Production Of Biofuel From Lignocellulosic Biomass

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Abstract

Bioethanol is the most common biofuel, accounting for more than 90% of total biofuel usage. There is a need for environmentally sustainable energy sources due to increase in industrial development. Bioethanol (ethanol from biomass) is an attractive, sustainable energy source to fuel transportation. Pretreatment is the first step to degrade the biomass component. There are different types of methods of pretreatment one of which dilute acid pretreatment. Dilute acid can open up the biomass structure for subsequent processing. Most of the technology has also been developed for converting the second largest biomass fraction, Cellulose and hemicellulose, into ethanol. After pretreatment enzymatic hydrolysis is used to ferment pre-treated biomass either by using simultaneous saccharification or by further acid treatment. Simultaneous saccharification and fermentation (SSF) process is favored for producing ethanol from the major fraction of lignocellulosic biomass, cellulose, because of its low cost potential. A range of acid pretreatment of biomass was made and the pre-treated biomass samples were fermented with Saccharomyces cerevisiae. The sample that was pre-treated with 3% dilute sulphuric acid gave an ethanol yield of 4.9 g l^{-1} *respectively. The remaining fraction, containing mostly lignin, can be burned as boiler fuel to power the conversion process and generate extra electricity to export.*

New developments and approaches of biofuel production from kinds of lignocellulosic biomass in conversion technology enhance the ethanol production and have reduced the projected price of ethanol in the present scenario. Biofuels are expected to reduce dependence on imported petroleum with associated political and economic vulnerability, reduce greenhouse gas emissions and other pollutants, and revitalize the economy by increasing demand and prices for agricultural products. In future, biofuels should ideally create the environmental, economic and social benefits to the communities and reflect energy efficiency.

Keywords: *Bioethanol, Biomass, Ethanol, Pretreatment, fermentation, hydrolysis.*

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1. 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. 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 of different kinds such as Lignocellulosic, Algal Biomass. Lignocellulosic materials are the world's most widely available low-cost renewable resources to be considered for ethanol production. 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. Renewable energy can and should play an important role for solving the problems arising as a consequence of the enormous demand of fossil fuels required by the global economy. One of the most relevant renewable resources is the lignocellulosic biomass, which is an abundant and cheap feedstock that can be utilized for energy production. In particular, lignocellulosic biomass (mainly agricultural and forestry residues and agro-industrial wastes) can be converted into renewable liquid biofuels as ethanol. Ethanol can be used as a unique fuel for motor engines, and as an oxygenate for gasoline.

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 reported by *Demirbas A in 2008*. 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 emissions [21].

Composition of Biomass: The term "lignocellulosic biomass" is used when referring to higher plants, softwood or hardwood. The main components of the lignocellulosic materials are cellulose, hemicellulose and lignin. Cellulose is a major structural component of cell walls, and it provides mechanical strength and chemical stability to plants. Solar energy is absorbed through the process of photosynthesis and stored in the form of cellulose [14]. Hemicellulose is a copolymer of different C5 and C6 sugars that also exist in the plant cell wall. Lignin is polymer of aromatic compounds produced through a biosynthetic process and forms a protective layer for the plant walls. Lignocellulosic biomass is made up of three main components: cellulose, hemicellulose, and lignin. Cellulose and hemicellulose, like starch, are made up of sugars. Unlike starch, the sugars in cellulose and hemicellulose are difficult to get at. The plant cell walls containing hemicellulose, cellulose, and lignin form an extremely complex structure that is very difficult to break down. There are four main steps to break down the biomass into its sugars: pretreatment, enzymatic hydrolysis, fermentation, and distillation. The purpose of the overall process is to obtain as much sugar from the biomass as possible the more sugar, the greater the amount of ethanol produced. The lignin does not contain any sugar, but can be burned to produce heat and electricity. Inside the lignocellulose complex, cellulose retains the crystalline fibrous structure and it appears to be the core of the complex. Hemicellulose is positioned both between the micro- and the macro fibrils of cellulose. Lignin provides a structural role of the matrix in which cellulose and hemicellulose is embedded [5].

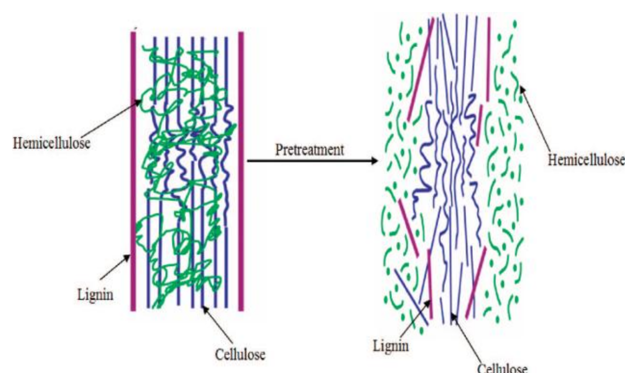

Figure: 1 Effect of Pretreatment of Lignocellulosic Biomass

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Cellulose: Cellulose is the β-1,4-polyacetal of cellobiose (4-O-β-D-glucopyranosyl-D-glucose). Cellulose is more commonly considered as a polymer of glucose because cellobiose consists of two molecules of glucose. The chemical formula of cellulose is $(C_6H_{10}O_5)n$. Lignocelluloses are a class of biomass that consists of three major compounds cellulose, hemicellulose and lignin. It also includes water and a small amount of proteins and other com-pounds, which do not participate significantly in forming the structure of the material [14]. Cellulose is derived from D-glucose units, which condense through β(1→4)-glycosidic bonds. This linkage motif contrasts with that for α (1→4)-glycosidic bonds present in starch, glycogen, and other carbohydrates. Cellulose is a straight chain polymer: unlike starch, no coiling or branching occurs, and the molecule adopts an extended and rather stiff rod-like conformation, aided by the equatorial conformation of the glucose residues. The multiple hydroxyl groups on the glucose from one chain form hydrogen bonds with oxygen atoms on the same or on a neighbor chain, holding the chains firmly together side-by-side and forming *micro fibrils* with high tensile strength. This strength is important in cell walls, where the micro fibrils are meshed into a carbohydrate *matrix*, conferring rigidity to plant cells. Compared to starch, cellulose is also much more crystalline [4].

Hemicellulose: The term "hemicellulose" is applied to the polysaccharide components of plant cell walls other than cellulose, or to

polysaccharides in plant cell walls which are extractable by dilute alkaline solutions. Hemicelluloses comprise almost one-third of the carbohydrates in woody plant tissue. The chemical structure of hemicelluloses consists of long chains of a variety of pentoses, hexoses, and their corresponding uronic acids. Hemicelluloses may be found in fruit, plant stems, and grain hulls. Although hemicelluloses are not digestible, they can be fermented by yeasts and bacteria. The polysaccharides yielding pentoses on hydrolysis are called pentosans. Xylan is an example of a pentosan consisting of D-xylose units with 1β→4 linkages. Hemicelluloses are polysaccharides in plant cell walls that have beta-(1-->4)-linked backbones with an equatorial configuration. Hemicelluloses include xyloglucans, xylans, mannans and glucomannans, and beta-(1-->3, 1-->4)-glucans. These types of hemicelluloses are present in the cell walls of all terrestrial plants, except for beta-(1-->3,1-->4)-glucans, which are restricted to Poales and a few other groups [14].

Lignin: Lignin or lignin is a complex chemical compound most commonly derived from wood, and an integral part of the secondary cell walls of plants and some algae [9]. Lignin's are high-molecular-weight, insoluble plant polymers, which have complex and variable structures. They are composed essentially of many methoxylated derivatives of benzene (phenylpropanoid alcohols, also called monolignols), especially coniferyl, sinapyl and coumaryl alcohols [11].

Pretreatment: Lignocellulosic biomass is the most abundant renewable resource on earth and has attracted continuing efforts to produce fuels and chemicals for a long time [10]. Production of ethanol from lignocellulosic biomass contains three major processes, including pretreatment, hydrolysis, and fermentation. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its sub microscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars [3]. Since lignocelluloses biomass is naturally recalcitrant to enzymatic hydrolysis, pretreatment is essential to improve its enzyme digestibility and also to obtain solubilised sugar. The pretreatment methods employed are physical, chemical and biological. The physical pretreatment like ball milling and compression milling decreases the degree of crystallinity and also molecular weight of cellulose [17]. The dilute acid hydrolysis of certain softwood species is found to be suitable for obtaining maximum soluble sugars [7].

2. Material and Methods:

Lignocellulosic biomass such as Sugarcane bagasse, wheat straw, wheat bran, rape straw was taken from rural agricultural area. The media is prepared by adding 4.5 per cent (w/v), 1.5 per cent yeast extract, 1 per cent glucose, 0.25 per cent NH_4Cl, 0.05 per cent thiamine dichloride, 0.2 per cent K_2HPO_4, 0.05 per cent $MgSO_4.7H_2O$, 0.01 per cent $CaCl_2$ and 0.05 per cent KCl and was sterilized at 121°C for 15 minutes. Then actively growing fungal cultures were inoculated and incubated for 10 days on a rotary shaker (150 rpm). The cultures were harvested, filtered and clarified by centrifugation at 7800 rpm for 20 min to remove mycelia and the clear supernatant was used as enzyme source. *Saccharomyces cerevisiae* was used for the fermentation of lignocellulosic biomass. For the prepared substrate, nutrient supplementation of peptone (0.1%), $MgSO_4$ (0.03%), $CaCl_2$ (0.04%), KH_2PO_4 (0.02%), $(NH_4)_2SO_4$ (0.14%) and urea (0.03%) was done. Then agar grown fungal cultures were inoculated and incubated for 7 days. After incubation, the amount of reducing sugar released was estimated by DNS method by *Miller* [12].

Acid Pretreatment: Dilute-acid pretreatment is a commonly used method of increasing the digestibility of lignocellulosic biomass. While it is difficult for the average lab to achieve the prescribed conditions for standard pretreatment, this protocol is a reduced severity treatment which is achievable using a standard autoclave. Dilute acid process is the oldest technology for converting cellulose biomass to ethanol. In simple terms, acid catalyzes the breakdown of long hemicelluloses 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 [21]. Dilute acid pretreatment (2 – 3.0% sulfuric acid, 110-160 ºC) of native lignocellulose also can be used to increase the conversion rate of cellulosic biomass. Pretreatment of different lignocellulosic biomass was done in the following manner: - (a) Prepare biomass samples as necessary (grinding, blending, etc.), weigh out 1g of biomass into a pre weighed aluminum tray (do replicates of this). (b) Place the trays in a 105°C oven overnight. (c) 3% (w/w) sulfuric acid was used for the pretreatment. 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 3% sulfuric acid to each sample. (d) Cap all flasks with foil. Autoclave at 121°C for 30 min on the liquid cycle. (e) 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 30 min.(f) Wash the biomass with 200ml water using the filter. Maintain pH at 5.0, and dilute it for enzymatic hydrolysis.

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3. Hydrolysis and Fermentation: Pure culture of the yeast was prepared on Agar Media by keeping the plate at 37^0C for at least 4 days and was maintained on YPD (yeast extract /peptone/ dextrose) agar slants sealed with sterile mineral oil at room temperature (25 °C). The strain was sub cultured to YPD agar slants and incubated at 25 °C for 3 days and then used to inoculate preculture broths. The preculture broth was prepared by inoculating sucrose broth with a loop full of the cultured yeast and when the density of the yeast cells in the liquid medium was adequate, i.e. a 24 h suspension of *S. cerevisiae* at OD 660 = 0.6, was used as the inoculums in the fermentation medium.

Anaerobic batch fermentation of 200 ml broth media consisting of pre-treated and hydrolyzed biomass was carried out in order to convert the released sugars into ethanol, the conversion process being accomplished by the enzymes released by *Saccharomyces cerevisiae*. The pH of the solution was brought to 5 by adding required amount of 5 N NaOH to accommodate yeast growth. The volume of the broth was brought to 200 ml by adding required amount of distilled water. The hydrolyzed material was completely sterilized by autoclaving (120°C, 15 psi pressure and 30 min) before inoculating the yeast. Detoxification of the hydrolyzed material is not necessary as most of the toxins are detoxified due to the pretreatment and hydrolysis process (heat and chemicals) [13]. The mixture can be directly utilized as a broth for fermentation without additional detoxification methods. The fermentation was carried out in a closed conical flask at temperature of 32^0C, agitation rate at 110- rpm in shaker incubator. The mouths of the flasks were tightly sealed to maintain anaerobic condition and an outlet was provided to release CO_2. The other end of the outlet was dipped in lime water to confirm the release of CO_2 as it turns lime water milky. Triplicate fermentation broths of same composition were prepared and incubated in the same conditions. The fermentation was continued for 9 days and samples were taken from each of the three broths on each alternate day for analysis to get triplicate results [13].

Analytical Analysis: The ethanol was estimated by colorimetric method as described by *Caputi et al.* [2]. Simple Distillation of the broth after fermentation was done to recover ethanol from it. Triplicate samples of the distillate were analyzed to estimate the amount of ethanol. Cellulose estimation was done by Anthrone method as explained by Sadasivam and Manickam in Biochemical Methods [15]. The sugar estimation was done by was done by Dinitrosalisylic acid (DNS) method [15].

4. Result and Discussion: Sugarcane bagasse, rape straw, corncob and Wheat bran biomasses were taken for the pre-treatment by dilute sulphuric acid**.** The biomasses were taken in equal amount for pre-treatment. After pre-treatment the pH of the substrate was adjusted at pH 5 and analysis for sugar determination was done by DNS method, and it was found 68, 52, 65 and 39 g/l for Sugarcane bagasse, rape straw, corncob and Wheat bran (Fig:4). The fermentation process started for 24 hours at 30^0C in incubator as described in the method. After hydrolysis the result was obtained as in Table: 1.

The final ethanol production from glucose was calculated by the general equation of ethanol fermentation. Since according to chemical equation 92 g of ethanol is produced from 180 g of glucose unit, which means 1 g of glucose gives 0.51 g of alcohol.

Table 1: Ethanol production by fermentation with yeast.

SN.	Raw Materials	Sugarcane Bagasse	Rape Straw	Corn cob	Wheat bran
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, (gl^{-1})	1.49	1.32	1.28	0.92
4	Final ethanol (gl^{-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

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According to above reaction the concentration of ethanol was found 34.6, 26.5, 31.5 and 22.9 g/l for Sugarcane bagasse, rape straw, corncob and Wheat bran as shown in Table 1. For the comparative result of concentration of ethanol the sugar concentration was made equal for each biomass and it is observed that ethanol concentration for 50 g/l of sugar of biomasses were found 25.4g/l for Sugarcane bagasse and rape straw, by utilizing 49.8g/l of glucose concentration during fermentation, while 21.2g/l was found for wheat bran by utilizing 41.5 g/l glucose as shown in Fig: 2 and Fig: 3 Similarly 24.2 g/l ethanol was found for corncob. Since yeast is able to ferment only hexoses from the pre-treated biomasses so that the yield of ethanol was found low in concentration, for better result of yield of ethanol the yeast strain is needed.

The main hydrolysis product of cellulose is glucose, whereas the hemicelluloses give rise to several pentose's and hexoses [18], [19]. However, high lignin content blocks enzyme accessibility, causes end product inhibition, and reduces the rate and yield of hydrolysis. In addition to lignin, cellobiose and glucose also act as strong inhibitors of cellulase [8].

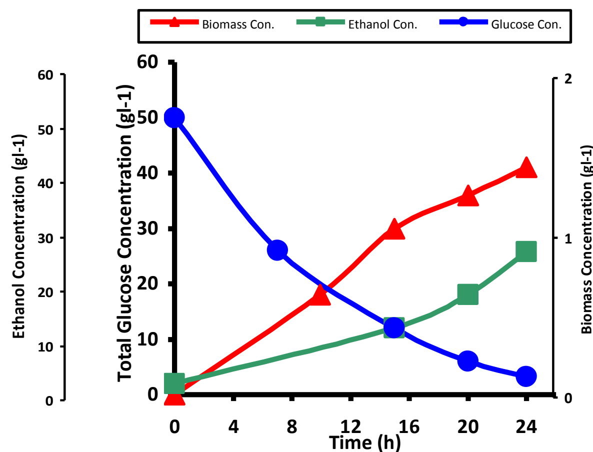

Fig 2: Fermentation of 50 gL^{-1} Glucose (Bagasse) by Yeast (temp 28-30^{0}C, pH5)

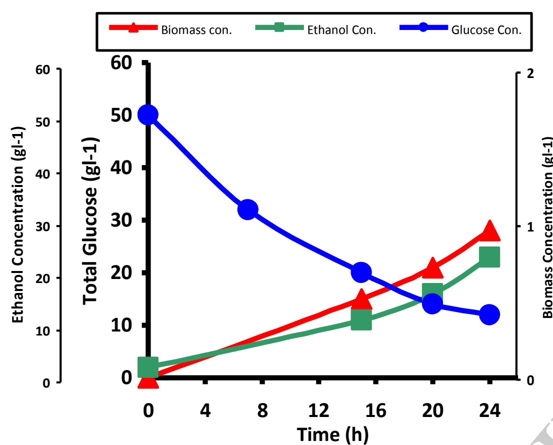

Fig 3: Fermentation of 50 gL^{-1} Glucose (Wheat bran) by Yeast (temp 28-30^{0}C, pH5)

Maximum Ethanol fermentation was found in 24 hours at and pH of 4.0 to 5.0 which was found similar as concluded by [19]. 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. *Tian et al.* [20] reported *Saccharomyces cerevisiae* efficiently converts both glucose and mannose into ethanol, but is unable to convert xylose into ethanol. Other yeast species, e.g. *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus*, have been found to be highly efficient xylose-fermenting strains that can be used in ethanol production [1]. However, these yeasts have a relatively low ethanol yield and inhibitor tolerance. For enhanced production of sugar and ethanol, it is essential to optimize the composition of culture media and process conditions. There are several factors that affect enzymatic hydrolysis of cellulose including substrates, cellulase activity and reaction conditions (temperature and pH).

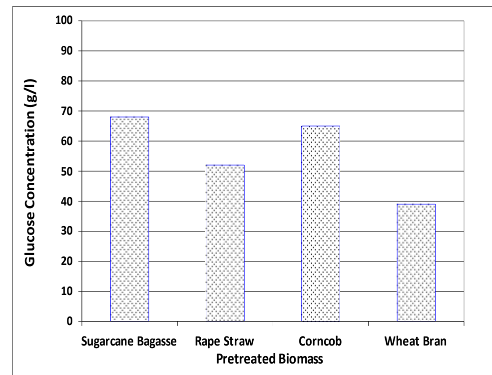

Fig: 4 Concentration of Glucose after pretreatment of equal amount of biomasses

Yeast is the main source of *Saccharomyces cerevisiae*. This enzyme is able to ferment only Hexoses but it cannot able to ferment Pentoses, this is because the major sugars from cellulosic biomass are not just glucose but also xylose with ratio glucose to xylose being approximately 2 or 3 to 1. It is generally agreed that unless both glucose and xylose from the cellulosic biomass can be fermented however nearly all of the fermentative yeasts including *Saccharomyces cerevisiae* are found to be unable to ferment xylose to ethanol or utilize the pentose sugar for growth. In order to solve the above described problem, we have taken not only enzyme to ferment xylose but also to be able for the effective co fermentation of both glucose and xylose simultaneously so that mixed sugar will be fermented as fast as possible and to easily convert *Saccharomyces cerevisiae* strains.

When we use the *Saccharomyces yeasts* the production of ethanol decreases because of no effect upon xylose fermentation, but the amount of glucose consumed completely to produce ethanol as shown in Fig: 5. But in presence of *Saccharomyces yeasts* strain the concentration of ethanol yield becomes more as compared to the ethanol yield concentration by un engineered *Saccharomyces yeasts.* It is clear that, only *Saccharomyces yeasts* are not sufficient to ferment both glucose and xylose as shown in Fig: 5. In Fig: 5 the fermentation of Xylose remains un effected, while ethanol concentration was found 47g/l in 30hours of fermentation, while in presence of yeast strain, the maximum ethanol concentration was found 60g/l in 30 hours, as shown in Fig: 6.

The enzyme growth medium is also one of the important factors for the enzyme growth and for better result. The only YEP is not a favorable condition for better enzyme growth, but the YEP medium with Xylose is more favorable for the better enzyme growth. This allows the genetically engineered yeasts to maintain their plasmids in YEPX (YEP with 2% xylose) medium without the use of antibiotic genetic in this discovery has made it possible for engineered yeasts to grow in rich medium (YEPX) without losing their xylose

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fermentation capability, this also allows engineered *Saccharomyces yeasts* cultured in YEPX during early stages of growth to be cultured in YEPD as concluded by [6].

Effect of pH is one of the effective parameter for the better yield of ethanol production. The effect of pH on the kinetics of glucose and pentose fermentation was studied using a pH of 4, 5, 5.5 and 6 in a batch culture at 30^{0}C, these data are given in Table 2.

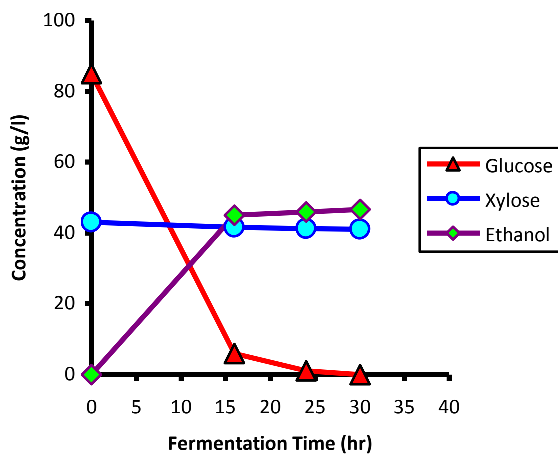

Fig: 5 Fermentation of glucose and xylose by the *Saccharomyces yeasts.*

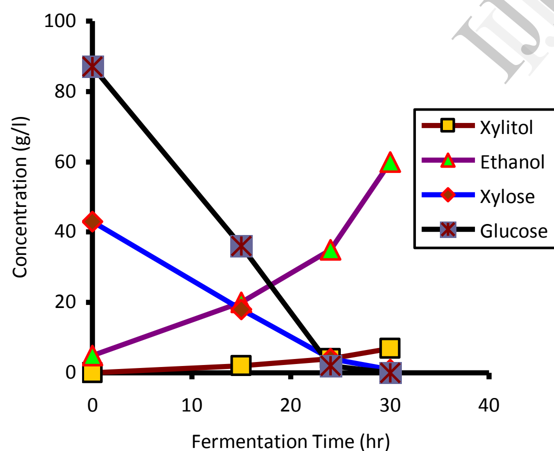

Fig: 6 Fermentation of glucose and xylose by *Saccharomyces yeasts* strain

To determine the effect of pH on ethanol fermentation different samples containing 100 gm sugars were fermented at different pH. pH 5-6 was found more favorable for effective fermentation and yield of ethanol. In the figure 7 it was clear that at pH 4.0 the maximum sugar utilization was found 90.72 % in 24 hours. Hence pH change is also an effective parameter but inoculums size is also an effective parameter for enzyme growth, the inoculums size of the experiment was found to have the highest impact on the process of fermentation by the organism, whereas pH of the medium had the least impact among all the selected optimization parameters. Various parameters, apart from having an individual effect on the process, also interacted among them. These interactions may be independent of individual effect.

Table 2: Effect of pH on fermentation on Pentoses and Hexoses by yeast

Kinetic & Overall Parameters	pH			
	4.0	**5.0**	**5.5**	**6.0**
Specific growth rate (μ) h^{-1}	0.11	0.14	0.15	0.15
Specific Ethanol productivity (qp) gg-1h-1	3.6	4.40	4.30	4.20
Specific substrate uptake rate (q_s) $gg^{-1}h^{-1}$	7.86	8.58	8.55	8.50
Cell Yield, $Y_{X/S}$ (gg^{-1})	0.013	0.016	0.01	0.01
Ethanol Yield ,Y p/s (g/g)	0.44	0.50	0.50	0.49
Final Biomass Con. g/l	1.20	1.53	1.66	1.76
Final Ethanol Con. g/l	40.00	47.50	48.0	48.0
Substrate Utilized (%)	90.72	94.80	95.9	96.9
Fermentation Efficiency* (% of theoretical)	90.0	97.8	98.1	98.8
Fermentation Time (h)	24	24	24	24

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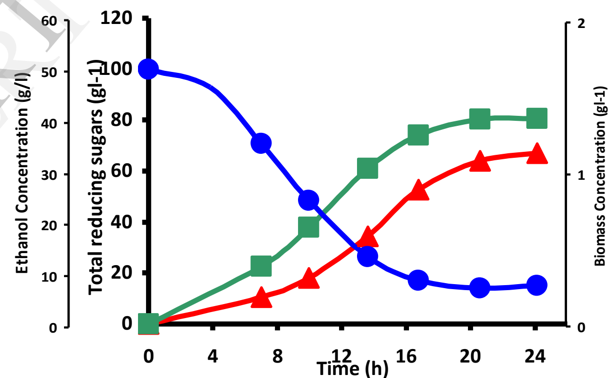

Fig. 7: Fermentation of 100 gl^{-1} sugar by yeast strain (temp 30°C, pH4) (•) total reducing sugars, (■) ethanol, (▲) biomass.

5. Conclusion: Pretreatment is the initial step to degrade the biomass content. Pretreatments are usually focused on lignin and hemicelluloses removal, cellulose crystallinity reduction and accessible surface area increase. 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. By the experimental analysis 3% (w/w) of acid was found optimum. In presence of higher sugar concentration Furfural, a pentose degradation product and 5-hydroxymethylfurfural HMF, a hexose degradation product and lignin degradation products are formed which decreases

the ethanol yield and production. Detoxification, Activated charcoal etc. are the suitable methods to remove inhibitors from the substrate. Eenzymatic hydrolysis and Fermentation is the second step of pretreated biomass. Hydrolysis process are done in different ways as with dilute sulphuric acid autoclaving at 121^{0}C for one hour, but in this step there may be the chance of formation of inhibitors or other compound, so it may be done carefully. Enzymatic hydrolysis of the pretreated biomass is an effective for the degradation of lignocellulosic biomass under optimum pH and conditions. Fermentation of hydrolysed biomass was done with *S. Cerevisiae* at 28-30^{0}C and at optimum pH. Yeast strain is one of the effective parameter which is genetically engineered for higher ethanol yield by fermentation.

The use of enzymes in the hydrolysis of cellulose is more advantageous than use of chemicals, because enzymes are highly specific and can work at mild process conditions. Despite these advantages, the use of enzymes in industrial applications is still limited by several factors: the cost of enzymes isolation and Purification is high; the specific activity of enzyme is low degrading enzymes. As consequence, the process yields increase at raising the enzymatic proteins dosage and the hydrolysis time (up to 2 days) while, on the contrary, decrease at raising the solids loadings.

Effective parameters of fermentation are also the important features that justified the conditions and maximum 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. At different time of fermentation for hydrolysed biomass 24 hours was found optimum. The fermentation media YEPX or YEPD was found optimum than YEP for the growth of enzymes for fermentation. Addition of nutrient supplementation as KNO_3, phosphates and sulphates increases the growth of enzymes as well as alcohol concentration.

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