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Conversion of Sugarcane Bagasse into Biofuel

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ABSTRACT

There is increasing demand for bioethanol fuel in all over the world now days because they allow the mitigation of green house gases, provides mean of energy independence and may even offer new employment possibilities. The aim of our project is to produce low cost ethanol by using waste like sugarcane bagasse (SB). In this study, ethanol was produced without using expensive commercial enzyme from sugarcane bagasse. Sugar analysis is done by Molisch's & Fehling's test. The percentage yield of synthesized biofuel in our case is 50%.

1. Introduction

Brazil is the biggest producer of sugarcane in the world. The sugarcane is basically consists of stem and straw. The sugarcane stem are milled to obtain cane juice, which is used for sugar or alcohol production. Sugarcane bagasse, the major by- product of the sugarcane industry, is an economically viable and very promising raw bagasse for bioethanol production (Rabelo et al., 2011; Badshah et al., 2012). Bioethanol reduces air pollution and allow mitigation of green house gases provides means of energy independence and may even offer new employment possibilities. Sugarcane bagasse is chemically composed of cellulose, hemicellulose and ligin. Cellulose and hemicellulose fractions are composed of mixture of carbohydrates polymers. Lignocellulosic materials need some pretreatment methods to alter the structure for greater accessibility for conversion of cellulose into glucose units. Main pretreatment methods are ; Milling, pyrolysis, Ammonia Fiber explosion (AFEX), stem explosion, acid or alkaline pretreatment and biological pretreatment. In this study dilute acid hydrolysis has been used for pretreatment of bagasse. The dil. Sulfuric acid pretreatment can achieve high reaction rates and improve Cellulose hydrolysis. The amount of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis. Ethanol can be produced by variety of microorganisms. Cellulose-to-ethanol bioconversion can be conducted by various anaerobic thermophilic bacteria, such as *Clostridium thermocellum* (Ingram et al., 1987), Engineered *Escherichia coli* (Millichip and Doelle, 1989) *Zymomonas* (Matthew et al., 2005), as well as by some fungi including *Monilia sp.* (Saddler and Chan, 1982), *Neurospora crassa* (Gong et al., 1981), *Trichoderma viride* (Ito et al., 1990), *Neurospora sp.* (Yamauchi et al., 1989), *Zygosaccharomyces rouxii* (Pastore et al., 1994), and *Paecilomyces sp.* (Gervais and Sarrette, 1990). In this study ethanol was produced using *Saccharomyces cerevisiae* enzyme. The aim of our project is to produce low cost ethanol by using waste sugarcane bagasse.

2. Materials and Methods

2.1 Lignocellulosic biomass

Sugarcane bagasse was purchased from the local industry. The biomass was taken and dried to remove all the moisture present in it and then milled into the powder form with the help of grinder.

2.2 Microorganism

Saccharomyces Cerevisiae was obtained from microbiology laboratory, DSCW.

2.3 Pretreatment

Pretreatment of sugarcane bagasse was done by acid hydrolysis method. The grind sugarcane bagasse samples were taken nearly 6-7gm in 250ml conical flasks and add 6ml of dil. Sulfuric acid of conc. 0.3M in two of the flasks. 10ml of dil. Sulfuric acid of conc. 0.5M is added to the remaining two flasks. These samples were soaked in dil. Sulfuric acid for 24 hrs. The flasks were then capped with cotton plugs. Before adding any micro-organisms to the above prepared samples, pH of the samples must be adjusted because the micro-organisms will die in hyper acidic or basic medium. The pH of around 5-5.5 is maintained. After pH adjustment the samples were sterilized in Autoclave MODEL No BRVA0050 for 30 min at 15psi, 394K.

2.4 Methodology Opted for Ethanol production

The medium used for ethanol fermentation composed of 8gm sugar (Dextrose), 0.2gm yeast extract, 0.04gm Magnesium sulfate, 0.2gm Ammonium sulfate, 100ml distilled water. In this 100ml media, 0.5gm of *Saccharomyces cerevisiae* is added in a 250ml conical flask. This flask is placed in Incubator for 24hrs. The 10ml of this medium is added in each of the four sterilized samples in Laminar flow MODEL No BR-58-03 under aseptic conditions. These flasks are placed again in Incubator at 308K for 24 hrs. After termination of the fermentation period, ethanol produced was estimated and the ethanol yield was calculated by using following formula as described in Yoswathana and Phuriphipat (2010).

$$\text{Ethanol Yield} = \frac{\text{Measured Ethanol in Sample}}{\text{*Theoretical Ethanol (g)}}$$

*Theoretical Ethanol = amount of initial sugar content (g) in fermentation solution x 0.5

2.5 Ethanol Analysis

The samples from conical flasks undergoing fermentation in Incubator are taken in test tubes in Laminar flow under the aseptic conditions. These samples were filtered to remove the biomass. The mixture of reducing sugars and ethanol was left behind. The ethanol content was measured by double beam UV- VIS spectrophotometer LABTRONICS MODEL- LT-2700 and its absorbance was noted down at 197nm wavelength.

2.6 Sugar Analysis

Reducing sugars were analyzed by the Molisch's & Fehling's test. Molisch's test- In a test tube add 1ml of solution, add 2-3 drops of α-napthol solution. Pour dropwise conc. H_2So_4 using dropper along the sides of the test tube. Fehling's test- In a test tube, add 1ml of solution, add equal amount of Fehling A & Fehling B and placed in a boiling water bath for few minutes. When the contest of the test tube starts boiling, mix them together.

2.7 Fourier Transform Infra red Spectroscopy of substrates

FTIR was used to check the chemical changes in the samples. (Irfan et al., 2011b). Mixture of sample and KBr (5% sample: 95% KBr) were passed into a disk for Fourier Transform Infrared Spectroscopy measurement. The spectrum was recorded with 32 scans in the frequency range of 4000-400 cm^{-l} with a resolution of 4 cm^{-l}.

2.8 Scanning Electron Microscopic (SEM) Study

SEM characterize the morphology of sugarcane bagasse. It produces the images of a sample by scanning the surface with a focuesd beam of electrons.

3. Results and Discussion

3.1 Characterization

In this study sugarcane bagasse was used for ethanol production by Saccharomyces cerevisiae in 250ml flask at 308K for two days of fermentation period. For ethanol production, pretreatment of substrate was first step which increases the accessible surface area and modify the ligin structure. So this substrate was first subjected to physical pretreatment which is size reduction and forming into powder form and then applied to chemical pretreatment with dil. Sulfuric acid followed by pressurized heating at 394K for 30 min. After that the pretreated biomass was analyzed by Fourier Transform infrared spectroscopy to check the structural changes created by this pretreatment technique.

Figure 1 showed the FTIR spectroscopic analysis of sugarcane bagasse. chemical bonds absorb infrared energy at specific frequencies (or wavelength/wave number), therefore the basic structure of compound can be determined by the spectral location of their IR adsorptions. FTIR spectra showed that sugarcane bagasse having functional groups of standard polymer α - cellulose and coirpith - lignin and the IR spectra of the adsorbent was found almost same. The FTIR spectroscopic analysis indicated broad bands observed at 3421cm^{-1} and 3441cm^{-1}, representing bonded –OH groups. The bands observed at about 2926.11 cm^{-1} could be assigned to the aliphatic C-H group. The peaks described in the region of 1600-1590 cm^{-1} represents skeletal vibrations of benzene ring. The peaks observed in the 1420-1300 cm^{-1} are attributed to C=C-H in plane bending indicating several bands in cellulose and xylose. At a wave number 1263.42 cm^{-1} which may be due to $–CH_2$ wagging and twisting indicating several bands in deoxysugars complex bands. The peak observed at 1120 cm^{-1} represents C-O and C-O-C stretching complex bands: appear simpler in polysaccharides.

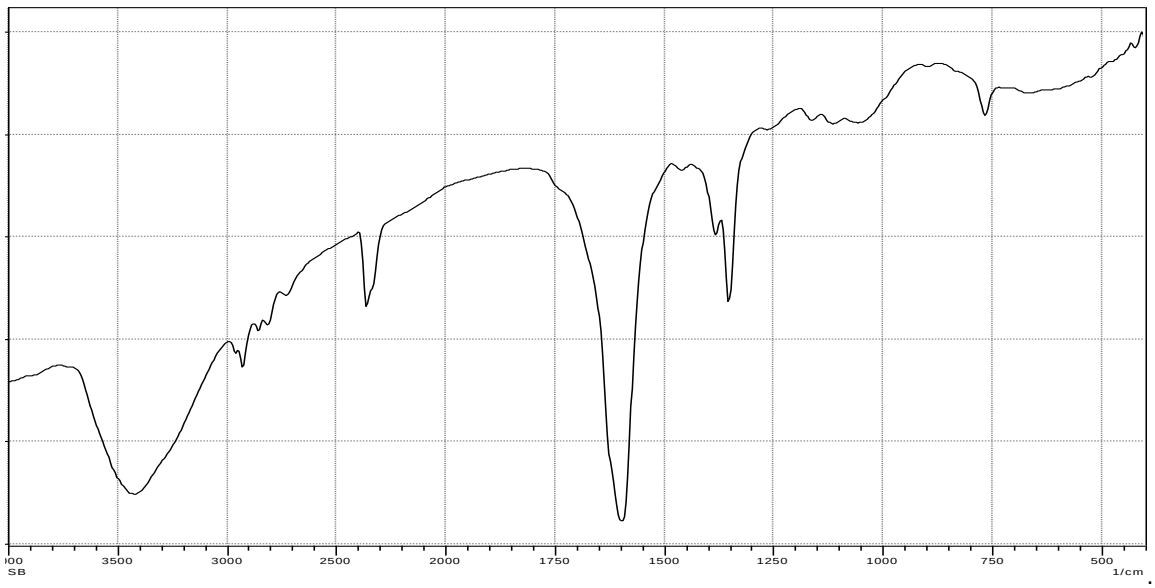
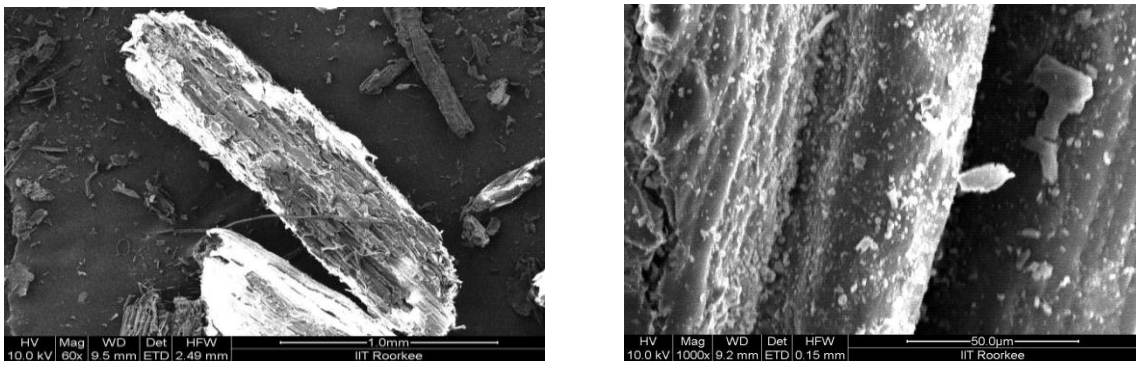


Figure 1. FTIR spectrum of SB.

The SEM pictures of typical SB sample at different magnifications were taken to analyze the morphology of the material more clearly. Micrographs show that the SB particles had fibrous character. These pictures (figure 2.a) reveal that the SB samples have compacted layer character. In which thin layers of cellulosic was material laying one on one. These fibers were of generally ~1.0 mm in length and of ~ 0.35 mm diameter. The presence of small pores on the surface showed rudimentary pore network. The second micrograph (Figure 2.b) depicted that some very small particles of nearly 0.5 - 2.0 µm in size were adhered to the surface to the large size fibers. These small grains may also take part in the adsorption process.



(a) (b)

Figure 2. SEM image of Sugarcane Baggase at different Magnifications

The amount of ethanol production from bagasse by Saccharomyces cerevisiae was 2.5 ml. The absolute ethanol mixed with water and an absorbance of 0.159nm was found in the UV-Vis Spectrophotometer. Results indicates that ethanol production was observed by sugarcane bagasse with ethanol yield of 0.50. Initially 8gm sugar was used. By considering the more amount of sugar, we obtain more amount of ethanol.

Sugar analysis was done by Molisch's & Fehling's test. In Molisch's test violet colour is observed In Fehling's test yellow colour is observed.it indicates the presence of reducing sugar.

4. Conclusion

Results of this study indicates that pretreatment is necessary. The sugars produced by acid hydrolysis was readily converted into ethanol by using Saccharomyces cerevisiae. Sugarcane bagasse was found to be very effective substrate for ethanol production and it is low cost phenomenon.

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