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RESEARCH ARTICLE

BIOETHANOL PRODUCTION FROM CELLULOSIC MATERIALS

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ABSTRACT

In the present study, pretreated sugarcane bagasse is used as the substrate for bioethanol production using cellulase and various yeast strains including thermotolerant strains by simultaneous saccharification and fermentation method at the optimized process conditions. Various pretreatment techniques namely dilute and concentrated sulphuric treatment, sodium hydroxide combined with high pressure steam treatment and steam autoclaving treatment are conducted for mechanically pretreated sugarcane bagaasse (milled ~ 100 mesh, 0.15 mm) for different time intervals (5, 10 and 15 min). The objective of the treatment step is to reduce the lignin and hemicellulose without altering the cellulose content because cellulase enzymes are highly specific in the cellulose hydrolysis reactions. From the results, the acid and alkali treatment techniques are not suitable for the enzymatic hydrolysis of pretreated sugarcane bagasse due the decreased cellulose content and also the hemicellulose and lignin content was significantly reduced. The effect of cellobiose concentration is studied by conducting batch experiments at different initial cellobiose concentrations namely 5, 10, 15, 20, and 25 g/l at the hydrolysis temperatures of 45°C with an initial enzyme loading of 15 FPU/g bagasse and with initial pH of 5.5 and with a hydrolysis period of 120h as constant. From the experimental results it is observed that the cellobiose show a strong inhibitory effect. The effect of temperature on ethanol fermentation is studied by conducting batch experiments at different incubation temperatures namely 45°C for different yeast strains. The results show the maximum ethanol concentration of 3.82 g/l is obtained at an optimum temperature of 35 °C when *S.cerevisiae* is used for a fermentation period of 120h. This separate hydrolysis with fermentation gives a lower yield when compared to SSF process and may be due to the inhibitory action of glucose and cellobiose in the cellulose hydrolysis.

INTRODUCTION

Biofuels are referred to as liquid or gaseous fuels for the transport sector that are predominantly produced from biomass. Bio-fuels are generally considered as offering many priorities, including sustainability, reduction of greenhouse gas emissions, regional development, social structure and agriculture, security of supply (Reijnders, 2006). World wide energy consumption has increased 17 fold in the last century and emissions of CO_2, SOx and NOx from fossil fuel combustion are primary causes of atmospheric pollution (Ture et al. 1997). Non-renewable energy sources, i.e. fossil fuels represent the most exploited forms of energy today and is forecasted that at the present rate of production fossil fuels would be exhausted in the next century. Hence there is a need to develop and implement viable technologies for the production of alternative renewable energy and feedstock. Today many of the technologies for the production of alternative fuels such as bioethanol are not competitive with the cheap fossils fuels available. Despite this, some commercial interest and research continues because of the abundance of raw materials and the prediction that the energy economics will change the near future to favor bio-fuels. Bioethanol is a promising renewable energy source, eco-friendly and causes maximum reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels. But the production of bioethanol from other sources except molasses are more complicated due to the presence of hemicellulose and lignin and it faces lot of problems such as low ethanol yield, more fermentation time, use of single microorganisms, cost involved in pretreatment step and quality of ethanol for commercial production. Bioethanol is conventionally produced from cane molasses or wheat molasses by a single step fermentation of yeast since the molasses availability is limited there is an immediate need for search of alternate sources for production of bioethanol. The promising alternative sources are lignocellulosic biomasses such as sugarcane bagasse, rice straw, wheat straw, wood. The transformation of such biological resources as energy-rich crops (like sugarcane

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conditioning or pretreatment of the feedstocks for fermenting organisms to convert them into bioethanol. This product has to be dehydrated in order to be utilized as an oxygenate for gasoline, the trade form in which bioethanol is mostly employed in the transportation sector. The complexity of this process partly explains why fuel ethanol has not played a leading role in comparison to cheaper oil derived fuels. Only during in the last two decades due to rising environmental concerns and to the periodic crises in some of the larger oil exporting countries, has bioethanol become a viable and realistic alternative in the energy market (Cardona and Sanchez, 2007).

Therefore the development of cost-effective technologies for fuel ethanol production is a priority for many research centers, Universities and private firms, and even for different governments. Due to the large amount of existing and not completely developed technologies for the production of ethanol (especially from lignocellulosic biomass), the application of process engineering tools is required. Process engineering applied to the production of fuel ethanol includes the design of new innovative process configurations aimed at reducing ethanol production costs (Cardona and Sanchez, 2007). The processing and utilization of lignocellulosic biomass is complex, differing in many aspects from crop-based ethanol production. One important requirement is an efficient microorganism able to ferment a variety of sugars (pentoses, and hexoses) as well as to tolerate stress conditions.

Bioethanol is mainly produced from fermentation of sugar containing materials such as molasses, sugarcane (cane juice or cane syrup), serial crops, sugar beet and sweet sorghum. Recent biotechnological developments have led to an increased focus on utilization of lignocellulosic biomass as a resource for the production of liquid fuels and other chemicals. Multiple biomass substrates have been identified to hold a great potential due to their high content of cellulose and hemicellulose, combined with an abundant annual production (Wiselogel et al. 1996). The main challenge in the conversion of biomass into ethanol is the pretreatment step. Due to the structure of the ligocellulosic complex, the pretreatment is required for its degradation, the removal of lignin, the partial or total hydrolysis of the hemicellulose, and the decrease in the fraction of crystalline cellulose related to the amorphous cellulose, the most suitable form for the subsequent hydrolysis step. In this step, the cellulose undergoes enzymatic hydrolysis in order to obtain glucose that is transformed into ethanol by process microorganisms. Eventually, the sugars released during the hydrolysis of hemicellulose can be converted into ethanol as well. Industrially, the pretreated material is mainly thought to be hydrolyzed and fermented in two different steps: separate hydrolysis and fermentation (SHF); or in one single step: simultaneous saccharification and fermentation (SSF). A few microbial species such as *Neurospora, Monilia, Paecilomyces* and *Fusarium* sp. have been reported to hold the ability to ferment cellulose directly to ethanol (Singh et al. 1992). Consequently, the involved technologies are more complex leading to higher ethanol production costs compared to cane, beet or corn. However, the fact that many lignocellulosic materials are by-products of agricultural activities, industrial residues or domestic wastes offers huge possibilities for the production of fuel ethanol at large scale as well as its global consumption as a renewable fuel. It is considered that lignocellulosic biomass will become the main feedstock for ethanol production in the near future.

MATERIAL OF MATERIALS

Culture maintenance

The yeast cultures *Candida wickerhamii, Pachysolen tannophilus, Saccharomyces cerevisiae, Kluyveromyces marxianus var. marxianus* and *Kluyveromyces fragilis* are maintained on yeast extract-malt extract-agar (YMA) medium. The liquid medium for the growth of inoculum is yeast extract-malt extract-peptone-glucose medium (YMP). The composition levels of the medium components and the nutrient levels. Steam pretreatment content was measured by Saddler et al., 1982. Dilute Sulphuric acid (H_2SO_4) treatment was measured by Han and Callihan, 1974. Concentrated Sulphuric acid (H_2SO_4) treatment Sodium hydroxide (NaOH) pretreatment were determinate respectively according to the methods of Han and Callihan, 1974. Sodium chlorite ($NaClO_2$) pretreatment was measured by Rajoka, 2000. Enzymatic hydrolysis of steam pretreated bagasse was Quantitatively determined by analytical methods (Vlasenko et al., 1997)

Estimation of extractives sugarcane bagasse

The extractives are measured by exhaustive Soxhlet extraction of the biomass using ethanol as solvent. Bagasse sample is weighed accurately (10g) and transferred into a 250ml round bottom flask with this 100 ml of ethanol is added. The reflux condenser is attached and digested for 3 hours over a heating mantle. After 3 hours the material is washed with distilled water and oven dried for 1 hour.

Estimation of cellulose

3 ml of acetic nitric reagent is added to 1 gram of the sample in a test tube and mixed in a vortex mixture. The tube is placed in a water bath at 100°C for 30 minutes, cooled and then centrifuged for 15 – 20 minutes. The supernatant liquid is discarded; the residue is washed with distilled water. Then 10 ml of 67 % sulphuric acid is added and allowed to stand for 1 hour. 1 ml of from the above solution is diluted to 100 ml. To 1 ml of this diluted solution, 10 ml of chilled Anthrone reagent is added and mixed well. The tubes are heated in boiling water bath for 10 minutes, cooled and the intensity of color is measured spectrophotometrically at 630 nm in UV/Vis-Double Beam Biospectrophotometer using blank as reference. Similar procedures are followed for the other concentration containing known quantity of cellulose.

Estimation of hemicelluloses

To 1gm of the powdered sample in a refluxing flask 10ml of cold Neutral Detergent Solution is added. With this 2ml of Decahydronaphthalene and 0.5g of Sodium sulphite is added. The mixture is warmed to boiling point and is refluxed for about 60min. The contents are filtered through sintered glass crucible (G-2) by suction and washed with hot water. Finally two washings are given

with acetone. Then the residue is transferred to a crucible, dried at 100°C for 8hr. The crucible is cooled in a desiccator and weighed (Goering and Vansoest, 1975). Calculation:Hemicellulose = Neutral detergent fibre (NDF) – Acid detergent fibre (ADF)

Estimation of lignins

1g of powdered sample in a round bottom flask is placed with 100ml of acid detergent solution. The mixture is then boiled to 5 – 10 min and cooled to avoid foaming as boiling begins. It is refluxed for 1h after the onset of boiling. To maintain the even level the boiling is adjusted to slow. The container is removed, swirled and filtered the contents through a preweighed sintered glass crucible (G-2) by suction and is washed with hot water twice. Finally the residue is washed with acetone until the filtrate is colorless. Dried at 100° C for overnight and weighed after cooling in a desiccators (Goering and Vansoest, 1975) Calculation; the ADF content is expressed in percentage as i.e., W/S x 100, where W is the weight of the fiber and S is the weight of the sample. Estimation of Total Reducing Sugars was measured by Miller, 1972. Estimation of glucose content was calculated by the method of Krishnaveni et al. 1984. Estimation of Total Carbohydrate content was measure by Dubios et al. 1956.

Estimation of Cellulase enzyme activity

1.0 ml 0.05 M Na-citrate (pH 4.8) is added to a test tube of volume at least 25 ml.0.5 ml enzyme is diluted in citrate buffer with Whatman No. 1 filter paper strip (1.0 x 6.0 cm or ~ 50 mg). Two dilutions are made of each enzyme sample for investigation. One dilution should release slightly more and one slightly less than 2.0 mg (absolute amount) of glucose (= reducing sugars as glucose) in the reaction conditions. Enzyme solution containing filter paper strip is incubated 50°C for 60 min. Then 3.0 ml of DNS reagent is added and mixed well and boiled for exactly 5.0 min in a vigorously boiling water bath. 1ml of 40% Rochelle salt solution is added when the contents of the tubes are still warm. 20ml of double distilled water is added with the content. The intensity of dark red color product is spectrophotometrically read at 510 nm UV/Vis-double beam Biospectrophotometer using blank as reference. Similar procedures are followed for the other enzyme concentrations.

Estimation of Cellobiase

In a test tube1.0 ml of enzyme solution is diluted in citrate buffer (pH – 4.8). At least two dilutions are made of each enzyme sample investigated because one dilution should release slightly more and one slightly less than 1.0 mg (absolute amount) of glucose in the reaction conditions.1.0 ml cellobiose stock solution is added to the enzyme solution and mixed well and incubated at 50°C for exactly 30 min. After which the reaction is terminated by immersing the tube in a boiling water bath for exactly 5.0 min. Then the tube is cooled in a cold water bath and the glucose formed is determined spectrophotometrically by using Glucose oxidase-peroxidase method (Ghose, 1987).

Estimation of cellmass

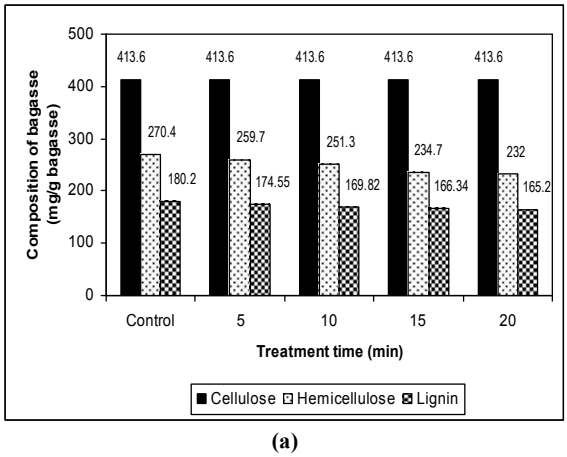
Centrifuge tubes are well washed and dried in an oven to remove all the moisture. Weights of empty dry centrifuge tubes are found out using electronic balance. 10 ml of the broth is taken in the centrifuge tube and centrifuged for 20 min. The settled biomass is made free of water and it is kept in the oven to remove all moisture. The weights of centrifuge tubes with the biomass are found out by electronic balance. The weight of the cell mass is found from the difference in measured weights (Narasimha et al. 2006)

Estimation of ethanol

The concentration of ethanol is estimated using NUCON 5765 gas chromatography (GC) with a flame ionization detector (FID) and CHROMATOPAK (10% Carbowax 20M) column (3m length and 1/8 mm diameter) using N_2 as the carrier gas at the rate of 30 µl per minute. Hydrogen and compressed air are used as fuel gas and are regulated at a rate of 30 and 300 µl per minute respectively. Flame is ignited at the flame ionization detector port. The injector, detector and oven temperatures are programmed. After reaching the stability, when the oven temperature and detector, injector temperature are at the programmed temperature, a sample is injected from fermentation flask into injector port by using a micro syringe (1 – 10 µl). The oven temperature is held at 80°C. The injector and detector temperature is maintained at 200°C. Injection volume is 1µL and 0.760 g/ml absolute ethanol is used as the internal standard. The peak eluted is noted (using WINACDS 6.2 software) and by knowing the area of peak, the concentration of ethanol is calculated using calibration chart. A view of the Gas chromatography used for this study is shown in Fig. 4.2 and the ethanol analysis.

RESULT AND DISCUSSION

Natural cellulose is a crystalline polymer generally associated with hemicellulose and lignin which is found to be highly resistant and difficult for the bioconversion. Lignin interferes with the cellulose hydrolysis because it acts as a physical barrier that prevents the contact of cellulase to cellulose. Therefore pretreatment is necessary to achieve the maximum yield of ethanol.



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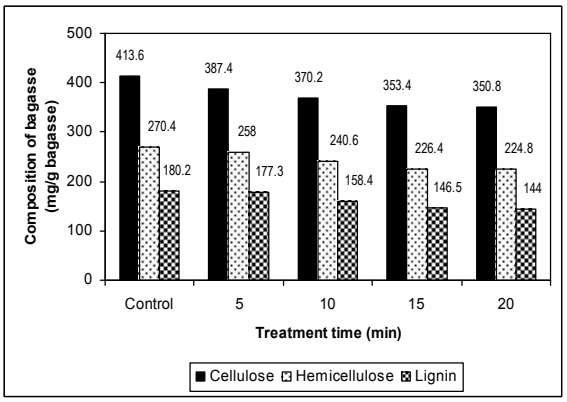
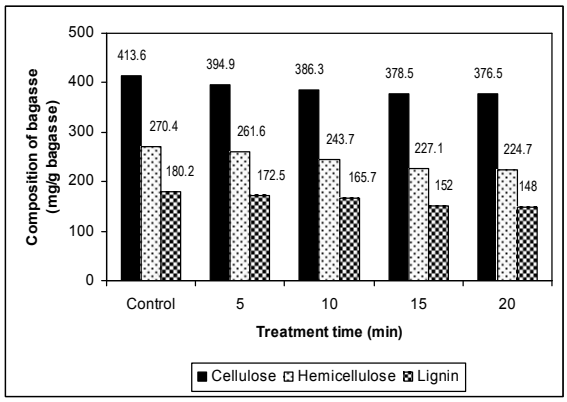


(b)

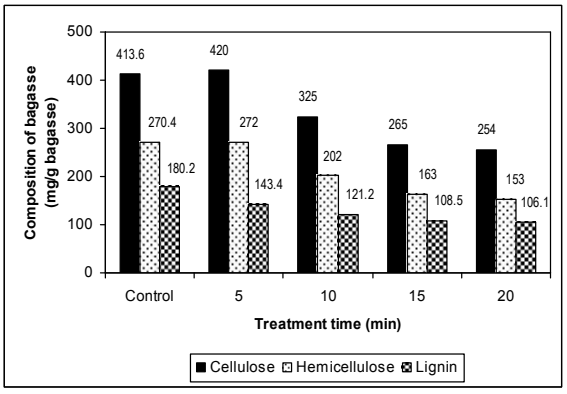
Fig. 1. Effect of pretreatment on bagasse with *a)* Steam treatment *b)* H_2SO_4 [1% v/v]



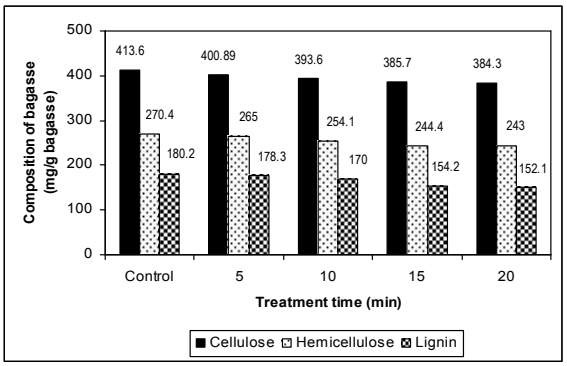
(c)

Fig.2.Effect of pretreatment on bagasse with a*)* H_2SO_4 [50% v/v] b*)* NaOH [1% w/v] c*)* NaOH [10% w/v]

The effect of steam on treatment of bagasse (particle size of 0.15 mm) is well studied by conducting using high pressure steam (15 psi, 121°C) and the pretreatment time is varied form 5 to 20 mins. A maximum reduction of 0.00% of cellulose, 15.45% of hemicellulose and 9.82% of lignin were obtained for a treatment time of 20 min. The total sugar content is increased by 4.38% due to the possible conversion of cellulose polymers into monomers and dimmers. The cellulose content is not changed even when the steam treatment time increased upto 20 minutes and also the hemicellulose and lignin content is significantly reduced by about 15.45 and 9.82% respectively. Generally cellulose (6-carbon) sugars would not degrade at this steam pressure and temperature (Fig.1a).



(a)



(b)

The effect of dilute sulphuric acid (1% v/v) on treatment of bagasse (particle size of 0.15 mm) is studied by are conducted using 1% (v/v) dilute sulphuric acid and the pretreatment time is varied form 5 to 20 mins. A maximum reduction of 15.71% of cellulose, 18.60% of hemicellulose and 22.41% of lignin were obtained for a treatment time of 20 min. The total sugar content is increased by 13.71% due to the possible conversion of cellulose polymers into monomers and dimmers (Fig.1b). The effect of concentrated sulphuric acid (50% v/v) on treatment of bagasse (particle size of 0.15 mm) is well studied by conducting experiments are conducted using 50% (v/v) concentrated sulphuric acid and the pretreatment time is varied form 5 to 20 mins.

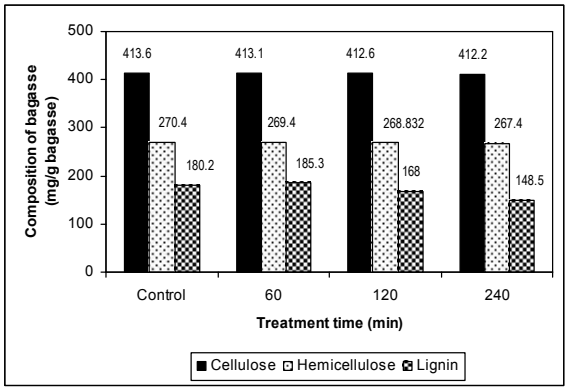


Fig. 3. Effect of pretreatment on bagasse with $NaClO_2$ [1.5g/g bagasse]

A maximum reduction of 47.07% of cellulose, 48.74% of hemicellulose and 45.33% of lignin were obtained for a treatment time of 20 min. The total sugar content is increased by 63.71% due to the possible conversion of cellulose polymers into monomers and dimmers. The effect of sodium hydroxide (1% w/v) on treatment of bagasse (particle size of 0.15 mm) is well studied by conducting experiments are conducted using 1% (w/v) sodium hydroxide and the pretreatment time is varied form 5 to 20 mins. A maximum reduction of 7.80% of cellulose, 11.42% of hemicellulose and 17.42% of lignin were obtained for a treatment time of 20 min (Fig.2a, 2b and 2c). The total sugar content is increased by 7.87% due to the possible conversion of cellulose polymers into

(10% w/v) on treatment of bagasse (particle size of 0.15 mm) is well studied by conducting experiments are conducted using 10% (w/v) sodium hydroxide and the pretreatment time is varied form 5 to 20 mins. A maximum reduction of 9.69% of cellulose, 18.82% of hemicellulose and 22.19% of lignin were obtained for a treatment time of 20 min. The total sugar content is increased by 10.01% due to the possible conversion of cellulose polymers into monomers and dimmers. There is no saturation occurs in percentage decrease of cellulose content during the treatment time with 10% alkali. It is found from the comparative pretreatment results of alkali treatment that the hemicellulose and lignin contents of the 10% alkali treated bagasse are significantly lower than the 1% alkali treated bagasse. The effect of sodium chlorite (1.5g/ g bagasse) on treatment of bagasse (particle size of 0.15 mm) is well studied by conducting experiments are conducted using sodium chlorite and the pretreatment time is varied form 5 to 20 mins. A maximum reduction of 0.38% of cellulose, 0.85% of hemicellulose and 27.19% of lignin were obtained for a treatment time of 20 min. The total sugar content is increased by 11.82% due to the possible conversion of cellulose polymers into monomers and dimmers. The cellulose and hemicellulose content is not changed even when the steam treatment time increased upto 20 minutes and lignin content is significantly reduced (Fig.2a, 2b and 2c)

Effect of cellulase activity on hydrolysis of pretreated bagasse

The effect of enzyme activity on hydrolysis of bagasse is well studied by the experiments are conducted at different enzyme activities of 5 FPU/ g bagasse, 10 FPU/ g bagasse, 15 FPU/ g bagasse and 20 FPU/ g bagasse, keeping the other conditions constant (initial substrate concentration, 50 g/l, initial pH – 5.5 and hydrolysis time 120 h).

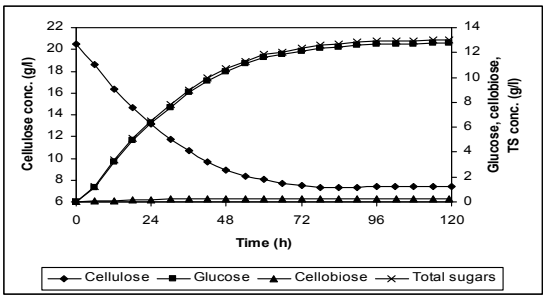


Fig.4. Enzymatic hydrolysis of steam treated sugarcane bagasse at the cellulase activity of 20 FPU /g bagasse and at 45°C.

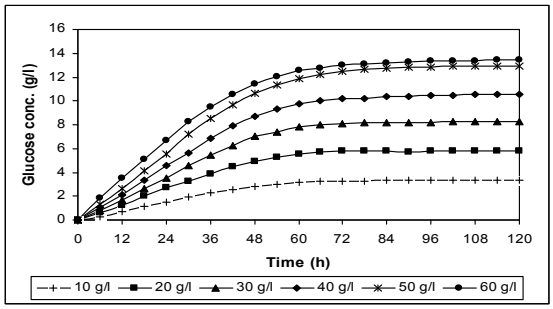


Fig.5. Effect of substrate concentration on enzymatic hydrolysis of

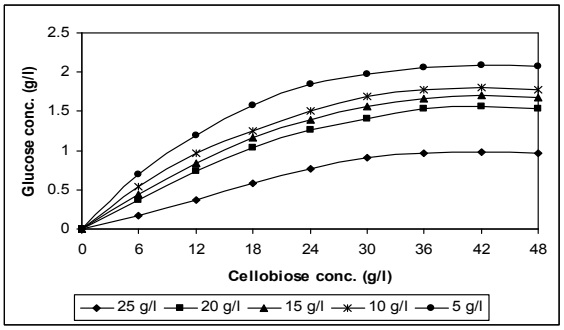
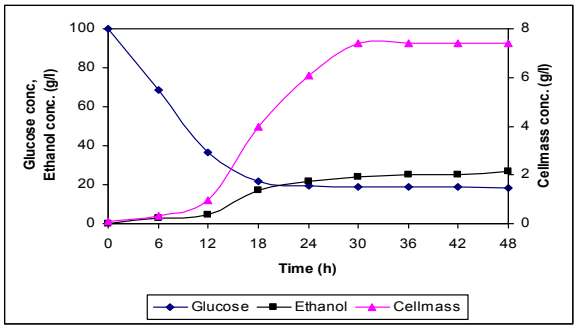


Fig.6. Effect of cellobiose concentration in the enzymatic hydrolysis of cellobiose at 45°C and at 15 FPU/ g bagasse

For each enzyme loading the experiments are conducted at temperatures 45°C keeping the other conditions constant (initial substrate concentration, 50 g/l, initial pH – 5.5 and hydrolysis time 120 h). Experimental results of the enzymatic hydrolysis of pretreated bagasse at different cellulase activities and at different hydrolysis temperatures. From the results it is found that a maximum product concentrations of 12.82 g/l, 0.258 g/l and 13.1 g/l of glucose, cellobiose and total reducing sugar concentration respectively at the highest hydrolysis temperature of 45°C (Fig. 4,5,and 6).



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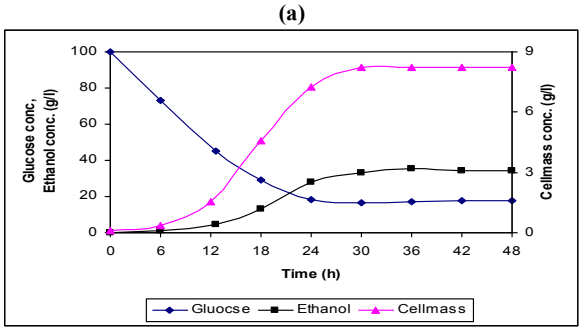

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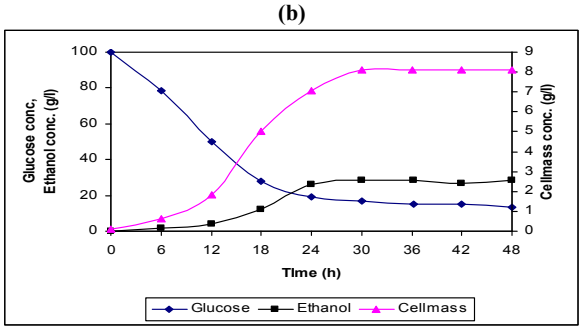

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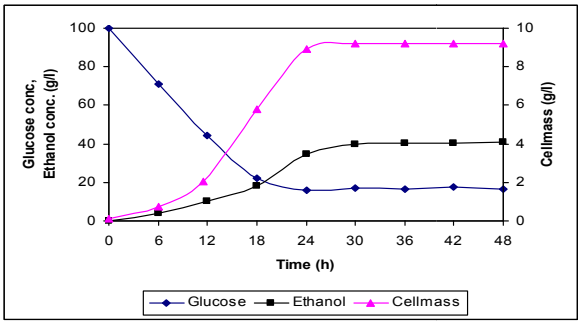

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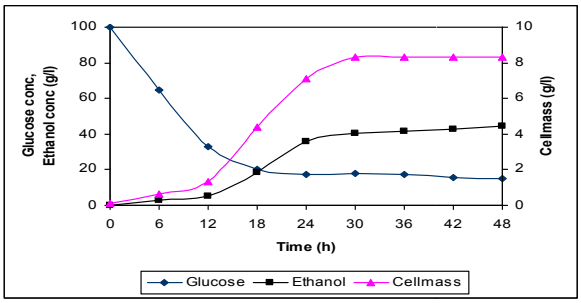

(e)

Fig.7. Effect of temperature on glucose consumption, cellmass concentration and ethanol concentration at 45°C using (a) *C.wickerhamii (b) S .cerevisiae (c) P.tannophilus* (d) *K.marxianus var. marxianus (e) K fragilis*

The effect of cellulase activities for substrate utilization, product formation (glucose, cellobiose and total reducing sugar concentration) at different hydrolysis temperatures and (Fig. 4, 5, and 6). show the comparative result of product concentrations (glucose, cellobiose and total reducing sugars) at different enzyme activities at 45°C. Due to tough crystalline structure, the enzymes currently available require several days (about 4 – 5 days) to achieve good results. More over enzymatic hydrolysis of natural lignocellulosic materials is a very slow process because cellulose hydrolysis is hindered by structural parameters of the substrate, such as lignin and hemicellulose content, surface area, and cellulose crystallinity. Generally, enzymatic cellulose degradation is characterized by a rapid initial phase followed by a slow secondary phase that may last until all substrate is consumed.

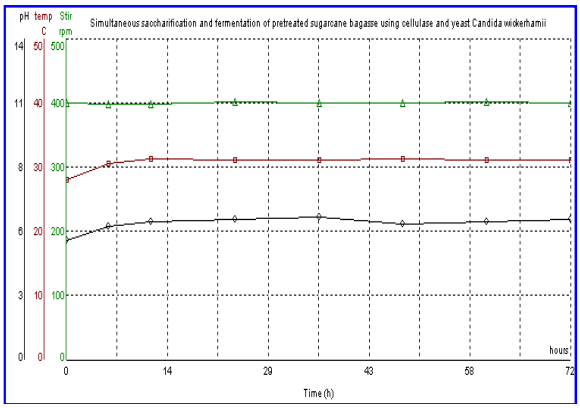

Fig. 8. Bioethanol production from pretreated bagasse

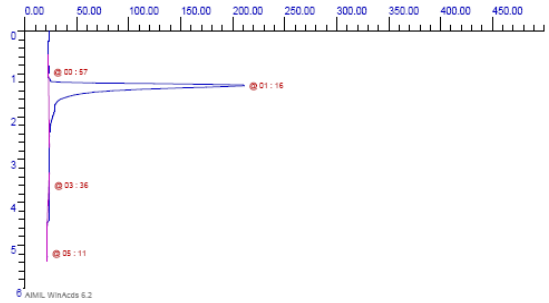

Fig. 9. Gas Chromatographic (GC) analysis of ethanol produced by the pretreated sugarcane bagasse using cellulase and yeast *S.cerevisiae*

It is evident from the results, as the enzyme loading is increased from 5 FPU/ g bagasse to 20 FPU/ g bagasse the percentage conversion to glucose in increased from 7.0 g/l to 12.82 respectively at 45°C. Also a high degree of bagasse conversion to sugar concentration (about 10.5%) is obtained when a high FPU activity is used (20 FPU/ g bagasse). This correlation of increased hydrolysis at higher enzyme concentration is not surprising as the relation have been reported in several studies with pretreated sugarcane bagasse. A maximum glucose yield of 12.82 g/l is obtained for the enzyme loading 20 FPU/ g bagasse. Since the difference in percentage conversion of cellulose is marginal for enzyme loadings 15 FPU/ g bagasse and 20 FPU/ g bagasse which produced 12.51 g/l and 12.82g/l of glucose concentration respectively.

Such a non-linearity may indicate a limit to the availability of surface area (and the associated cellulase binding sites) dictated by the size and amount of cellulose present, such that the number of enzyme binding sites becomes limiting at higher enzyme loadings. Since the cellulase enzyme preparation appears to be one of the most expensive materials needed for a lignocellulose to ethanol conversion, it may be advantageous to optimize the efficiency of ethanol production per unit of cellulose enzyme. Thus using such high enzyme concentrations per gram substrate (20 FPU/ g bagasse) is not economically justified and economic feasibility must be considered. Accordingly, in further studies cellulase loading of 15 FPU/ g bagasse is chosen optimum loading for the production of bioethanol from steam treated bagasse by Simultaneous Saccharification and Fermentation (SSF) process (Fig. 8 and 9).

Effect of temperature on ethanol fermentation by yeast strains

Temperature is one of the most important parameter in the production of ethanol since enzymatic hydrolysis and glucose fermentation rates are depend upon the temperature. Generally the fermentation temperature has a greater influence on the rate of fermentation. As the fermentation temperature increase the rate of growth as well as the rate of product formation increase. But there is a limitation for bioprocesses a higher temperature may not favor the growth, the cells may die, the enzymes may denature and the rate of product formation may be affected. Moreover all fermentations are exothermic in nature, the extent of heat released depends on the nature

conditions etc. Hence a complete temperature controls at an optimum temperature will certainly enhance the ethanol production. As the increases, the ethanol production increase intern the yeast activity, the growth rate and the glucose consumption rate increases to maximum at the optimum temperature with a further increase in temperature beyond the optimum temperature may result in the cell viability and inturn affects the cellmass and the ethanol production.

The effect of temperature on ethanol fermentation is studied by conducting batch experiments at different incubation temperatures namely 45°C for different yeast strains namely *Candida wickerhamii* MTCC*3013, *Pachysolen tannophilus* MTCC *1077, *Saccharomyces cerevisiae* MTCC 172*, Kluyveromyces marxianus var. marxianus* MTCC 1389 and *Kluyveromyces fragilis* MTCC 095 by keeping initial substrate concentration (100 g/l), initial pH (5.5), inoculum age (24 h old culture) and agitator speed (400 rpm) as constant for 120h fermentation period. At lower incubation temperature, the activity of yeast cells can be maintained longer and the final ethanol concentration in the fermentation broth is greater than at a higher temperature (Fig.7). This phenomenon is common in thermo tolerant yeasts, in that a higher temperature lowers the yeast's viability and its tolerance to ethanol (it is generally accepted that the inhibitory effect of ethanol increases with increasing temperature).

In order to choose the optimum conditions, the maintenance of cellulase and yeast's activities during SSF of pretreated bagasse must be taken into consideration. For this reason, the effect of temperature on glucose fermentation by different yeast strains is studied in the range of 45°C. The parameters measured are glucose utilization, ethanol production and cell growth and the effect of incubation temperature on glucose consumption, cellmass concentration and ethanol production using different yeast strains including thermo tolorant strains.

From it is observed that yeast cells readily consumed glucose to produce ethanol in the range of 40°C at an initial glucose concentration of 100 g/l. At 30°C and 35°C, glucose is consumed within 12h of incubation without an apparent lag phase. The ethanol yield reached 32.7g/l (approximately 64% of theoretical value), 32 g/l (63%), 38.6 g/l (76%), 46.3 g/l (90%) and 44 g/l (86%) using *C.wickerhamii, P.tannophillus, S.cerevisiae, K.marxianus var. marxianus* and *K.fragilis* respectively at 40°C for 48h fermentation period. In contrast to ethanol yield, the growth is highest at 35°C and decreased as the incubation temperatures increase in all the cases. This decrease in biomass yield is typical in thermotoleratnt yeasts. Anderson et al. 1996 observed the decrease in yeast viability at 43°C and higher. The maximum ethanol production of 46.3 g/l is obtained at 40°C by *K.marxianus* on 120h fermentation. At higher temperatures the death rate of cells might be more and might cause lesser growth and less ethanol production.

The incubation temperature for hydrolysis of sugarcane bagasse and for SSF of sugarcane bagasse is chosen as 38°C to maintain yeast activity and good saccharification activity during the prolonged period of incubation. There are distinct advantages in high temperature (40 to 50°C) fermentation by yeasts. These include faster rates of substrate consumption and product (ethanol) formation facilitation of ethanol recovery, and considerable savings on capital and running costs of refrigerated temperature control.

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