



## Production of bio products from agriculture biomass

Aafia Islam<sup>1\*</sup>, Hania Naeem<sup>2</sup>

<sup>1</sup>University Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University Rawalpindi, 46300, Pakistan

<sup>2</sup>Department of Biosciences, COMSATS University Islamabad, 45550, Pakistan.

\* [aafiaislam37@gmail.com](mailto:aafiaislam37@gmail.com)

### 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 use as alternative source of existing fuels. Butanol may be used as a fuel in an internal combustion engine and it is more similar to gasoline. Butanol is a drop-in fuel and thus works in vehicles designed for use with gasoline without modification. Both n-butanol and isobutanol have been studied as possible fuels. Butanol is produced from starch- or sugar-based material such as corn grain, sugar cane or from cellulosic feed stocks. Biofuel can play a great role in Pakistan because country is oil dependent. Second generation cellulosic biofuels offer a solution to reduce carbon emissions of traffic as well as generation of energy for domestic and commercial uses. A study was conducted to develop a approach for the management of agriculture as well as other organic wastes utilization for production of alcoholic fuels. Therefore, cellulosic materials like wheat and rice straws as well as fruit wastes were used in this study. Samples were analyzed for different parameters. Biological and chemical pretreatments were compared for each substrate. Efficiency of microbial enzymes for saccharification of agricultural substrates was evaluated. It is expected that outcome of this study will help to increase production of biofuels and to reduce burden of imported fossil fuels.

Received date: 12 July 2025

Revised date: 27 July, 2025

Accepted date: 4 August 2025

Published date: 15 August 2025

**Copyright:** © 2025 by the authors. Submitted for possible open access publication under the terms and conditions of the GAUS.

**ISSN:** 0000-000

**Keywords:** Fossil fuels, Bio butanol, Biomass, Greenhouse gases, Climatic changes

### 1. Introduction

Exploration of sources for alternate energy have been increased because of increasing concerns about energy security and climate change. The transportation sector plays a significant role for emission of greenhouse gases due to uses of fossil fuels, However, replacement of oil derived fuels such as ethanol or butanol could reduce environmental impacts and give advantages on social as well as economical levels Various alternatives to generate sustainable biofuels are being investigated. Biological energy resources are like bioelectricity, biogases, biodiesel and bio alcohols. Among these sources, bio-alcohol shows a great potential to reduce the emission of greenhouse gases, decrease the dependence on fossil fuel and act as a chemical feedstock and fuel for transport (Dhamole *et al.*, 2015). The production of alcoholic fuels has been improved extremely because many countries are trying to reduce the import of oil, improving the quality of air and growing rural economics. The global ethanol production is 51,000 million liters (Renewable Fuels Association, 2007). Ethyl

alcohol has some advantages as a fuel as it has higher oxygen contents. The higher oxygen level permits improved oxidation of hydrocarbons with successive reduction in aromatic compounds and carbon monoxide emission. Ethanol has greater octane rating properties (Thomas and wong, 2001).

Biomass is a vital energy resource in Pakistan because of agriculturally based country. The biomass produced in livestock and agriculture sector in the form of animal waste and crop remaining as sugarcane bagasse and rice husk (Amiri *et al.* 2014). Second generation biomass is mainly composed of lignocellulosic material. Lignocellulosic biomass is more plentiful organic substance on earth and consists of cellulose (35-50%), hemicellulose (20-35%) and lignin (5-30%) (Huber *et al.*, 2006). Various renewable energy resources include different agricultural substances like green leaves, fruit shells, straws, nut shells and fruit seeds. Most commonly used feedstocks are wheat straw, wheat bran, corn stover, corn steep liquor and apple pomace (Ejezi *et al.*, 2006). Now a day, agricultural waste is used for the production of biofuels like biodiesel, bioethanol, biohydrogen and methane as compared to energy crops because they have competition with food crops. As huge amount of agro waste is available and have discarding problem so, alternate option is the utilization of lignocellulosic biomass in order to reduce the competition between fuel and food (Mahro and Timm, 2007). The grasses are considered as reliable substance for extraction of ethanol. The utilization of perennial grasses is advantageous and possibly it further decreases the cost for the production of ethanol and its use as fuel (Gomez *et al.*, 2008).

The grasses can be grown all over the year worldwide, particularly in subtropical as well as tropical countries. Cogon grass has been exploited to rise the soil stability and as fodder, it is recognized as worst weed and it is known as pest by almost 73 countries in all over 35 crops. The roots of cogon grass have secondary metabolites which have medical importance. It is known as perennial grass and could be cultivated in any soil which usually considered as unfit for production of crops. Cellulose is a major sugar in wood; it is broken down by bacteria available in gut of termite and finally converted into various products including fatty acids and alcohol like ethanol etc. (Kim and Dale. 2005). *Clostridium*, genus of rod shaped gram positive bacteria member of which found in soil, water and intestinal tract. *Clostridium acetobutylicum* ferments sugar to a mixture of organic solvents like acetone, butanol and ethanol. *Saccharomyces cerevisiae* (known as baker's yeast) single celled eukaryotes which is frequently used in fermentation process for production ethanol and other alcoholic products. Therefore, current study was undertaken for Chemical and biological analysis of cellulosic biomass for various parameter required for alcohol fuels like biobutanol production

## 2. Materials and Methods

### 2.1 Collection of Agricultural Substrates

Various samples of wheat and rice straws as well as, peel wastes were collected from various areas. About 1 Kg samples of each sample were collected in fine plastic bags. The samples were shaded followed by sun and oven dried for overnight at 55 °C. The samples were converted into fine powder form by electric grinder and passed through 40 mesh standard size sieve. The powder form of samples was saved in fine plastic bags duly labeled with the name and were stored in refrigerator at 4°C till further uses.

### 2.2 Proximate Analysis of Samples

All samples were analyzed for ash contents, volatile matter, crude protein, crude fiber, crude fat and wet as well as dry-weight. The standard methods were used for the estimation of total solids and moisture contents by drying at 105 °C to remove moisture from the samples (AOAC, 1990).

### 2.3 Chemical Analysis of Raw Biomass

The cellulose content of sample was estimated by using reported method. The hemicellulose was determined by computing ADF (Acid Detergent Fiber) and NDF (Neutral Detergent Fiber) differences. The lignin contents were determined by standard method as reported by AOAC (1990).

### 2.4 Chemical Pretreatment

For chemical pretreatment two chemicals were used such as acid ( $\text{H}_2\text{SO}_4$ ) Pretreatment experiment was performed by using  $\text{H}_2\text{SO}_4$  (1.0, 1.5 and 2%) at diverse temperatures such as 100 °C, 110 °C and 120 °C for different times durations (15, 30, and 45 minutes). Solid sample (10 %) (w/v) in reagent bottle was utilized during experiment. After pretreatment, the vacuum filtration assembly was used for filtration of sample in each bottle and the contents were emptied on filter paper. After filtration, the solid washed away with 300 ml distilled water in order to neutralize the pH. The filter paper was then dried at 105 °C and weighed.



**Figure 1. Biomass**

## 2.5 Enzymatic Hydrolysis

The biomass after pretreatment 5% (w/v) was hydrolyzed with cellulose and  $\beta$ -glucosidases at 50 °C and 160 rpm for 72 hours in a water bath shaker with 0.05 M buffer (sodium citrate) at 4.8 pH. Cellulases having activity of (30FPU g<sup>-1</sup>). The samples were withdrawn from reagent bottle after every 12 hours to determine the concentration of sugar. After enzymatic hydrolysis,  $\text{H}_2\text{SO}_4$  ( $\mu\text{l}$ ) was added. Un-hydrolyzed sample was separated by centrifuging for 10 minutes at 13,500g. Supernatant was collected by means of syringe filters for sugar analysis by dinitrosalicylic acid (DNS) method. The amount of sugar was analyzed by p-hydroxybenzoic acid hydrazide (PAHBAH) method. By using the concentration 1Mm-25mM of xylose the standard curve was drawn. Then by comparing the standard sugar concentration, the amount of sugar in pretreated sample was determined. The best pretreatment condition was selected after enzymatic hydrolysis process. The sample containing higher amount of released sugar was further selected for fermentation process. The solid biomass was stored at 4 °C which was then used for fermentation process. (Demirbas, 2001; Iram *et al.*, 2021; Maria *et al.*, 2021).

## 1.6 *Clostridium acetobutylicum* function for butanol

The clostridium specie *Clostridium acetobutylicum* was maintained at -20°C. Enzymatically hydrolyzed sample was then used for fermentation. The pH was maintained at 6.5 with NaOH. 1ml *C. acetobutylicum* spores were added in 100 ml enzymatically hydrolysed solution in reaction bottle for separate hydrolysis and fermentation. These reaction bottles were placed in shaking incubator having 120 rpm at 37 °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 ).

### 1.7 Analysis of sugar and alcohol by using HPLC

All the samples and standard solution of glucose was passed through the 0.22  $\mu\text{m}$  filter prior to analysis. About 20  $\mu\text{l}$  of sample was injected through injection loop into HPLC system. In order to analyze the glucose, enzymatically hydrolyzed samples were run in the gradient mode for 10 minutes (Shields and Cathcart.2010; Sluiter *et al.*, 2008; Tao *et al.*, 2014; Zhao *et al.*, 2012).

## 3. Results

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 $\pm$ 0.48	7.83 $\pm$ 0.25	8.15 $\pm$ 0.24	8.17 $\pm$ 0.33	34.45 $\pm$ 0.43	5.36 $\pm$ 0.2
Rice straw	89.15 $\pm$ 0.26	7.19 $\pm$ 0.27	7.16 $\pm$ 0.24	9.15 $\pm$ 0.33	36.45 $\pm$ 0.45	6.25 $\pm$ 0.3
Peel(wastes)	92.43 $\pm$ 0.47	8.56 $\pm$ 0.35	5.95 $\pm$ 0.23	5.92 $\pm$ 0.43	34.86 $\pm$ 0.36	4.76 $\pm$ 0.4

Analysis of organic wastes samples

**Table 2 Chemical analysis (%) of biomass samples**

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

Mean  $\pm$  standard deviation (n=3) NDF = Neutral Detergent Fiber and ADF=Acid Detergent Fiber

### 3.2 Dilute H<sub>2</sub>SO<sub>4</sub> pretreatment

The samples of the various biomass were pretreated with dilute acid 1, 1.5 and 2% concentration, an autoclave at temperature of 105, 120 and 135°C for the period of 15, 30 and 45 minutes. The temperature 120 °C is considered best for both the samples while the retention time of 15 minutes was suitable for peel wastes and 30 minutes for cogon grass at the concentration of 1.5% and 1% respectively, these are the optimized conditions that was used for enzymatic experiment (Figures 2-5).

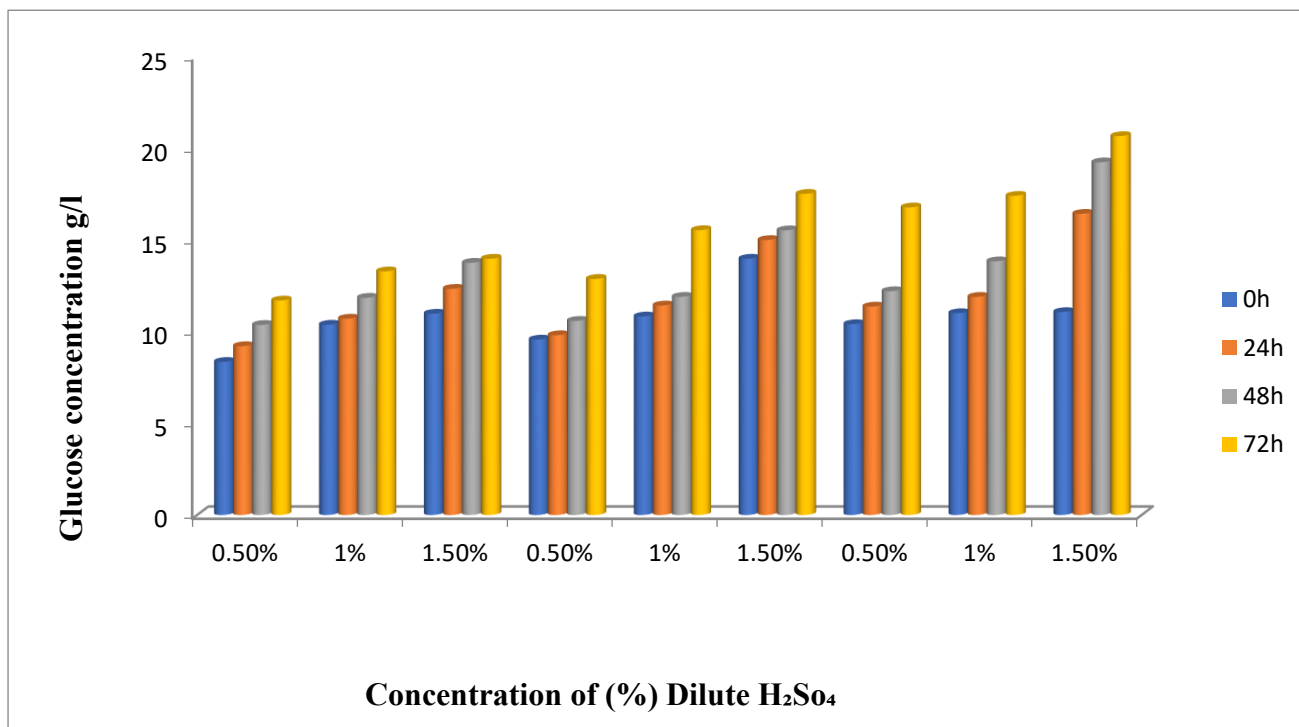


Figure 2 Glucose yield obtained from Wheat straw at H<sub>2</sub>SO<sub>4</sub> pretreatment conditions at 120°C

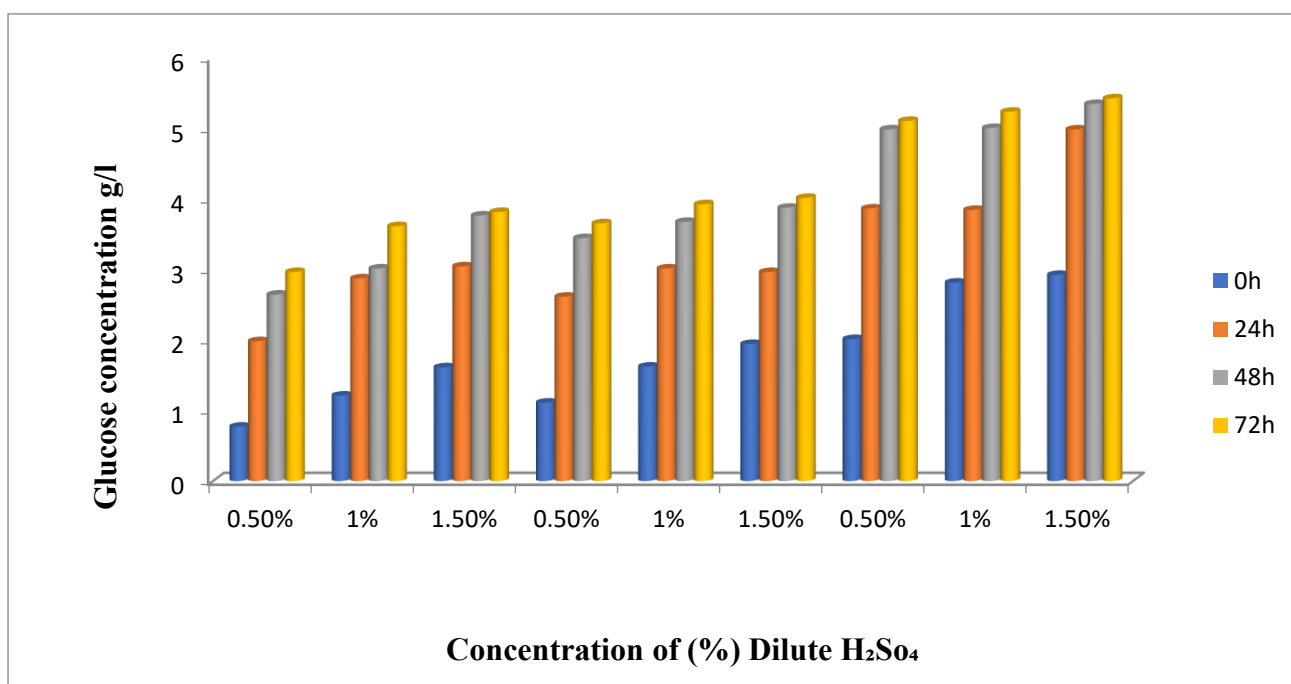
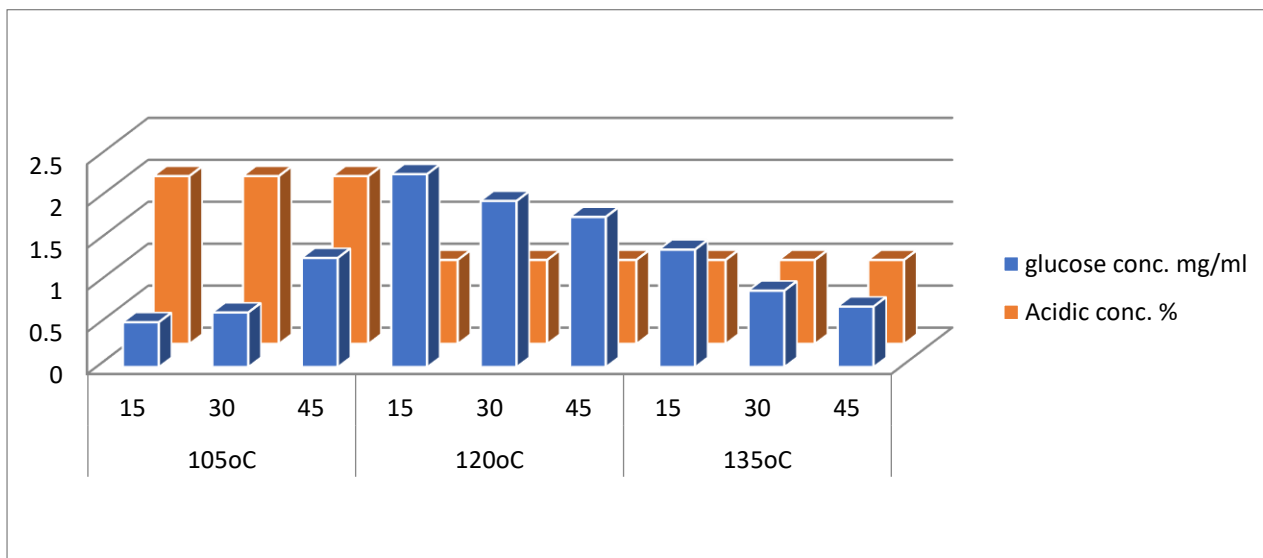
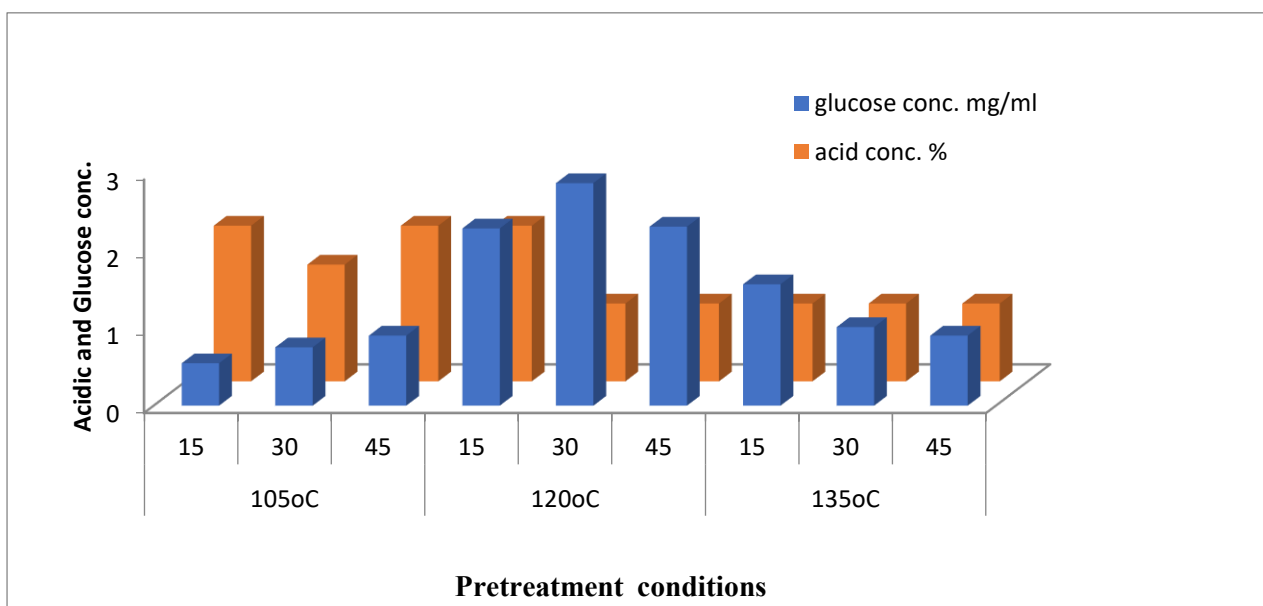


Figure 3 High glucose yield obtained from Rice straw at H<sub>2</sub>SO<sub>4</sub> pretreatment conditions at 110 °C



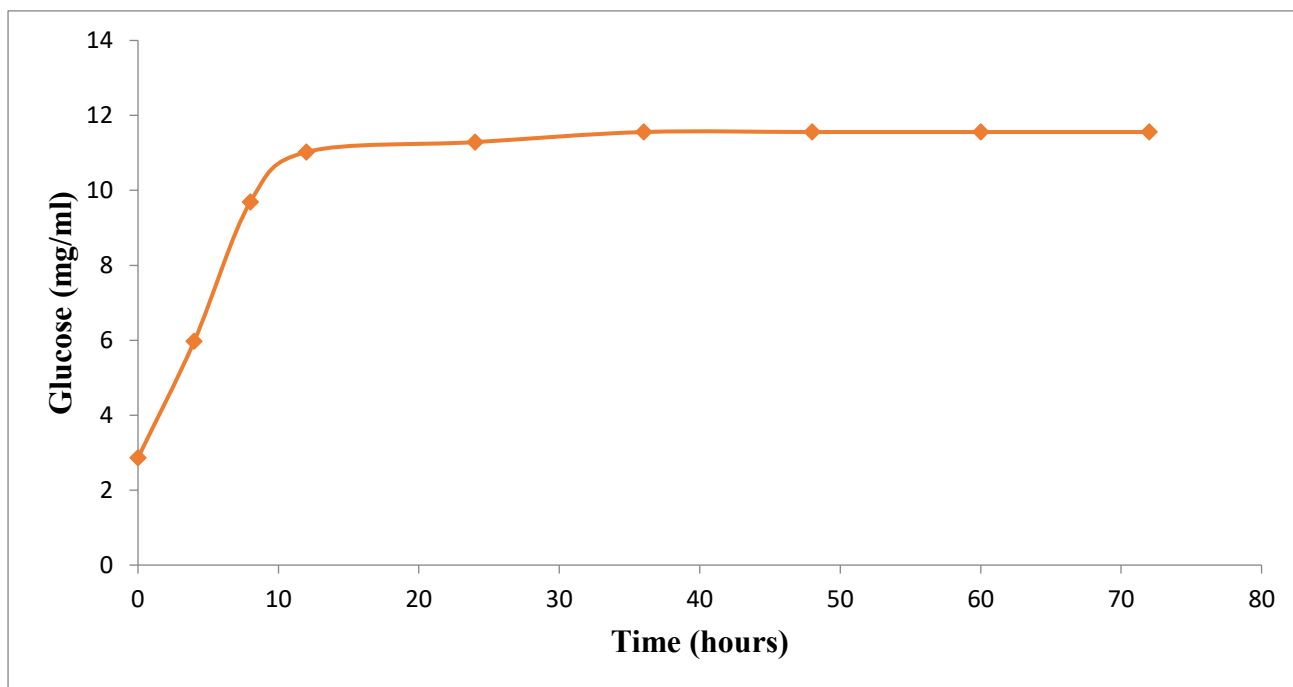
**Figure 4 Comparison of glucose concentration at various temperatures after dilute acid pretreatment**



**Figure 5 Comparison of glucose concentration at various temperature**

### 3.3 Saccharification of biomass samples with enzymes

Higher maximum amount of glucose was released from agro waste up to 36 hours so there was no need to run experiment for more hours (Fig. 6). This released sugar, can than further be used for fermentation experiments (Becerra *et al.*, 2015; Dheeran *et al.*, 2012; Garcia *et al.*, 2011). Glucose (11.55 mg/ml) released during the first 48 hours after the addition of enzyme and after that sugar released and became constant as observed from the straight line (Fig.6).



**Figure 6 Enzymatic Saccharification of sugar from biomass**

### 3.4 Fermentation

Significantly higher concentration of butanol was obtained from different substrates as shown in Tables 3 to 5. As the time period increases, glucose concentration was reduced but ethanol concentration was enhanced but up to certain time limit. However, after 72 hours glucose concentration was not sufficient to maintain the ethanol production. Higher cellulosic but lower lignin contents were found and these contents make agrowaste a better candidate for alcohol fuel production (Gregg and saddler, 1996; Hanifeng *et al.*, 2015; Jiang *et al.*, 2015).

### 3.5 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 is mostly 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 hydroxymethyl 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* H<sub>2</sub>SO<sub>4</sub> 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 agro-waste by *Clostridium acetobutylicum* 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

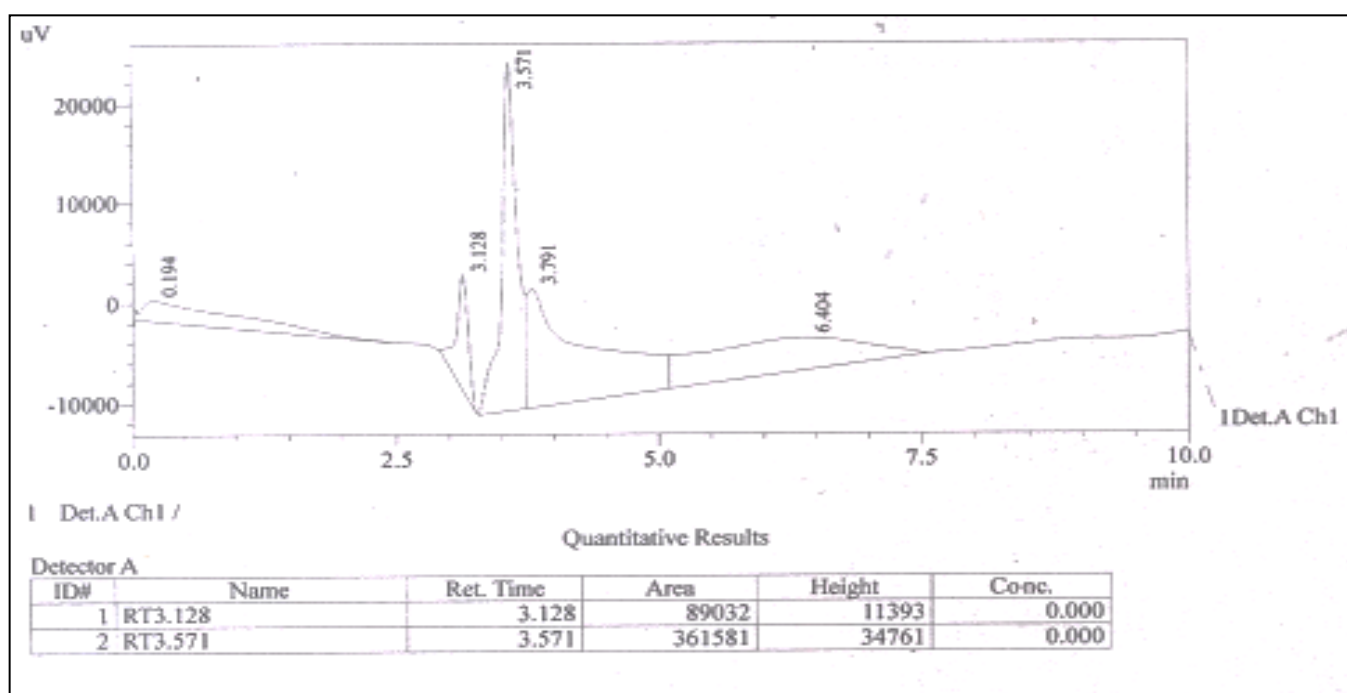
### 3.6 HPLC Analysis of reaction mixtures

The enzymatically hydrolyzed samples of acidic pretreatment of wheat and rice straws etc., were further analyzed by HPLC. For this purpose, the samples those have shown higher amount of glucose at optimized conditions were used for analysis. The samples those were withdrawn at different time periods during enzymatic hydrolysis, then these were centrifuged at 14,000 rpm, at 4 °C for 15 minutes. Supernatant was separated and then filtered by using 0.22 µm syringe filter. An aliquot of the sample (500 µl) was diluted with 1ml methanol to bring the concentrations of the samples within the range of calibration curve. Methanol was used due to the solubility of the sugars. The identification of peak as based on the retention time  $t_R$ . Identification of glucose in three samples i.e. wheat straw, rice straw and corn stover were confirmed by the known standard injected through HPLC and its only one prominent peak was observed at a retention time of 3.255 minutes (Table 6 and Fig.7).



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

Components	Retention time (min)	Concentration (mg/ml)	
		Rice straw	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

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

#### 4. Discussion

For the production of alcoholic fuels (Butanol and Ethanol) from lignocellulosic feedstock required various technological steps like acid or alkali pretreatment, saccharification and fermentation. To accomplish an cost effective production of biofuels, proper adjusting of all units of system is of a great importance. In the past different countries significantly improved alcoholic fuels production by refining different process like pretreatment, enzymatic hydrolysis, fermentation, and higher level of ethanol recovery (Zhao, 2012). The popular cases of

biomass-based fuels production in developed countries may be good references for the developing countries. In addition, many novel ideas, such as biorefinery and the concept of oriented conversion of classified composition have been investigated for ethanol production. Similar technology are also applicable for butanol production from lignocellulosic biomass (García *et al.*, 2011; Demirbas, 2001). The cost of fuels may further decreases when it will produce at industrial scale and efficient combination of these processes will result in competitive biofuel production from plant biomass, which is currently not being utilized effectively (Talo *et al.*, 2014). Fermentation of available sugars in cellulosic biomass have potential to provides important products like acetone, butanol, ethanol and similar other alcohols, that could be used as liquid fuels. Mostly available source of biomass containing carbohydrates are wood wastes, agriculture crops like wheat, rice and cotton straws, corn covers, sorghum straws, fruit and vegetable wastes and similar other substrates (Iram *et al.*, 2021). Cellulose is considered as major sugar for alcohol (fuel) production and cellulose is complex sugar present in plants materials. This complex cellulosic material is break down into smaller units with help of acid treatment and enzymatic hydrolysis as well as bacterial/ fungal fermentation. These forms of alcohols is important because that may use as fuels. Therefore, biofuels may provide solution of combating climate change, as it help to reduce level of carbon emission release from traffic etc. Therefore, various order of alcoholic fuels production from cellulosic substrates was obtained. Among all substrates of biomass used straws has provided better yields of alcoholic fuels as compared to others material used. However, amount of acetone, butanol and ethanol produced depends on nature of cellulosic biomass used as well as various distillation process conducted after fermentation for purification of this type of alcohols (Maria *et al.*, 2021).

## 5. Conclusion and Future recommendations

Renewable and sustainable energy resources are the best alternative of conventional fuels and energy sources. Bioconversion of lignocellulosic biomass into alcoholic fuels (butanol and ethanol) provides a sustainable and economical pathway. While, a deep understanding of fundamentals of various pretreatment processes and development of more efficient and economical fermentation processes needs continuing efforts. Moreover, the development of cost-effective detoxification, more efficient microbial strains are required. The process of integration and optimization to reducing energy consumption as well as to increase yields replace currently available fossil fuels those are already in process of depletion. Therefore, scientists all over the world are observing different cost-effective methods for alternative sources of energy especially by using cellulosic biomass. It is expected that these types of research work could be an important phenomenon for the development of country by using indigenous resources in future. Future research should focus on developing efficient pretreatment, fermentation, and detoxification methods alongside robust microbial strains to enhance biofuel production from lignocellulosic biomass. Such advancements will reduce reliance on depleting fossil fuels and promote sustainable energy development.

**Supplementary Materials:** Not Applicable.

**Author Contributions:** Both authors equally contribute.

**Funding:** There is no funding for this project

**Institutional Review Board Statement:** Not Applicable.

**Informed Consent Statement:** Not Applicable.

**Data Availability Statement:** Data will be available on request

**Acknowledgments:** The authors gratefully acknowledge the support and facilities provided by the University Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University Rawalpindi, Pakistan, which made this research work possible.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

- Amiri, H., Karimi, K., & Zilouei, H. (2014). Organosolv pretreatment of rice straw for efficient acetone, butanol, and ethanol production. *Bioresource Technology*, 152, 450–456. <https://doi.org/10.1016/j.biortech.2013.11.064>
- AOAC. (1990). *Official methods of analysis of the AOAC* (15th ed., Method 920.85). Association of Official Analytical Chemists.
- Becerra, M. M. E., Cerdan, M. I., & Gonzalez-Siso, M. I. (2015). Biobutanol from cheese whey. *Microbial Cell Factories*, 14(27), 1–13. <https://doi.org/10.1186/s12934-015-0206-8>
- Demirbas, A. (2001). Biomass resource facilities and biomass conversion processing for fuels and chemicals. *Energy Conversion and Management*, 42(11), 1357–1378. [https://doi.org/10.1016/S0196-8904\(00\)00137-](https://doi.org/10.1016/S0196-8904(00)00137-)
- Dhamole, P. B., Mane, R. G., & Feng, H. (2015). Screening of non-ionic surfactants for enhancing biobutanol production. *Applied Biochemistry and Biotechnology*, 177(1), 1–10. <https://doi.org/10.1007/s12010-015-1734-8>
- Dheeran, P., Nandhagopul, N., Kumar, S., Jaiswal, Y. K., & Adhikari, D. K. (2012). A novel thermostable xylanase of *Paenibacillus macerans* 11PSP3 isolated from the termite gut. *Journal of Industrial Microbiology and Biotechnology*, 39(1), 1–10. <https://doi.org/10.1007/s10295-011-0999-2>
- Ezeji, T. C., Qureshi, N., & Blaschek, H. P. (2007). Bioproduction of butanol from biomass: From genes to bioreactors. *Current Opinion in Biotechnology*, 18(3), 220–227. <https://doi.org/10.1016/j.copbio.2007.04.002>
- García, V. J., Pääkilä, H. O., Muurinen, E., & Keiski, R. L. (2011). Challenges in biobutanol production: How to improve the efficiency? *Renewable and Sustainable Energy Reviews*, 15(2), 964–980. <https://doi.org/10.1016/j.rser.2010.11.008>
- Gomez, L. D., Steele-King, C. G., & McQueen-Mason, S. J. (2008). Sustainable liquid biofuels from biomass: The writing's on the wall. *New Phytologist*, 178(3), 473–485. <https://doi.org/10.1111/j.1469-8137.2008.02422.x>
- Gregg, D., & Saddler, J. N. (1996). A techno-economic assessment of the pretreatment and fractionation steps of a biomass-to-ethanol process. *Applied Biochemistry and Biotechnology*, 57–58(1), 711–727. <https://doi.org/10.1007/BF02941704>
- Haifeng, S. L., Gang, H. M., & Furong, T. (2015). A biorefining process: Sequential, combinational lignocellulose pretreatment procedure for improving biobutanol production from sugarcane bagasse. *Bioresource Technology*, 187, 149–160. <https://doi.org/10.1016/j.biortech.2015.03.126>
- Huber, G. W., Iborra, S., & Corma, A. (2006). Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chemical Reviews*, 106(9), 4044–4098. <https://doi.org/10.1021/cr068360d>
- Iram, B., Faryal, K., Hania, N., & Nazish, M. (2021). Bioconversion of plant-based biomass into ethanol by using fermentation process. *Journal of Biomaterial and Bioproducts Technology*, 1(2). Retrieved from <http://jbtt.org>
- Jiang, Y. J., Liu, W., Jiang, Y. Y., & Yang, S. (2015). Current status and prospects of industrial bioproduction of n-butanol in China. *Biotechnology Advances*, 33(7), 1493–1501. <https://doi.org/10.1016/j.biotechadv.2014.11.002>
- Kathleen, F. H., Petersen, A. M., Gottumukkala, L., Mandegari, M., Naleli, K., & Görgens, J. F. (2018). Simulation and comparison of processes for biobutanol production from lignocellulose via ABE fermentation. *Biofuels, Bioproducts and Biorefining*, 12(6), 1015–1033. <https://doi.org/10.1002/bbb.1917>
- Kim, S., & Dale, B. E. (2004). Global potential of bioethanol production from wasted crops and crop residues. *Biomass and Bioenergy*, 26(4), 361–375. <https://doi.org/10.1016/j.biombioe.2003.08.002>
- Mahro, B., & Timm, M. (2007). Potential of biowaste from the food industry as a biomass resource. *Engineering in Life Sciences*, 7(5), 457–468. <https://doi.org/10.1002/elsc.200720210>

- Maria, A., Zafar, M., Mushtaq, A., Shazia, S., Shaista, J., & Nabila, N. (2021). Recent trends in biodiesel production: Challenges and advances. *Journal of Biomaterial and Bioproducts Technology*, 1(3). Retrieved from <http://jbtt.org>
- Moretti, R., & Thorson, J. S. (2008). A comparison of sugar indication enables a universal high-throughput sugar-1-phosphate nucleotidyltransferase assay. *Analytical Biochemistry*, 377(2), 251–258. <https://doi.org/10.1016/j.ab.2008.03.001>
- Qureshi, N., & Blaschek, H. P. (2000). Butanol production using *Clostridium beijerinckii* BA101 hyperbutanol producing mutant strain and recovery by pervaporation. *Applied Biochemistry and Biotechnology*, 84–86(1), 225–235. <https://doi.org/10.1385/ABAB:84-86:1-9:225>
- Shields, P., & Cathcart, L. (2010). Oxidase test protocol. *ASM Microbe Library*. <http://www.microbelibrary.org>
- Sluiter, A., Hames, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). Determination of structural carbohydrates and lignin in biomass (Laboratory Analytical Procedure, NREL/TP-510-42618). National Renewable Energy Laboratory.
- Tao, L., He, X., Zhang, M., & Aden, A. (2014). Comparative techno-economic analysis and reviews of n-butanol production from corn grain and corn stover. *Biofuels, Bioproducts and Biorefining*, 8(3), 342–361. <https://doi.org/10.1002/bbb.1462>
- Thomas, V., & Kwong, A. K. (2001). Ethanol as a lead replacement: Phasing out leaded gasoline in Africa. *Energy Policy*, 29(13), 1133–1143. [https://doi.org/10.1016/S0301-4215\(00\)00133-2](https://doi.org/10.1016/S0301-4215(00)00133-2)
- Xue, C., Zhao, J. B., Lu, C. C., Yang, S. T., & Bai, F. W. (2012). High-titer n-butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping. *Biotechnology and Bioengineering*, 109(11), 2746–2756. <https://doi.org/10.1002/bit.24550>
- Zhao, X. Q., Zi, L. H., Bai, F. W., Lin, H. L., Hao, X. M., & Li, X. M. (2012). Bioethanol from lignocellulosic biomass. In X. Q. Zhao (Ed.), *Advances in Biochemical Engineering/Biotechnology* (Vol. 128, pp. 25–51). Springer. [https://doi.org/10.1007/10\\_2012\\_127](https://doi.org/10.1007/10_2012_127)