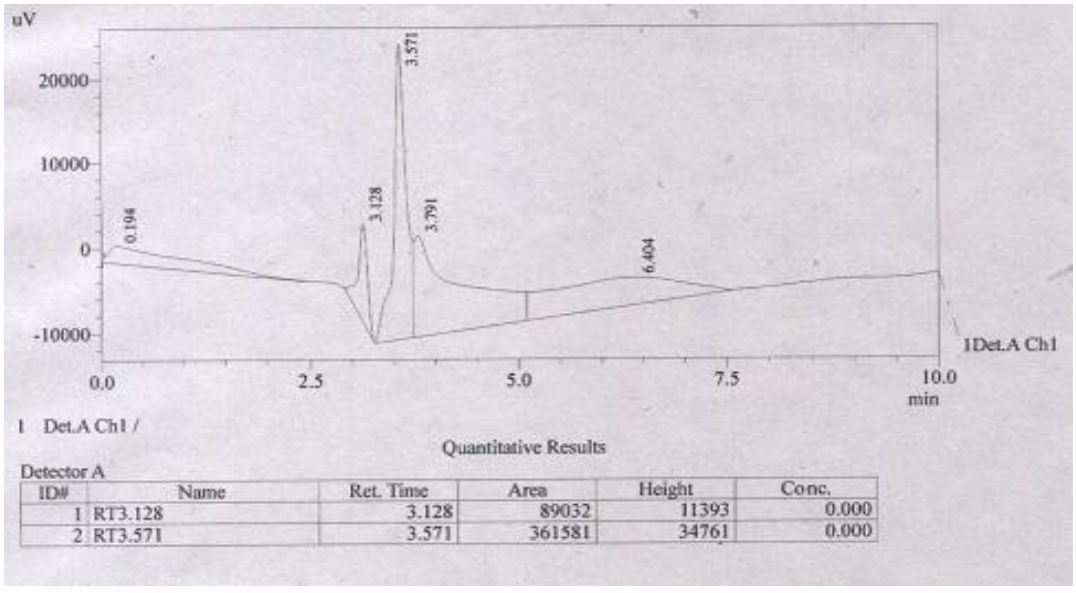

Figure 7. Chromatogram of Wheat straw hydrolysate sugar at acidic pretreatment. Peak of glucose (retention time, 3.128).

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

Butanol and ethanol, two alcoholic fuels, are made from lignocellulosic feedstock via a series of technical processes that include saccharification, fermentation, and acid or alkali pretreatment. Accurately modifying all system components is crucial to achieve cost-effective biofuel production. Pretreatment, enzymatic hydrolysis, fermentation, and ethanol recovery levels were all greatly enhanced in the past by various nations, allowing them to produce alcoholic fuels at a better standard (Zhao, 2012). Emerging nations may learn a lot from the successful examples of biomass-based fuels generation in industrialized nations. Furthermore, several innovative concepts have been explored for ethanol generation, including biorefinery and the notion of directed conversion of categorized content. García et al. (2011) and Demirbas (2001) found that butanol may be produced from lignocellulosic biomass using comparable technologies. When these technologies are combined efficiently, they will lead to the competitive production of

biofuels from plant biomass, which is now underutilized, and the cost of fuels might drop even more (Talo et al., 2014).

Important products such as acetone, butanol, ethanol, and related alcohols might be produced by fermentation of sugars found in cellulosic biomass. These products could be used as liquid fuels. Iram et al. (2021) lists wood waste, agricultural crop straws (such as wheat, rice, and cotton), maize covers, sorghum straws, fruit and vegetable waste, and similar substrates as the most common sources of carbohydrate-containing biomass. One of the most important sugars found in plant materials, cellulose is also a key ingredient in making alcohol, a fuel. Fermentation by bacteria or fungi, in conjunction with acid treatment and enzymatic hydrolysis, reduces this complex cellulose substance to smaller components. The potential use of these alcohols as fuels makes them significant. Consequently, biofuels have the potential to be a weapon in the fight against climate change by lowering the amount of carbon emissions caused by transportation and other human activities. Consequently, several orders of alcoholic fuels were produced from cellulosic substrates. When compared to other biomass substrates, straws have produced the highest yields of alcoholic fuels.The quantity of acetone, butanol, and ethanol generated, however, is dependent on the kind of cellulosic biomass used and the specific distillation processes that are employed to purify these alcohols after fermentation (Maria et al., 2021).

5. 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. The creation of more cost-effective and efficient fermentation processes, as well as a thorough familiarity with the principles of different pretreatment methods, need ongoing research. In addition, there has to be research into more efficient microbial strains for detoxification that doesn't break the bank. In order to replace the presently available fossil fuels, which are already dwindling in supply, the optimization and integration process is being used to decrease energy consumption and boost yields. Scientists 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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