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Synthesis and Study on Feasibility of Ethanol Production from Leachate of Pretreatment of Sugarcane Bagasse

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Abstract

Cashew nut shell (CNS), on the other hand, has been studied extensively for pyrolysis product distribution and oil characteristics. The total oil extracted from CNS accounts to more than 95% of theoretical oil yield. The oil has unique characteristics unlike other pyrolysis oil, with a heating value of 40 MJ/kg, comparable to fuel oil and double to that of other pyrolysis oils. The unique structure found having long (C6-C15) linear chain as a head and phenolic group in the tail, in CNS oil, gives the excellent solubility in diesel, as well as in other biomass pyrolysis oils. The oil has an amazingly high stability at room temperature.

A blending of high proportion of CNS oil / cardanol has been found to improve the properties like calorific value, viscosity and pH of bagasse pyrolysis oil, while the stability of the blend is being taken care by addition of as low as 5 wt. % ethanol in combination.

Key words: *CNS, Ethanol, Glucose, Biomass, Sugarcane bagasse*

1. Introduction

Ethanol has a very large use in industry especially as a solvent and potential use as a liquid. The unique characteristic of ethanol has been found in making pyrolysis oil very stable with time [1-5]. Although fermentation is used for production of industrial alcohol, and for a fuel especially in Brazil, most ethanol is made as a by-product of the petroleum industry. In petroleum industries, breaking molecules of crude oil (cracking) to sizes suitable for petrol release a lot of ethylene ($CH_2=CH_2$), which by adding a molecule of water, is converted to ethanol CH_2CH_2OH. Ethanol produced this way costs less than fermentation production. Fermentation production of ethanol can be economically competitive in special circumstances. The main cost of production of ethanol by fermentation is the raw material: sugar or starch. In order to get the costs down, there has been a lot of attention given to using 'waste biomass' which costs nothing of source. Such waste includes rice straw, sugarcane bagasse, and the forestry wastes such as small brances, saw dust etc. All these mainly composed of carbohydrates, which theoretically converted to near half its weight to ethanol [6-9]. In this paper, pretreatment of bagasse through leaching has been resorted to for selective removal of ash. This is accompanied by removal of other organic components like sugars, extractives, hemicellulose etc. The leachate has enough carbon to support the growth of organisms and to convert this carbon source into desired chemicals like ethanol. In view of this, the following study investigates the potential of ethanol production via fermentation of the leachates [10-14].

2. Principle of ethanol fermentation

The carbohydrate present in the biomass is not in a form suitable for fermentation. First, it must be broken down to its constituent sugars. Again not all these sugars are fermented by yeasts. In particular, xylose,

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which may be as much 30% of the total, is untouched. The most frequently used yeast for fermenting ethanol in industrial process is Saccharomyces Cerevisiae, which has proved to be very robust and well suited to the fermentation of lignocellulosic hydrolysates (same species used for bread making and some wines and beers). It can ferment glucose, fructose, sucrose but not pentoses like xylose. Other less common yeasts do ferment xylose but not glucose [15-18]. Genetic engineering has been able to modify microorganism so that they are able to ferment all sugars in waste biomass to ethanol. Some effort has gone into taking genes from these other yeasts that are responsible for xylose utilization and putting them into *S. Cerevisiae*. This has had only moderate success. Bacteria which are simpler and are called prokaryotes are also been used instead the complex eukaryote type yeasts. A few bacteria, mainly Zymomonas Mobilis ferment glucose, fructose and sucrose to ethanol but like yeast S. Cerevisiae it also don't ferment xylose. Many other bacteria, among which most common is Escherichia Coli, ferment xylose but don't make ethanol as end product. Thus, it is easy to transfer genes between bacteria by genetic engineering, and there are two possible routes by which a genetically engineered bacterium can be constructed which will ferment all sugars to make ethanol [19-23]. The first is to take the xylose-utilizing gene from *E. Coli* and transfer them to *Z. Mobilis*. This has been done with reasonable success. On the other hand, it is reported to be much simpler to take the genes that are responsible for Z. Mobilis making ethanol, and transfer them to *E.* Coli. (or similar xylose using organism). This transgenic E.Coli has been reported to be capable of converting both glucose and xylose to ethanol in surprisingly high yield [24-28]. However, the present work restricts to a very preliminary study on the feasibility of ethanol production from the leachates, resulting from pretreatment of bagasse, using the commercially available yeast in India.

The commercial enzymatic process for the production of ethanol can be divided into four major steps like i) pretreatment, ii) hydrolysis, iii) fermentation and iv) distillation. The schematic flow sheet (Figure 1) of a wood to ethanol process is as follows [29-32].

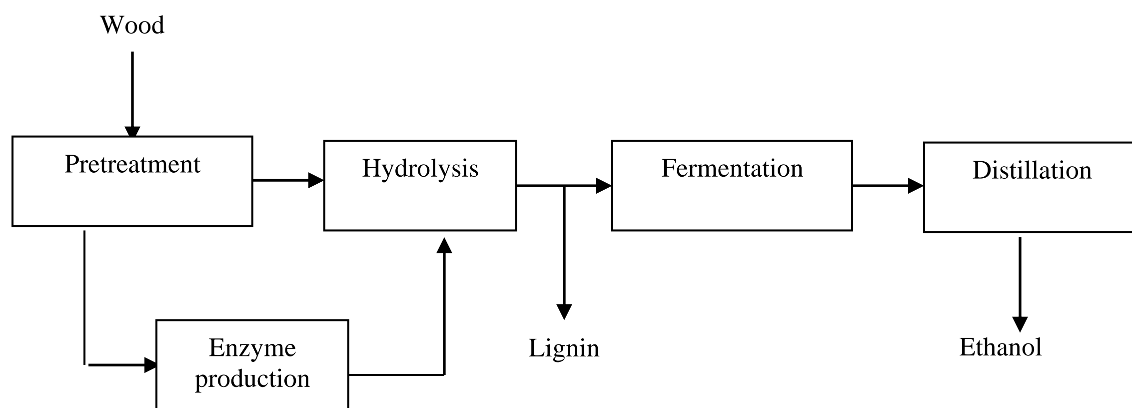

Fig.1: Schematic of Commercial Ethanol Making Process

In the pretreatment stage, the cellulose and hemicellulose is made accessible to the enzymes through some kind of physical or chemical process. Physical process involves milling or grinding of biomass into a very fine powder as pretreatment step. This process increases the external surface area of the cellulose and

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thereby facilitating enzymatic hydrolysis [33-38]. The second step is the enzymatic hydrolysis in which the cellulose is converted to monomeric glucose. In the fermentation step, the liberated sugars during both pretreatment and hydrolysis are fermented to ethanol. Hexoses are generally fermented by S. Cerevisiae. The technique for fermentation of pentoses (i.e. xylose) is essentially still at the laboratory stage, although significant advances have been made in the modification of pentose fermenting microorganism as mentioned earlier. The final step is ethanol distillation to get 94 wt. % as the end product, which can be further processed to pure ethanol. In the present study the leachates, which results from different pretreatment processes of finely powered sugarcane waste, was fermented for ethanol by venu et al. [39-42].

3. Experimental Methods

3.1 Microorganism and culture media

In the present study the leachates, which results from different pretreatment processes of finely powdered sugarcane waste, was fermented for ethanol by yeast S. Cerevisiae. Yeast strains S. Cerevisiae (Distillers yeast) provided by Burns Philip India Ltd (Kegaon-Uran, India) was used for the study [43-47]. The strain was maintained on culture medium containing glucose 20g/L, peptone 5g/L, yeast extract powder 5g/L, and agar 25g/L.

3.2 Ethanol fermentation with leachate

The ethanol fermentation was carried out in the leachate medium. The water leachates (pH 3-4) were first neutralised with 0.1 N NaOH solutions. Ethanol fermentation medium was prepared by adding peptone (Nitrogen source) and yeast extract powder, with a concentration of 5g/L each, in the neutralised leachate medium and was autoclaved. The culture maintained on slants was transferred to 100ml (in 500ml flask) of culture media. This culture medium, containing 20g/L glucose, 5g/L peptone, 5g/L yeast extract powder), was used to inoculate 50ml (in 250 ml flask) of fermentation media. This was used as a seed culture for experimental work [48-51]. All experiments were carried out at 30^0C on orbital shaker at 250 r.p.m. Biotransformation was initiated by addition of 2 ml of seed culture to the fermentation media and 1 ml. of sample was withdrawn at known time intervals for analysis. Each of the samples taken in regular time interval was then centrifuged at 6000 r.p.m for 10 minutes. The supernatant was collected in eppendrof tubes and stored at 4^0C for ethanol and glucose estimation. The centrifuged organisms were washed with saline water (0.9% aqueous NaCl solution) and finally it was resuspended in saline water making the volume same as initial.

3.3 Estimation of cell concentration

Each sample (centrifuged organism supended in saline) was diluted with saline water to desired concentration range and mixed in a vortex to make homogeneous solution. Then, the absorbance was measured at 600nm. on UV/Visible spectrophotometer (Jasco model V-530). For calibration of dry cell weight of yeast, 5ml of cell suspensions with known absorbance values were centrifuged and dried in an oven at 80^0C to constant weight. This procedure was repeated thrice and average values were used for calibration [52-56].

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3.4 Estimation of glucose concentration

For glucose estimation, 1 ml of each sample (supernatant of each centrifuged sample diluted to desired range of concentration) was added to 3 ml. of o-toluidine reagent (8% v/v o-toluidine in glacial acetic acid). This was vortexed and placed in a boiling water bath for 20 minutes. The resulting solution was cooled and the absorbance was read at 630 nm. A series of standards was made with concentration of glucose (dextrose) in the range 0.05-0.25 g/L and absorbance was measured. Calibration curve was obtained by plotting absorbance against concentration of standard samples. The equation for the line of best fit for glucose calibration found is given in following section. This method measures the reducing sugar glucose exclusively among a mixture of saccharides. O- Toluidine in the presence of glacial acetic acid reacts quantitatively with aldehyde [>CHO] groups of aldohexoses to yield glycohexyl amines and schiff's base. Aldopentoses, maltose and lactose give similar reactions but are very less reactive. A blue green colour is obtained which is estimated spectrophotometrically at 630nm [57-59].

3.5 Estimation of ethanol concentration

Ethanol concentration was estimated using a MAK series 911M Gas Chromatograph (GC) with 0.5 inch steel Porapak Q column of 2m length. The column has a mesh size of 80/100. The GC conditions are as follows: Oven temperature 180^{0}C (isothermal), Injector temperature 220^{0}C, Flame Ionisation Detector (FID) temperature 220^{0}C. 3 µL sample (supernatant of each centrifuged sample) was injected for each run. Nitrogen was used as the carrier gas with a flow rate of 30ml/min. The calibration curve was obtained using standard ethanol solutions of known concentration [60-63].

4. Results and discussion

All the leachates were analysed for glucose content and the results are presented in **Table 1**. It shows that, an average of 55 % of total weight loss of biomass during leaching process is contributed by glucose and the rest might be due to pentoses like xylose and other sugars. The glucose concentration of leachate being high (78%) in case of pretreatment with higher biomass to water ratio [64-66].

Table 1: Sugar analysis of leachates

Treatment No.	Wt. of glucose (g/L) in leachate	% Sugar (glucose) loss w.r.t total wt. loss in leaching
I	7.5	50.00
II	12.90	57.30
III	35.31	78.00
IV	22.11	53.06
V	15.08	56.55

All the leachates were first neutralised with 0.1 N NaOH solution and tested for the viability of cell growth. In case of 5M HCl and HF pretreatment leachate, there was no growth of cell, which may be attributed to higher salinity of the leachate on neutralisation with NaOH solution. On the other hand, NaF produced on neutralising HF treated leachate inhibits the TCA and glycolysis pathways i.e. the central metabolic pathways of cell growth. Finally, leachate resulting from special 24- hour water leaching of bagasse was

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taken for fermentation study. Fermentation tests were carried out with four set of substrates, viz. with control, 20g/L glucose solution, 24 hour water leachate (Treatment II, WL2), water leachate of special water treatment (Treatment III, WL3, and with the leachate of Treatment III supplemented with additional 20g/L glucose. The rate of production of ethanol as well as the rate of consumption of glucose and rate of cell growth for all the above mentioned substrates has been presented in **Figure 2, Figure 3** respectively. It is very interesting to note that maximum conversion of glucose to ethanol is ~ 38-40% of original glucose concentration. The calibration curve obtained for estimation of cell concentration, glucose concentration and ethyl alcohol concentration by GC are presented in **Figure 2** and **3** respectively.

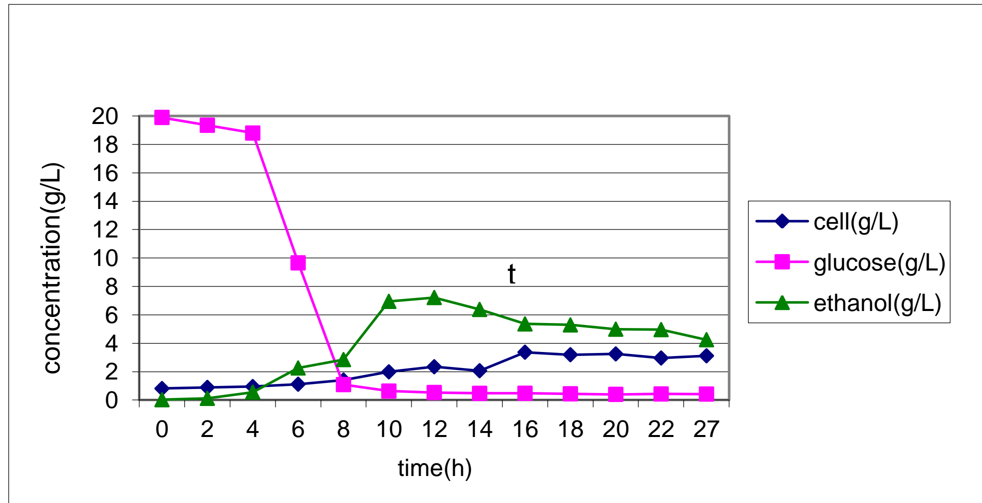

Figure 2: Kinetics of control (2% Dextrose) fermentation

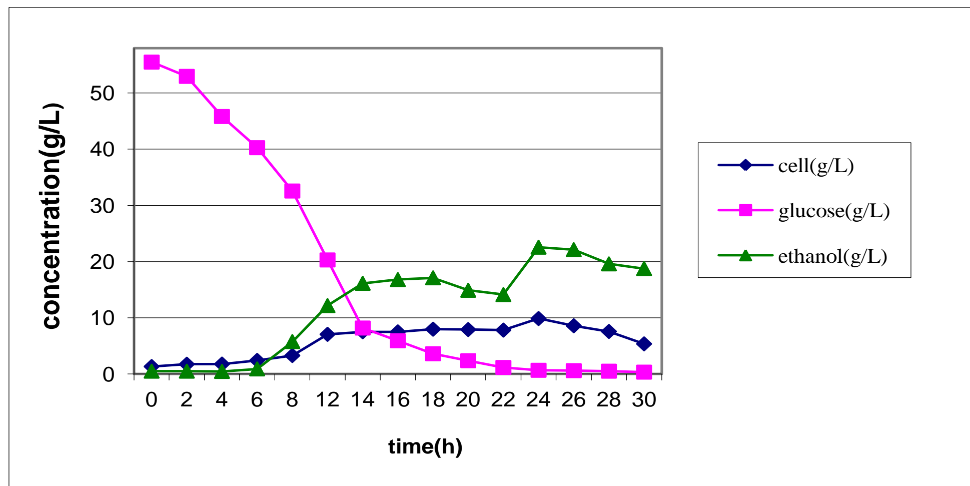

Figure 3: Kinetics of mixture of treatment III- leachate and 2% dextrose fermentation

5. Conclusion

The leachate of all the pretreatment processes have high sugar contents, but ethanol fermentation was not feasible in acid treated leachates due to high salinity of neutralized leachates which inhibits the TCA cycle and glycolysis pathways of fermenting microorganism. The high sugar content of water leaching process

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carried with lowest possible water and for longer period (24 hours) shows a promising result of a maximum yield of ethanol 13.5 g/l with 38% conversion of sugar. The fermentation process can be made more economic by supplemented the leachate with glucose or other ethanol making substrate and hence increasing the ethanol concentration in the final product. The ethanol in turn can be used to stabilize the oil produced from pretreated (water leached) bagasse. Technically based on simple calculations, i.e. if oil yield from 24hour water leached bagasse is 31% and 5% ethanol is required for stabilizing the oil, the water leachate when fermented produces enough ethanol for stabilization of the oil. This shows the potential of integrated loop type approach for stable, less viscous bio-oil product.

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