USES OF BIOMASS FOR EXTRACTION OF ENVIRONMENTALLY FRIENDLY ALCOHOLIC FUEL

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ABSTRAT

Researchers set out to find a way to manage agricultural and other organic wastes so that they may be turned into alcoholic fuels like bioethanol and bio butanol. Consequently, this research made use of cellulosic materials such as straws from wheat, cotton, and rice, as well as maize stover, fruit waste, and cogon grass. Every substrate was subjected to a comparison of chemical and biological pretreatments. Bacterial enzymes were tested for their efficiency in saccharifying agricultural substrates. This research concludes that these bacterial enzymes may hydrolyze agricultural waste in addition to pure substrates. This research is anticipated to contribute to the growth of biofuel production while simultaneously decreasing the reliance on foreign currency for fossil fuel imports.

Key words; Bioethanol, Biomass, Green house gases, Climatic changes

Introduction

Worries about energy security and global warming have prompted a surge in research into potential alternative energy sources. Use of fossil fuels in transportation is a major contributor to greenhouse gas emissions; however, ethanol and other oil-derived fuel alternatives might lessen these effects while also providing social and economic benefits (Humbird et al., 2011). Diverse options for producing environmentally friendly biofuels are now under study. Biodiesel, bioalcohols, bioelectricity, and biogases are examples of biological energy resources. According to Dhamole et al. (2015), bioalcohol has the ability to lessen our reliance on fossil fuels, lower emissions of greenhouse gases,

and serve as both a chemical feedstock and a transportation fuel.As a result of several nations' efforts to lessen their reliance on oil imports, enhance air quality, and boost rural economies, bioethanol production has seen tremendous improvements. According to the Renewable Fuels Association (2007), the world's ethanol output stands at 51,000 million liters. The greater oxygen concentration of ethanol gives it certain fuel benefits. With more oxygen in the air, hydrocarbons may be oxidized more efficiently, leading to a decrease in aromatic compounds and emissions of carbon monoxide. Thomas and Kwong (2001) found that ethanol has better octane rating attributes.

Pakistan is mostly an agricultural nation, making biomass a crucial energy resource for the country. Animal manure, crop remnants such as sugarcane bagasse and rice husk, and other biomass generated by the livestock and agricultural sectors (Amiri et al., 2014; Chaudhry et al., 2009). The majority of second-generation biomass comes from lignocellulosic materials, which are the most abundant organic substances on Earth. These materials include cellulose, hemicellulose, and lignin, with proportions of 35–50%, 20–35%, and 5–30%, respectively (Huber et al., 2006).Demirbas (2001) lists a variety of agricultural materials that may be used as renewable energy sources, including straws, green leaves, fruit shells, nut shells, and fruit seeds. Ejeziet al. (2006) lists maize stover, apple pomace, wheat straw, bran, and corn steep liquor as the most popular feedstocks.Instead of growing energy crops, which compete with food crops, agricultural waste is used to produce biofuels such as biodiesel, bioethanol, biohydrogen, and methane. Using lignocellulosic biomass as an alternative to dispose of the large amounts of agrowaste that are now accessible is one way to lessen the food-fuel conflict (Mahro and Timm, 2007). The grasses are regarded as a dependable material for ethanol extraction. The usage of long-lived grasses has several benefits, one of which is the potential reduction in production and fuel costs associated with ethanol (Gomez et al., 2008).

Cogon grass, scientifically known as Imperatacylindrica, is a year-round crop that thrives in both tropical and subtropical regions of the globe. Although it has its uses (such as fodder and improving soil stability), cogon grass is considered a pest in 35 different crops in over seventy-three nations and the worst weed in the world. There are

medicinally significant secondary metabolites in the roots of the cogon grass plant. It is possible to grow this kind of grass, which is known as perennial grass, on soil that is otherwise unsuitable for farming. Cogon grass has the potential to be a renewable energy source's raw material (Lin and Lee, 2011).Coastal Bermuda grass, or Cynodondactylon, is a perennial grass with a high cellulosic content and potential for ethanol generation. Due to its low selling price or high rate of waste, coastal Bermuda grass is an ideal raw material for ethanol production. Because of its greater biomass content and ability to convert entire carbs into bioethanol, Bermuda grass has emerged as the most promising alternative to maize as a source for ethanol production. Tropical and subtropical regions are the most common habitats for Bermuda grass. Although some of its roots have reached depths of less than 60 cm, the plant may achieve a height of 1-30 cm and a root system that can reach depths of up to 2 meters (Sun and Cheng, 2005).While the maximum height for some Bermuda grass species is about 15-20 cm, others may reach far over 1 m. The North African continent, southern Europe, Asia, and Australia are just a few of the numerous continents where Bermuda grass may grow natively (Sluiteret al., 2008).

Due to their prevalence as organic waste in Pakistan, the following agrowaste samples were chosen for this investigation: straws from wheat, cotton, and rice; cogon grass, maize stover, and fruit peels. Corn is an essential grain, right up there with wheat and rice. It is planted on 1,130 hectares and produces 4.695 million tons per year. Every year, cotton is sown as the second main crop. Rice, the third most significant crop, is grown on 28,911 hectares and yields 70,05 metric tons per year (PES, 2014–2015). According to many studies, the agricultural area of Sanghar in Pakistan's Sindh province produces 2.7 million tons of garbage each year. This includes rice straw, rice husk, canola straw, wheat straw, cotton stalks, cotton bagasse, and sugarcane remnants. Roughly three quarters to four in ten of these feedstocks end up in the fire. According to the UN Environment Program (2011), these materials might be used to generate electricity without compromising food supplies or other domestic resources.There is an abundance of maize (Zea mays), the greatest cereal stover, and it is also a great feed for cattle. All portions of the maize plant have many uses, it is often burnt in fields in many regions of Pakistan before planting the following crop, and it may be grazed off if

necessary (Kim and Dale, 2004). One of the planet's largest microbial populations is found in termite stomach. The termites' ability to break down the wood's complex carbohydrates into smaller molecules is a function of the bacteria living in their digestive system. A number of byproducts, including fatty acids and alcohols like ethanol, are produced when the bacteria found in termites' digestive systems break down cellulose, a primary sugar in wood (Kim & Haltzapple, 2005).Soil, water, and the intestines are all home to the rod-shaped gram-positive bacteria that make up the genus Clostridium. The fermentation process of sugar by *Clostridium acetobutylicum* produces a blend of organic solvents, including ethanol, butanol, and acetone.The single-celled eukaryotic organism *Saccharomyces cerevisiae*, more often known as baker's yeast, is a common ingredient in the fermentation process that yields ethanol and other alcoholic beverages.

MATERIAL AND METHODS

Collection of Agricultural Substrates

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

Proximate Analysis of Samples

Wet and dry weight, ash content, volatile matter, crude protein, crude fiber, and crude fat were all factors examined in each sample (AOAC, 1990). After drying the samples at 105 ºC to eliminate moisture, the usual procedures for estimating total solids and moisture contents were followed (Sluiter, 2005).

Chemical analysis of raw biomass

We used the previously described approach to determine the sample's cellulose content. To find

the hemicellulose, the differences between acid detergent fiber (ADF) and neutral detergent fiber (NDF) were computed.The AOAC-reported standard technique was used to determine the lignin contents (1990).

Analytical Procedures

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

Chemical Pretreatment

Two chemicals, an acid (H2SO4) and an alkali (NaOH), were used for the chemical treatments. Researchers conducted a series of pretreatment experiments with varying concentrations of H2SO4 and NaOH (1.0, 1.5, and 2%), subjecting the samples to temperatures of 100 ℃, 110 ℃, and 120 ℃ for periods of 15, 30, and 45 minutes, respectively. For this experiment, we used a solid sample (10% w/v) in a reagent bottle. Following pretreatment, the sample was filtered in each container using the vacuum filtration assembly, and the contents were poured onto filter paper. After filtering, 300 milliliters of distilled water were used to remove the solid and bring the pH level back to neutral. After that, it was weighed and the filter paper was dried at 105 ℃.

Figure 1. Biomass

Enzymatic Hydrolysis

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

Maintaining the *Saccharomyces cerevisiae* strain at 4°C was done using YPD agar medium, which consisted of yeast extract 1% (w/v), peptone 2% (w/v), and glucose 2% (w/v). The yeast cells were cultured in a 5-milliliter tube of YPD media with 0.9% (w/v) NaCl at 30°C for 16 hours on a rotary shaker set at 100 r.p.m., following the protocol described by Alfanoro (2002).

Separate hydrolysis and fermentation

The experiment included growing C. thermocellum in a medium containing glucose yeast extract for 48 hours. After that, 10% of the inoculum was added to 50 mL of fermentation media that had already been saccharified. The mixture was then let to sit at room temperature for three days (Jiang et al., 2015).An anaerobic fermentation experiment was conducted for 72 hours at 50ºC and 120 rpm. The fractional distillation technique was used in a fractional distillation device based on boiling point to separate acetone, butanol, methanol, and the remaining mixture after the fermentation reaction was complete. Condensation and subsequent separation of butanol are possible because of its higher boiling point (118 ºC) compared to water (100 ºC). According to

Kathleem et al. (2018), ethanol may be condensed before water since its boiling point is lower, at 78.3 ºC.

Statistical analysis

Data generated through various analysis were statically analyzed for mean, standard deviation etc.,

Results and Discussion

Results regarding isolation of bacteria , chemical analysis of biomass samples as well as fermentation of sugars into acetone- butanol- ethanol are given in the following sections.Termites are considered as good source of various useful bacteria isolates those have industrial applications . These isolates are found to have good potential for conversion of various sugars into alcoholic products . Therefore in current study acetone- butanol - ethanol (ABE) were produced from organic wastes material of agriculture and municipal sources by using termite based bacterial isolates (Figures 2-3).

Biological Pretreatment

Results displayed in table 3 indicates amount of sugar released by different bacteria isolates. It was observed that bacterial isolates 9x (xylanase enzyme)has provided higher amount of sugar (27.84$\pm$0.48mM/l) from wheat straw (Table 3), which was higher than all other substrates analyzed.

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

Bacteri	**Corn stover**	**Wheat straw**

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

Pretreatment of biomass samples

END PRODUCT ANALYSIS

Simultaneous Sccharification and Fermentation

It was observed that there are variation for growth of different isolates on differentsubstrates that might be due to availability of amount sugars and other similar byproducts

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

	Acetate	Formate	Lactate	Ethanol	Acetate	Formate	Lactate	Ethanol
Isolate 9x	1.15±0.06	_	1.41±0.18	5.73±0.28	3.04±0.65	_	1.65±0.79	3.34±0.41
Isolate 10	1.28±0.14	_	3.44±0.34	6.98±0.58	1.55±0.28	1.24±0.17	6.14±0.55	5.99±0.26
Isolate 31	1.29±0.34	1.98±0.39	8.57±0.59	9.21±0.54	1.72±0.07	1.63±0.28	3.58 ±0.26	6.43±0.49

Various Fermentation products

Biomass analysis

Data in table 6 represents various parameter found in biomass samples. Whereas ligno-cellulosic contents of the samples are given in table 7. It was observed that Cogon grass has higher cellulosic contents as compared to other substrates used for analysis.

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

Analysis of organic wastes samples

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_4Concentration	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

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%. Both samples performed best when subjected to a temperature of 120 ℃ for 15 minutes with peel wastes and 30 minutes with cogon grass at concentrations of 1.5% and 1%, respectively, according to the optimized conditions utilized in the enzymatic experiment (Figures 6-7).

FERMENTATION

Table 9 shows that whilst peel wastes create 7.4% ethanol, cogon grass produces 10%. The concentration of glucose decreased while the concentration of ethanol increased with increasing time, up to a specific point. But even with a 72-hour glucose concentration, ethanol synthesis stalled. The higher cellulose content and reduced lignin percentage of cogon grass make it a more favorable choice for ethanol generation as compared to peel wastes.

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

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

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

% age values of various parameters of biomass samples.

Dilute sulfuric acid pretreatment of Peel wastes

The peel waste had a glucose content of 27.33±2.15%, as shown in Table 10. After the therapy, the glucose content rose (Figures 10 -11). After 30 minutes of treatment with a diluted acid concentration of 1.8% at a reaction temperature of 110 °C, the solid portion of the samples produced a higher amount of glucose. The results showed that the pretreatment glucose contents were significantly improved with moderate temperature and acid concentration. Talo et al. (2014) also found that orange peel hydrolyzes similarly at low temperatures.

Percentage decrease in xylose content in solid fraction of peel wastes after H_2SO_4Pretreatment

The xylose level in the peel wastes was 15.37%±1.13 according to Table 10, and it was shown to be lower following pretreatment. Figure 11 shows the optimal conditions for achieving lowest xylose in the solid fraction, which include an acid concentration of 1.10% and an incubation temperature of 110 °C for 30 minutes.It is feasible to remove all traces of hemicellulose using diluted acid pretreatment (Sun and Cheng, 2005). The solubilization of xylose reaches its peak at moderate temperatures, according to Wyman et al. (2005).

Percentage increase in lignin content in solid fraction after H_2SO_4pretreatment

The optimal acid dosage is 2.0% for 37 minutes at 125 °C, as shown in the contour plot (Figures 10 and 11), which is sufficient to provide the least amount of lignin in the solid fraction. The reaction may have produced a 7.32 percent rise in lignin content, but the actual numbers were in a declining sequence. After being pretreated with H2SO4, xylose was greatly removed, leading to an apparent rise in lignin concentration. Solubilization of lignin increases with increasing reaction time to determine maximum value, assuming temperature remains constant.

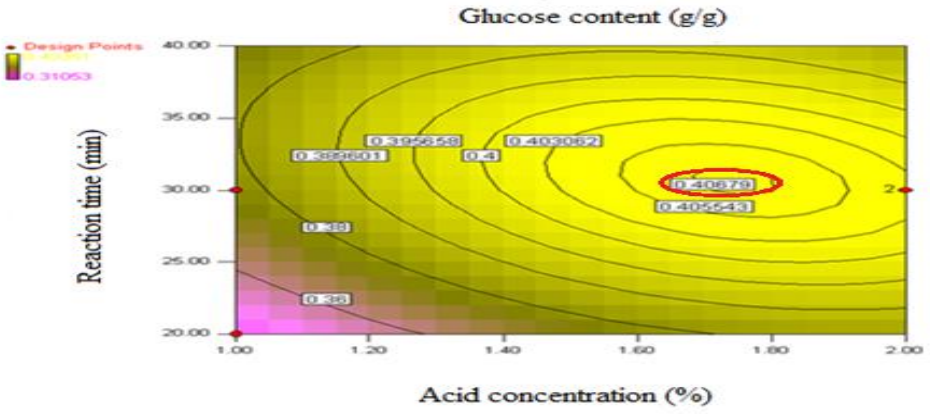

Figure 10; Contour plot for glucose content in relation to acid concentration and reaction time

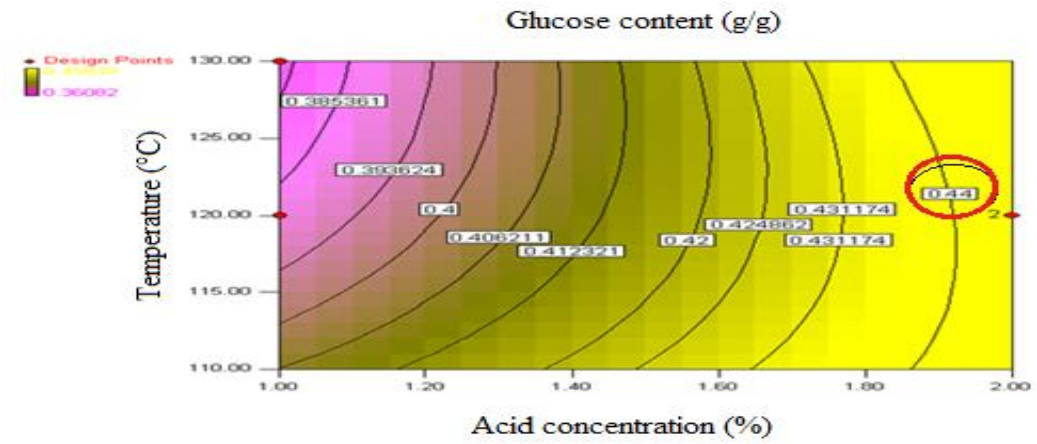

Figure 11; Contour plot for glucose content in relation to acid concentration and temerature

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

Substrate	$Glucose_{SF}$ (2.5g/50mL)	$Glucose_{SF}$ (50g/L)	Glucose(g/L)	$Rate_{sac}$ (%)	$Time_{OPT}$(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

There was no buildup of sugar like cellobiose, even though cellobiose was present in the reaction mixture, which led to increased saccharification (80.54%). In addition, Xue et al. (2012) noted that celllase efficiency was improved (since cellubioses were not present), leading to more sugar recovery during enzymatic hydrolysis.

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

Various parameters of bioma

Analysis of Sugar after Pretreatment and Enzymatic Hydrolysis

The experiment included 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. Pretreatment of lignocellulose is essential for sugar production because it breaks down lignin and makes carbs more accessible to bacteria and enzymes (Figures 12-13). These substrates have been subjected to two distinct pretreatment procedures. Physical preparation included first grinding the substrates to a powder and then passing them through a sieve with a mesh size of 80 to decrease particle size. Following that, these surfaces underwent chemical preparation. Table 12 shows the results of the chemical pretreatment conditions that were used to disrupt the structure of the lignocellulosic biomass.

Dilute Acid Pretreatment

The samples of agricultural waste were pretreated with varying amounts of sulphuric acid H2SO4. After being prepared with a 20% (w/v) solid loading slurry, the samples were autoclave heated to temperatures of 100, 110, and 120 ºC. With sulphuric acid concentrations of0.5%,1%, and 1.5%, the reaction was carried out at retention times of10,15, and 20 minutes. Three different amounts of sulphuric acid were applied to the substrate before to each temperature. Using the same temperature and reaction time, a sample was pretreated three times. Three samples were pretreated in 100 ml reagent bottles at a time for a total of nine treatments. Figure 12 shows that 81 treatments of three samples were carried out at three different temperatures in a total of nine

tests to determine the optimal conditions for acidic pretreatment.

Dilute Alkali Pretreatment.

Pretreatment of agrowaste samples was done using diluted alkali. In order to find the optimal conditions that may provide the highest possible glucose production, we experimented with various temperatures, retention times, and sodium hydroxide (NaOH) concentrations. Before being heated in an autoclave at 100, 110, or 120 ºC for 10, 15, or 20 minutes of reaction time, the samples were prepared with a 20% w/v solid loading slurry. Various amounts of sodium hydroxide were used for pretreating the sample. The appropriate conditions for basic pretreatment were checked by doing 81 treatments on three samples at three different temperatures, which resulted from a total of nine tests ($9 \times 9 = 81$).

Spectrophotometric analysis and Comparison of sugar production in three agrowaste samples

After 72 hours of enzymatic hydrolysis, wheat straw yielded better glucose in all 9 studies. Increasing the concentration of H2SO4 from 0.5 to 1.5% resulted in an increase in sugar, as seen throughout the experiment. Twenty minutes of retention time, as opposed to ten or fifteen minutes, resulted in a greater glucose production in all trials. The ideal conditions for the acidic pretreatment of wheat straw were 120 ºC, 20 minutes of retention time, and 1.5% sulphuric acid (Figure 14). Glucose levels peaked at this level. Increasing the temperature during the alkali pretreatment conditions led to a larger glucose production; specifically, a yield of 120 ºC was reported. Meanwhile, using a 1.5% concentration of NaOH resulted in a large glucose output at the same temperature (Figure 16). When the duration of enzymatic hydrolysis was raised from 0 to 48 hours, the sugar production rose. However, when the time was increased to 72 hours, the sugar concentration was drastically reduced.It is likely that the synthesis of inhibitors by greater acid concentrations was responsible for the drop in glucose concentration. The highest concentration of reducing sugars (7.73 g/L) was achieved at 120ºC with a 1% NaOH concentration and a 15-minute reaction period.

Pretreatment of rice straw with an acid at 110 ºC, 1.5% acid concentration, and a retention duration of 10 minutes resulted in a greater glucose production (Figure 15).In the case of alkaline pretreatment, the ideal conditions for rice straw analysis are a temperature of 100 ºC, a sodium hydroxide concentration of 0.5 percent, and a retention duration of 20 minutes. Figure 17 shows that after 72 hours of enzymatic hydrolysis, a higher yield was achieved.The acidic pretreatment

resulted in a high glucose yield at 120ºC, 1.5% H2SO4, and a reaction duration of 15 minutes (Figures 13–14). A temperature of 100 ºC, a concentration of sodium hydroxide of 1.5%, and a retention duration of 20 minutes were the ideal conditions for maize stover under the alkaline pretreatment condition (Figure 18).

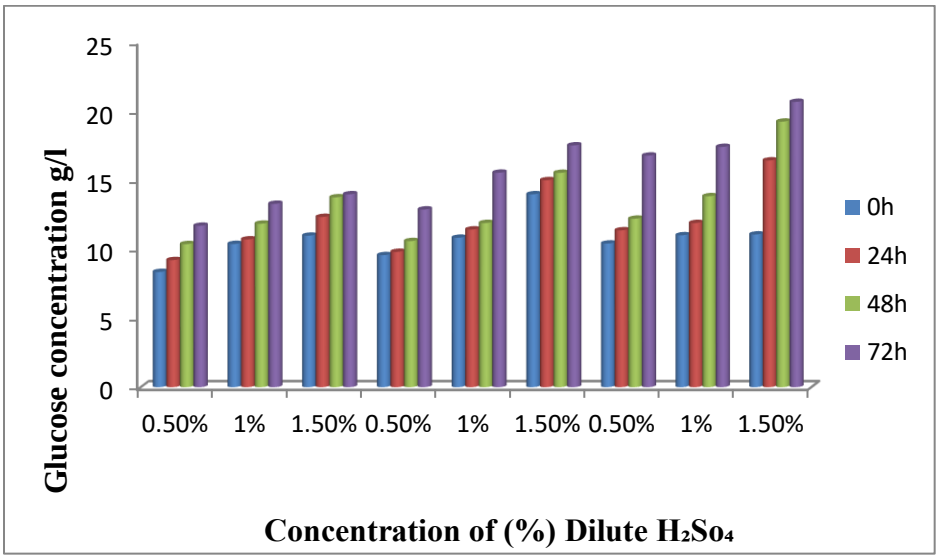

Figure 14. Glucose yield obtained from Wheat straw at H_2SO_4 pretreatment conditions at 120^{0}C

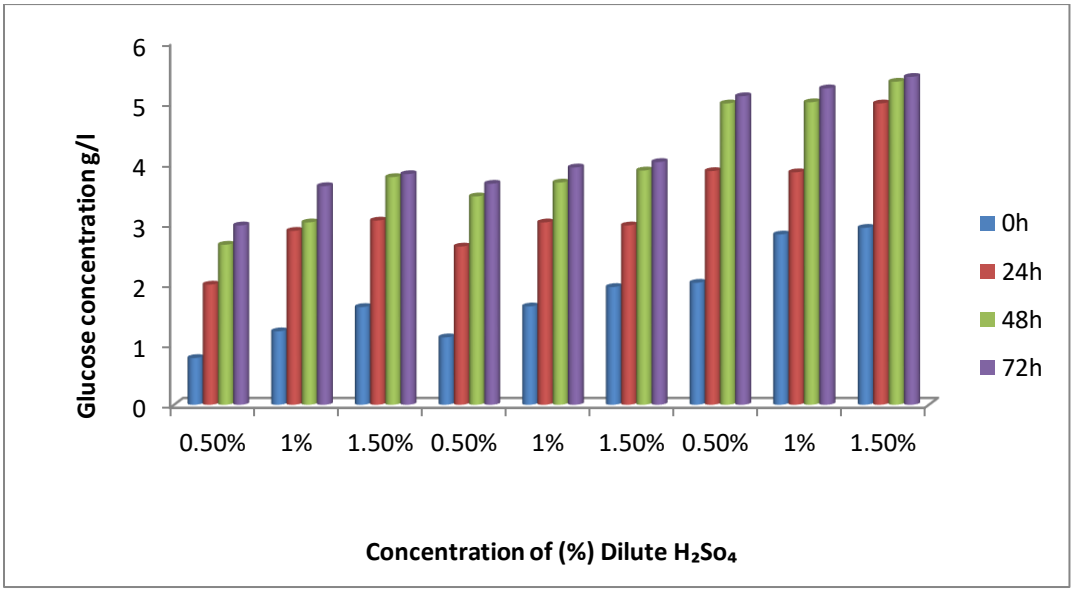

Figure 15. High glucose yield obtained from Rice straw at H_2SO_4 pretreatment conditions at 110 ^{o}C

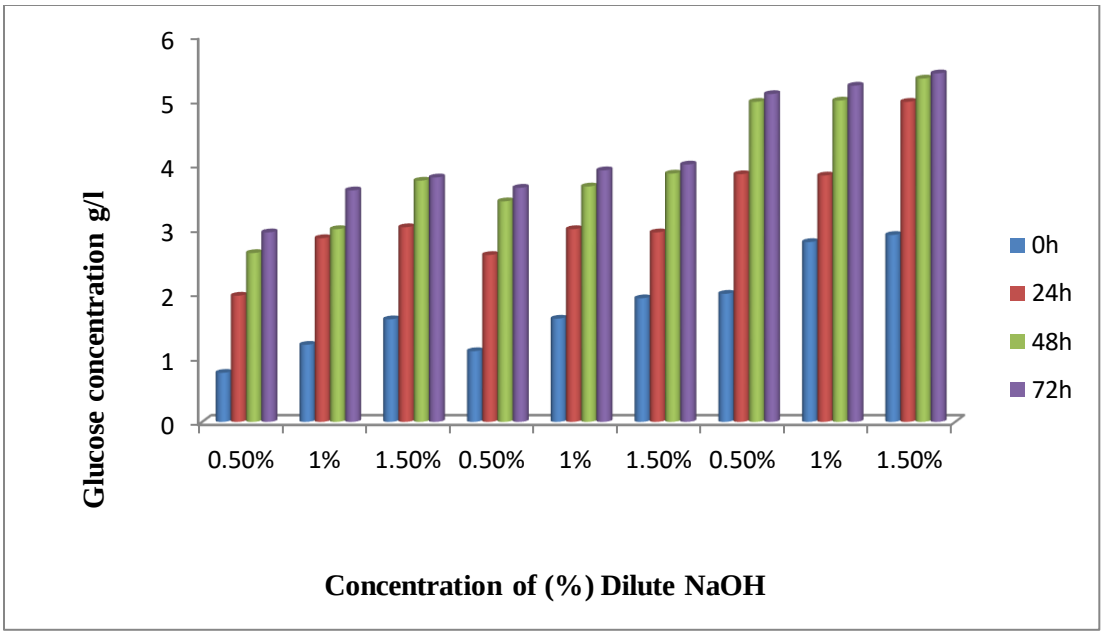

Figure 16. High glucose yield obtained from Wheat straw byNaOH pretreatment conditions at 120 °C

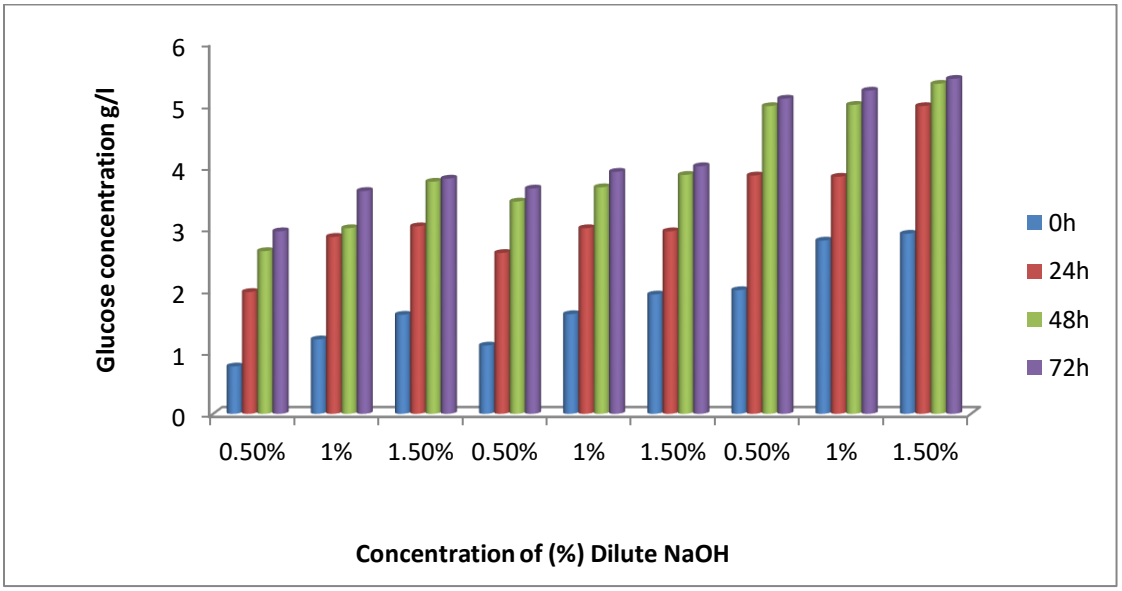

Figure 17. Glucose yield obtained from Rice straw at NaOH pretreatment conditions at 100 °C

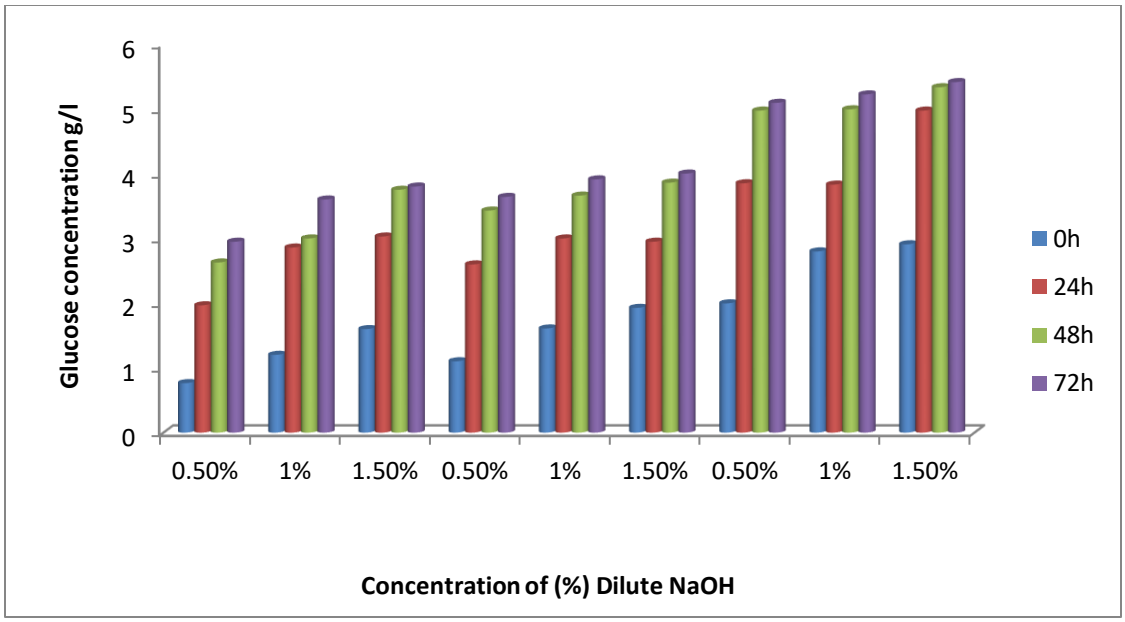

Figure 18. Glucose yield obtained from Corn stover byNaOH pretreatment conditions at 100 ºC

HPLC Analysis of Enzymatic Hydrolysate

Further analysis was conducted using high-performance liquid chromatography (HPLC) on samples of acidic and alkaline pretreatment of wheat, rice, and maize stover that had been hydrolyzed by enzymes. To achieve this goal, we only analyzed samples that have previously shown a greater concentration of glucose under these ideal circumstances. Following enzymatic hydrolysis, samples were removed at various intervals and centrifuged at 14,000 rpm for 15 minutes at 4 ºC.A 0.22 µm syringe filter was used to separate the supernatant. The sample concentrations were adjusted to fall within the calibration curve range by diluting 500 µl of the sample with 1 milliliter of methanol. The sugars' solubility necessitated the use of methanol. Before analysis, the samples and glucose standard solution were all passed through a 0.22 µm filter. The HPLC system was supplied with about 20µl of the agrowaste sample via the injection loop. After enzymatic hydrolysis, samples were subjected to a 10-minute gradient run for glucose analysis (Shields and Cathcart, 2010).

Using the retention time, tR, to determine the peak.Table 13 and Figures 19–20 show that the recognized standard 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.

Table 10; 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

Fermentation with*Clostridium acetobutylicum*

The main outcome of this kind of fermentation is called ABE (acetone, butanol, and ethanol) fermentation. Many writers have already observed that the fermentation process's acetone, butanol, and ethanol ratio is essentially 3:6:1. Comparing *Clostridium acetobutylicum* to alkaline pretreatment conditions, it was calculated that the former produces more butanol.. Although alkaline pretreatment conditions are best for butanol production because the chances for the production of fermentation inhibitors are very low. But in this experiment the reason for low butanol production might be due to low quantity of glucose obtained at alkaline conditions.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 corn stover. 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 and corn stover. 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.. It is concluded that wheat straw is a superior fermentation substrate probably fermentation stimulatory chemicals are present in wheat straw.

Table 11; Proximate analysis of straws samples

Substrates	Dry matter%	Moisture%	Crude protein%	Crude fat%	Crude fiber%	Ash%
Corn stover	91	5.32	7	2.9	2.5	3
Wheat straw	92.8	7.2	17.5	3.6	15	23.5
Rice straw	90.8	5.40	4.37	1.9	11	24

Analysis of biomass samples

Table 12;Chemical analysis of straws samples

Samples	Cellulose %	Hemicellulose%	Lignin%
Corn stover	30	21	7
Wheat straw	40	25	13
Rice straw	35	22	20

Chemical analysis of biomass samples

***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 120rpm 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 highyield of butanol (Tables 16 -18).

Nevertheless, clostridium species may metabolize glucose and xylose, the primary end

products of acidic/alkaline pretreatments and enzymatic hydrolysis, during growth and acetone-butanol-ethanol (ABE) fermentation (Qureshi and Blaschek, 2000; Moretti and Thorson, 2008).Common bacteria used in ABE fermentation include *C. acetobutylicum* and *C. beijerinckii*. Batch, fed-batch, and continuous fermenters all employ different biomass samples to make ABE, however in recent years, researchers from different nations have looked at different strains, parent microbes, growing conditions, and growth medium. table 16–18

Table 13; 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
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

It took a number of technical processes, including saccharification, fermentation, and acid or alkali pretreatment, to convert lignocellulosic feedstock into alcoholic fuels like butanol and ethanol. Achieving cost-effective biofuel production relies heavily on fine-tuning all system parts. 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). Developed nations' well-known examples of biomass fuel generation might serve as useful pointers for less developed nations. Biorefinery and the notion of orientated conversion of categorized composition are only two of the numerous innovative approaches to ethanol production that have been studied. García et al. (2011) and Demirbas (2009) found that butanol can be produced from lignocellulosic biomass using comparable technologies. Producing fuels on an industrial scale and combining these processes efficiently will lead to competitive biofuel production from plant biomass, which is presently underutilized (Talo et al., 2014).

Fermentation of the sugars present in cellulosic biomass has the potential to provide valuable byproducts such as acetone, butanol, ethanol, and other alcohols with comparable properties that might be utilized as fuel. The majority of carbohydrate-containing biomass comes from a variety of sources, including agricultural crops (such as wheat, rice, cotton, and sorghum), maize stalks, sorghum straws, fruit and vegetable scraps, and similar substrates. One of the most important sugars found in plant materials, cellulose is also a key ingredient in making alcohol, a fuel. Acid treatment, enzymatic hydrolysis, and bacterial/fungal fermentation all play a role in breaking down this complex cellulose substance into smaller pieces. The potential use of these alcohols as fuels makes them significant. As a result, biofuels have the potential to (1) mitigate climate change by reducing the amount of carbon emissions from sources such as transportation (2). The increasing demand for fossil fuels and energy may be met by switching to biofuels. This would help to secure the energy supply and combat the rising fuel prices throughout the world.Because they help reduce waste while making use of natural resources, biofuels are a great illustration of

how the circular economy may solve problems. The present investigation included the production of bioethanol and biobutanol from a variety of cellulosic materials. The results showed a variety of orders for the synthesis of alcoholic fuels from cellulosic substrates. When compared to other biomass substrates, straws have produced the highest yields of alcoholic fuels.The quantity of acetone, butanol, and ethanol generated, however, is dependent on the kind of cellulosic biomass used and the specific distillation processes employed to purify these alcohols after fermentation.

CONCLUSION

In spite of a dismal supply situation, the country's energy consumption is projected to triple by 2050. The ideal replacement for traditional fuels and power sources is renewable and sustainable energy resources because of the same reason. A sustainable and cost-effective method is the bioconversion of lignocellulosic biomass into ethanol and butanol, two alcoholic fuels. However, there has to be persistent effort to fully comprehend the basics of different pretreatment procedures and to create fermentation systems that are both more efficient and less expensive. Additionally, more efficient microbial strains need to be developed for detoxification that is both cost-effective and environmentally friendly. We can replace the presently available fossil fuels, which are already depleting, with a process of integration and optimization that reduces energy use and increases yields. Research into alternative energy sources, particularly those using cellulosic biomass, is thus ongoing on a global scale. Research of this kind has the potential to play a significant role in the future of the country's growth as it makes use of its own resources..

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Publications out of this work

1. IramBatool , Muhammad Gulfraz, Muhammad JavaidAsad, FaryalKabir, SobiaKhadam and Asma Ahmad. 2018. Cellulomonas sp. Isolated from termite Gut for saccharification and fermentation of agriculture biomass. Bioresources. 13 (1) 752-763. Impact factor 1.5

2. Irambatool, Muhammad JavaidAsad, M, Shareez Ahmad, Raja Tahir Mahmood , Hinagul, LubnaNisar, InamulHaq, Imran Bodhlah and MuhammdGulfraz.2016. Ethanol Production from agricultural residues by simultaneoussaccarification and fermentation process (SSF) by using termites and *Saccharomyces cerevisiae*. Advances in Environmental Biology, 10 (7) 107-115. Impact factor 0.5
3. Abdul Rehman, Muhammad Gulfraz ,GhazalaKaukab Raja, Muhammad InamulHaq and Zahid Anwar.2015. A Comprehensive Approach to Utilize an Agricultural Pea peel (Pisum 2 sativum) Waste as a Potential Source for Bio-ethanol Production. Romanian Journal of Biotechnology Letters. 3(2): 10422-10430. Impact factor 0.5

Doctoral and Master of philosophy research work conducted on similar aspects

1. IramBatool PhD scholar 2018 (Regd. 04- arid-912). Extraction of Bioethanol from agriculture wastes by using Termites gut flora through fermentation
2. Abdul Rehman PhD scholar 2014 (Regd. 03-arid-825) . Screening of Second Generation Biomass and optimization of operating variable for the production of ethyl alcohol by using Fermentation Fungal strain.
3. ZunaraRazaM.phil scholar 2017 (Regd. 13-arid-2012). Bioconversion of second generation cellulosic biomass into Ethanol and Butanol through Fermentation process.
4. SheerenSadafFarooqi M. Phil scholar 2015 (Regd. 12-arid- 1345) Extraction of Bioethanol and Biobutanol from Agriculture Biomass.
5. ShaamaSamanFarooqi M. Phil scholar 2014 (Regd. 12-arid- 1345) Evaluation of Cannabis sativa for Extraction of Bioethanol by using Nitric acid and Potassium hydroxide pretreatment.
6. SummerakasuarM.phil scholar 2013 (Regd. 08- aird-1113). Bioconversion of Cellulosic Biomass of grasses wastes in to ethanol.
7. SumariaKhadiumM.phil scholar 2013 (Regd. 09- aird-1286). Utilization of Fruit wastes for extraction of bioethanol
8. FaryalKabir M. Phil scholar 2012 (Regd. 08- aird-942). Study of Lignocellulosic biomass from agrowastes for production of Biobutanol

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