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EXTRACTION AND UTILIZATION OF VARIOUS ALCOHOLS TO REPLACE FOSSIL FLUES AND TO MINIMIZE CLIMATIC EFFECTS

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ABSTRACT

Bio fuels appeared to be more climate-friendly than burning fossil fuels. There are different types of alcoholic fuels that can be produced easily and may used as alternative source of existing fuels. Butanol may be used as a fuel in an internal combustion engine and it is more similar to gasoline. Butanol is a drop-in fuel and thus works in vehicles designed for use with gasoline without modification. Both n-butanol and isobutanol have been studied as possible fuels. Butanol is produced from starch- or sugar-based material such as corn grain , sugar cane or from cellulosic feed stocks Biofuel can play a great role in Pakistan because country is oil dependent. Second generation cellulosic biofuels offers a solution to reduce carbon emissions of traffic as well as generation of energy for domestic and commercial uses. A study was conducted to develop a approach for the management of agriculture as well as other organic wastes utilization for production of alcoholic fuels. Therefore cellulosic materials like wheat and

rice straws as well as fruit wastes were used in this study. Samples were analyzed for different parameters. Biological and chemical pretreatments were compared for each substrates. Efficiency of microbial enzymes for saccharification of agricultural substrates was evaluated. It is expected that outcome of this study will help to increase production of biofuels and to reduce burden of imported fossil fuels.

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

INTRODUCTION

Exploration of sources for alternate energy have been increased because of increasing concerns about energy security and climate change. The transportation sector plays a significant role for emission of greenhouse gases due to uses of fossil fuels, However, replacement of oil derived fuels such as ethanol or butanol could reduce environmental impacts and give advantages on social as well as economical levels .Various alternatives to generate sustainable biofuels are being investigated. Biological energy resources are like bioelectricity, biogases, biodiesel and bio alcohols. Among these sources, bioalcohol shows a great potential to reduce the emission of greenhouse gases, decrease the dependence on fossil fuel and act as a chemical feedstock and fuel for transport (Dhamole et al., 2015).The production of alcoholic fuels has been improved extremely because many countries are trying to reduce the import of oil, improving the quality of air and growing rural economics. The global ethanol production is 51,000 million liters (Renewable Fuels Association, 2007). Ethyl alcohol has some advantages as a fuel as it has higher oxygen contents. The higher oxygen level permits improved oxidation of hydrocarbons with successive reduction in aromatic compounds and carbon monoxide emission. Ethanol has greater octane rating properties (Thomas and wong, 2001).

biomass is a vital energy resource in pakistan because of agricultural based country. the biomass produced in livestock and agriculture sector in the form of animal waste and crop remaining as sugarcane bagasse and rice husk (amiri et al. 2014). second generation biomass is mainly composed of lignocellulosic

material. lignocellulosic biomass is more plentiful organic substance on earth and consists of cellulose (35-50%), hemicellulose (20-35%) and lignin (5-30%) (huber et al., 2006).various renewable energy resources include different agricultural substances like green leaves, fruit shells, straws, nut shells and fruit seeds . most commonly used feedstocks are wheat straw, wheat bran, corn stover, corn steep liquor and apple pomace (ejezi *et al*., 2006).now a day, agricultural waste is used for the production of biofuels like biodiesel, bioethanol, biohydrogen and methane as compared to energy crops because they have competition with food crops. as huge amount of agro waste is available and have discarding problem so, alternate option is the utilization of lignocellulosic biomass in order to reduce the competition between fuel and food (mahro and timm, 2007). the grasses are considered as reliable substance for extraction of ethanol. the utilization of perennial grasses is advantageous and possibly it further decreases the cost for the production of ethanol and its use as fuel (gomez et AL*.,* 2008).

the grasses can be grown all over the year worldwide, particularly in subtropical as well as tropical countries. cogon grass has been exploited to rise the soil stability and as fodder, it is recognized as worst weed and it is known as pest by almost 73 countries in all over 35 crops. the roots of cogon grass have secondary metabolites which have medical importance. it is known as perennial grass and could be cultivated in any soil which usually considered as unfit for production of crops.

Cellulose is a major sugar in wood , it is broken down by bacteria available in gut of termite and finally converted into various products including fatty acids and alcohol like ethanol etc.(kim and dale. 2005).*clostridium* **,**genus of rod shaped gram positive bacteria member of which found in soil, water and intestinal tract. *clostridium acetobutylicum* ferments sugar to a mixture of organic solvents like acetone , butanol and ethanol. *saccharomyces cerevisiae* (known as baker s years) single celled eukaryotes which is frequently used in fermentation process for production ethanol and other alcoholic products. therefore current study was

undertaken for chemical and biological analysis of cellulosic biomass for various parameter required for alcohol fuels like biobutanol production

MATERIAL AND METHODS

Collection of Agricultural Substrates

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

Proximate Analysis of Samples

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

Chemical analysis of raw biomass

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

Chemical Pretreatment

For chemical pretreatment two chemicals were used such as acid (H_2SO_4) Pretreatment experiment was performed by using H_2SO_4 (1.0, 1.5 and 2%) at diverse temperatures such as 100 °C , 110 °C and 120 °C for different times durations (15, 30, and 45 minutes). Solid sample (10 %) (w/v) in reagent bottle

was utilized during experiment. After pretreatment, the vacuum filtration assembly was used for filtration of sample in each bottle and the contents were emptied on filter paper. After filtration, the solid washed away with 300 ml distilled water in order to neutralize the pH. The filter paper was than dried at 105 °C and weighed.

Figure 1. Biomass

Enzymatic Hydrolysis

The biomass after pretreatment 5% (w/v) was hydrolyzed with cellulose and β-glucosidases at 50 °C and 160 rpm for 72 hours in a water bath shaker with 0.05 M buffer (sodium citrate) at 4.8 pH. Cellulases having activity of (30FPU g-1).

The samples were withdrawn from reagent bottle after every 12 hours to determine the concentration of sugar. After enzymatic hydrolysis, H_2SO_4 (µl) was added. Un-hydrolyzed sample was separated by centrifuging for 10 minutes at 13,500g. Supernatant was collected by means of syringe filters for sugar analysis by dinitrosalicylic acid (DNS) method .The amount of sugar was analyzed by p-hydroxybenzoic acid hydrazide (PAHBAH) method. By using the concentration 1Mm-25mM of xylose the standard curve was drawn. Then by comparing the standard sugar concentration, the amount of sugar in pretreated sample was determined. The best pretreatment condition was selected after enzymatic hydrolysis process. The sample containing higher amount of released sugar was further selected for fermentation process. The solid biomass was stored at 4 °C which was then used for fermentation process (Demirbas, 2001; Iram et al., 2021; Maria et al., 2021).

***Clostridium acetobutylicum* function for butanol**

The clostridium specie *Clostridium acetobutylicum*was maintained at at -20^{0}C. Enzymatically hydrolysed sample was then used for fermentation. The pH was maintained at 6.5 with NaOH. 1ml *C. acetobutylicum*spores were added in 100ml enzymatically hydrolysed solution in reaction bottle for separate hydrolysis and fermentation. These reaction bottles were placed in shaking incubator having 120 rpm at 37^{0}C for 72 hours. The butanol concentration was determined by alcohol meter after 72 hours, and values were expressed as % butanol obtained from wheat straw, from rice straw and from corn stover. Among these three substrates wheat straw produced higher yield of butanol (Xue et al., 2012).

ANALYSIS OF SUGAR AND ALCOHOL BY USING HPLC

All the samples and standard solution of glucose was passed through the 0.22 µm filter prior to analysis. about 20 µl of sample was injected through injection loop into hplc system. in order to analyze the glucose, enzymatically hydrolyzed samples were run in the gradient mode for 10 minutes (shields and cathcart.2010; sluiter et al., 2008; tao et al., 2014; zhao et al., 2012).

RESULTS AND DISCUSSION

Results regarding chemical analysis of biomass samples as well as fermentation of sugars into acetone- butanol- ethanol are given in the following sections. therefore in current study acetone- butanol - ethanol (abe) were produced from organic wastes material of agriculture and municipal sources by using bacterial fermentation.

Biomass analysis

Data in table 1 represents various parameter found in biomass samples. Whereas ligno-cellulosic contents of the samples are given in table 2. It was observed that wheat straw has higher cellulosic contents as compared to other substrates used for analysis.

Table 1. Proximate analysis (%) of biomass samples

Substrate	Dry Matter	Moisture	Crude Protein	Crude Ash	Crude Fiber	
Wheat straw	91.12±0.48	7.83±0.25	8.15 ±0.24	8.17±0.33	34.45±0.43	
Rice straw	89.15±0.26	7.19±0.27	7.16 ±0.24	9.15±0.33	36.45±0.45	
Peel(wastes)	92.43±0.47	8.56±0.35	5.95 ±0.23	5.92± 0.43	34.86±0.36	

Analysis of organic wastes samples

Table 2. Chemical analysis (%)of biomass samples

Substrate	NDF	ADF	Hemicellulose	Cellulose	Lignin
Wheat straw	83.4±0.58	57.85±0.26	28.14 ±0.25	28.16±0.36	24.46±0
Rice straw	84.16±0.27	54.13±0.26	26.15 ±0.23	27.16±0.43	26.48±0
Peel(wastes)	79.5±0.56	51.1±0.35	25.2±0.35	26.6±0.65	23.4±0.4

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

Dilute H_2SO_4 pretreatment

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

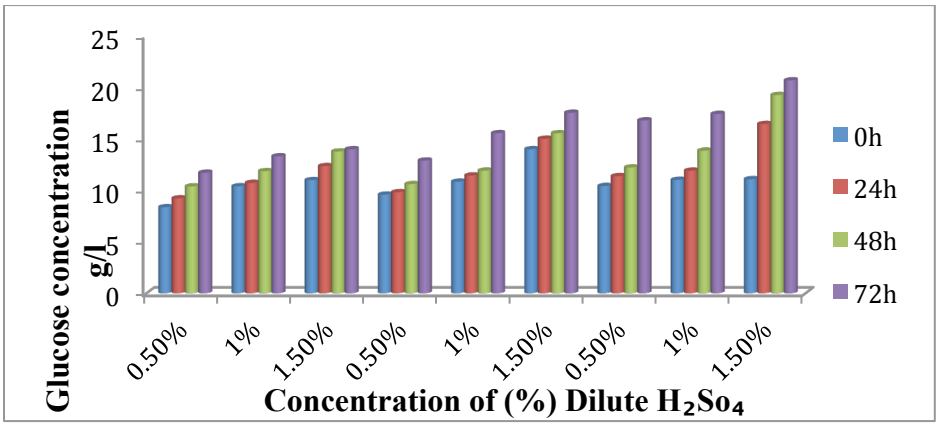

Figure 2. Glucose yield obtained from Wheat straw at H_2SO_4 pretreatment conditions at 120^{0}C

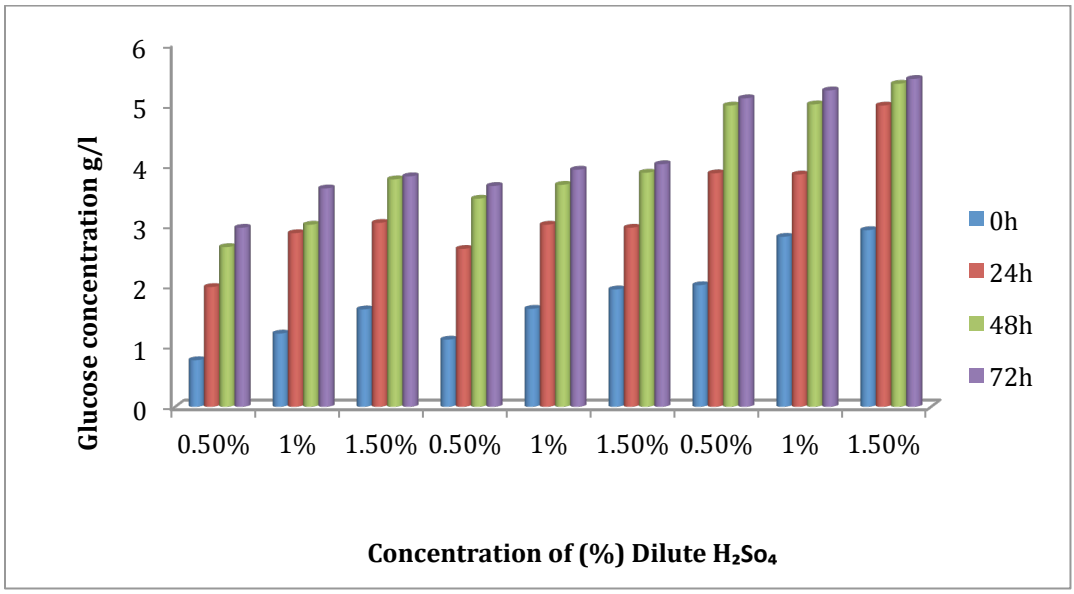

Figure 3. High glucose yield obtained from Rice straw at H_2SO_4 pretreatment conditions at 110 °C.

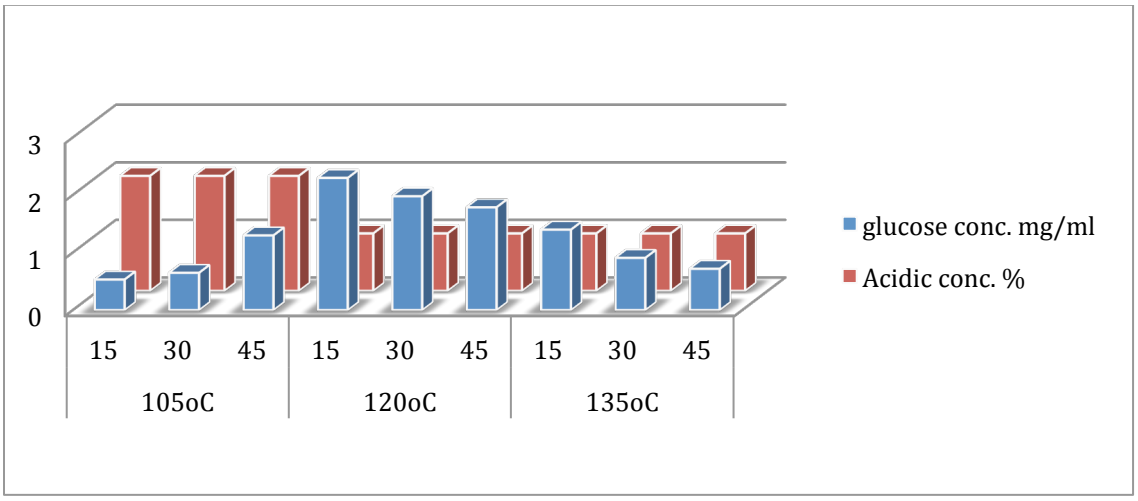

Figure 4. Comparison of glucose concentration at various temperatures after dilute acid pretreatment.

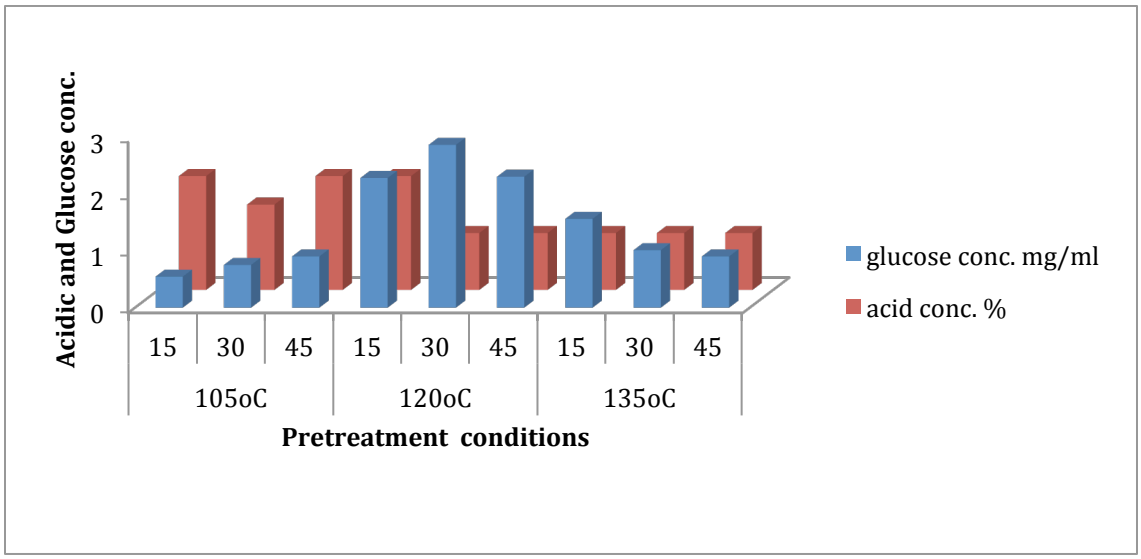

Figure 5. Comparison of glucose concentration at various temperature

Saccharification of biomass samples with enzymes

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

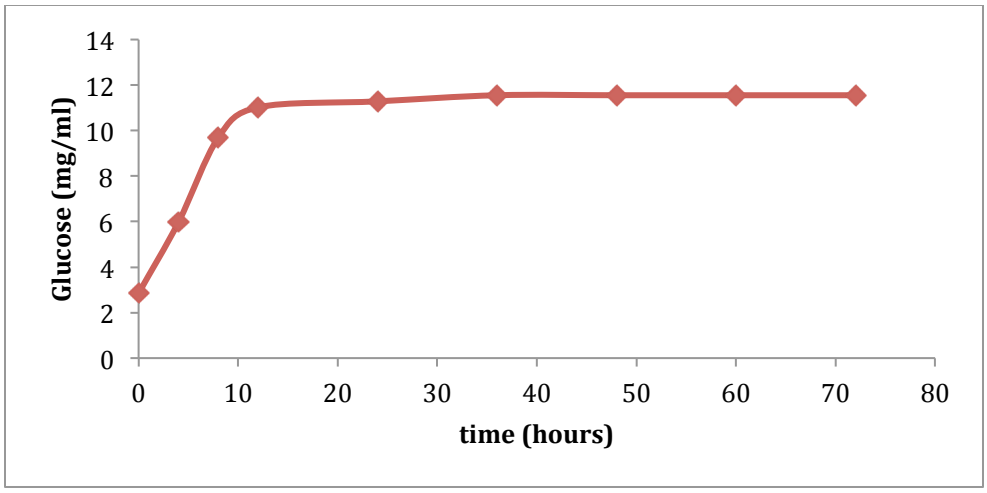

Figure 6. Enzymatic Saacharification of sugar from biomass

Fermentation

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

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 higher butanol quantity at acidic pretreatment Among three substrates the yield of butanol was high in wheat straw because of number of factors i.e. the amount of carbohydrate was high in wheat straw as compared to rice straw and peel waste . Low lignin content in wheat straw is responsible for high glucose yield as well as high yield of butanol than other two substrates. Wheat straw contains low lignin contents as

compared to rice straw. The higher butanol production from wheat straw may be due to its low lignin contents. Among three substrates wheat straw yields highest quantity of butanol at acidic pretreatment conditions. It was estimated from previous studies that wheat straw hydrolysate contain furfural and hydoxymethyl furfural that supported the production of biobutanol by fermentation (Kathleen et al., 2015; eMoretti and Thorson, 2008; Quershi and Blaschek, 2000).

Table 3. Acetone, Butanol and Ethanol production (%) from various agrowaste by

Clostridium acetobutylicum

Samples	Acetone%	Butanol%	Ethanol%
Wheat straw	1.5	6.3	1.8
Rice straw	1.2	6.2	1.3
Peel wastes	1.0	5.2	1.1

ABE production from Biomass samples

Table 4. Acetone, Butanol and Ethanol production from agrowaste by *Clostridium acetobutylicum* at H_2SO_4 pretreated samples

Samples	Acetone %	Butanol%	Ethanol%
Wheat straw	2.5	7.2	2.1
Rice straw	1.7	4.9	2.2
Peel waste	1.1	4.5	2.3

ABE production from Biomass sample

Table 5. Acetone, Butanol and Ethanol production from agrowaste by *Clostridium acetobutylicum*at NaOH pretreated samples

Samples	Acetone %	Butanol%	Ethanol%
Wheat straw	1.9	4.9	2.8
Rice straw	1.5	4.6	2.4
Peel waste	1.3	4.2	2.2

ABE production from Biomass samples

HPLC Analysis of reaction mixtures

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

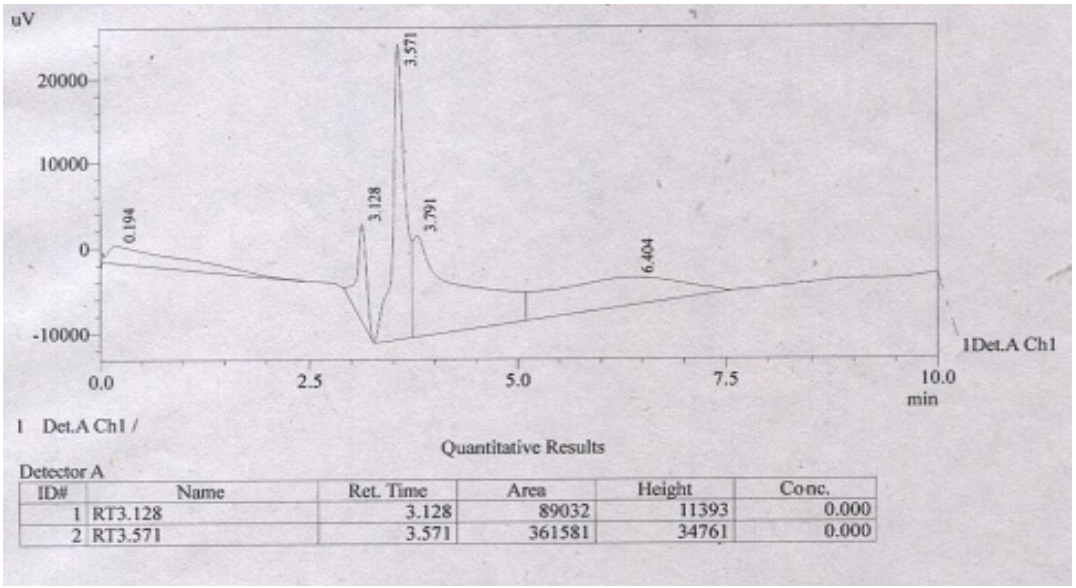

Figure 7. Chromatogram of Wheat straw hydrolysate sugar at acidic pretreatment. Peak of glucose (retention time, 3.128).

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

Components	**Retention time (min)**	**Concentration (mg/ml) Rice straw**	**Concentration (mg/ml) Wheat straw**
Glucose	8.6	22.52	28.3
Cellobiose	7.1	1.02	1.05
Xylose	11.6	4.3	5.6
Arabinose	12.0	1.4	1.8
Mannose	13.2	1.5	2.8
Galactose	15.5	1.2	1.5
Furfural	42.5	1.4	2.65
HMF	28	1.2	2.84

Analysis of sugar with HPLC

DISCUSSION

For the production of alcoholic fuels (Butanol and Ethanol) from lignocellulosic feedstock required various technological steps like acid or alkali pretreatment,

saccharification and fermentation. To accomplish an cost effective production of biofuels, proper adjusting of all units of system is of a great importance. In the past different countries significantly improved alcoholic fuels production by refining different process like pretreatment, enzymatic hydrolysis, fermentation, and higher level of ethanol recovery (Zhao, 2012). The popular cases of biomass based fuels production in developed countries may be good references for the developing countries . In addition many novel ideas, such as biorefinery and the concept of oriented conversion of classified composition have been investigated for ethanol production. Similar technology are also applicable for butanol production fromlignocellulosic biomass (García et al., 2011; Demirbas, 2001). The cost of fuels may further decreases when it will produce at industrial scale and efficient combination of these processes will result in competitive biofuel production from plant biomass, which is currently not being utilized effectively (Talo et al., 2014).

Fermentation of available sugars in cellulosic biomass have potential to provides important products like acetone, butanol, ethanol and similar other alcohols, that could be used as liquid fuels. Mostly available source of biomass containing carbohydrates are wood wastes , agriculture crops like wheat, rice and cotton straws , corn covers, sorghum straws, fruit and vegetable wastes and similar other substrates (Iram et al., 2021) . Cellulose is considered as major sugar for alcohol (fuel) production and cellulose is complex sugar present in plants materials. This complexcellulosic material is break down into smaller units with help of acid treatment and enzymatic hydrolysis as well as bacterial/ fungal fermentation. These forms of alcohols is important because that may use as fuels. Therefore biofuels may provide solution of combating climate change, as it help to reduce level of carbon emission release from traffic etc. Therefore various order of alcoholic fuels production from cellulosic substrates was obtained . Among all substrates of biomass used straws has provided better yields of alcoholic fuels as compared to others material used.However, amount of acetone, butanol and ethanol produced depends on nature of cellulosic biomass used as

well as various distillation process conducted after fermentation for purification of these type of alcohols (Maria et al., 2021).

CONCLUSION

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

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biofuel 1

by Maham misbah

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EXTRACTION AND UTILIZATION OF VARIOUS ALCOHOLS TO REPLACE FOSSIL FLUES AND TO MINIMIZE CLIMATIC EFFECTS

ABSTRACT

Bio fuels appeared to be more climate-friendly than burning fossil fuels. There are different types of alcoholic fuels that can be produced easily and may used as alternative source of existing fuels.

As it is more comparable to gasoline than to ethanol, butanol may be used as a fuel in internal combustion engines. As a drop-in fuel, butanol may be used in gasoline-powered cars without any adaptations. However, research on the viability of n-butanol and isobutanol as fuels has been mixed. Butanol is a fuel alcohol made from sugars and starches like maize grain, sugar cane, and cellulosic feed stocks.

Biofuel can play a great role in Pakistan because country is oil dependent. Second generation cellulosic biofuels offers a solution to reduce carbon emissions of traffic as well as generation of energy for domestic and commercial uses. A study was conducted to develop a approach for the management of agriculture as well as other organic wastes utilization for production of alcoholic fuels. Therefore cellulosic materials like wheat and rice straws as well as fruit wastes were used in this study. Samples were analyzed for different parameters. Biological and chemical pretreatments were compared for each substrates. Efficiency of microbial enzymes for saccharification of agricultural substrates was evaluated. Results from this research might lessen the need for costly fossil fuel imports and boost domestic biofuel production.

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

INTRODUCTION

When it comes to greenhouse gas emissions, the transportation sector is a major offender due to its reliance on fossil fuels. However, switching to alternatives like ethanol or biodiesel from oil could lessen environmental impacts and provide benefits on social and economic fronts as well (Malakar et al., 2020).

Multiple options for producing biofuels in a sustainable manner are under study. Energy that is generated from living things includes things like bioelectricity, biogases, biodiesel, and bioalcohols. Bioalcohol is one of these sources that has the potential to significantly cut down on greenhouse gas emissions, fossil fuel consumption, and transportation fuel and chemical feedstock needs (Chenubini ,

2010). There has been a dramatic increase in the manufacture of alcoholic fuels as a result of the worldwide push to decrease oil imports, improve air quality, and stimulate rural economies. Produced ethanol amounts to 51,000,000,000 liters worldwide (Renewable Fuels Association, 2007). The greater oxygen concentration of ethyl alcohol makes it a viable fuel alternative. Improved oxidation of hydrocarbons and subsequent decrease in aromatic compounds and carbon monoxide emission are made possible by the increased oxygen content. The octane rating of gasoline is lower than that of ethanol (Thomas and Wong, 2001).

For a nation like Pakistan, so dependent on agriculture, biomass fuels are essential. Sugarcane bagasse and rice husk are two examples of the biomass that farmers and livestock farmers generate (Malakar et al., 2020). The lignocellulosic materials are the principal components of the second generation biomass. The three main components of lignocellulosic biomass are cellulose (35%-50%), hemicellulose (20-35%), and lignin (5-30%). (Li et al., 2014). Leafy greens, fruit rinds, straws, nut husks, and fruit seeds are just few of the many agricultural commodities that may be converted into renewable energy. Wheat straw, wheat bran, maize stover, corn steep liquor, and apple pomace are some of the most popular feedstocks (Ejezi et al., 2006). Energy crops compete with food crops, thus nowadays biofuels like biodiesel, bioethanol, biohydrogen, and methane are produced from agricultural waste. Due to the abundance of both agricultural waste and the difficulty in disposing of it, one alternative solution that might help alleviate the tension between food and fuel is the use of lignocellulosic biomass (Mahro and Timm, 2007). Grass is a trusted source for ethanol production. Using perennial grasses to create ethanol and utilize it as fuel might be beneficial and reduce the price of both processes (Gomez et al., 2008).

The grasses can be grown all over the year worldwide, particularly in subtropical as well as tropical countries. Cogon grass has been exploited to rise the soil stability and as fodder, it is recognized as worst weed and it is known as pest by almost 73 countries in all over 35 crops. The roots of cogon grass have secondary metabolites which have medical importance. It is known as perennial grass and could be cultivated in any soil which usually considered as unfit for production of crops.

Cellulose is a major sugar in wood , it is broken down by bacteria available in gut of termite and finally converted into various products including fatty acids and alcohol like ethanol etc.(Kim and Dale. 2005).*Clostridium* ,genus of rod shaped gram positive bacteria member of which found in soil, water and intestinal tract. *Clostridium acetobutylicum* ferments sugar to a mixture of organic solvents like acetone ,

butanol and ethanol. *Saccharomyces cerevisiae* (known as baker s years) single celled eukaryotes which is frequently used in fermentation process for production ethanol and other alcoholic products. Therefore current study was undertaken for Chemical and biological analysis of cellulosic biomass for various parameter required for alcohol fuels like biobutanol production

MATERIAL AND METHODS

Collection of Agricultural Substrates

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

Proximate Analysis of Samples

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

Chemical analysis of raw biomass

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

Chemical Pretreatment

Pretreatment experiments were conducted using H2SO4-based acids at concentrations of 1%, 1.5%, and 2%, and temperatures ranging from 100 to 120 degrees Celsius (15, 30, and 45 minutes). The experiment made use of a solid sample contained in a reagent bottle at a concentration of 10%

(w/v). Each bottle's contents were dumped onto filter paper after being subjected to pretreatment using the vacuum filtration assembly. Following filtering, neutral pH was achieved by flushing away the solid with 300 cc of distilled water. After being dried at 105 degrees Celsius, the filter paper was measured.

Enzymatic Hydrolysis

The biomass was pretreated with 5% (w/v) ethanol and then hydrolyzed with cellulose and -glucosidases at 50 °C and 160 rpm in a water bath shaker containing 0.05 M buffer (sodium citrate) at 4.8 pH for 72 hours. Cellulases that can break down cellulose (30FPU g-1). Every 12 hours, a sample was taken from the reagent bottle and analyzed for sugar content. H2SO4 (l) was added after enzymatic hydrolysis. Separation of the un-hydrolyzed material was achieved by centrifugation at 13,500g for 10 minutes. The dinitrosalicylic acid (DNS) technique was used to analyze the sugar concentration in the supernatant that was collected using syringe filters. The p-hydroxybenzoic acid hydrazide (PAHBAH) technique was used to determine the exact quantity of sugar present. The standard curve was constructed using xylose concentrations between 1M and 25mM. The quantity of sugar in the unprocessed sample was calculated by comparing it to the standard sugar concentration. After an enzymatic hydrolysis procedure, the optimal pretreatment condition was determined. For the next step in the fermentation process, only the sample with the highest sugar release was chosen. We fermented the solid biomass after storing it at 4 degrees Celsius (Demirbas, 2001; Iram et al., 2021; Maria et al., 2021).

***Clostridium acetobutylicum* function for butanol**

The clostridium specie *Clostridium acetobutylicum*was maintained at at -20^{0}C. Enzymatically hydrolysed sample was then used for fermentation. The pH was maintained at 6.5 with NaOH. 1ml *C. acetobutylicum*spores were added in 100ml enzymatically hydrolysed solution in reaction bottle for separate hydrolysis and fermentation. These reaction bottles were placed in shaking incubator having 120 rpm at 37^{0}C for 72 hours. The butanol concentration was determined by alcohol meter after 72 hours, and values were expressed as % butanol obtained from wheat straw, from rice straw and from corn stover. Among these three substrates wheat straw produced higher yield of butanol (Xue et al., 2012).

Analysis of sugar and alcohol by using HPLC

Before analysis, both the samples and the glucose standard solution were filtered via a 0.22 m membrane. The HPLC system had around 20 l of sample put into it via the injection loop. Enzymatically hydrolyzed samples were analyzed for glucose using gradient mode for 10 minutes (Shields and Cathcart.2010; Sluiter et al., 2008; Tao et al., 2014; Zhao et al., 2012).

RESULTS AND DISCUSSION

Results regarding chemical analysis of biomass samples as well as fermentation of sugars into acetone-butanol-ethanol are given in the following sections. Therefore in current study acetone-butanol-ethanol (ABE) were produced from organic wastes material of agriculture and municipal sources by using bacterial fermentation.

Biomass analysis

Data in table 1 represents various parameter found in biomass samples. Whereas ligno-cellulosic contents of the samples are given in table 2. It was observed that wheat straw has higher cellulosic contents as compared to other substrates used for analysis.

Dilute H_2SO_4 pretreatment

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

Saccharification of biomass samples with enzymes

Since the highest quantity of glucose released from agricultural waste reached a plateau after 36 hours, further experimentation was unnecessary (Fig. 6). After the sugar has been extracted, it may be utilized in fermentation processes (Huang et al., 2015; Dheeran et al., 2012; Garcia et al., 2011). Within the first 48 hours after the enzyme was added, glucose was released at a rate of 11.55 mg/ml; after that, the rate of sugar release was constant, as shown by a straight line (Fig.6).

Fermentation

Tables 3–5 indicate that much greater butanol concentrations were achieved from a variety of substrates. Up to a point, the time period increased while the ethanol content remained constant or decreased. Nonetheless, the glucose content was insufficient to sustain the ethanol synthesis after 72 hours. Results showed that agrowaste was a superior option for alcohol fuel generation due to its higher cellulose and lower lignin concentrations (Gregg and saddler, 1996; ; Hanifeng et al., 2015; Jiang et al., 2015).

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 higher butanol quantity at acidic pretreatment Among three substrates the yield of butanol was high in wheat straw because of number of factors i.e. the amount of carbohydrate was high in wheat straw as compared to rice straw and peel waste . Low lignin content in wheat straw is responsible for high glucose yield as well as high yield of butanol than other two substrates. Wheat straw contains low lignin contents as compared to rice straw. The higher butanol production from wheat straw may be due to its low lignin contents. Among three substrates wheat straw yields highest quantity of butanol at acidic pretreatment conditions. It was estimated from previous studies that wheat straw hydrolysate contain furfural and hydoxymethyl furfural that supported the production of biobutanol by fermentation (Kathleen et al., 2015; eMoretti and Thorson, 2008; Quershi and Blaschek, 2000).

HPLC Analysis of reaction mixtures

Following enzymatic hydrolysis, HPLC analysis was performed on samples of acidic pretreated wheat and rice straws, etc. The samples chosen for this investigation were those that showed a greater increase in glucose under the best possible circumstances. After collecting samples at various intervals during enzymatic hydrolysis, they were centrifuged at 14,000 rpm, 4 0C, for 15 minutes. Separated supernatant was filtered via a 0.22 m syringe filter. To ensure that sample concentrations fell within the range of the calibration curve, a 500 l aliquot was diluted with 1 ml of methanol. Sugars' solubility in methanol necessitated its usage. Peak identification based on retention time (tR). Three samples, wheat straw, rice straw, and corn stover, were analyzed for the presence of glucose using high-performance liquid chromatography (HPLC), with the presence of glucose verified by the injection of a recognized standard. (Table 6 and Fig. 7).

DISCUSSION

Alcoholic fuels (such as butanol and ethanol) may be made from lignocellulosic feedstock using a number of technical processes, including acid or alkali pretreatment, saccharification, and fermentation. Correctly regulating all elements of the system is crucial for achieving efficient and economical biofuel production. Many nations have made great strides in the manufacturing of alcoholic fuels in the past, particularly in refining processes including pretreatment, enzymatic hydrolysis, fermentation, and increased ethanol recovery (Zhao, 2012). Developed nations' successful experiences with fuels made from biomass might serve as models for less developed nations. Ethanol production has also inspired the exploration of several cutting-edge concepts, such as biorefinery and the notion of directed conversion of categorized content. To make ethanol from lignocellulosic biomass, a similar set of processes may be used (Garca et al., 2011; Riberio, 2013). Production at industrial scale and the successful coupling of various techniques will lead to cost-competitive biofuel production from plant biomass, which is presently underutilized (Talo et al., 2014).

Fermentation of the abundant sugars in cellulosic biomass has the potential to yield key products like acetone, butanol, ethanol, and comparable other alcohols that might be utilized as liquid fuels. Wood scraps, agricultural crops including wheat, rice, and cotton straws, maize cobs, sorghum straws, fruit and vegetable scraps, and similar substrates are the most common sources of biomass with carbs (Iram et al., 2021). Cellulose is a complex sugar found in plant materials and is often regarded as the sugar of choice for the manufacture of alcohol (fuel). With the use of

acid treatment and enzymatic hydrolysis as well as bacterial/fungal fermentation, this complex cellulose substance is broken down into smaller pieces. These alcohols are useful because they can be converted into useful fuels. As a result, biofuels may provide a means of fighting climate change by lowering the overall quantity of carbon dioxide released as a result of vehicle use and other human activities. Thus, a hierarchy of alcoholic fuels was produced from cellulosic substrates. In comparison to other biomass substrates, straws provide the highest yields of alcoholic fuels. The quantity of acetone, butanol, and ethanol generated, however, varies according to the kind of cellulosic biomass utilized and the method of distillation used to purify the alcohols that have been created during fermentation (Maria et al., 2021).

CONCLUSION

When compared to traditional fuels and energy, renewable and sustainable options are superior. Making alcoholic fuels (ethanol, etc.) from lignocellulosic biomass is an environmentally friendly and financially viable option. Meanwhile, ongoing efforts are required to get a comprehensive grasp of the basics of different pretreatment procedures and to design more efficient and cost-effective fermentation techniques. Furthermore, more efficient microbial strains are necessary for the development of cost-effective detoxification. In order to replace the rapidly depleting fossil fuels that are presently in use, it is necessary to integrate and optimize methods of lowering energy usage while simultaneously increasing 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 phenomena for the development of country by using indigenous resources in future.

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