

Exploitation of Ethanol as an Alternative Fuel Source from Agricultural Residues

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Abstract: – Agroresidues are the main source of lignocellulosic biomass. Lignocellulosic biomasses are globally abundant, inexpensively-available and sustainable sources suitable for the development of bioethanol manufacturing.. A huge diversity of agroresidue is available around the world. Agricultural and agro-industrial residues such as sugarcane bagasse, rice hulls, wheat straw, rape straw, wheat bran, barley straw, and cassava stalks have the potential to be subjected to bioconversion processes.. Lignocellulosic biomass is a plant biomass and it is composed of cellulose, hemicelluloses and lignin. Biomass has been identified as a highly sought-after resource, due to its capacity to be used as a substitute for petroleum in the production of biofuels and chemicals.

Bio-ethanol is an attractive, sustainable energy source to fuel transportation , which is prepared by these agroresidues in different steps. Prior to degradation of biomass components, pretreatment is essential.. The pretreatment of biomass can be achieved through a variety of means; one such method being dilute acid pretreatment. This method is effective in enabling access to the internal structures of the biomass for further processing.. Most of the technology has also been developed for converting the second largest biomass fraction, Cellulose and hemicellulose, into ethanol.Following pretreatment, enzymatic hydrolysis can be utilized to ferment pre-treated biomass either through Simultaneous Saccharification (SS) or by additional acidic treatment. SSF is the favored method for creating ethanol from the major portion of lignocellulosic biomass, cellulose, as it exhibits cost-efficiency.l. A range of acid pre-treatment of biomass was made and the pretreated biomass samples were fermented with *Saccharomyces cerevisiae*. The sample, pre-treated with a 3% dilute sulfuric acid solution, yielded an ethanol concentration of 4.9 g l-1. The residual fraction, predominantly composed of lignin, can be utilized as boiler fuel in order to facilitate the conversion process and generate surplus electricity for exportation.

New developments and approaches of biofuel production from kinds of lignocellulosic biomass inconversion technology enhance the ethanol production and have reduced the projected price of ethanol in the present scenario. In a case study of production of ethanol from agroresidues such as rape straw, sugarcane bagasse in eastern U.P. was found 348 L and 360 L (approx) ethanol per dry ton biomass. Ethanol is used as E85, E10 as a motor fuel. The utilization of biofuels is predicted to diminish reliance on imported petroleum, which has the potential to reduce political and economic instability, and concurrently lower greenhouse gas emissions and other pollutants in the atmosphere. Moreover, an enhanced demand for agricultural products is likely to stimulate the fiscal climate.. In future, bio fuels should ideally create the environmental, economic and social benefits to the communities and reflect energy efficiency.

Keywords: Agroresidues, Biomass, Ethanol, Pretreatment, Fermentation, Hydrolysis.

Introduction: Climate change due to global warming and with an increasing demand of energy, volatile oil prices, and energy poverty have led to a search for alternative sources of energy A costeffective, just, and sustainable approach could be employed.. One of such type of fuel is biofuels. Biofuels have generated considerable energy and have been effective in mitigating climate change, thus prompting government and industrial stakeholders across the globe to increase their production. Ethanol is a liquid hydrocarbon derived from carbohydrates extracted from sugar-containing organic matter.. The production of ethanol has gained importance in last few years due to the increased dependency on oil and conventional fuels. The blending of ethanol with diesel or petrol can reach up to a maximum of 20% (Ramanathan, 2000). Currently, ethanol is generated utilizing molasses, a by-product of the sugar industry.. The cost of production increases as the demand for molasses has increased. Given the need for an alternate source of ethanol production, it is clear that common crops such as sugarcane, sweet sorghum, beet and potato are viable options. Furthermore, the utilization of agricultural crop residues, such as rape straw, rice straw, wheat straw and bagasse, is an attractive proposition due to their abundance of sugars and renewable nature, as well as their low cost. represent the ideal feedstock for their conversion into value-added products by biotechnological routes (Mussatto and Teixeira 2010). These agro-residues, like other lignocellulosic materials, are constituted primarily of lignin, hemicellulose and cellulose. The depolymerization of hemicellulose and cellulose into sugars serve as a major carbon source for microbes which can then be utilized in the production of xylitol, ethanol, organic acids, industrial enzymes, and other compounds (Carvalheiro et al. 2008; Mussatto and Teixeira 2010; Chandel et al. 2011a).

The utilization of biomass fuels in rural India accounts for more than 80 percent of total energy consumption, constituting a predominant element of energy consumption patterns. Biomass fuels utilized in rural areas include fuelwood, crop residues (including those from plantations) and livestock dung.. Fuelwood is the preferred and most dominant biomass source accounting for 54% of biofuels used in India (Ravindranath 1995). Scarcity and increasing prices of fuelwood have been altering the biofuel consumption. The scarcity of fuelwood has led to the utilization of dung and crop residues as an alternative energy source. The use of biofuels for domestic purposes has been associated with additional labor and detrimental health effects on female users (Smith KR. 1993). The natural energy resources such as petroleum and coal are being utilized at a rapid rate and these resources have been estimated to last over a few years. Due to dwindling of fossil fuel, microbial production of biofuel from organic by products has acquired significance in recent years, Biomass has been shown to have considerable promise as a raw material for gaseous fuels, liquid fuels and certain petrochemical intermediates (Correa et al, 1999; Yamada and Ono, 1999). The lignocellulosic agro residues mainly comprise of cellulose, hemicellulose and lignin (Kaur et al, 1998). The carbohydrate portion of the agro residues is approximately 60% by weight and is considered as a feedstock for conversion to sugars, which may be fermented to ethanol (Vlasenko et al, 1997).

In lignocellulose, the cellulose and hemicellulose are intimately entwined with lignin (Cowling and Kirk, 1976; Okeke and Obi, 1994). This lignin component acts as a physical obstacle (Lynch 1992) and thus, must be removed in order to render the carbohydrates suitable for further transformation. Therefore, to effectively utilize lignocellulose, it must first be subjected to pretreatment in order to increase the surface area, bulk density and decrease the cellulose crystallinity, thus rendering it amenable to hydrolysis (Gharpuray et al, 1983; Rao and Setta, 1983; Gould, 1984; David et al, 1985; Wood and Saddler, 1988; Viesturs et al, 1996; Nikolov et al, 2000). This pretreated lignocellulose, with freed cellulose, is now susceptible to enzymatic hydrolysis ((Nazhad et al, 1994; Wykvan, 1999) Enzymatic hydrolysis of pretreated lignocellulosics can produce mono- and oligomeric sugar solutions, which can subsequently be used in the production of fuels or chemicals such as ethanol (Nguyen and Saddler, 1991). Structural parameters play a role in determining the outcome of lignocellulose hydrolysis; the heterogeneous distribution of lignin in lignocellulosic residue creates physical impediments to homogeneous reactions, while the polar nature of lignin due to the presence of phenolic and alcoholic hydroxyl groups leads to the formation of intermolecular hydrogen bonds (Ghosh et al., 1983). The hydrolysis of native cellulose is a slow and difficult process, thus necessitating specific pretreatment processes to increase the enzymatic susceptibility. Unprocessed lignocellulose is broken down slowly by microorganisms due to its highly compact and stringent structure of (1, 4) linkages in the cellulose and due to the close association of lignin with cellulose and hemicellulose, leaving very few reactive sites for enzyme attachment (Bjerre et al, 1996).

The agriculturalists in less affluent nations are seeking alternative sources of revenue, and the production of biomass offers them supplemental profit. But it has negative implications of growing biomass for biofuel production. Current biofuels are often made from feedstock crops which also serve as food. Consequently, there is an interaction between food and fuel.. To produce biofuels, production of food is decreasing and many people are starving due to shortage of food, so that people of poorer countries are suffering from malnutrition and prices of foods are increasing in these countries. The production of biofuels often results in the disruption of habitats, leading to the fragmentation of ecosystems and a decrease in the total biodiversity, consequently affecting the delivery of essential services to our economies . and human life. Bioethanol, biodiesel, and biogas (comprised of methane and other hydrocarbons) are among the foremost biofuel sources of the first generation.. Biodiesel are obtained from edible oil or from animal oils which are transformed by a chemical process called transesterification. Biodiesel can be blended with fossil fuels without any motor's transformation and also can be used as pure biodiesel. Bioethanol are produced by the fermentation of sugars or starches from sugar cane, cassava, maize, potatoes, sorghum, sugar beet, wheat etc. Second-generation biofuels can be created from a multitude of non-food based sources including waste biomass, residuals, ligno-cellulosic and non-food cellulosic materials (which are made available through enzymatic cellulose breakdown), corn stover, wheat stalks, and energy crops. These biofuels can be produced through a biomass-to-liquid procedure, involving either fermentative or thermochemical conversion (mainly for biodiesel production). Algae fuel (oilgae) can also be classified as a third-generation biofuel.. Algae are feedstocks from aquatic cultivation for production of triglycerides to produce biodiesel. Second and third generation biofuels, otherwise known as advanced biofuels, have similar processing technologies to those used in biodiesel production from second-generation feedstocks. However, the commercialization of alcohol-based biofuels such as bio-propanol and bio-butanol is projected to occur no earlier than 2050, based on IEA 2008 studies.

Ethanol may be used as a fuel itself or in blends with petrol or diesel. It has a lower energy density than petrol or diesel, a higher octane number than petrol, and a much lower cetane number than diesel. It is highly miscible with petrol but not miscible with diesel. Ethanol-diesel blends use cetane enhancers and solubility improvers. Table 3 lists the important fuel properties of ethanol and compares these with petrol and diesel fuel.

Property	Ethanol	Petrol	Diesel
Composition, weight %			
C	52.2	85-88	84-87
H	13.1	12-15	13-16
O	34.7	0	0
Density, kg/m ³	794	750	825
Lower heating value, MJ/kg	26.7	42.9	43

Octane number	100	86-94	-
Cetane number	8	5-20	40-55
Reid vapour pressure (kPa)	15.6	55-103	1.4
Stoichiometric air/fuel ratio, weight	9:1	14.7:1	14.7:1
Boiling temperature, °C	78	80-225	188-343
Flash point, closed cup, °C	13	-42	74

Sources: JEC, 2005; Joseph, 2007

Table 1: Fuel properties of anhydrous ethanol and comparison with petrol and diesel fuel

Material and Methods: Lignocellulosic dry samples such as (Sugarcane bagasse, wheat straw, rape straw etc.) were taken from agricultural rural areas. All the biomass samples were dried in to oven and cut in to small pieces by milling. The process of obtaining ethanol from lignocellulosic materials necessitates the initial reduction of biomass into smaller fragments followed by acid or enzymatic hydrolysis to break down the biomass into monosaccharides.

Pretreatment: To produce biofuel from agroresidues pretreatment step is the necessary step to degrade the lignocellulosic biomasses in to cellulose, Hemicellulose and Lignin. Because hemicelluloses and cellulose present in the cell wall undergo lignifications, an effective and economic method must be used to separate cellulose and hemicelluloses from cell wall. To make monomeric sugar utilization from these residues various physical, chemical and biological pretreatments methods is necessary. Mechanical and thermal methods exist to treat agro-industrial residues, but these methods tend to require a high energy input which can increase the processing cost considerably. Physical pre-treatment can also make product separation for fermentation purposes expensive.

Chemical treatment is generally used to remove lignin content of agro residues. Chemical pretreatments by alkali or acid hydrolysis are common in paper and pulp industries to recover cellulose for paper production. Chemical pretreatment with acid or alkali is frequently utilized to enhance the surface area of agro-industrial residue, as the swelling and disruption of lignin facilitates bioconversion. However, these treatments can be costly and thus are not commonly employed.. Pretreatment is the pre step to release the components of lignocellulosic biomass. Agroresidues consist of lignocellulose that is compact, partly crystalline structure consisting of linear and crystalline polysaccharides cellulose, branched non cellulose and non-crystalline hetero polysaccharides (hemicelluloses), and branched (non crystalline) lignin (Glasser et al. 2000). 2-3% acid (H_2SO_4) was used for the pretreatment method. The acid-soaked biomass slurry was autoclaved at 121°C for 30 minutes, after which a centrifugation process was employed to separate the solid and liquid fractions. Before proceeding to the fermentation process, the pH of the centrifuged biomass was adjusted to 5 by adding an alkali.

Cellulose is made up of a linear polymer chain, which in turn consists of a series of hydro glucose units in glucan chains (Fig. 1). The hydro glucose units are held together by β -1-4 glycosidic linkages, producing a crystalline structure that can be broken down more readily to monomeric sugars. Another major component of the lignocellulose structure is hemicellulose, which is made up of various polysaccharides, namely, xylose, galactose, mannose and arabinose. The function of hemicelluloses has been proposed as a bonding agent between lignin and cellulose. Hemicellulose is a polymeric structure composed of L-arabinose, D-galactose, D-glucose, Dmannose, and D-xylose, containing both linear and branched components.

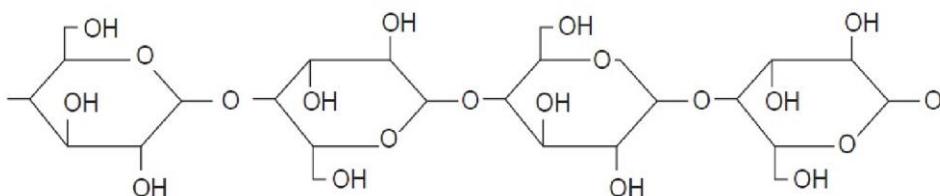


Fig. 1 Structure of cellulose

Microorganisms and Culture Media: we cultured yeast on PDA slants. Liquid medium for the preparation of the yeast was composed of 50 grams glucose, 5 grams yeast extract, 1 gram KH_2PO_4 , 0.3 grams NH_4Cl and 2 grams $MgSO_4 \cdot 7H_2O$ per litre (A. Edgardo et al, 2008). Following the introduction of the yeast slant into the medium, it was incubated in a shaker at 41°C and 130 rpm for 24 hours.. 2.5% (by volume) of this prepared yeast was inoculated for ethanol production. Fermentations were conducted in pH-controlled flaker fermentors, as described (Beall DS et al, 1991). Each 200ml working volume 500ml flask was outfitted with a pH probe, an infusion needle, a sampling needle, and a magnetic stir bar. We placed a six position magnetic-stirring plate underneath a water bath to drive the bar at 150rpm. A 0.05 M Phosphate buffer of pH 5.5 was utilized and a sugar concentration of 50 g/L was maintained for Glucose alone. The pre-defined volume of biomass culture was centrifuged and the cell pellet was re-suspended in the fermentor to achieve an initial Optical Density (600 nm) of 0.5. The values of fermentation properties, such as cell concentration, metabolic ethanol output, volumetric ethanol production rate and specific ethanol production rate, were determined according to Lau et al. (2008).

Analytical measurement: The concentrations of glucose, xylose, ethanol, glycerol and xylitol in the fermentation and culture experiments underwent analysis through highperformance liquid chromatography (HPLC). The HPLC system utilized was described by Lau et al. (2008). Cell densities were quantified with the aid of a UV/Vis Spectrophotometer (Beckmann Coulter

DU720) at a wavelength of 600 nm; the absorbance reading was transformed into g dry-wt/L. One unit of absorbance at 600 nm is equal to 0.31 and 0.47 g dry-wt/L for *Z. Mobilis* and *S. Cerevisiae* respectively.

Result and Discussion: Ethanol production from agro residues involves the pre-treatment with acid or alkali, scarification, fermentation using yeast, finally separation followed by distillation as shown in Fig.2. (Olsson and Hagerdal, 1996).

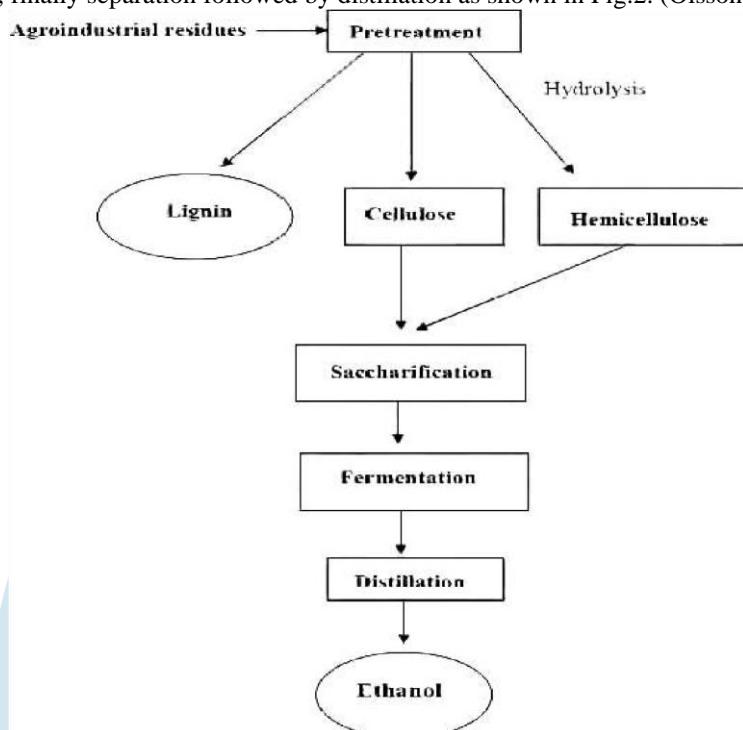


Fig.-2 Ethanol production from agro residues.

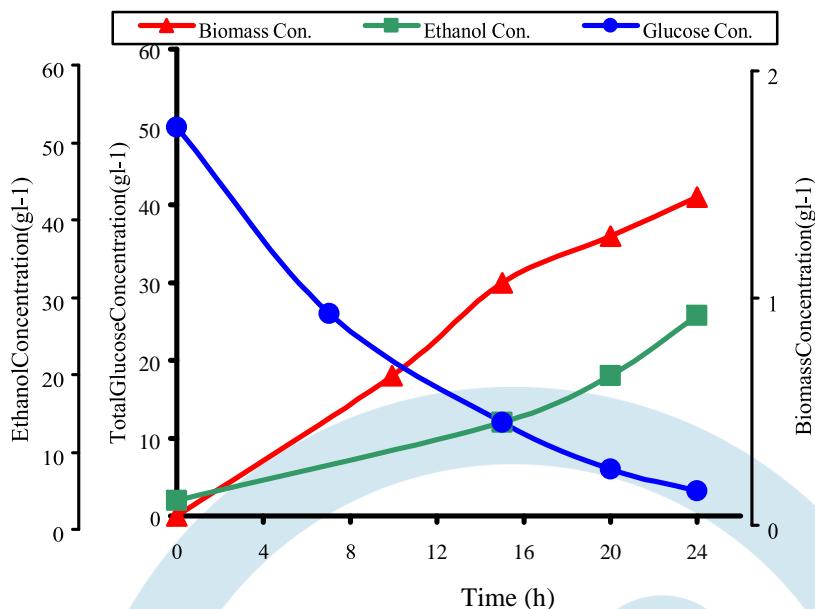
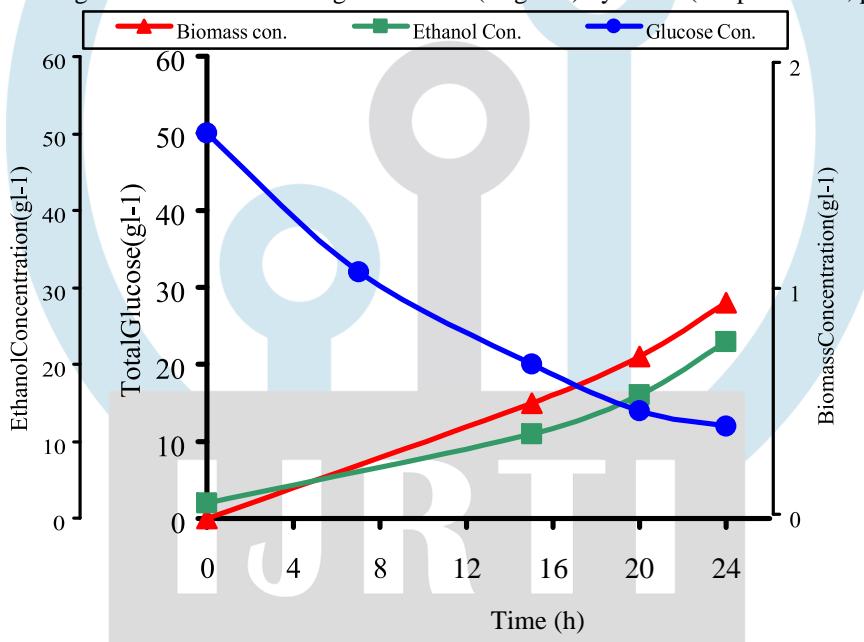
The biomasses were taken in an equivalent amount for pre-processing. Subsequent to preprocessing, the pH of the substrate was adjusted to pH 5, and the quantification of sugar was completed by the DNS method, yielding results of 68, 52, 65 and 39 g/l for Sugarcane bagasse, rape straw, corncob and Wheat bran (Fig:3). Upon hydrolysis, the results were tabulated in Table 1. Through the application of the general equation for ethanol fermentation, the final ethanol production from glucose was calculated. Utilizing the established chemical equation of 92 g of ethanol produced from 180 g of glucose, it can be determined that 1 g of glucose yields 0.51 g of alcohol..



The comparative analysis of ethanol concentrations of Sugarcane bagasse, rape straw, corncob and Wheat bran, after equalizing the sugar levels to 50 g/l, yielded an ethanol concentration of 25.4 g/l for Sugarcane bagasse and rape straw, utilizing 49.8 g/l of glucose concentration, while 21.2 g/l was obtained for wheat bran with 41.5 g/l glucose as presented in Figures 3 and 4, respectively. Additionally, 24.2 g/l ethanol was obtained for corncob. Results presented in Table 2 demonstrate that the initial ethanol concentrations of Sugarcane bagasse, rape straw, corncob and Wheat bran were 34.6, 26.5, 31.5 and 22.9 g/l, respectively.

SN.	Raw Materials	Sugarcane	Rape Straw	Corn cob	Wheat bran
		Bagasse			
1	Biomass yield $Y_{X/S}$ (gg ⁻¹)	0.015	0.014	0.040	0.010
2	Ethanol yield $Y_{P/S}$ (gg ⁻¹)	0.36	0.29	0.33	0.26
3	Final biomass, (g l ⁻¹)	1.49	1.32	1.28	0.92
4	Final ethanol (g l ⁻¹)	34.6	26.5	31.5	22.9
5	Substrate utilized, (%)	95.90	90.80	93.52	85.42
6	Fermentation efficiency (% of theoretical)	99.01	96.5	89.2	84.6
7	Fermentation time, (h)	24	24	24	24

Table 2: Ethanol production by fermentation with yeast.

Fig: 3 Fermentation of 50 gL^{-1} Glucose (Bagasse) by Yeast (temp $28\text{-}30^{\circ}\text{C}$, pH5)Fig: 4 Fermentation of 50 gL^{-1} Glucose (Wheat bran) by Yeast (temp $28\text{-}30^{\circ}\text{C}$, pH5)

It has been observed that the yield of ethanol from pre-treated biomasses was found to be low in concentration due to yeast's capacity to only ferment hexoses. In order for improved ethanol yields, a suitable yeast strain is required.. Cellulose undergoes hydrolysis to yield primarily glucose, while hemicelluloses lead to the production of multiple pentose and hexose molecules. Nevertheless, high lignin concentrations impede enzyme access, resulting in a decreased rate and yield of hydrolysis due to end product inhibition.. In addition to lignin, cellobiose and glucose also act as strong inhibitors of cellulase (Knauf M., 2004 et al). Maximum Ethanol fermentation was found in 24 hours at and pH of 4.0 to 5.0 which was found similar as concluded by (Taherzadeh, M.J. et al). However, optimal conditions may change with fermentation time. This improvement was adopted for further fermentation by using yeast strain in 28-30 hours at pH 5.

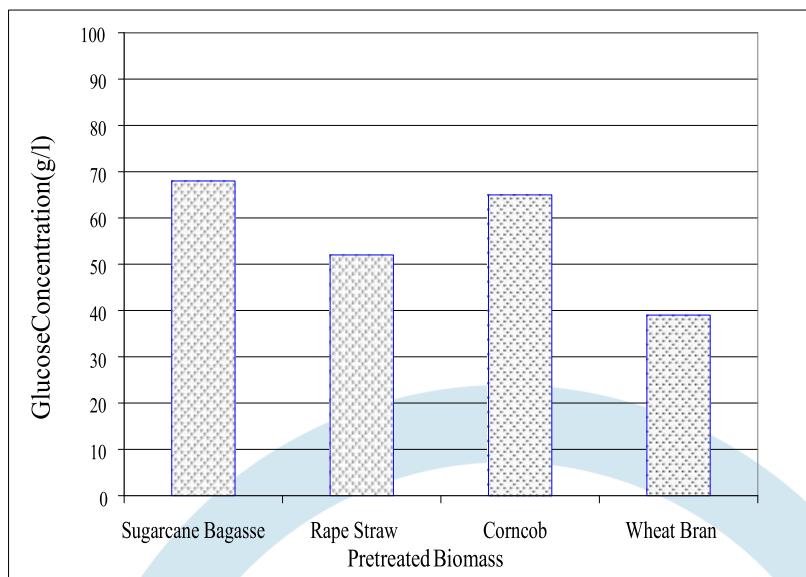


Fig: 5 Concentration of Glucose after pretreatment of equal amount of biomasses

Conclusion: Biomass is subjected to pretreatment prior to its degradation. Pretreatment with acid autoclaving at 121°C for 30 min, is an effective and cheap parameter in comparison to enzymatic degradation and other methods. The acid pretreatment exhibited a more pronounced effect on the sugar liberation through enzymatic hydrolysis.. An increase in acid severity in terms of concentration resulted in higher sugar releases. By the experimental analysis 3% of acid was found optimum. The second step in the pretreatment of biomass involves enzymatic hydrolysis and fermentation. Dilute sulfuric acid autoclaving at 1210C for one hour is a common hydrolysis method, though it carries the risk of producing inhibitors or other compounds, requiring caution. Enzymatic hydrolysis of pretreated biomass is a reliable strategy for efficient degradation of lignocellulosic biomass under appropriate pH and other conditions. The effective parameters of fermentation are essential components that are necessary to ensure optimal production of Alcohol.. In presence of higher sugar concentration the ethanol concentration decreases. In case of different pH ethanol fermentation is more favourable at pH 5-6. Similarly higher temperature for fermentation decreases the concentration of ethanol. Analysis of hydrolyzed biomass revealed that 24 hours of fermentation produced the highest yield.. The fermentation media YEPX or YEPD was found optimum than YEP for the growth of enzymes for fermentation. The addition of nutritional supplements such as potassium nitrate, phosphates, and sulphates has been found to result in an increased growth of enzymes and higher alcohol concentration.

Biofuel as ethanol in the blended form with petrol is also beneficial in the reduction of environmental pollution. Ethanol forms a carbon dioxide cycle, as combustion of ethanol releases carbon dioxide, which is then taken up by plants during photosynthesis to form cellulose - thereby completing the cycle. The production of ethanol is an energy-efficient process, relying solely on renewable energy sources and thus not contributing to additional carbon dioxide emissions in the atmosphere. As demonstrated by Wyman and Hinman (1990), the exhaust emissions from ethanol have a lower toxicity than those of petroleum-based sources. Consequently, ethanol is an environmentally beneficial source of energy.. Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect.

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