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Acid And Alkaline Pretreatment Of Lignocellulosic Biomass To Produce Ethanol As Biofuel

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Abstract: Ethanol obtained from biomass has potential to become an important sustainable transportation fuel in the near future. Renewable energy sources, such as lignocellulosic biomass, are environmentally friendly because they emit less pollution without contributing net carbon dioxide to the atmosphere. Among kinds of biomass, lignocellulosic biomass is a very useful feedstock to economically produce environmentally friendly biofuels. The main goal of the research described in this paper is to investigate the process of pretreated biomass, effect of inhibitors and ethanol fermentation as an efficient bio fuel source for the society, as well as to examine whether it is used and beneficial for the society, which helps to control the global warming. Processing of lignocellulosic biomass to ethanol consists of four major operations: (a) Pretreatment (b) Acid or enzymatic hydrolysis (c) Fermentation and (d) Ethanol separation/purification. In this paper alkali and acid method of pretereatment was used to examine their effect on ethanol production. Effective parameters such as pH, Nutrient media and sugar concentration also effected the enzymatic growth and production of ethanol from lignocellulosic biomasses.

Production of ethanol from agricultural and forestry residues, municipal solid waste, energy crops, and other forms of lignocellulosic biomass could improve energy security, reduce trade deficits, decrease urban air pollution, and contribute little, if any, net carbon dioxide accumulation to the atmosphere. The use of production of ethanol is more beneficial and economical because most of the lignocellulosic agricultural wastes are converted in to ethanol as biofuel in urban areas. Biofuels should ideally create the environmental, economic and social benefits to the communities in India, but modification in process and car engine should be needed. Bioethanol is also made from different Algal biomass in the terms of third generation of biofuel. In fact, not only are they environmentally friendly, but they have also been shown to get just as good of gas mileage as petroleum based fuels. It will eventually totally replace petroleum fuels, but that remains to be seen. Ethanol is made from agricultural wastes is less polluting than diesel and oil based fuels, and gets better gas mileage than many of the alternatives.

Keywords: Bio ethanol, Biomass, Pretreatment, Inhibitors, fermentation, hydrolysis, sugar.

Introduction:

Biomass is a renewable energy resource derived from the carbonaceous waste of various human and natural activities. It is derived from numerous sources, including the by-products from the timber industry, agricultural crops, raw material from the forest, major parts of household waste and wood. Biomass is available in different forms in the country, it cannot be defined easily because all things, which are dead and produces energy on combustion is a part of biomass either it may consist different thing. Liquid fuels are prepared by those types of biomass which consist starch, cellulose and another different forms of carbohydrates followed by fermentation in a feasible conditions. Biomass is an important source of energy and the most important fuel worldwide after coal, oil and natural gas. Biofuels are referred to liquid, gas and solid fuels predominantly produced from biomass. Lignocellulosic materials are the world's most widely available low-cost renewable resources to be considered for ethanol production.

Biofuel is a renewable energy source produced from natural (biobased) materials, which can be used as a substitute for petroleum fuels. The most common biofuels, such as ethanol from corn, wheat or sugar beet and biodiesel from oil seeds, are produced from classic food crops that require high-quality agricultural land for growth. However, bioethanol is a petrol additive/substitute that can be produced from plentiful, domestic, cellulosic biomass resources such as herbaceous and woody plants, agricultural and forestry residues, and a large portion of municipal and industrial solid waste streams. The best biofuels from biomasses are bioethanol and biodiesel. Production of bioethanol from biomass is one way to reduce both the consumption of crude oil and environmental pollution. There is also a growing interest in the use of vegetable oils for making biodiesel, which is less polluting than conventional petroleum diesel fuel[16,17]. Bioethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. Bioethanol is one of the cheapest biofuel which is produced by different types of lignocellulosic biomass. Bioethanol is produced from different resources of biomass by using different process, such as pretreatment, hydrolysis and fermentation. Production of ethanol from agricultural and biodegradable wastes provides a variable solution to multiple environmental problems simultaneously creating sink for waste and renewable energy production as well. Using ethanol-blended fuel for automobiles can significantly reduce petroleum use and greenhouse gas emissionsm[27].

Alkaline pretreatment is basically a delignification process, in which a significant amount of hemicellulose is solubilised as well. The action mechanism is believed to be saponification of intermolecular ester bonds cross linking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. Alkaline pretreatment also removes acetyl and various uronic acid substitutions on hemicellulose that reduce the accessibility of hemicellulose and cellulose to enzymes[2]. Several types of acids, concentrated or diluted, can also be used, such as sulphurous, sulphuric, hydrochloric, hydrofluoric, phosphoric, nitric and formic acid for the pretreatment and acid hydrolysis of lignocellulosic biomass. Sulphuric and hydrochloric acids are the most commonly used catalysts for hydrolysis of lignocellulosic biomass[14]. Dilute acid process is the oldest technology for converting cellulose biomass to ethanol. The main step is essentially hemicellulose hydrolysis. In simple terms, acid catalyzes the breakdown of long hemicellulose chains to form shorter chain oligomers and then to sugar monomers that the acid can degrade. However, because hemicellulose is amorphous, less severe conditions are required to release hemicellulose sugars[28-30]. The goal of the pretreatment process is to break down the lignin structure and disrupt the crystalline structure of cellulose, so that the acids or enzymes can easily access and hydrolyze the cellulose[15,16,20]. Pretreatment can be the most expensive process in biomass-to-fuels conversion but it has great potential for improvements in efficiency and lowering of costs through further research and development[13,17]. Pretreatment is an important tool for biomass-to-biofuels conversion processes and is the subject of this review article. The digestibility of NaOH-treated hardwood was reported to increase from 14% to 55% with a decrease of lignin content from 24-55% to 20%. However, no effect of dilute NaOH pretreatment was observed for Lignocelluloses with lignin content greater than 26%[19]. Dilute NaOH pretreatment was also found to be effective for the hydrolysis of straws with relatively low lignin contents of 10-18% [1].

Material and Methods:

Lignocellulosic dry samples (Sugarcane baggase, 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 method of producing ethanol from lignocelluloses involves reducing the size of biomass to smaller particles prior and using acid or enzyme treatments to hydrolyze the biomass to sugars. Some of the reducing sugars are glucose, galactose, lactose and maltose. The Nelson-Somogyi method is one of the classical and widely used methods for the quantitative determination of reducing sugars[24].

For the fermentation of pretreated, wet biomass was mixed with distilled water to obtain a solution with 10% solid content and then divided in 250-ml flasks containing 100 ml of slurry with pH 5. Yeast enzymes were growth in PDA slant in incubator. The fermentation process started with the addition of 1.0 mL of the activated yeast culture, 0.30 g of yeast extract, and 0.1 g of KH_2PO_4 into the hydrolyzates in each flask. Fermentation was conducted at 30°C for 24-36 hours in an incubator shaker operating at 150 rpm under anaerobic conditions.

Alkaline Pretreatment:

Alkaline pretreatment of lignocellulosic materials causes swelling, leading to decreased crystallinity, increased internal surface area, disruption of the lignin structure, and separation of structural linkages between lignin and carbohydrates[10,11]. Alkali pretreatment refers to the application of alkaline solutions such as NaOH, $Ca(OH)_2$ (lime) or ammonia to remove lignin and a part of the hemicelluloses, and efficiently increase the accessibility of enzyme to the cellulose.

For the alkaline hydrolysis of the samples three alkaline solution NaOH, $Ca(OH)_2$ of equal concentration were taken and each lignocellulosic biomasses were treated as in the following manner

Ground biomass 3g of each crop was placed in a 1000ml Erlenmeyer flask and 300 ml of 3% (w/v) NaOH solution was added to it. The flask was cotton plugged and autoclaved at 121ºC for 30 min. The material obtained after treatment was dark in colour which was then filtered through muslin cloth and washed under running distilled water until no colour was visible in the wash water. The neutralized residue was pressed manually to remove excess water and used for the enzymatic hydrolysis. Small portion of the treated biomass was dried in the oven at 70ºC for 24 h and ground to fine particle size in a Laboratory Mill for the compositional analysis studies.

Acid Pretreatment: Acid pretreatment is the one of the pretreatment method which is used to degrade the biomass component in to fermentable sugars by further acid hydrolysis or using enzymatic hydrolysis. There are two types dilute and concentrated acid treatment is used for the biomass degradation. The advantage of the acid hydrolysis is that acids can penetrate lignin without any preliminary pre-treatment of biomass, thus breaking down the cellulose and hemicelluloses polymers to form individual sugar molecules. The main advantage of the dilute hydrolysis process is the low amount of acid required (2-3%). However this process is carried out at high temperatures to achieve acceptable rates of cellulose conversion. The high temperature increases the rates of hemicellulose sugars decomposition thus causing the formation of toxic compounds such as furfural and 5-hydroxymethyl-furfural (HMF).Detoxification, activated charcole, Calcium hydroxide over-liming etc are the process used to remove inhibitors[3-5,32]. The acid concentration used in the concentrated acid hydrolysis process is in the range of 10-30%. The process occurs at low temperatures, producing high hydrolysis yields of cellulose (i.e. 90% of theoretical glucose yield). However, this process requires large amounts of acids causing corrosion problems to the equipments.

For the pretreatment with acid the following process has been used:- Prepare biomass samples as necessary (grinding, blending, etc.) Weigh out 3g of biomass into a pre weighed aluminium tray (do replicates of this). Be sure to record the weight of the biomass as well as the weight of the tray. Place the trays in a 105°C oven overnight. Prepare 3% (w/w) sulphuric acid. Once the moisture content is determined, weigh the amount of wet biomass correlating to 1.5g dry mass to a 125ml flask. (Record this weight and repeat for all samples). Apply 45ml of 0.8% sulphuric acid to each sample. Autoclave it at 121°C for 30 min. Centrifuge the obtained at 1500 rpm for 10 minutes. Separate the solid part, wash with distilled water and again make slurry by adding known concentration of acid, autoclaved again at 121°C for 30min. immediately after the cycle is finished, remove the samples and filter it. Wash the biomass with 200ml water using the filter. Maintain pH at 5.0, and dilute it for enzymatic hydrolysis.

Result and Discussion:

To determine the composition of biomass after Acid, Alkaline biomass samples of different kinds, Determination of Structural Carbohydrates and Lignin in Biomass'' and ''Determination of Ash in Biomass,'' refined by NREL researchers[23]. The Sugar concentrations of the biomasses were measured by HPLC also. Contents of three main components of cellulose, hemicelluloses and lignin in the initial biomasses was found, are shown in Table 1. The other components of the initial biomass samples were extractives, ash and protein. The biomasses taken were contained 55 to 62 % cellulose after acid treatment of biomasses. The low lignified corncob and rice straw contained 8 to 15% lignin and 60 to 62% cellulose. Besides the corncobs had the highest content of hemicelluloses, 12% after acid treatment of samples.

Study of the enzymatic hydrolysis of the initial biomass samples showed that initial biomass(untreated) sample cannot be considered as appropriate feedstock for enzymatic hydrolysis due to insufficient yield of reducing sugars. Low utilization rate of the biomass moreover increasing lignin content worsened enzymatic digestibility of the samples. The biomass samples were pretreated with 3% sulphuric acid for acidic study, and the cellulose % was found in the range of 55-62%, while hemicelluloses % was found 6-12%. The biomasses taken were contained 15-31% of lignin after acidic treatment and enzymatic hydrolysis. The cellulose % was found maximum by using 3% sodium hydroxide for alkaline study at moderate temperatures. The biomasses taken were contained 62-78% Cellulose after alkaline treatment; it was found maximum 78% in rice straw and minimum 62% in wheat bran sample. The comparative results are shown in Fig: 1, 2 for kinds of biomasses.

Table: 1 Percentage of Cellulose, Hemicellulose and Lignin in the Biomass sample Pretreated with Acid (AC), Alkali (AL)

Overall Parameters	**Rice Straw**		**Corncobs**		**Barley Straw**		**Wheat Bran**	
	AC	AL	AC	AL	AC	AL	AC	AL
Cellulose (%)	62	78	60	73	55	65	58	62
Hemicelluloses (%)	7	15	12	17	7	16	6	14
Lignin (%)	15	5	20	8	26	12	31	16
Yield of Reducing Sugar (%)	49	82	45	78	40	65	34	46

The final result showed some improvement of the enzymatic digestibility of the acid pretreated biomass in comparison with untreated samples. However the digestibility characteristics were insufficient, the utilization rate of biomass was in the range 30-42% and yield of reducing sugars was in the range 34-49% for acid pretreated biomass and 46-82% for alkali treated biomass. It is clear that after acidic pretreatment of biomasses and in case of alkali pretreatment of the biomass caused decreases of both of hemicelluloses and lignin as in Table: 1 and Fig: 1,2. Simultaneously increasing cellulose content was observed which should promote the enzymatic digestibility of the pretreated biomass.

Hence the experiment showed that, pretreatment of the biomass samples with dilute alkali solution caused considerable rise of the utilization rate and yield of sugars after enzymatic hydrolysis of alkali pretreated biomass in comparison with acid pretreated samples. This result is in agreement with the findings of other researchers[31].

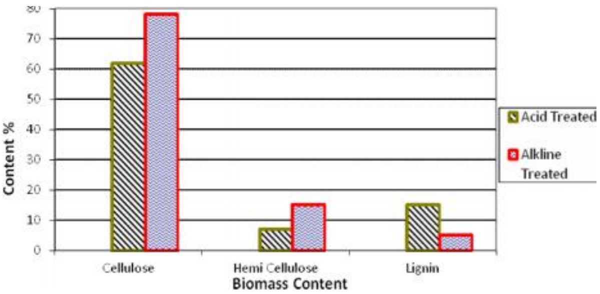

Fig. 1: Percentage of Cellulose, Hemicelluloses and lignin in Rice Straw

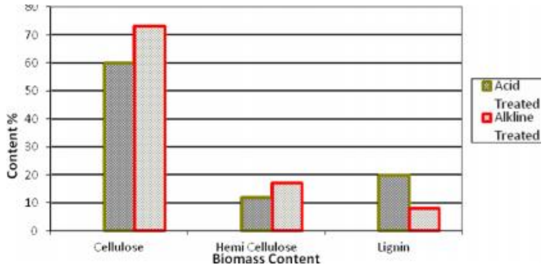

Fig. 2: Percentage of Cellulose, Hemicelluloses and lignin in Corn Cobs

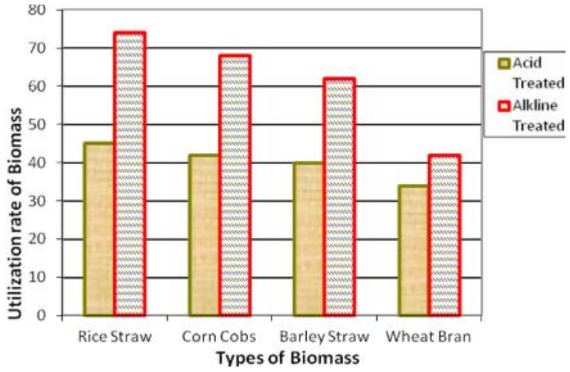

Fig. 3: Utilization rate of biomass (UR) after enzymatic hydrolysis of untreated, Acid and Alkali pretreated biomass sample

From the previous studies it was noted that increased acid concentration used for the pretreatment purpose enhanced the sugar release during enzymatic hydrolysis. But it was found optimum at 3%. Fermentation studies also proved that optimum acid concentration yielded more amount of alcohol. Alcohol content of about 8.9 gl^{-1} (Table 2) was detected in the 48 hours of fermentation broth containing the hydrolyzed sample pretreated with 3% sulphuric acid. This study proved that sugars released by means of enzymatic hydrolysis of pretreated corncob are of fermentable category and alcohol can be produced efficiently using a suitable fermenting strain *Saccharomyces cerevisiae.*

Hence it is clear that at 3% of acid sample ethanol yield was found maximum at optimum temperature and pH value. Fig. 4 represents the data of maximum alcohol yield and cellulose conversion. On the basis of above discussion we were also used different kinds of biomasses and their results of cellulose conversion from the following Table: 3 represents the ethanol yield at optimum acid concentration of 3% and optimum temperature and other conditions as discussed for corncob.

Table2: S1 to S7 – Biomaas samples of Alcohol Production on Acid Pretreated corncob through Fermentation with *Saccharomyces cerevisiae.*

S No	Sample-Acid used for Pretreatment w/w (%)	Ethanol gl^{-1}
S1	0.0	1.4
S2	0.5	3.8
S3	1.0	4.9
S4	1.5	5.6
S5	2.0	6.8
S6	2.5	7.7
S7	3.0	8.9

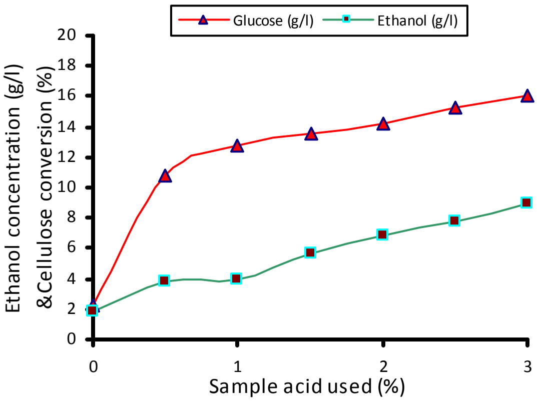

Figure: 4 Comparison of total Cellulose conversion (%) and Ethanol fermentation with *Saccharomyces cerevisiae* at different sample used of acid.

Table 3: Alcohol Production on (3.0%) acid pretreated kinds of biomass through Fermentation with *Saccharomyces cerevisiae.*

SNo	Types of biomass samples	Ethanol gl-1
1	Corncob	8.9
2	Cassava	7.4
3	Wheat Straw	6.2
4	Rape Straw	7.9

According to Table: 3, it is concluded that the maximum ethanol yield was found 8.9g/l in corncob and minimum ethanol yield was found in wheat straw (6.2 g/l). These data shows that degradation of biomass by acid pretreatment and enzymatic hydrolysis followed by fermentation gives sustentative yield of ethanol production.

To check the pretreatment process three sample of alkaline solution were also taken in the manner of same concentration. 3% alkaline using different basic solutions, results are shown by alkali pre-treatment with NaOH solution has the most impact on sugarcane bagasse structure (Table 4). Furthermore regarding lower molecular weight of NaOH in comparison with KOH and $Ca(OH)_2$, using this base is equal to less consumption of material while at the same time the resulted solid has the better composition for ethanol production. Compositional analysis of pretreated bagasse shows that residual solid is rich in glucose. Therefore pretreatment effect in this condition is dissolution of lignin and partially hemicelluloses. Hence it was found that Alkaline pretreatment with NaOH is more optimum than KOH and $Ca(OH)_2$. The percentage of glucose by alkali pretreatment with NaOH was found 58%, which was maximum than pretreatment by KOH (54%) and by $Ca(OH)_2$ (40%). Therefore, NaOH solution was chosen for pretreatment of other biomasses for ethanol production. After this pre-treatment, solid composition was found 58% (w/w) glucose. On behalf of the method the following results was obtained after pretreatment of kinds of biomasses as in (Table 4).

Table 4: Effect of different basic solutions on sugarcane baggase composition

SN.	Base	g/g of Solids	% Glucose in solid after pretreatment
1	NaOH	0.4	58
2	KOH	0.56	54
3	$Ca(OH)_2$	0.74	40

From the data it is concluded that alkaline hydrolysis of different kinds of biomass is more effective parameter than others. Alkaline hydrolysis is so very effective in comparison to acid hydrolysis. Fig: 5 show the ethanol yield by fermentation with Saccharomyces cerevisiae at optimum temp 30^0C and Optimum pH 5.

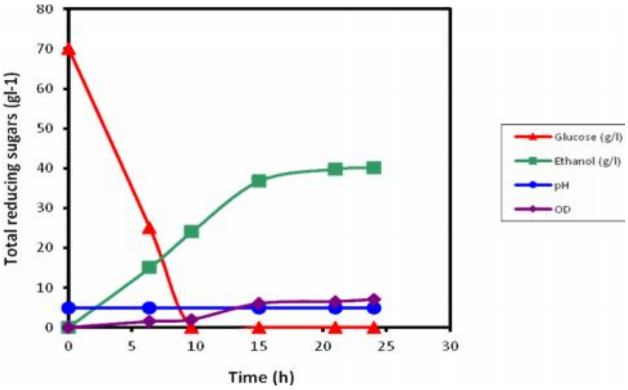

Fig 5: Fermentation of 70 gl^{-1} Glucose medium (Bagasse) by Saccharomyces cerevisiae (temp 30°C, pH5)

Conclusion:

The biomass pretreatment and the intrinsic structure of the biomass itself are primarily responsible for its subsequent hydrolysis. The conditions employed in the chosen pretreatment method will affect various substrate characteristics, which, in turn, govern the susceptibility of the substrate to hydrolysis and the subsequent fermentation of the released sugars. Therefore, pretreatment of biomass is an extremely important step in the synthesis of biofuels from lignocellulosic biomasses, and there is a critical need to understand the fundamentals of various processes, which can help in making a suitable choice depending on the structure of the biomass substrate and the hydrolysis agent. Pretreatment is the initial step to degrade the biomass content. Pre-treatment's are usually focused on lignin and hemicelluloses removal, cellulose crystallinity reduction and accessible surface area increase. Without pretreatment it is not possible to convert biomass content in to reducing sugars and further fermented in to ethanol as biofuel. Pretreatment of lignocellulosic and algal biomass can be held in three ways Acid, Alkali and enzymatic pretreatment. Pretreatment with acid autoclaving at 121°C for 30 min, is an effective and cheap parameter in comparison to enzymatic degradation and other methods. Acid pretreatment had a greater influence on the sugar release through enzymatic hydrolysis. An increase in acid severity in terms of concentration resulted in higher sugar releases. By the experimental analysis 3% by volume of acid was found optimum.

Acid pretreatment is effective but in the controlled concentration, because at higher concentration is the cause of formation of inhibitors which inhibits the fermentation process in to ethanol. In this study and work, optimum acid concentration was found 2-3%. Alkaline hydrolysis is also effective for pretreatment of biomass but after alkaline hydrolysis further enzymatic hydrolysis is need for better result. Alkaline pretreatment with 3% NaOH solutions was found optimum than KOH and Lime Water $Ca(OH)_2$, Pretreatment. Alkaline hydrolysis reduces the lignin component during degradation which enhances the alcohol production in comparison to acidic pretreatment because at high concentration of acid sugar concentration decreases, due to formation of inhibitors. In presence of higher sugar concentration Furfural, a pentose degradation product and HMF as inhibitors 5-hydroxymethylfurfural, a hexose degradation product and lignin degradation products are formed which decreases the ethanol yield and production. Detoxification is the method to avoid the formation of inhibitors.

Compared with acid processes, alkaline processes cause less sugar degradation, and many of the caustic salts can be recovered and/or regenerated. Sodium, potassium, calcium, and ammonium hydroxides are suitable alkaline pretreatment agents. Of these four, sodium hydroxide has been studied the most[9,18,25]. Alkali pretreatment can be carried out at ambient conditions, but pretreatment times are on the order of hours or days rather than minutes or seconds. Dilute NaOH treatment of lignocellulosic materials has been found to cause swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure[11,12]. Hence it is cleared that dilute acid hydrolysis of biomass yields higher sugar concentration but at high concentration of acid decreases the sugar concentration decreases and in case of alkaline hydrolysis the lignin component is decreases, it was also satisfied by some of the researcher[26].

The choice of the pretreatment technology used for a particular biomass depends on its composition and the byproducts produced as a result of pretreatment. These factors significantly affect the costs associated with a pretreatment method. There have been some reports comparing various pretreatment methods for biomass[28-30,21,22]. The pretreatments were compared after enzyme treatment using a cellulase enzyme system. The acid or water impregnation followed by steam explosion of agricultural waste/straw was the best pretreatment in terms of the resulting glucose concentration in the liquid hydrolysate after enzymatic hydrolysis.

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