

Analysis of Bioethanol production from Newspaper by *Saccharomyces cerevisiae* and *Zymomonas mobilis*

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Abstract - As the world transportation is totally dependent on the petroleum fuels, the demand of the non-conventional energy is increasing day by day. So it's the time to look for approach other than tradition methods for the production of Bioethanol. Keeping the facts of both the waste management and energy crisis Bioethanol production from waste Lignocellulosic biomasses offers the cheaper and remarkable contribution. Newspaper as a lignocellulosic materials was selected for study of Bioethanol production. The alkaline pretreatment of the substrate was carried out, amount of the reducing sugar was calculated using DNSA method. The hydrolysate was then subjected to fermentation using *Saccharomyces cerevisiae* and *Zymomonas mobilis* (MTCC 2428), and ethanol concentration was estimated by using potassium dichromate method.

Index Terms - Lignocellulosic material, ethanol, *Saccharomyces cerevisiae*, *Zymomonas mobilis* and fermentation.

I. INTRODUCTION

In the last few years due to rapid growth of population and industrialization has resulted in consumption of the fossil fuels at a higher rate all over the world. As the demand of energy is increasing and are being utilized at a rapid rate, thus creating a concern over the depletion of fossil resources in upcoming decades, have directed the interest of the researchers to develop the fuels from biomass resources [1]. Biofuels offers the better replacement of petroleum based fuels and have emerged as an attractive alternative. They also reduce the greenhouse gas emission which will be helpful in providing the clean atmosphere. According to an estimate, in 1998 Brown *et al.* reported that the utilization of Bioethanol as an additive fuel could reduce carbon dioxide emissions by 60-90 % relative to conventional petroleum fuels [2]. Among various Biofuels, most widely used liquid biofuel is Bioethanol. [3-4]. Ethanol has a property to reduce emissions of gases like carbon monoxide, various hydrocarbon and nitrous oxide which are the main causes of environmental pollution. It has a high octane number which enables the engine of motor vehicles to run at faster rate and thus provides superior performance to the vehicles[5].

In the production of Bioethanol different kind of raw materials are conveniently used and they are generally divided into three main groups: sugars, starches, and cellulose materials. Sugars substrates (like cane or sweet sorghum juice, molasses), starch substrate (like corn, cassava, potatoes, and root crops), lignocellulosic materials like straw agricultural residues, waste paper sludge, paper mills and waste sulfite liquor, wood [6].

Lignocellulose is the most abundant polysaccharide present on the earth and has greater benefits because of its wide availability, low cost and no competence with foods. It is made up of mainly of cellulose and hemicelluloses. Cellulose is a linear and crystalline organic polymer consisting of repeating units of glucose bonded by β -1,4 glycosidic bonds [7]. Hemicellulose is a heteropolymer consist of pentose and hexose sugar, it also contains xylose, galactose and glucose, and mannose[8]. While Lignin is a complex of chemical compound. Lignin is an aromatic chemical compound are bonded together by a set of linkages to form a very complex matrix[9].

Among various lignocellulosics substance, the recycled books, magazines and newspaper, office paper, tissues paper etc. have contributed to the production of Bioethanol by the fermentation process[10].

The frequent recycling of newspaper has resulted in low grade quality of the paper and recycling of paper needs the high processing cost. In the present time, the majority of waste paper is either incinerated or land filled, thus rising several problems to the environment. Waste paper contains hemicelluloses cellulose and lignin and several studies reported that the fermentation of the newspaper can be utilized in ethanol production [11] and thus it is a better approach to minimize the waste and to obtain the fuels.

The significant advantage of using the lignocellulosic biomass is that they are not directly linked with the food crops and available at a cheaper cost hence there is no concern over the global security of food[12]. Ethanol production involves the pretreatment of the raw material, hydrolysis of cellulose and hemicelluloses to obtain simple sugar and then the conversion of the sugar into ethanol by microorganisms. The product is recovered by distillation.

The purpose of this study to utilize the waste material for the ethanol production with the help of micro-organism at low cost.

II. MATERIAL AND METHODS

Collection of raw material

Newspapers were collected in polythene bags locally in Gwalior, Madhya Pradesh. Newspapers were then cut into small pieces using scissors and were soaked in water for 24 hours (1 Kg newspaper: 20 Liter water). They were then air dried and were grounded using a grinder and were kept at room temperature for further analysis.

Microorganisms

Zymomonas mobilis (MTCC 2428) culture was procured from NCIM, Pune, Maharashtra and was maintained on RM Agar Medium containing Glucose 20g/l, Yeast extract 10.0g/l, KH_2PO_4 2.0g/l, Agar 15.0g/l and pH was Adjusted to 6.0. It was sub-cultured and stored at 4°C. *Saccharomyces cerevisiae* was obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, Haryana. The culture was routinely grown on Yeast Extract Peptone Dextrose (YPD) agar plates (pH 4.5) at 30°C for 48 h. The YPD medium contained Yeast extract 3.0 g/l, Peptone 5.0 g/l, Glucose 10.0 g/l and Agar 20 g/l.

III. PRETREATMENT OF THE RAW MATERIAL

The newspapers were pretreated with 0.1 M NaOH at 121°C and 15 psi pressure for 1 hr in the ratio of 1:10 to substrate and NaOH solution. The pretreated waste paper was washed with tap water and excess water is removed by squeezing. Again the substrate dried in oven at 65°C for 24 hours, stored at room temperature for further use. Amount of reducing sugars were measured after the pretreatment.

IV. DETERMINATION OF GLUCOSE CONCENTRATION BY DINITRO SALYSILIC ACID (DNS) METHOD

The estimation of sugar quantity in the pretreated substrate was determined by Dinitro salysilic acid (DNS) method using Glucose Standard Curve[13]. 1 ml of the sample was mixed with 3 ml of DNS reagent in a test tube and the reaction mixture was heated at 90°C for 5-15 minutes till red-brown color was developed. 1 ml of 40% Na-K Tartarate solution was then added. Test tubes were cooled at room temperature and optical density of the reaction mixture was measured at 540 nm by UV spectrophotometer.

V. FERMENTATION

The fermentation was done in 250 ml flask containing fermentation medium and newspaper hydrolysate. The 200 ml of the medium was prepared and 2 g of the hydrolysate was added and the flask was autoclaved at 121°C for 15 min at 15psi pressure. Culture was introduced in the flask and the static culture was maintained in incubator for 48 h. The content of the ethanol was assayed by standard dichromate test.

VI. ESTIMATION OF ETHANOL

The ethanol content in the fermented broth was determined with the help of dichromate method, where ethanol produced was converted to acid by reaction with dichromate. The fermentation broth was taken and then centrifuged to obtain the supernatant (cell free broth). It was diluted to a reaction mixture of 10 ml. 2mL of Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) (3.37 g/100 mL) was added to the mixture and absorbance was measured on a spectrophotometer at 600 nm[14].

VII. RESULTS AND DISCUSSION

The pretreated newspaper was analyzed to obtain the sugar concentration. The amount of the sugar present in the sample was calculated by using Glucose as standard. The results were obtained by measuring the absorbance in UV spectrophotometer, amount of the Reducing Sugar was found is 256.56 mg/l. After 2 days of fermentation, ethanol estimation was done, it was observed that *Saccharomyces cerevisiae* and *Zymomonas mobilis* produced 22% and 18% of ethanol respectively when compared with ethanol standard graph. From the results, it was concluded the yeast *Saccharomyces cerevisiae*. has more potential to ferment the newspaper than *Zymomonas mobilis* and gives higher ethanol concentration. This study has shown that newspaper can be converted to Bioethanol.

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