Journal of Biomaterials and Bio-products Technology
6(3): 2026



Research Article

Application of Fermentation for Production of Bioethanol by Using Organic Waste Material

Iram Batool and Nisha Nawaz
Department of Biotechnology, University of Narowal
nishanawaz772@gmail.com

Corresponding author:Iram06ali@gmail.com

Abstract

Processing waste biomass to create bio-based goods for sustainable development is gaining popularity worldwide. A key element in evaluating the financial viability of bio refineries is the efficient conversion of biomass components for the production of biofuels and value-added bio chemicals. A method for managing agriculture and using other organic wastes to produce alcoholic fuels like bioethanol and biobutanol was created in the current study. Thus, cellulosic materials such as cogon grass, fruit waste, maize stover, and straws from wheat, cotton, and rice were employed in this investigation. For every substrate, a comparison was made between chemical and biological pretreatments. It was determined how well bacterial enzymes scarified agricultural materials. It is determined that these bacterial enzymes can hydrolyze fresh substrates as well as break down agricultural waste. The results of this study are anticipated to contribute to the development of more biofuels and lessen the burden of foreign exchange that is now used to import fossil fuels from other nations.

Key words; Bioethanol, Biofuels, Greenhouse gases, Climatic changes

Received: 2 August 2026 **Accepted**: 22 Sept 2026 **Published**: 30 September 2026
Citation: Batool, I and N. Nawaz (2026). Application of fermentation for production of bioethanol by using organic waste material. 6 (3):106-120
This is an open-access article distributed under the terms of the **Creative Commons Attribution License (CC BY)**.

Graphical Abstract



1: Introduction

Concerns over climate change and energy security have led to a surge in the investigation of alternative energy sources. Due to the usage of fossil fuels, the transportation industry contributes significantly to greenhouse gas emissions; nevertheless, switching to fuels derived from oil, such ethanol, might have positive social and economic effects in addition to reducing environmental consequences (Humbird et al., 2011). Numerous options for producing sustainable biofuels are being researched. Bioelectricity, biogas, biodiesel, and bioalcohols are examples of biological energy sources. Among these sources, bioalcohol has significant promise for lowering greenhouse gas emissions, reducing reliance on fossil fuels, and serving as a chemical feedstock and transportation fuel (Dheeran et al., 2012).

Since many nations are working to cut back on oil imports, bioethanol production has greatly improved, improving air quality and boosting rural economies. 51,000 million liters of ethanol are produced worldwide (Renewable Fuels Association, 2007). Due to its increased oxygen concentration, ethanol has some benefits as a fuel. Better hydrocarbon oxidation is made possible by the increased oxygen content, which also leads to a subsequent decrease in aromatic compounds and the release of carbon monoxide. The octane rating qualities of ethanol are higher (Thomas and Kwong, 2001).

Pakistan is an agriculturally oriented nation, making biomass an essential energy resource. Animal waste and crop residues such as rice husk and sugarcane bagasse make up the biomass generated in the livestock and agricultural sectors (Amiri et al. 2014; Chaudhry et al., 2009). The primary component of second-generation biomass is lignocellulosic material. Cellulose (35–50%), hemicellulose (20–35%), and lignin (5–30%) make up lignocellulosic biomass, which is the most abundant organic material on Earth (Huber et al., 2006).

Different agricultural materials such as green leaves, fruit shells, straws, nut shells, and fruit seeds are examples of renewable energy supplies (Demirbas, 2001). According to Ejezie et al. (2006), the most often utilized feedstocks are apple pomace, maize stover, corn steep liquor, wheat bran, and wheat straw. In contrast to energy crops, which compete with food crops, agricultural waste is now utilized to produce biofuels such as biodiesel, bioethanol, biohydrogen, and methane. Due to the abundance of agricultural waste and its disposal issues, using lignocellulosic biomass is an alternative strategy to lessen the conflict between food and fuel (Mahro and Timm, 2007). Grass is regarded as a dependable material for ethanol extraction. According to Gomez et al.

(2008), the usage of perennial grasses is beneficial and may even lower the cost of producing ethanol and using it as fuel.

It is possible to cultivate Cogon grass *(Imperatacylindrica)* year-round, especially in tropical and subtropical regions. Although cogon grass is considered the worst weed and a pest in over 73 nations across more than 35 crops, it has been used to increase soil stability and as feed. Cogon grass roots contain secondary metabolites that are useful in medicine. Commonly referred to as perennial grass, it may be grown in any soil that is typically deemed unsuitable for crop development.

One potential use for cogon grass is as a raw material for renewable energy sources (Lin and Lee, 2011). The perennial grass *Cynodondactylon*, often known as Coastal Bermuda grass, has a greater cellulosic content and may be utilized to produce ethanol. Because it is either sold for extremely low prices or is typically discarded, coastal Bermuda grass is a great raw material for ethanol production. Because of its greater biomass concentration and ability to convert entire carbs into bioethanol, Bermuda grass has been shown to be the most promising source for ethanol production when compared to corn. The world's tropical and subtropical regions are home to the majority of the Bermuda grass. According to Sun and Cheng (2005), it may grow to a height of 1 to 30 cm and has roots that reach up to 2 meters deep, albeit some of them only reach 60 centimeters below the surface. While certain Bermuda grass species can grow as short as 15 to 20 cm, others can grow as long as 1 m. Bermuda grass grows natively on numerous continents, including Australia, Asia, southern Europe, and North Africa (Sluiter et al., 2008).

Since these are the primary organic wastes in Pakistan, a variety of agricultural waste samples were chosen for the current study, including maize stover, cogon grass, and fruit peels, as well as wheat, cotton, and rice straws. Corn is the third essential cereal after rice and wheat. It grows across 1130,000 hectares and produces 4.695 million tons a year. Cotton is the second most important crop and is grown every year. Rice, the third most significant crop, is grown on 2891 thousand hectares and yields 7005 thousand tons per year (PES, 2014-15). According to several studies, Sanghar, an agricultural area in Pakistan's Sindh province, produces 2.7 million tons of garbage, including rice straw, rice husk, canola straw, wheat straw, cotton stalks, cotton bagasse, and sugarcane remnants.

Nearly 75–85% of these feedstocks are burned up and not used. Therefore, these materials might be utilized to produce energy without affecting domestic resources like food (UNEP, 2011). The best cereal stover and most plentiful animal feed is maize (Zea mays). All sections of maize can be used for various reasons, although in many parts of Pakistan, it is often burnt in fields before the following crop is sown. Otherwise, it can be grazed off (Kim and Dale, 2004). Termites have one of the largest concentrations of microorganisms on the planet.

Termites rely on microorganisms in their digestive system or stomach to break down the complex sugars in wood into simpler ones. According to Kim and Haltzapple (2005), cellulose, a main component in wood, is broken down by bacteria found in termites' guts and ultimately transformed into a variety of chemicals, such as fatty acids and alcohol like ethanol. Soil, water, and the digestive system are home to rod-shaped, gram-positive bacteria belonging to the genus Clostridium. Acetone, butanol, and ethanol are among the organic solvents produced when sugar is fermented by Clostridium acetobutylicum. A common single-celled eukaryotic organism used in fermentation processes to produce ethanol and other alcoholic products is *Saccharomyces cerevisiae*, sometimes referred to as baker's years. In order to evaluate different biomass materials for the extraction and purification of alcoholic solvents that may be used as biofuels, a study was undertaken

2. Materials and Methods

2.1: Collection of Agricultural Substrates

Samples of rice and wheat straws, together with peel debris, were gathered from different locations. Samples weighing approximately 1 kg apiece were gathered in delicate plastic bags. The samples were shaded, sun-dried, and then oven-dried at 55 °C for a whole night. After being ground into a fine powder using an electric grinder, the samples were run through a 40 mesh standard size sieve. Samples in

powder form were kept in refrigerators at 4°C until they were needed again, in little plastic bags that were properly labeled with their names.

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 (AOAC, 1990). By drying the samples at 105 oC to eliminate moisture, the conventional techniques were applied to estimate the total solids and moisture contents (Sluiter, 2005).

2.3: Chemical analysis of raw biomass

The stated method was used to estimate the sample's cellulose content. Calculating the discrepancies between ADF (Acid Detergent Fiber) and NDF (Neutral Detergent Fiber) allowed for the determination of the hemicellulose. According to the AOAC (1990), the lignin contents were ascertained using a conventional procedure.

2.4: Analytical procedures

Using the previously described approach, the fermentation products, such as ethanol, acetone-butanol, monomer sugars (hexoses and pentoses), and their bioproducts, were identified (Haifeng et al., 2015).

2.5: Chemical Pretreatment

Two chemicals, such as acid (H2SO4) and alkali (NaOH), were utilized for chemical treatments. Using H2SO4 and NaOH (1.0, 1.5, and 2%) at various temperatures (100, 110, and 120 degrees Celsius) and for varying amounts of time (15, 30, and 45 minutes), a pretreatment experiment was conducted. During the experiment, a 10% (w/v) solid sample was used in a reagent bottle. Following pretreatment, the sample was filtered in each container using a vacuum filtration assembly, and the contents were poured onto filter paper. Following filtering, 300 milliliters of distilled water were used to wash away the solid in order to balance the pH. After drying at 105 °C, the filter paper was weighed.

2.6: Enzymatic Hydrolysis

In a water bath shaker with 0.05 M buffer (sodium citrate) at 4.8 pH, the biomass was hydrolyzed with cellulose and β-glucosidases for 72 hours at 50 °C and 160 rpm following a 5% (w/v) pretreatment. Cellulases with 30FPU g-1 activity. To find the sugar content, samples were taken out of the reagent vial every twelve hours. Enzymatic hydrolysis was followed by the addition of H2SO4 (µl). Centrifuging the un-hydrolyzed material at 13,500g for 10 minutes was used to separate it. Syringe filters were used to collect the supernatant for the dinitrosalicylic acid (DNS) technique of sugar determination. The p-hydroxybenzoic acid hydrazide (PAHBAH) technique was used to determine the quantity of sugar.

The standard curve was created using xylose concentrations ranging from 1 mM to 25 mM. The quantity of sugar in the pretreatment sample was then calculated by comparing the standard sugar concentration. Following the enzymatic hydrolysis procedure, the optimal pretreatment condition was chosen. For the fermentation procedure, the sample with the highest amount of liberated sugar was chosen. After being held at 4 °C, the solid biomass was utilized for the fermentation process. YPD (yeast extract 1% (w/v), peptone 2% (w/v), and glucose 2% (w/v)) agar medium was used to keep the *Saccharomyces cerevisiae* strain at 4°C. In accordance with (Alfenoro, 2002), yeast cells were cultured in a 5-mL tube of YPD media containing NaCl 0.9% (w/v) at 30°C for 16 hours on a rotating shaker (100 r.p.m.).

2.7: Separate Hydrolysis and Fermentation

C. thermocellum was cultivated in a broth medium containing glucose yeast extract for 48 hours in order to do the fermentation experiment. A 10% inoculum was then added to 50 mL of fermentation medium that included the previously saccharified solution, and the mixture was allowed to sit at room temperature for three days (Jiang et al., 2015). The fermentation experiment was conducted in anaerobic conditions for 72 hours at 50°C and 120 rpm. Based on the boiling point, the fractional distillation procedure in a fractional distillation apparatus eliminated the methanol, butanol, ethanol, and acetone from

the mixture that was left over after the fermentation reaction was finished. Butanol may be separated after condensing because it has a higher boiling point (118 °C) than water (100 °C). Ethanol can condense quicker than water because it has a lower boiling point (78.3 °C) than water (Kathleem et al., 2018).

2.8: Statistical analysis

The mean and standard deviation of the data produced by different analyses were statistically examined.

3. Result and discussion

The next sections present the findings from the chemical examination of biomass samples, the isolation of bacteria, and the fermentation of carbohydrates into acetone, butanol, and ethanol. Termites are thought to be a good source of several beneficial bacteria isolates with industrial uses. It has been discovered that these isolates have a strong potential for turning different sugars into alcoholic products. Thus, termite-based bacterial isolates were used in the current investigation to manufacture acetone-butanol-ethanol (ABE) from organic waste materials from urban and agricultural sources (Tables 1-15 and Figures 1-5).

3.1: Biological Pretreatment

The amount of sugar released by the various bacterial strains is shown in Table 3. In comparison to all other substrates examined, it was shown that bacterial isolates 9x (xylanase enzyme) produced a greater amount of sugar (27.84+0.48mM/l) from wheat straw (Table 3).

Table1.Chemical pretreatment of biomass samples with different concentrations (%) of NaOH and H_2SO_4,to release of sugars (%).

Substrates	Chemicals					
	H_2SO_4 concentration			NaOH concentration		
	1%	2%	3%	1%	2%	3%
Wheat straw	15.38±1.24	19.74±1.25	6.38±0.86	14.71±0.46	15.95±0.08	16.85±0.15
Corn stover	14.57± 0.18	13.73±1.12	4.27±0.81	13.69±0.46	13.65±0.08	13.82±0.14
Cotton stalk	1.53±0.04	1.35±0.05	0.28±0.02	0.86±0.06	0.87±0.11	0.97 ±0.01
Rice straw	16.85±0.15	15.38±0.17	3.44±0.15	15.07±0.17	14.32±0.28	13.39±0.59

Table 2. Various fermentation products (mM/l) obtained from biomass samples

Bacterial Isolates	Corn stover				Wheat straw			
	Acetate	Formate	Lactate	Ethanol	Acetate	Formate	Lactate	Ethanol
Isolate 9x	1.15±0.06	_	1.41±0.18	5.73±0.28.0	3.04±0.65	_	1.65±0.79	3.34±0.41
Isolate 10x	1.28±0.14	_	3.44±0.34	6.98±0.58	1.55±0.28	1.24±0.17	6.14±0.55.0	5.99±0.26.0
Isolate 31x	1.29±0.34	1.98±0.39	8.57±0.59	9.21±0.54.0	1.72±0.07.0	1.63±0.28.0	3.58 ±0.26	6.43±0.49

3.2: Various Fermentation products Biomass analysis

A variety of parameters included in biomass samples are represented by the data in Table 6. On the other hand, table 7 lists the samples' ligno-cellulosic compositions. Cogon grass was found to contain more cellulosic material than other substrates utilized in the investigation

Table 3:Proximate analysis (%) of biomass samples

Substrate	Dry Matter	Moisture	Crude Protein	Crude Ash	Crude Fiber
Peel(wastes)	92.41±0.48	7.53±0.34	7.93 ±0.23	5.91± 0.45	33.87±0.33
Cogon grass	93.11±0.27	6.89±0.26	5.12 ±0.21	9.18±0.34	35.41±0.42

Table 4. Chemical analysis (%) of biomass samples

Substrate	NDF	ADF	Hemicellulose	Cellulose	Lignin
Peel(Fruit wastes)	79.6±0.51	52.1±0.31	26.3±0.34	29.6±0.67	21.5±0.43
Cogon grass	82.06±0.72	48.41±0.42	29.6±0.52	34.2±0.83	15.32±0.25

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

Table 5; Recovery of solid mass (%) due to treatment with Acid under various conditions.

Pretreatment conditions.		Total solid recovery (g/100g dry biomass)	
Time (min.)	H_2SO_4 Conc.	Peel (wastes)	Cogon grass
15	1.0	63.52±0.20	76.86±0.61
	1.5	62.02±0.13	74.24±0.43
	2.0	57.11±0.34	70.33±0.25
30	1.0	56.61±0.43	68.86±0.48
	1.5	56.07±0.22	67.10±0.35
	2.0	52.37±0.20	64.76±0.24
45	1.0	53.07±0.32	64.62±0.42
	1.5	52.11±0.51	64.02±0.36
	2.0	51.23±0.44	63.50±0.56

Dilute H_2SO_4 pretreatment

The samples of the different biomass were pretreated with diluted acid at concentrations of 1, 1.5, and 2%. They were then autoclaved for 15, 30, and 45 minutes at 105, 120, and 135 degrees Celsius. The ideal conditions for the enzymatic experiment were 120 °C for both samples and 15 minutes for peel wastes and 30 minutes for cogon grass at concentrations of 1.5% and 1%, respectively (Figures 1-5).

3.3: Fermentation

As seen in Table 9, cogon grass yields 10% ethanol whereas peel waste yields 7.4%. The concentration of glucose decreased with increasing time, whereas the concentration of ethanol increased, but only for a limited amount of time. But after 72 hours, the concentration of glucose was insufficient to sustain the manufacture of ethanol. When cogon grass's higher cellulosic but lower lignin levels were compared to peel wastes, it was discovered that the former was a better option for producing ethanol.

Table 6. Ethanol production from Cogon grass and peel wastes samples

Sample	Ethanol Production (% v/w)
Cogon Grass	10.5
Peel wastes	7.4

3.4: Dilute sulfuric acid pretreatment of Peel wastes

Peel waste had a glucose content of 27.33±2.15%. Following therapy, the glucose concentration rose (Figures 10-11). After 30 minutes of treatment with a diluted acid concentration of 1.8% and a reaction temperature of 110 °C, the solid portion of the samples produced a greater amount of glucose. It was demonstrated that a moderate temperature and a high concentration of acid are essential for increasing the amount of glucose present during pretreatment. Talo and colleagues (2014) obtained similar results on the hydrolysis of orange peel by acid at low temperatures. Percentage reduction in xylose concentration following H_2SO_4 pretreatment in the solid component of peel wastes. The amount of xylose in peel wastes was 15.37%±1.13, and it was found to have decreased following pretreatment. The best conditions for achieving the least

amount of xylose in the solid fraction were determined to be an acid concentration of 1.10 percent and an incubation temperature of 110 °C for 30 minutes. Hemicellulose may be completely removed with diluted acid pretreatment (Sun and Cheng, 2005). According to Wyman et al. (2005), xylose solubilization reaches its peak at moderate temperatures.

3.5: Percentage rise in the solid fraction's lignin concentration following H2SO4 pretreatment

A contour map (Figure 1a,b) further shows that the smallest amount of lignin in the solid fraction may be obtained with an optimal acid dosage of 2.0% for 37 minutes at 125 °C. Despite the reaction's apparent 7.32% rise in lignin content, the actual values were in decreasing order. The significant elimination of xylose during H2SO4 pretreatment was the cause of the apparent rise in lignin concentration. The solubilization of lignin increases with increasing reaction time to determine the maximum value if the temperature remains constant. Although cellobiose was present in the reaction mixture, there was no sugar buildup, which is why a greater saccharification rate of 80.54 percent was attained. According to Xue et al. (2012), the absence of cellubioses actually improved the efficacy of celllases, which leads to a larger sugar recovery following enzymatic hydrolysis.

Table 7; Sugars and other products (%) obtained from grasses

Substrate	Glucose	Xylose	Lignin	Dry matter	Moisture	Ash
Cogon grass	32.36. ±1.14	18.85 ±1.18	6.93 ±0.44	90.33±1.85	9.67±0.54	5.77±0.46
Peal wastes	27.32. ±2.15	15.37±1.13	4.75±0.54	92.46 ±1.24	8.56 ±0.55	4.89 ±0.58

Table 8; Maximum Sugar yields after enzymatic hydrolysis of substrates at pH 4.8, 50 °C, 120 rpm.

Substrate	GlucoseSF (2.5g/50mL)	GlucoseSF (50g/L)	Glucose(g/L)	Ratesac (%)	TimeOPT (hours)
Cogon grass	1.10	22.00	17.72	80.54	72
Peal wastes	0.98	18.5	14.7	78.35	72

* SF = Solid fraction Y = Yield Sac = saccharification Opt = optimum

Table 9 .Chemical analysis of various crops samples

Parameters	Cotton stalks	Corn stove
Moisture contents	6.5	7.0
Volatile Matter	77.0	75.0
Fixed Carbon	9.5	19.5
Ash contents	8.7	6.0
Crude Fiber	31.0	32.0
Ether extract	1.8	2.5
Crude Protein	4.2	3.8
Cellulose	34.5	33.6
Hemicellulose	29.5	32.5
Lignin	14.8	18.5

3.6: Analysis of Sugar after Pretreatment and Enzymatic Hydrolysis

In this investigation, sugar was produced by enzymatic hydrolysis of three distinct substrates corn stover, wheat straw, and rice straw—in a 500 mL Erlenmyer flask at 50 °C for three days. In order to produce sugar, lignocellulose must be pretreated in order to break down lignin and improve the accessibility of enzymes and microorganisms to carbs. On these substrates, two different kinds of pretreatment techniques have been used. To minimize the size of the particles, these substrates were first crushed into a fine powder and then run through an 80 mesh filter as part of the physical pretreatment process. Following that, these substrates underwent a chemical processing. Acidic and basic pretreatment (chemical

pretreatment) conditions were used to break the structure of lignocellulosic biomass.

3.7: Dilute Acid Pretreatment

Samples of agricultural waste were pretreated using varying amounts of sulfuric acid H2SO4. Samples were cooked in an autoclave to 100, 110, and 120 °C after being prepared with a solid charge of 20% (w/v) slurry. Three distinct sulfuric acid concentrations—0.5, 1, and 1.5%—and retention times of 10, 15, and 20 minutes were used to conduct the reaction. Three distinct sulfuric acid concentrations were used to pretreat the substrate at each temperature. At the same temperature and reaction time, the sample was pretreated three times. Three samples underwent a total of nine pretreatments in 100ml reagent bottles. To determine the ideal conditions for acidic pretreatment, 81 treatments of three samples were carried out at three different temperatures in a total of nine tests.

3.8: Dilute Alkali Pretreatment

Agrowaste samples were pretreated with diluted alkali. To optimize the conditions that might provide the highest amount of glucose, several concentrations of sodium hydroxide (NaOH) were utilized at varying temperatures and retention times. The samples were autoclaved for 10, 15, and 20 minutes at 100, 110, and 120 °C after being prepared with a solid charge of 20% w/v slurry. 0.5%, 1%, and 1.5% sodium hydroxide concentrations were utilized to pretreat the material. In order to determine the ideal conditions for basic pretreatment, a total of nine tests (9× 9 = 81) were conducted, resulting in 81 treatments of three samples at three different temperatures. Spectrophotometric analysis and Comparison of sugar production in three agrowaste samples Following 72 hours of enzymatic hydrolysis, wheat straw produced higher glucose yields in each of the nine studies. During the experiment, it was found that the amount of sugar grew together with the H2SO4 concentration, which went from 0.5 to 1.5%. A greater yield of glucose was also seen in all studies at a retention duration of 20 minutes as opposed to 10 and 15 minutes. The ideal conditions for the acidic pretreatment of wheat straw were 120 °C, 20 minutes of retention, and 1.5% sulfuric acid. The glucose levels peaked at this level. By raising the temperature during the alkali pretreatment conditions, a greater yield of glucose was observed at 120 °C. Meanwhile, using a 1.5% concentration of NaOH at the same temperature produced a significant yield of glucose. The sugar production rose when the enzymatic hydrolysis time was extended from 0 to 48 hours; however, the sugar concentration decreased when the duration was extended to 72 hours. The formation of inhibitors by increased acid concentrations was most likely the cause of the drop in glucose concentration. At 120°C, maximum reducing sugars (7.73 g/L) were achieved with a 1% NaOH concentration and a 15-minute reaction period. When acidic pretreatment conditions were applied, such as 110 °C, 1.5% acid concentration, and a 10-minute retention period, rice straw demonstrated a greater glucose output. When alkaline pretreatment is used, the ideal conditions for rice straw analysis are temperature (100 °C), sodium hydroxide concentration (0.5%), and retention period (20 minutes). Following 72 hours of enzymatic hydrolysis, a higher yield was achieved. A high yield of glucose was achieved during the acidic pretreatment at a temperature of 120°C, a concentration of 1.5% H2SO4, and a reaction duration of 15 minutes. During alkaline pretreatment, the ideal conditions for maize stover were 100°C, 1.5% sodium hydroxide concentration, and 20 minutes of retention time.

3.9:HPLC Analysis of Enzymatic Hydrolysate

Using HPLC, the enzymatically hydrolyzed samples of the acidic and alkaline pretreatment of maize stover, rice straws, and wheat straws were further examined. The samples with greater glucose levels under ideal circumstances were chosen for examination in this regard. After being extracted at various points throughout the enzymatic hydrolysis process, the samples were centrifuged for 15 minutes at 14,000 rpm at 4 °C. After the supernatant was separated, a 0.22 µm syringe filter was used to filter it. A 500 µl aliquot of the sample was diluted with 1 ml of methanol to bring the sample concentrations within the calibration curve's range. The sugars' solubility led to the usage of methanol. Before analysis, all samples and the glucose standard solution were run through a 0.22 µm filter. Approximately 20 µl of the agrowaste sample was introduced into the HPLC system via an injection loop. Enzymatically hydrolyzed samples were processed in the gradient mode for ten minutes in order to measure the glucose (Shields and Cathcart, 2010).

The retention time (tR) is used to identify the peak. Using a recognized standard injected using HPLC, the identification of glucose in three samples—wheat straw, rice straw, and maize stover was verified. At a retention time of 3.255 minutes, a single, noticeable peak was seen (Table 13-16 and Figures 1-6).Table 10; Analysis of wheat and rice straws samples for sugars with HPLC Using HPLC, the enzymatically hydrolyzed samples of the acidic and alkaline pretreatment of maize stover, rice straws, and wheat straws were further examined. The samples with greater glucose levels under ideal circumstances were chosen for examination in this regard. After being extracted at various points throughout the enzymatic hydrolysis process, the samples were centrifuged for 15 minutes at 14,000 rpm at 4 °C. After the supernatant was separated, a 0.22 µm syringe filter was used to filter it. A 500 µl aliquot of the sample was diluted with 1 ml of methanol to bring the sample concentrations within the calibration curve's range. The sugars' solubility led to the usage of methanol. Before analysis, all samples and the glucose standard solution were run through a 0.22 µm filter. Approximately 20 µl of the agrowaste sample was introduced into the HPLC system via an injection loop. Enzymatically hydrolyzed samples were processed in the gradient mode for ten minutes in order to measure the glucose (Shields and Cathcart, 2010).

The retention time (tR) is used to identify the peak. Using a recognized standard injected using HPLC, the identification of glucose in three samples—wheat straw, rice straw, and maize stover—was verified. At a retention time of 3.255 minutes, a single, noticeable peak was seen (Table 13-16 and Figures 1-6).Table 10; Analysis of wheat and rice straws samples for sugars with HPLC

Table 10. HPLC analysis of sugars and degradation products in rice straw and wheat straw hydrolysates.

Components	Retention time (min)	Conc. (mg/ml) Rice straw	Conc. (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

3.8: Fermentation with*Clostridium acetobutylicum*

ABE (acetone, butanol, and ethanol) fermentation is the main byproduct of this kind of fermentation. According to previous reports by several authors, the ratio of acetone, butanol, and ethanol throughout the fermentation process is around 3:6:1. According to estimates, *Clostridium acetobutylicum* produces more butanol under acidic pretreatment conditions than under alkaline ones. However, because there is very little probability of fermentation inhibitors being produced, alkaline pretreatment conditions are ideal for butanol synthesis. However, the low amount of glucose collected in this experiment under alkaline circumstances may be the cause of the poor butanol synthesis. A variety of variables, such as the higher quantity of carbohydrates in wheat straw compared to rice straw and maize stover, contributed to the higher butanol output among the three substrates. The greater yield of butanol and glucose compared to the other two substrates is caused by the low lignin concentration of wheat straw. Compared to maize stover and rice straw, wheat straw has a lower lignin level. Wheat straw may produce more butanol since it has less lignin. Under acidic pretreatment conditions, wheat straw produces the most butanol of the three substrates. Previous research estimated that the hydrolysate of wheat straw included furfural andhydoxymethyl furfural, which aided in the fermentation-based generation of biobutanol. The conclusion is that wheat straw is an excellent substrate for fermentation, most likely due to the presence of fermentation-stimulating compounds.

Table 10. HPLC analysis of sugars and degradation products in rice straw and wheat straw hydrolysates.

Component	Retention time min)	R. straw (mg/mL)	W.straw(mg/mL)
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	28.0	1.2	2.84

Table 12; Chemical analysis of straws samples

Samples	Cellulose %	Hemicel-lulose%	Lignin%
orn stover	30	21	7
heat straw	40	25	13
ice straw	35	22	20

3.9: *Clostridium acetobutylicum* function for butanol

The species of Clostridium The temperature of Clostridium acetobutylicum was maintained at -20°C. The sample that had been hydrolyzed by enzymes was then utilized for fermentation. NaOH was used to keep the pH at 6.5. For independent hydrolysis and fermentation, 1 milliliter of C. acetobutylicum spores was introduced to 100 milliliters of enzymatically hydrolyzed solution in a reaction container. For 72 hours, these reaction bottles were kept at 37°C in a shaking incubator with a rotation speed of 120 rpm. After 72 hours, the butanol concentration was measured using an alcohol meter, and the results were reported as the percentage of butanol in corn stover, rice straw, and wheat straw. Wheat straw yielded the highest amount of butanol among all three substrates (Tables 13–16).

But during growth and acetone-butanol-ethanol (ABE) fermentation, Clostridium species may metabolize glucose and xylose, the primary end products of acidic/alkaline pretreatments and enzymatic hydrolysis (Qureshi and Blaschek, 2000; Moretti and Thorson, 2008). ABE fermentation typically uses two important microbes: C. acetobutylicum and C. beijerinckii. To generate ABE, however, researchers from several nations have recently examined a variety of strains, their parent microbes, culture conditions, and growth media for a range of biomass samples utilized in batch, fed batch, and continuous fermenters. Tables 13–16

Table 13; Acetone, Butanol and Ethanol production (%) from various agrowaste by Clostridium acetobutylicum

Samples	Ace-tone%	Butanol%	Etha-nol%
Wheat straw	1.5	6.3	1.8
Rice straw	1.2	6.2	1.3
Corn stover	1.0	5.2	1.1

ABE production from Biomass samples

Table 14; 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
Corn stover	1.1	4.5	2.3

ABE production from Biomass samples

Table 15; 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
Corn stover	1.3	4.2	2.2

ABE production from Biomass samples

DISCUSSION

Saccharification, fermentation, and acid or alkali pretreatment are some of the technical processes needed to produce alcoholic fuels (butanol and ethanol) from lignocellulosic feedstock. It is crucial to properly modify every system unit in order to achieve cost-effective biofuel production. By improving various processes such as pretreatment, enzymatic hydrolysis, fermentation, and increased ethanol recovery, some nations have in the past greatly increased the production of alcoholic fuels (Zhao, 2012). The well-known examples of biomass-based fuel generation in wealthy nations might serve as useful models for underdeveloped nations. Furthermore, a number of innovative concepts have been studied for the manufacture of ethanol, including the biorefinery and the notion of directed conversion of categorized content. Similar technologies may also be used to produce butanol from lignocellulosic biomass (Demirbas, 2009; García et al., 2011). When fuels are produced on an industrial scale, their cost may drop even further. When these techniques are combined, competitive biofuel production from plant biomass—which is currently

underutilized—will be achieved (Talo et al., 2014).

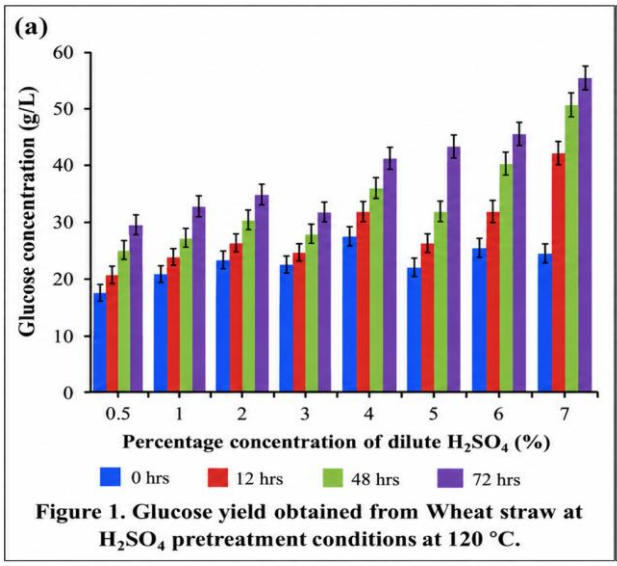


Figure 1. Glucose yield obtained from Wheat straw at H_2SO_4 pretreatment conditions at 120 °C.

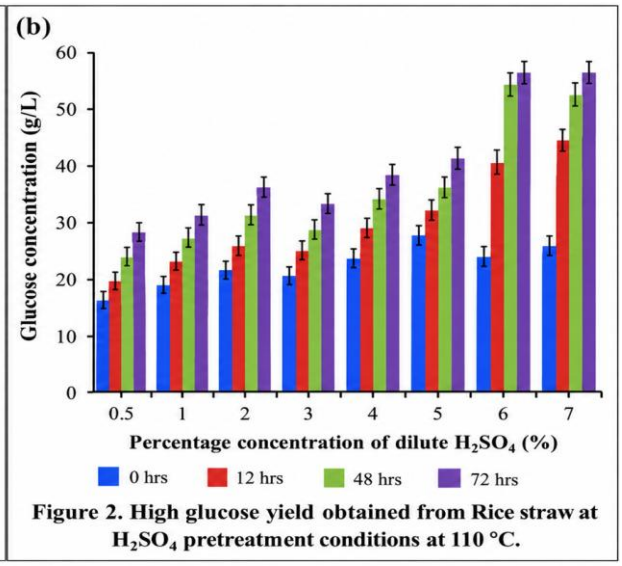


Figure 2. High glucose yield obtained from Rice straw at H_2SO_4 pretreatment conditions at 110 °C.

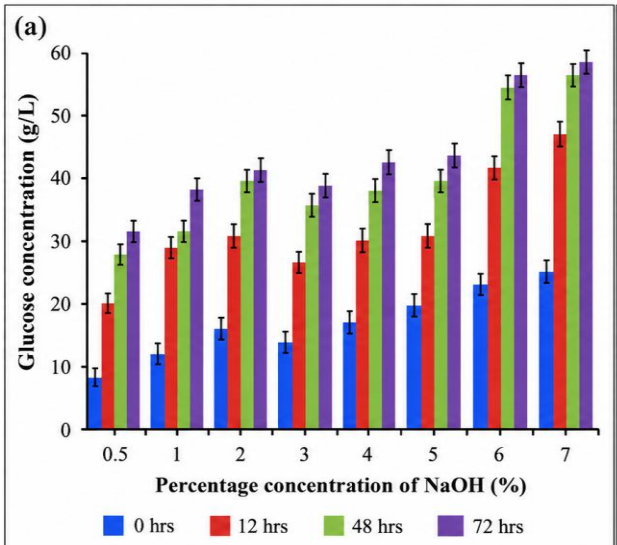


Figure 3. High glucose yield obtained from Wheat straw by NaOH pretreatment conditions at 120 °C.

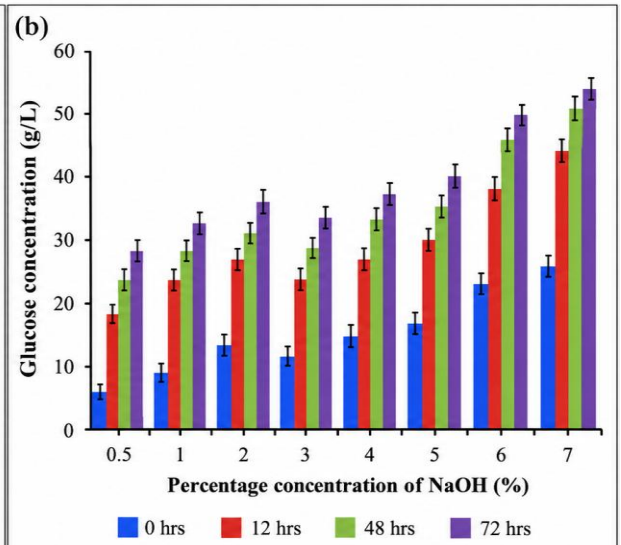


Figure 4. Glucose yield obtained from Rice straw at NaOH pretreatment conditions at 100 °C.

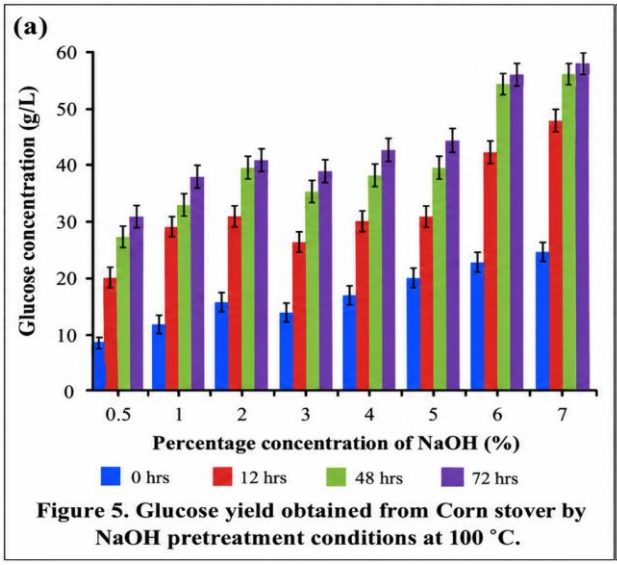


Figure 5. Glucose yield obtained from Corn stover by NaOH pretreatment conditions at 100 °C.

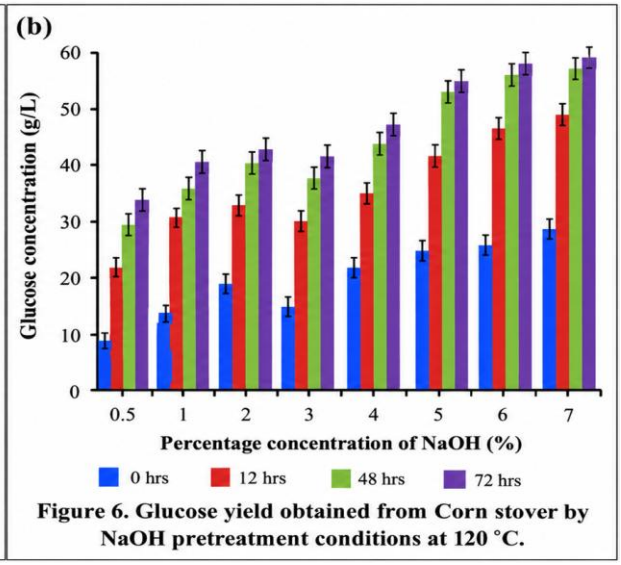


Figure 6. Glucose yield obtained from Corn stover by NaOH pretreatment conditions at 120 °C.

Important products including acetone, butanol, ethanol, and other alcohols that may be utilized as liquid fuels could be produced by fermenting the abundant sugars in cellulosic biomass. Wood wastes, agricultural crops including wheat, rice, and cotton straws, maize covers, sorghum straws, fruit and vegetable wastes, and similar substrates are the most readily available sources of biomass that include carbs. Cellulose is a complex sugar found in plant materials and is thought to be a primary sugar used in the synthesis of alcohol (fuel). Enzymatic hydrolysis, bacterial/fungal fermentation, and acid treatment all aid in the breakdown of this complex cellulose substance into smaller pieces. These types of alcohol are significant because they might be used as fuel. As a result, biofuels might assist (1) fight climate change by lowering the amount of carbon emissions released by vehicles, etc. (2). Biofuel can meet the increasing demand for energy and fossil fuels (3). It also secures the energy supply by addressing the developing global fuel issues (4). Biofuels are a great example of a circular economy solution since they reduce waste and use natural resources. In the current work, bioethanol and biobutanol were produced using a variety of cellulosic materials. Thus, several orders of alcoholic fuels were produced from cellulosic substrates. Straws have produced superior yields of alcoholic fuels as compared to other materials employed as biomass substrates. However, the kind of cellulosic biomass utilized and the different distillation processes carried out after fermentation to purify these types of alcohols determine how much acetone, butanol, and ethanol are generated. By 2050, the nation's energy needs are predicted to triple, but the supply situation is unimpressive. The ideal substitute for traditional fuels and energy sources is renewable and sustainable energy resources because of comparable circumstances. A cost-effective and sustainable method is the bioconversion of lignocellulosic biomass into alcoholic fuels (ethanol and butanol). However, ongoing efforts are required to create more cost-effective and efficient fermentation methods as well as to get a thorough grasp of the principles of various pretreatment procedures. Furthermore, more effective microbial strains are needed to achieve cost-effective detoxification.

5. Conclusion

Currently accessible fossil fuels that are already depleting are replaced by integration and optimization processes that lower energy consumption and boost yields. As a result, researchers worldwide are looking at many affordable approaches for alternative energy sources, particularly those that use cellulosic biomass. These kinds of studies are anticipated to play a significant role in the nation's future growth through the utilization of domestic resources.

Author contribution: Conceptualization IB, Methodology NN, Software IB, Formal analysis IB, Investigation IB, Data curation NN, Writing original draft NN, writing review and editing NN and IB

Funding: No funding was received for this project

Declaration: it is to declare that AI is used to generate graphical abstract

Conflict of Interest: Authors declare no conflict of interest.

Reference:

Amiri, H., Karimi, K., and 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.2013.11.038

AOAC International. (1990). *Official methods of analysis of the Association of Official Analytical Chemists* (15th ed., Method 920.85, p. 780). Association of Official Analytical Chemists.

Becerra, M., Cerdán, M. E., and González-Siso, M. I. (2015). Biobutanol from cheese whey. Microbial Cell Factories, 14, Article 27. https://doi.org/10.1186/s12934-015-0200-1

Chaudhry, A., Raza, R., and Hayat, S. A. (2009). Renewable energy technologies in Pakistan: Prospects and challenges. Renewable and Sustainable Energy Reviews, 13(6–7), 1657–1662. https://doi.org/10.1016/j.rser.2008.09.025

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)00137-0

Demirbas, A. (2009). Biorefineries: Current activities and future developments. *Energy Conversion and Management, 50*(11), 2782–2801. https://www.sciencedirect.com/science/article/pii/S0196890409002519

Dheeran, P., Nandhagopal, N., Kumar, S., Jaiswal, Y. K., and Adhikari, D. K. (2012). A novel thermostable xylanase of *Paenibacillus macerans* IIPSP3 isolated from the termite gut. *Journal of Industrial Microbiology and Biotechnology, 39*(6), 851–860. https://doi.org/10.1007/s10295-012-1093-1

Ezeji, T. C., Qureshi, N., and 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., Päkkilä, J., Ojamo, H., Muurinen, E., and 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., and McQueen-Mason, S. J. (2008). Sustainable liquid biofuels from biomass: The writing's on the walls. New Phytologist, 178(3), 473–485. https://doi.org/10.1111/j.1469-8137.2008.02422.x

Su, H., Liu, G., He, M., and Tan, F. (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.2015.03.107

Huber, G. W., Iborra, S., and 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

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D. D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., and Dudgeon, D. (2011). *Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: Dilute-acid pretreatment and enzymatic hydrolysis of corn stover* (NREL/TP-5100-47764). National Renewable Energy Laboratory. https://doi.org/10.2172/1013269

Jiang, Y., Liu, J., Jiang, W., Yang, Y., and 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.2014.10.007

Haigh, K. F., Petersen, A. M., Gottumukkala, L., Mandegari, M., Naleli, K., and Görgens, J. F. (2018). Simulation and comparison of processes for biobutanol production from lignocellulose via ABE fermentation. *Biofuels, Bioproducts and Biorefining, 12*(6), 1023–1036. https://doi.org/10.1002/bbb.1917

Kim, S., and Dale, B. E. (2004). Global potential 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

Kim, S., and Holtzapple, M. T. (2005). Lime pretreatment and enzymatic hydrolysis of corn stover. Bioresource Technology, 96(18), 1994–2006. https://doi.org/10.1016/j.biortech.2005.01.014

Lin, Y.-S., and Lee, W.-C. (2011). Simultaneous saccharification and fermentation of alkali-pretreated cogongrass for bioethanol production. *BioResources, 6*(3), 2744–2756. https://bioresources.cnr.ncsu.edu/resources/simultaneous-saccharification-and-fermentation-of-alkali-pretreated-cogongrass-for-bioethanol-production/

Mahro, B., and 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.200620206

Moretti, R., and Thorson, J. S. (2008). A comparison of sugar indicators enables a universal high-throughput sugar-1-phosphate nucleotidyltransferase assay. *Analytical Biochemistry, 377*(2), 251–258. https://doi.org/10.1016/j.ab.2008.03.018

Pakistan, Ministry of Finance. (2015). *Pakistan Economic Survey 2014–15*. Government of Pakistan. https://www.finance.gov.pk/

Qureshi, N., and Blaschek, H. P. (2000). Butanol production using Clostridium beijerinckii BA101, a hyper-butanol-producing mutant strain, and recovery by pervaporation. In Applied biochemistry and biotechnology (pp. 225–235). Humana Press. https://doi.org/10.1007/978-1-4612-1392-5_16

Shields, P., and Cathcart, L. (2010). *Oxidase test protocol*. American Society for Microbiology. https://asm.org/protocols/oxidase-test-protocol

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). Determination of structural carbohydrates and lignin in biomass (NREL/TP-510-42618). National Renewable Energy Laboratory.

Sun, Y., and Cheng, J. J. (2005). Dilute acid pretreatment of rye straw and bermudagrass for ethanol production. *Bioresource Technology*, *96*(14), 1599–1606. https://doi.org/10.1016/j.biortech.2004.12.022

Tokuda, G., and Watanabe, H. (2007). Hidden cellulases in termites: Revision of an old hypothesis. *Biology Letters*, *3*(3), 336–339. https://doi.org/10.1098/rsbl.2007.0073

Tao, L., Tan, E. C. D., McCormick, R., Zhang, M., Aden, A., He, X., and Zigler, B. T. (2014). Techno-economic analysis and life-cycle assessment of cellulosic isobutanol and comparison with cellulosic ethanol and *n*-butanol. *Biofuels, Bioproducts and Biorefining*, *8*(1), 30–48. https://doi.org/10.1002/bbb.1431

Tao, L., He, X., Tan, E. C. D., Zhang, M., and 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., and Kwong, A. (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(01)00041-6

United Nations Environment Programme. (2011). *A project to make clean energy a reality for households in a rural region of Pakistan*. https://www.unep.org/

Xue, C., Zhao, J. B., Lu, C. C., Yang, S. T., and 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.24563

Zhao, X. Q., Bai, F. W., Lin, H. L., Hao, X. M., and others. (2012). Bioethanol from lignocellulosic biomass. In Advances in biochemical engineering/biotechnology (Vol. 128, pp. 25–51). Springer. https://doi.org/10.1007/10_2011_129