CHEMICAL ANALYSIS OF BIOMASS FOR ALCOHOL AND ITS CONVERSION INTO FUELS

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ABSTRACT

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 like bioethanol and biobutanol. Therefore, cellulosic materials like wheat, cotton and rice straws, corn stover, fruit wastes and cogon grass were used in this study. Biological and chemical pretreatments were compared for each substrate. It was determined how well bacterial enzymes saccharified agricultural materials. Findings indicate that these bacterial enzymes may hydrolyze agricultural waste in addition to pure substrates. With any luck, this study's findings will encourage more biofuel production and lessen the need to spend foreign currency on fossil fuel imports.

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

1. 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). Many different ways to produce environmentally friendly biofuels are being studied. Biodiesel, bioalcohols, bioelectricity, and biogases are examples of biological energy resources. Bioalcohol has the most promise among these sources for lowering carbon emissions, weaning society off

fossil fuels, and serving as both a chemical feedstock and a transportation fuel (Dhamole et al., 2015).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. Worldwide, 51,000,000,000 liters of ethanol are produced each year (Renewable Fuels Association, 2007). Due to its increased oxygen concentration, ethanol has some benefits as a fuel. An increase in oxygen levels allows for more efficient hydrocarbon oxidation, which in turn reduces aromatic compounds and carbon monoxide emissions. 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. Biomass from the agricultural and livestock industries, including rice husks and sugarcane bagasse (Amiri et al., 2014; Chaudhry et al., 2009). Lignocellulosic material makes up the majority of second generation biomass. The most abundant organic substance on Earth is lignocellulosic biomass, which contains cellulose (35-50%), hemicellulose (20-35%), and lignin (5-30%), according to 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. According to Ejeziet al. (2006), the most popular feedstocks are maize stover, apple pomace, wheat straw, and corn steep liquor.Biodiesel, bioethanol, biohydrogen, and methane are some of the biofuels made from agricultural waste instead of energy crops, which compete with food crops. One potential solution to the disposal issue of the vast amounts of agricultural waste is to make use of lignocellulosic biomass, which may help alleviate the conflict between food production and fuel production (Mahro and Timm, 2007). When it comes to extracting ethanol, grasses are a dependable material. The usage of long-lived grasses has several benefits, one of which is the potential reduction in production and fuel costs associated with ethanol (Gomez et al., 2008).

The Cogon grass (*Imperatacylindrica*) can be grown all over the yearworldwide, 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. The cogon grass could be utilized as a raw material for renewable source of energy (Lin and Lee, 2011).The *Cynodondactylon* (Coastal

Bermuda grass) is perennial grass that has the higher cellulosic content and can also be used for ethanol production. The excellent raw material for yield of ethanol is coastal Bermuda grass as it is either sold at a very cheap price or is wasted in most of the cases. Comparison of corn and Bermuda grass has shown that most potential source for production of ethanol is Bermuda grass because of higher contents of biomass and conversion of whole carbohydrates into bioethanol. Bermuda grass is predominantly present in tropical and subtropical part of the world. It reaches 1-30 cm in height and have deep roots up to 2m into the ground, however various roots penetrated less than 60 cm under-ground (Sun and Cheng, 2005).Even though some species of Bermuda grass can grow up to 15-20 cm, others may reach a height of greater than 1m long. Bermuda grass can naturally grow in many continents such as North Africa, southern Europe, Asia and Australia (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. Produced from an area of 1,130 hectares, it has an annual output of 4,695 million tons. Cotton is grown every year and is the second main crop. With an annual production of 705,000 metric tons and a cultivation area of 28,911,000 hectares, rice ranks third among the most significant crops (PES, 2014–2015). Sanghar is an agricultural area in Pakistan's Sindh province. According to several research, 2.7 million tons of waste materials, including rice straw, canola straw, wheat straw, cotton stalks, cotton bagasse, and sugarcane remnants, are grown there. 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. You may graze it off or burn it before planting your next crop in many regions of Pakistan. Plus, there are various uses for every component of the maize plant (Kim and Dale, 2004). Among all the places on Earth, termite gut contains the densest concentration of microbes. 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. Among the many sugars found in wood is cellulose, which termites use to produce a number of byproducts, such as fatty acids and alcohols like ethanol (Kim and Haltzapple, 2005).Soil, water, and the intestines are all home to the rod-shaped gram-positive bacteria that make up the genus Clostridium. A combination of organic solvents such as

acetone, butanol, and ethanol is produced when Clostridium acetobutylicum ferments sugar.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.

2. MATERIAL AND METHODS

2.1. Collection of Agricultural Substrates

Straws from wheat and rice, together with peel trash, were gathered from different locations. In little plastic bags, researchers gathered around 1 kilogram of each sample. After being sun-dried for a few hours, the samples were placed in an oven set at 55 °C for the night. After being ground into a fine powder using an electric grinder, the samples were strained through a standard size 40 mesh 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.

2.2. Proximate Analysis of Samples

The following parameters were measured in all samples: ash content, volatile matter, crude protein, crude fiber, crude fat, and wet and dry weight (AOAC, 1990). Total solids and moisture contents were estimated using the conventional procedures, which included drying the samples at 105 ºC to eliminate moisture (Sluiter, 2005).

2.3. Chemical analysis of raw biomass

Using the previously described procedure, we were able to determine the sample's cellulose content. Computing the differences between Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) allowed us to identify the hemicellulose.As previously reported by AOAC (1990), the lignin contents were determined using a conventional technique.

2.4. Analytical procedures

According to Haifeng et al. (2015), the aforementioned approach was used to identify the fermentation products, which included hexoses and pentoses as monomer sugars, acetone-butanol, ethanol, and their bioproducts.

2.5. Chemical Pretreatment

Humic acid (H_2SO_4) and sodium hydroxide (NaOH) were the two chemicals used for the chemical treatments. In the pretreatment experiment, various concentrations of H_2SO_4 and NaOH (1.0, 1.5, and 2% by weight) were subjected to varying temperatures (100, 110, and 120 ℃) for varying lengths of time (15, 30, and 45 minutes, respectively). In the course of the experiment, a solid sample containing 10% (w/v) of the reagent was used. Following pretreatment, the sample was filtered in each container using the vacuum filtration assembly, and the contents were poured onto filter paper. To get the pH level back to neutral after filtering, 300 ml of distilled water was used to wash away the solid. After being dried at 105 ℃, the filter paper was weighed.

Figure 1. Biomass

2.6. Enzymatic Hydrolysis

After undergoing a 5% (w/v) pretreatment, the biomass was subjected to hydrolysis with cellulose and β-glucosidases in a water bath shaker with a 0.05 M buffer (sodium citrate) at a pH of 4.8 for 72 hours. Cellulases with activity of (30FPU g-1). In order to find the sugar content, samples were taken from the reagent bottle every 12 hours. Hydrogen peroxide (µl) was added after the enzymatic hydrolysis. The unhydrolyzed material was separated by subjecting it to a 10-minute centrifugal force of 13,500 g. The sugar analysis was done using the dinitrosalicylic acid (DNS) technique, and the supernatant was collected using syringe filters.The p-hydroxybenzoic acid hydrazide (PAHBAH) technique was used to determine the sugar content. The standard curve was produced using xylose concentrations ranging from 1Mm to 25mM. The quantity of sugar in

the pretreatment sample was then assessed by comparing it to the reference sugar concentration. Following the enzymatic hydrolysis procedure, the optimal pretreatment conditions were chosen. To proceed with the fermentation process, only the samples with the highest amounts of released sugar were chosen. Before being used in the fermentation process, the solid biomass was kept at 4 °C.

The *Saccharomyces cerevisiae* strain was cultured at 4°C in YPD agar medium, which contains yeast extract 1% (w/v), peptone 2% (w/v), and glucose 2% (w/v). A 5-milliliter tube of YPD medium containing 0.9% (w/v) sodium chloride was used to cultivate yeast cells for 16 hours at 30℃ on a rotary shaker (100 r.p.m.) in accordance with (Alfenoro, 2002).

2.7. Separate Hydrolysis and Fermentation

In a fermentation experiment, C. thermocellum was cultured in a broth 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).Anaerobic conditions were maintained for 72 hours throughout the fermentation experiment, with temperatures of 50ºC and speeds of 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 are viable options for butanol due to its greater 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.

2.8. Statistical analysis

The results of the several analyses were subjected to statistical testing for measures like mean, standard deviation.

3. 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 sources of various useful bacteria isolates that 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).

3.1. 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±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
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±0.0 5	0.28±0. 02	0.86±0.0 6	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

3.2. END PRODUCT ANALYSIS

3.2.1. 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

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	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

3.3. Various Fermentation products

3.3.1. 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

3.4. Dilute H_2SO_4 pretreatment

Pretreatment of the biomass samples was done using diluted acids at concentrations of 1, 1.5, and 2%. The samples were then autoclaved at temperatures of 105, 120, and 135oC for 15, 30, and 45 minutes, respectively. The ideal conditions for the enzymatic experiment were a temperature of 120 °C, a retention time of 15 minutes for the peel wastes and 30 minutes for the cogon grass, with concentrations of 1.5% and 1%, respectively (Figures 6-7).

3.5. Fermentation

As stated in Table 9, a 10% yield from cogon grass and a 7.4% yield from peel wastes make up the ethanol. There was a time limit beyond which the concentration of ethanol increased and the concentration of glucose decreased. After 72 hours, however, the concentration of glucose was too low to keep the ethanol synthesis going. 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**	**Moisture**	**Ash**

				matter		
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.

3.6. Dilute sulfuric acid pretreatment of Peel wastes

The peel waste had a glucose content of 27.33±2.15%, as shown in Table 10. Following therapy, the glucose content rose (Figures 10–11). By subjecting the solid portion of the samples to a diluted acid concentration of 1.8% for 30 minutes at a temperature of 110 ℃, a greater amount of glucose was produced. In order to improve the glucose contents during pretreatment, it was shown that a combination of moderate temperature and acid concentration is crucial. Talo et al. (2014) also made a similar discovery about the acid hydrolysis of low-temperature orange peel.

3.6.1. Percentage decrease in xylose content in solid fraction of peel wastes after H2SO4 Pretreatment.

According to Table 10, the xylose concentration in the peel wastes was 15.37%±1.13. Following processing, a reduction in this amount was seen. To get the lowest amount of xylose in the solid fraction, the ideal conditions were an acid concentration of 1.10% and an incubation temperature of 110 ℃ for 30 minutes (Figure 11).According to Sun and Cheng (2005), hemicellulose may be completely removed with diluted acid pretreatment. The solubilization of xylose reaches its peak at moderate temperatures, according to Wyman et al. (2005).

3.6.2. Percentage increase in lignin content in solid fraction after H2SO4pretreatment

Figures 10 and 11 provide contour plots that show the optimal acid dosage of 2.0% for 37 minutes at 125 ℃ is sufficient to get the minimal amount of lignin in the solid fraction. The reaction's output showed a 7.32 percent rise in lignin content, however 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.

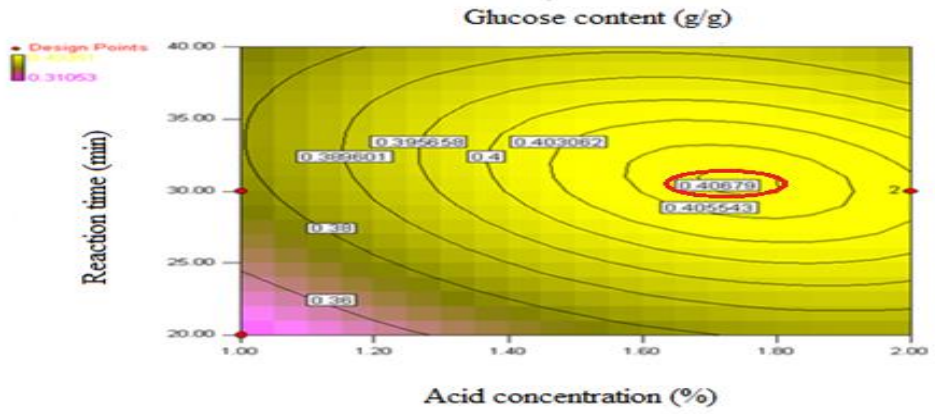

Figure 10; Contour plot for glucose content in relation to acid concentration and reaction time

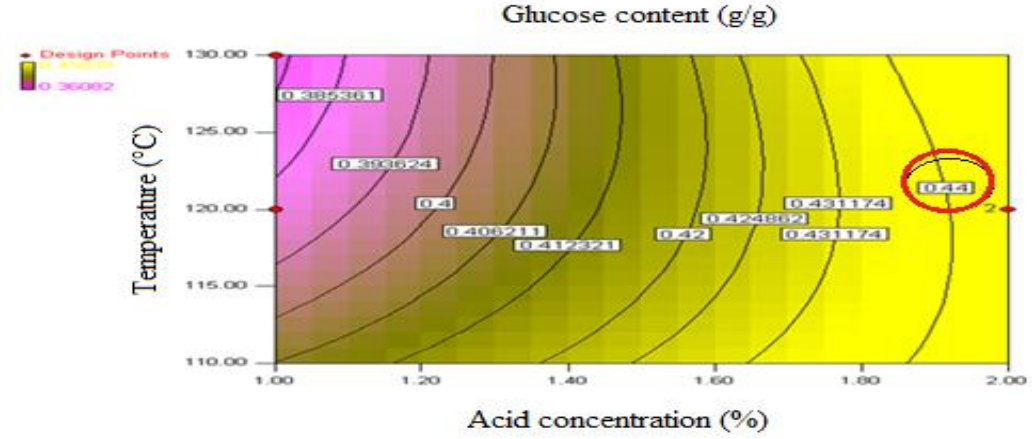

Figure 11; Contour plot for glucose content in relation to acid concentration and temerature

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

Even though cellobiose was present in the reaction mixture, significant saccharification (80.54%) was accomplished since no sugar-like cellobiose buildup occurred. Further, Xue et al. (2012) noted that celllase efficacy was improved (since cellubioses were not present), leading to greater 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 biomass

3.7. Analysis of Sugar after Pretreatment and Enzymatic Hydrolysis

Enzymatic hydrolysis was used in this work to produce sugar from three distinct substrates: maize stover, wheat straw, and rice straw. The process was carried out in a 500 mL Erlenmyer flask at 50 ℃ for three days. 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. During physical pretreatment, the substrates were first reduced in size by grinding them into a fine powder

and then passing them through a sieve with a mesh size of 80. Afterwards, chemical pretreatment was applied to these substrates. The lignocellulosic biomass was subjected to acidic and basic pretreatment (chemical pretreatment) conditions in order to disrupt its structure (Table 12).

3.8. Dilute Acid Pretreatment

In order to prepare the samples of agricultural waste, various quantities of sulphuric acid H2SO4 were used. After being prepared with a 20% (w/v) solid loading slurry, the samples were autoclave heated to temperatures of 100, 110, and 120 ºC. Three distinct concentrations of sulphuric acid (0.5, 1, and 1.5%) were used in the reaction, which was carried out at retention times of 10, 15, and 20 minutes. We used three different amounts of sulphuric acid to pretreat the substrate at each temperature. Using the same temperature and reaction time, a sample was pretreated three times. Each of the nine samples underwent pretreatment in a 100 ml reagent container. In order to determine the optimal conditions for acidic pretreatment, a total of nine tests were conducted ($9 \times 9 = 81$), with three samples treated at three distinct temperatures (Figure 12).

3.9. 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. The sample was pretreated using varying doses of sodium hydroxide, ranging from 0.5% to 1.5%. In order to determine the optimal conditions for basic pretreatment, a total of nine tests were conducted, yielding 81 treatments on three samples at three distinct temperatures.

3.10. Spectrophotometric analysis and Comparison of sugar production in three agrowaste samples

In all nine trials, after 72 hours of enzymatic hydrolysis, wheat straw produced better glucose yields. 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. Figure 14 shows the optimal settings

for acidic pretreatment of wheat straw, which include 120 ºC, 20 minutes of retention time, and 1.5% sulphuric acid. There was a maximum concentration of glucose at this point. The glucose yield was found to be highest at 120 ºC, and it was found that raising the temperature under alkali pretreatment conditions further improved the yield. Figure 16 shows that using a 1.5% concentration of NaOH resulted in a high yield of glucose at the same temperature. 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. Higher acid concentrations likely caused the development of inhibitors, which led to a drop in glucose concentration. At 120ºC, with a 1% NaOH concentration and a 15-minute reaction period, the maximum reducing sugars (7.73 g/L) were achieved.

Figure 15 shows that under acidic pretreatment conditions with a temperature of 110 ºC, an acid concentration of 1.5%, and a retention duration of 10 minutes, rice straw had a greater glucose output.Temperature (100 ºC), sodium hydroxide concentration (0.5%), and retention period (20 minutes) are the ideal conditions for analyzing rice straw when alkaline pretreatment is utilized. Figure 17 shows that the yield increased after 72 hours of enzymatic hydrolysis.High glucose yields were achieved after acidic pretreatment at 120ºC, 1.5% H2SO4, and 15 minutes of reaction time (Figures 13–14). At an alkaline pretreatment condition, the ideal parameters for maize stover were a temperature of 100 ºC, a concentration of sodium hydroxide of 1.5%, and a retention duration of 20 minutes (Figure 18).

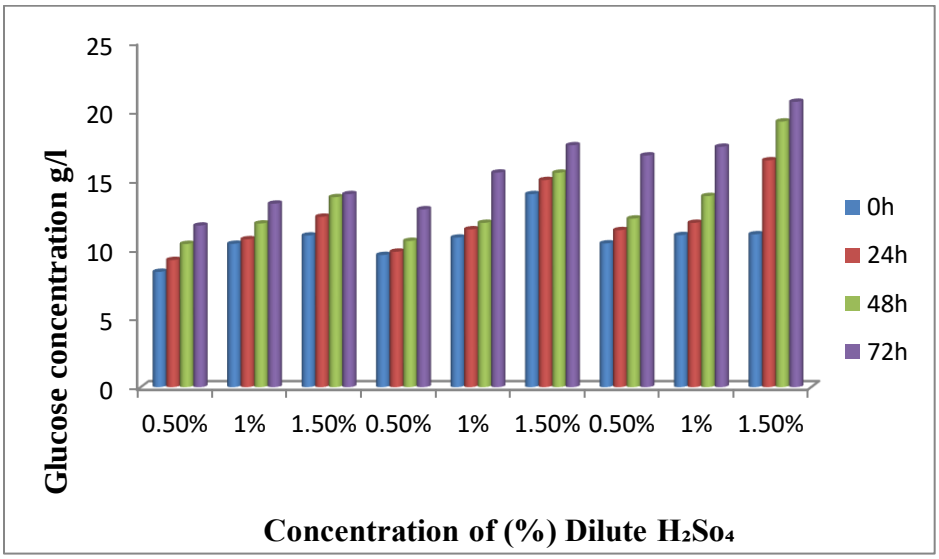

Figure 14. Glucose yield obtained from Wheat straw at H_2SO_4 pretreatment conditions at 120^0C

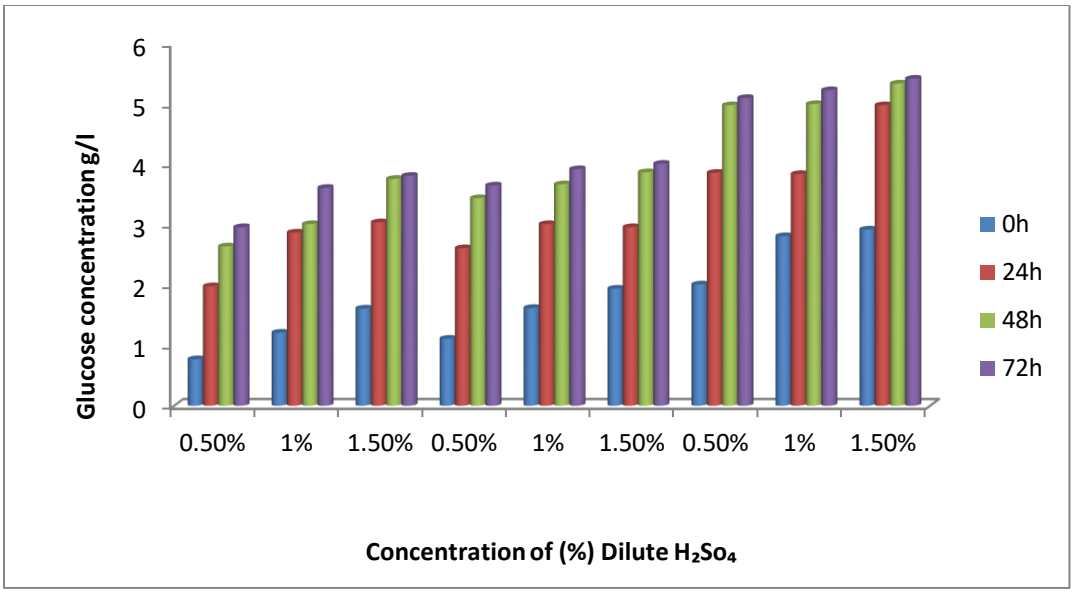

Figure 15. High glucose yield obtained from Rice straw at H_2SO_4 pretreatment conditions at 110 °C

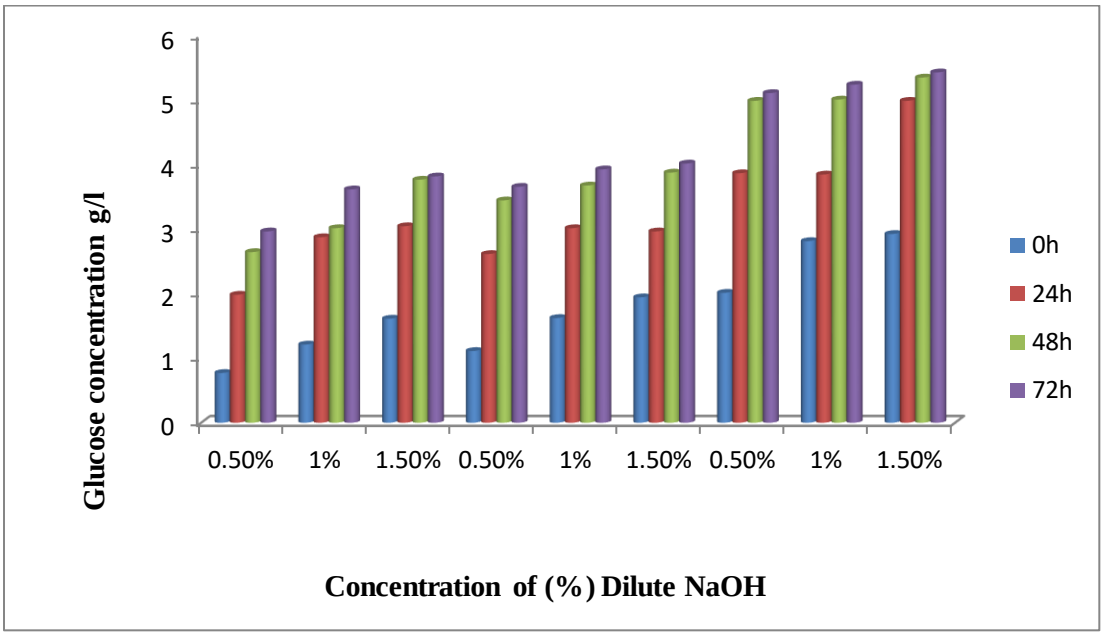

Figure 16. High glucose yield obtained from Wheat straw byNaOH pretreatment conditions at 120 °C

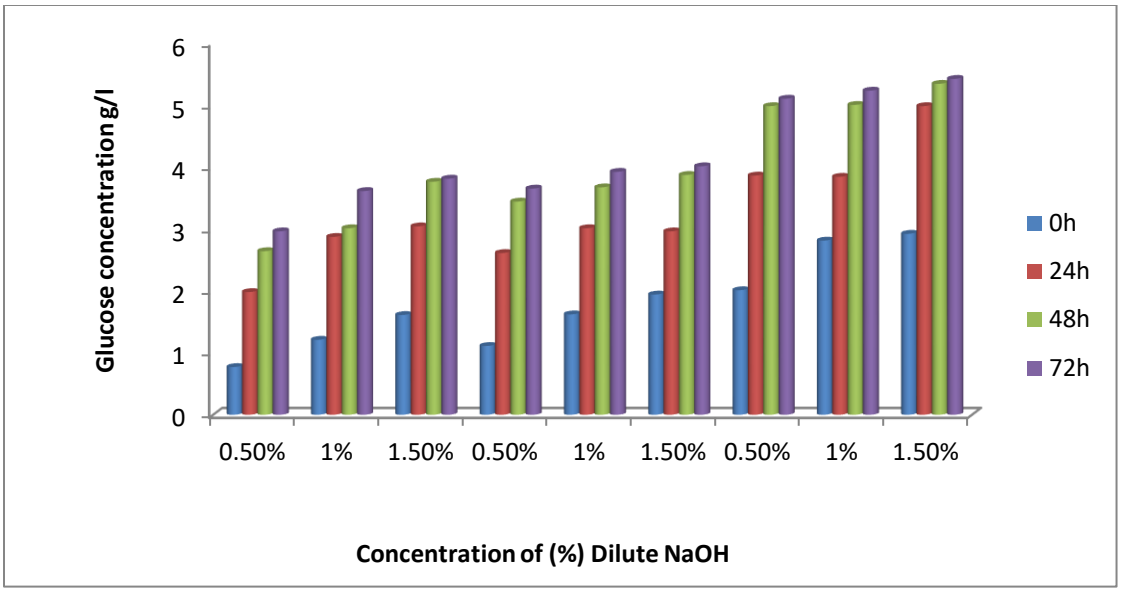

Figure 17. Glucose yield obtained from Rice straw at NaOH pretreatment conditions at 100 ºC

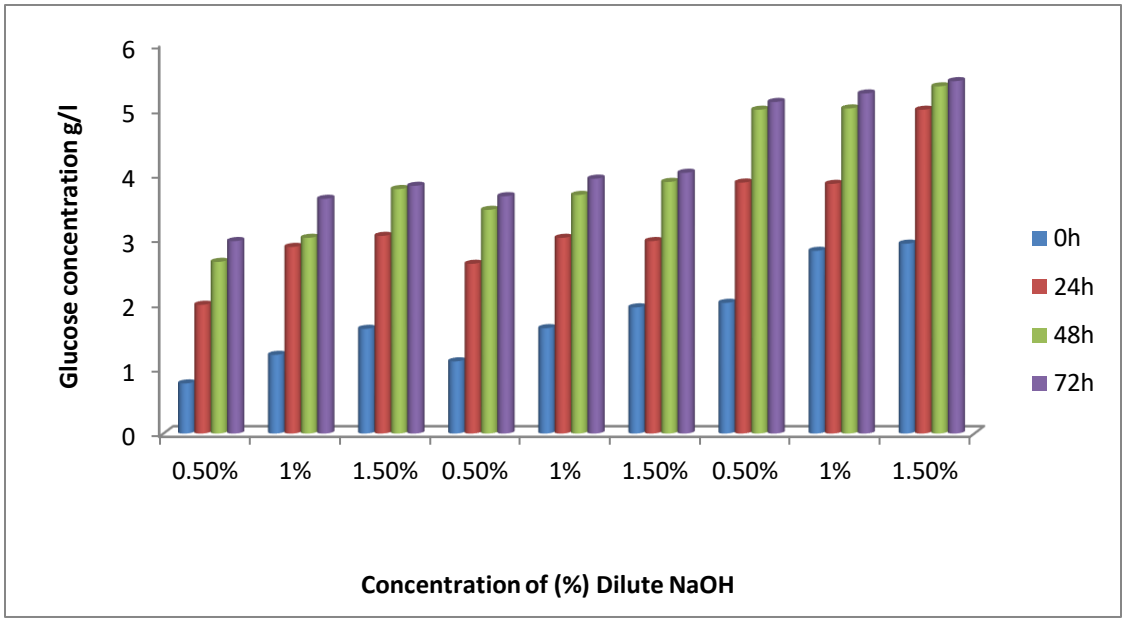

Figure 18. Glucose yield obtained from Corn stover byNaOH pretreatment conditions at 100 ºC

3.11. 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. The samples that demonstrated a larger quantity of glucose under optimal circumstances were chosen for this reason. 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. In order to get the sample concentrations within the range of the calibration curve, 500 µl of the sample was diluted with 1 ml of methanol. The sugars' solubility necessitated the use of methanol. Before analysis, the 0.22 µm filter was used to pass all the samples and the glucose standard solution. A volume of about 20µl of agricultural waste was introduced into the HPLC system 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, the peak may be identified.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

3.12. Fermentation with*Clostridium acetobutylicum*

The major product of this type of fermentation is known as ABE (acetone, butanol and ethanol) fermentation. The ratio of the acetone, butanol and ethanol in the fermentation process ismostly 3:6:1 as reported earlier by many authors. It was estimated that *Clostridium acetobutylicum* yields higherbutanol quantity at acidic pretreatment conditions as compared to alkaline pretreatment conditions. 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

3.13. Chemical analysis of biomass samples

3.13.1. *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).Two main types of bacteria often used in ABE fermentation are Candida acetobutylicum and Candida beijerinckii. Yet, in order to create ABE, researchers from different nations have been studying different strains, parent microbes, growing conditions, and growth medium for diverse biomass samples in batch, fed batch, and continuous fermenters in recent years. Figures 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

4. DISCUSSION

Butanol and ethanol, two alcoholic fuels, are made from lignocellulosic feedstock via a series of technical processes that include saccharification, fermentation, and acid or alkali pretreatment. Accurately modifying all system components is crucial to achieve cost-effective biofuel production. According to Zhao (2012), several nations have made great strides in the manufacture of alcoholic fuels by honing various processes such as pretreatment, enzymatic hydrolysis, fermentation, and achieving a greater percentage of ethanol recovery. Emerging nations may learn a lot from the successful examples of biomass-based fuels generation in industrialized nations. Furthermore, several innovative concepts have been explored for ethanol generation, including biorefinery and the notion of directed conversion of categorized content. The manufacture of butanol from lignocellulosic biomass may also be achieved using comparable technologies (García et al., 2011; Demirbas, 2009). When these technologies are combined

efficiently, they will lead to the competitive production of biofuels from plant biomass, which is now underutilized, and the cost of fuels might drop even more (Talo et al., 2014).

Important products such as acetone, butanol, ethanol, and related alcohols might be produced by fermentation of sugars found in cellulosic biomass. These products could be used as liquid fuels. Wood chips, agricultural crop residues (such as wheat, rice, and cotton), maize stalks, sorghum straws, fruit and vegetable scraps, and similar substrates are the most common sources of carbohydrate-containing biomass. One of the most important sugars found in plant materials, cellulose is also a key ingredient in making alcohol, a fuel. Fermentation by bacteria or fungi, in conjunction with acid treatment and enzymatic hydrolysis, reduces this complex cellulose substance to smaller components. The potential use of these alcohols as fuels makes them significant. Since biofuels aid in reducing levels of carbon emission release from transportation and other sources, they may provide a solution to (1) the problem of climate change (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.Biofuels are a great example of a product that may help solve the problems associated with the circular economy as they reduce waste while making use of natural resources. The present investigation included the production of bioethanol and biobutanol from a variety of cellulosic materials. Consequently, several orders of alcoholic fuels were produced from cellulosic substrates. When compared to other biomass substrates, straws have produced the highest yields of alcoholic fuels.The composition of the cellulosic biomass and the distillation processes used to purify the alcohols after fermentation determine the quantity of acetone, butanol, and ethanol that are generated.

5. 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. The creation of more cost-effective and efficient fermentation processes, as well as a thorough familiarity with the principles of different pretreatment methods, need ongoing research. In addition, there must be research into more efficient microbial strains for detoxification that doesn't

break the bank. We can replace the presently available fossil fuels, which are already depleted, with a process of integration and optimization that reduces energy use and increases yields. 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.

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7. Publications out of this work

8. 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
9. 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
10. 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. Summerakasuar M.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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