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Production of Biofuel From Paper Sludge By Simultaneous Saccharification And Fermentation.

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ABSTRACT

Paper is commonly used in our daily life. It is used to store information and to maintain documents. There are several paper mill industries all over India which produce large amount of paper and hence, huge amount of waste is being generated. A problem to be solved by the present study is, how to utilize the waste paper sludge for ethanol production process, where ethanol yield or productivity would also be maintained, thus improving the cost-efficiency of the process. The study relates to a process of producing ethanol by fermentation, said process comprising a simultaneous saccharification and fermentation (SSF) step conducted at a temperature of above 34° C in the presence of a glucoamylase and thermo-tolerant yeast. The elevated fermentation or saccharification temperature means that less cooling is required after the initial liquefaction steps which are normally carried out at much higher temperatures. The process of the study comprised of recovery step of the produced ethanol confirmed by Gas Chromatography/Mass Spectroscopy (GC/MS). The percentage of alcohol obtained was found to be 2.28 %.

Keywords: waste paper, total reducing sugars, glucoamylase, simultaneous saccharification and fermentation, ethanol

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INTRODUCTION

Energy demand worldwide has resulted in the need for development of sustainable energy [1].Currently, the production of biofuel for automobiles used for personal and public transport is in focus. Investigations on producing biofuel has been increased as it can be an alternative for the conventional gasoline blends, bioethanol is an oxygenated fuel that contains 35% oxygen, reduces particulate and NOx emissions from combustion [2]. Domestic production and use of ethanol for fuel can decrease dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air pollution, and reduce global climate change and carbon dioxide build-up.

It is estimated that global production of ethanol has been increased from 13.12 billion of gallons in 2007 to 25.68 billion of gallons in 2015 [3]. The first-generation biofuels utilize agricultural sources like sugarcane and corn for ethanol production, which may result in the immoderate pressure on the global food supply. Hence, ethanol production from second generation biofuels such as fuels produced from mixed paper waste, paper sludge, cotton etc. can be utilized.

In pulp and paper industry, different types of solid waste and sludge are produced at different stages of the production processes [4]. As, reported by Balwaik and Raut (2011) [5], around 300 Kg of sludge is generated from every one ton of recycled paper. Recycled paper sludge is a cellulosic waste, where the pre-treatment process for the cellulose bioavailability is not required and has an added advantage of low cost collection and storage system of the raw materials compared to the first-generation biofuel [3].Also, recycled paper is composing approximately 50% of cellulose and has no commercial value hence, are deposited in the landfills, thus it becomes an attractive source of raw material for ethanol production [6]. In recent years saccharification along with simultaneous fermentation (SSF) process has said to improve hydrolysis rates and ethanol yields.

In the present study, we have focused on ethanol production by simultaneous saccharification and fermentation from the paper sludge using *Saccharomyces cerevisiae,* where paper sludge can be used as an effective alternative substrate for bioethanol production.

MATERIALS AND METHODS

Raw material

Waste paper sludge from the last sage of paper making was obtained from west coast paper mill Dandeli, Karnataka. The sludge was grey in colour and was solid with 30 - 40% of water content. The sample was collected aseptically into clean polythene bag and was mixed with anti- bacterial and anti-fungal enzyme to protect against bacterial and fungal contaminations. The anti- bacterial and anti-fungal enzyme was obtained from Alfanzyme Lifescience, Belgaum.

Chemicals used:

The main enzymes alpha-amylase, acidic cellulase and glucoamylase were obtained from Alfanzyme Lifescience Belgaum, and was stored in dark coloured chemical bottles.

Saccharomyces cerevisiae was obtained from Alfanzyme Lifescience, Belgaum and was stored in cool and dark place.

Pre-treatment of the substrate:

Paper sludge (500g) was diluted with distilled water in 1:6 dilutions. This mixture was mixed properly and was heated up to 85-90 ℃ in water bath and 5-6 mL of acidic cellulose was added and continuously agitated using magnetic bed homogenizer for 3-4 h until fine slurry was obtained. Later, the temperature was reduced to 50-60 ℃ and alpha-amylase was added and the temperature was maintained for 2 h with agitation. Then the temperature was reduced to 30-40 ℃ with continuous agitation upto 3-4 h.

Chemical analysis of the substrate:

Starch content of the substrate was estimated by Iodine [7]before the addition of the enzyme glucoamylase. Total reducing sugar (TRS) of the substrate was analysed by titrimetric method. The substrate was filtered using filter paper and to 50 mL of filtrate, 5ml of HCl was added and kept on hot plate at 68˚C for 4-5 min, and then was cooled to room temperature, 2-3 drops of phenolphthalein indicator was added, followed by the addition of 6N NaOH until pink colour appeared. The resulting solution was made upto 100 mL by adding distilled water and shaken well. Then, to 5 mL of each Fehling solution A and Fehling solution B, 20 mL of distilled water was added and the flask was kept on hot plate until boil and the resulting solutions were titrated using methylene blue indicator with brick red colour as end point.

The total reducing sugars were estimated using the formula,

$$TRS = \frac{5.128}{0.005 * FF * BR}$$

Where, FF (Fehling factor) = 0.96, BR (burette reading) = 51.2

Wash analysis:

To 500 mL of the substrate solution 4-5 g of dry yeast (*S. cerevisiae*) was added (with molasses) and was kept for fermentation at 37℃ for 24 h. Specific gravity, and temperature was checked before and after fermentation. After 24 h, the residual sugar was analysed by titrimetric method as mentioned above.

The residual sugar was analysed using the formula;

$$RS\ \% = \frac{5.18}{BR * FF * DF}$$

Where, FF (Fehling factor) = 0.9679
BR (burette reading) = 26.3
DF (dilution factor) = 0.25

GAS CHROMATOGRAPHY/MASS SPECTROSCOPY (GC/MS) ANALYSIS

Ethanol production was confirmed by GC/MS analysis. Using 80/120 carbopach B AW/6.6% PEG 20M column with nitrogen as the carrier gas.

ALCOHOL PERCENTAGE

The ash solution was diluted in 1:1 ratio, the foam developed was reduced by adding 2-3 drops of anti-foaming agent and the resulting solution was kept for distillation. Solution obtained by distillation was measured for % alcohol using hydrometer, simultaneously temperature was noted.

The percentage of alcohol obtained was calculated using the formula;

Alcohol % = 100 – syke's reading*0.57142

Where, Syke's reading = 96.0

RESULT AND DISCUSSION

Chemical analysis of the substrate:

A colour change was observed from grey to blue black upon addition of gram's iodine to the substrate indicating the presence of starch. The total reducing sugars estimated by titrimetric method was found to be 20.776mg.

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Wash analysis:

Specific gravity of the fermentation medium was found to be 0.4, whereas the sp. gravity of the fermentation medium before fermentation was found to be 1.10, and the pH of the medium was found to be 4.0. The Sp. Gravity of the resulting solution value is near to the standard values as per ASTM 4806 for the produced ethanol to be used as biofuel [8].

Residual sugar:

The amount of residual sugar present in the resulting wash solution was found to be 0.805 %.

Gas Chromatography/Mass Spectroscopy (GC/MS) analysis:

The retention time of ethanol on GC chromatogram was found to be 2.32 (Fig 1), further the presence of ethanol was confirmed by MS Spectra, where a peak was observed at45 (m/z) (Fig 2).

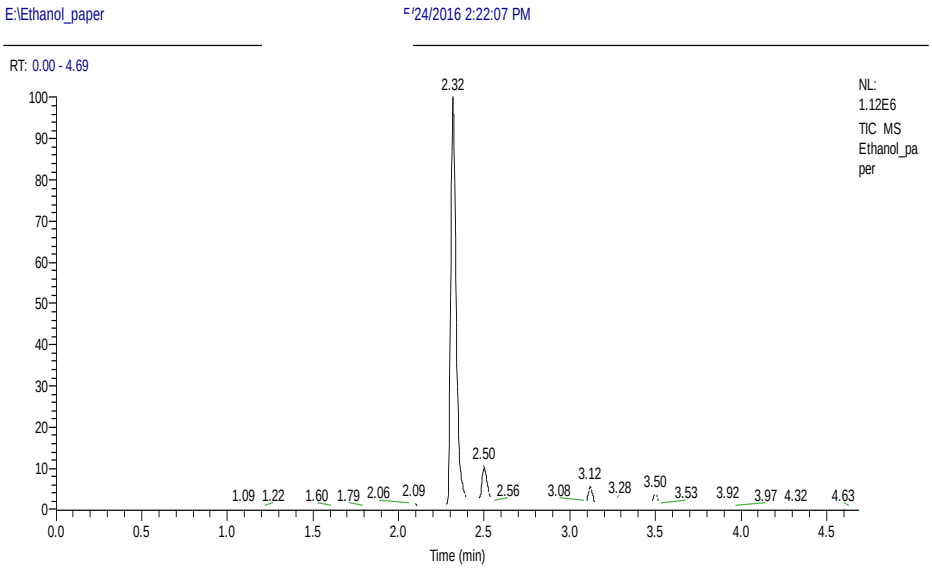

Fig 1: GC chromatogram of ethanol.

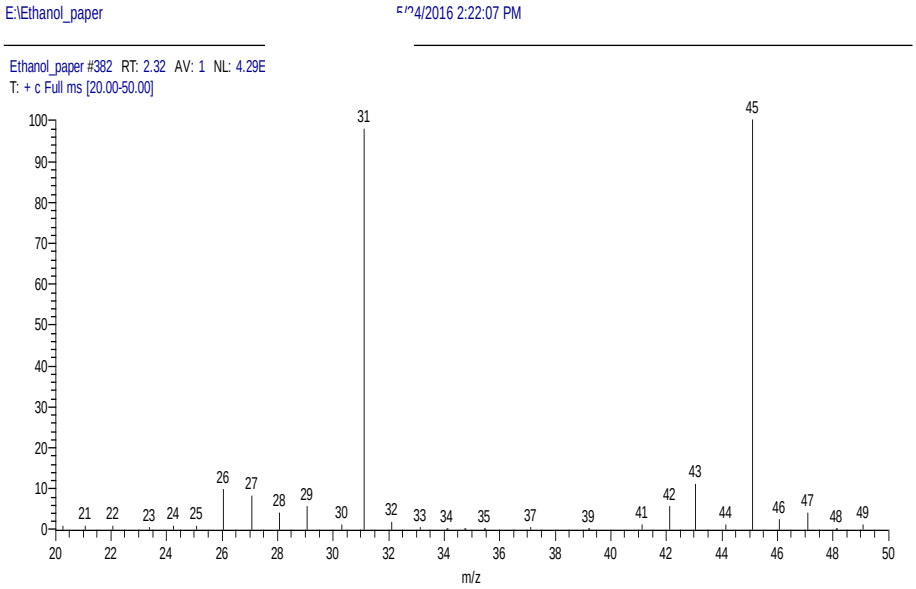

Fig 2: Mass spectra of ethanol.

Alcohol percentage:

The percent of alcohol obtained was found to be 2.28 %. Similar work performed by Alfonsín et al have showed the percent of alcohol obtained was 9.67 using 10 % inoculum. Thus, increasing the inoculum size or standardizing the fermentation conditions would help in further increase in alcohol production.

CONCLUSION

Paper sludge obtained from industry can be used as an alternative source of substrate, where simultaneous saccharification and fermentation process can be performed to produce ethanol. This method thus avoids the need of physical and chemical pre-treatment of the substrate, however, the amount the ethanol produced was less, butoptimizing the fermentation parameters may help in increased ethanol production.

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