PRODUCTION OF BIOETHANOL FROM WASTE PAPER

[1]RK Singh, [2]Suresh Chaluvadi and [3]BN Naik

[1,2,3]National Institute of Technology Rourkela, Rourkela-769008, Odisha, India

Email: [1]rksingh@nitrkl.ac.in, [2]sureshbiotechnology@gmail.com, [3]yathin.dthunder@gmail.com

Abstract

The generation of biofuels from wastes forms an attractive solution towards both waste management and energy generation via bio-fuels. Newspaper, which is a cellulosic feed stock, is emerging as an attractive option for the production of bio-ethanol because of lower feedstock costs, higher potential for fossil fuel displacement and also there will be a reduction in greenhouse gas emission as compared to production of ethanol from food grains. Experimental studies have been carried out to optimize the pre-treatment process for increasing the efficiency of enzymatic hydrolysis, the efficient conversion of cellulose to sugars from cellulose-degrading microorganisms and to convert the sugars released to ethanol by using fermentation process. Pre-treatment, hydrolysis, and fermentation are the steps involved in the production of bioethanol. The optimum glucose produced was 89.59 % yield at 80°C, 23 h, 1.76 % v/v with H_2SO_4pre-treatment and 58.02% yield at 23.6 h, 53°C, 0.5% w/v with NaOHpre-treatment. The enzyme cellulase was used for hydrolysis process, which helped in converting the cellulose to sugars and was analysed using Dinitrosalicilic acid. Fermentation method is used for the production of ethanol by using waste paper in the presence of microorganism *Pichiastipitis* NCIM 3498. The yield was estimated by using HPLC and it is found to be 6.61 g/L for H_2SO_4pre-treated paper, 4.37 g/L for NaOHpre-treated paper and 3.42 g/L for untreated paper. Structural changes of waste paper before and after pre-treatment with H_2SO_4 and NaOH were further investigated through Fourier transformed infrared spectroscopy (FTIR) and X-ray diffraction (XRD).

Keywords: Biomass, pretreatment, saccharification, paper, ethanol.

1. INTRODUCTION

Bioethanol produced from fermentation of carbohydrates present in sugar or starch crops such as corn, sugarcane, etc. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being utilized as a feed stock for ethanol production[1]. Ethanol is an eco-friendly alternative to petroleum-based fuel as it has lessergreenhouse gases emissions

Increase in the population over the last century lead to the increase in energy consumption worldwide. The global oil production would decline to 5 billion barrels from 25 billion barrels approximately. Due to this unavoidable depletion of the world petroleum resources in the coming years, the worldwide interested aroused in seeking an alternative non-petroleum based energy source. One of the best alternative fuels, in order to beat severely the energy crises, is from biofuel. From biologically carbon fixation the energy is derived from Biomass. The various factors like the need for increasing energy security, hikes and gaining the scientific and public attention the biomass are driven.

The bioethanol is one of the environment-friendly fuels because the ethanol contains more oxygenin contrast to hydrocarbon fuel [2].Also in comparison with the conventional gasoline, the blends of E10 resulted in 12-25% less emission of carbon monoxide. The sugarcane and corn are the

first generation bio-fuels. Due to a vast increase in the ethanol production using these crops they cause immoderate pressure on the global food supply. The second generation biofuels can be produced by means of wide range of sources like waste chicken feathers, cellulosic biomass food, and organic waste. The cellulosic biomass, such as agricultural residues and industrial wastes are the most abundant and cheap source of renewable energy in the world. The second generation biofuels may also include the fuels produced from mixed paper waste which is separated from the municipal solid waste, cash crops Jatropha, Honge, Cotton, Maize etc. can be utilized to produce bioethanol. The third generation biofuels can be produced from micro-organisms mainly Algae [3].

In developed and developing countries municipal wastes have become a severe problem during the last century, the shrinking of landfill capacity resulted in rising of landfill costs which are mainly due to the waste paper from the municipal waste. Because of the above concern the waste paper is used as a cheap source for the production of bioethanol. Due to the shrinking landfill capacity, the tighter environmental control exists on their siting operation, construction, and of the unwillingness of communities to have new landfill sites nearby. The tighter environmental regulations are responsible for the premature closure of existing landfills and higher costs for constructing new ones, Among the various components the municipal solid waste consists of food waste, wood, leaf, garden or yard trimmings, rubber, textile, leather, metals (ferrous metals or Non-ferrous metals), glass and major of paper and paper boards. About 35% to 40% by weight of the municipal solid waste is made of the paper [4].

Ethanol is a chemical compound that is present in a lot thing we use on a daily basis. Ethanol is used extensively as a solvent in the manufacture of varnishes and perfumes, as a preservative for biological specimens, in the preparation of essences and flavouring, in many medicines and drugs, as a disinfectant and as a fuel and gasoline additive. The first step is pretreatment of raw or shredded biomass. The goal of the pretreatment is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic biomass [5]. Various pretreatments like thermal, chemical, biological have been adapted, of which thermochemical was found to be most effective [5, 6]. Fermentation of sugars obtained from saccharification of carbohydrate polymers produce ethanol or biofuels[7, 8]. HPLC technique is used to measure the concentration of ethanol [9]. In this work, we performed the pretreatment of newspaper, saccharification of pretreated biomass, and fermentation.

2. MATERIALS AND METHODS

2.1. Raw material, Chemicals, and microorganisms

Waste Paper was collected from waste office paper, NIT Rourkela. Waste paper was cut into samples weighing a total of 50 grams. Grinding of waste paper was done for reducing its size up to 3-5mm, so that, mass transfer becomes effective and enzymes can easily bind the cellulose. The moisture content of the waste paper was measured and it was found to be 7.8 percent. Cellulase was gifted by Brenntag, India and used for enzymatic hydrolysis. The microorganism *Pichia stipites* NCIM 3498from NCIM, Pune was used in the fermentation process. The weighed sample was stored in air tight plastic bags and stored in a desiccator at room temperature until further analysis. This

microorganism *Pichia stipitis* strain was cultured in a YPD broth (yeast extract 10 g/L, bacteriological peptone 20 g/L, and glucose 20 g/L) with pH 6 and incubated at 30 °C and 130rpm for 24 h. Then agar slants were made by supplementing 2% agar in YPD media and maintained at 4 °C until use. H_2SO_4 and NaOH were used for the pretreatment of the paper and citric acid monohydrate and sodium azide were the chemicals procured from Merck.

2.2. Compositional analysis and pretreatment conditions

The paper composition was determined by National renewable energy laboratory (NREL) procedure [10].For pretreatment of paper with H_2SO_4, three parameters were selected temperature (35 - 80 °C), time (6 - 24 h) and acid concentration (1 - 4 %v/v). For pretreatment ofwaste paper with NaOH, three parameters were selected in the base concentration (1 – 2 %w/v), temperature (35 - 80 °C) and time (6 – 24 h).

3.4. Enzymatic Saccharification

Saccharification was done utilizing 10% biomass loading with 50ml citrate buffer in 150mL Erlenmeyer flasks kept at 45 °C and 120rpm for 72 h in an orbital shaker (Reico, Kolkata, India). A saccharification blend of 50mL constitutes 1g pretreated biomass, 120mg of cellulase catalyst, 0.05 mol l^{-1} citrate buffer of pH 4.8 and 11mg of sodium azide (antimicrobial substance). After completion of hydrolysis, the fluid was separated through cheesecloth. The hydrolysate tests were filtered utilizing syringe filters and analyzed for glucose utilizing HPLC [11].

3.5. Fermentation

The inoculum for fermentation was set up from agar slants of *Pichia stipitis* by taking a loopful of yeast in 30 ml YPD (yeast extract 1%, peptone 2% and dextrose 5%) of pH 6 and incubated at 30°C, 130rpm for 18 h under aerobic conditions. The inoculum volume is taken in such a way that the fermentation broth attains a OD of 0.5 at 600 nm. The calculated inoculum volume was centrifuged at 4500 rpm for 5 minutes and 0.5ml of sterile water was added to the pellet. Anaerobic fermentations were performed with and without supplementation of cysteine hydrochloride (0.5g/L) by taking 9.5ml hydrolyzate each in a glass tube for pretreated (NaOH and H_2SO_4) and untreated paper samples. All hydrolysates acquired after saccharification were used for this test. Moreover, these were supplemented with 1% yeast extract, 2% peptone and acclimated to pH 6. These tubes were kept for sterilization in an autoclave at 121°C and 15lbs for 15 min and inoculated with 0.5ml of inoculum after sterilization. The glass tubes were incubated at 30°C, 130rpm for 40 h under anaerobic conditions in an orbital shaker. The samples were gathered and sent for measurement of glucose and ethanol using HPLC after 40 h[12].

3.6. DNS Assay

The DNS method for estimating the concentration of reducing sugars in a sample Reducing sugars contain a free carbonyl group have the property to reduce many of the reagents. DNS reagent was prepared by dissolving 10.6 g of 3,5 dinitrosallicylic acid and adding 19.8 g of NaOH. Then adding slowly 306 g of sodium potassium tartrate, 7.6 ml of phenol and 8.3 g of sodium metabisulfite and dilute to a final volume of 1500 ml using distilled water. DNS reagent is stored in dark place as it

is light sensitive.After enzymatic hydrolysis, samples were centrifuged in Eppendorf tubes at 5000 rpm for 10 min to determine sugar concentration

3.7. Estimation of sugars and ethanol by HPLC

The biomass hydrolysates and fermentation samples were filtered through 0.20µm syringe filters with nylon membrane and all the samples containing acid were filtered through PTFE membrane before analysis with HPLC. A 20 µl sample volume was injected through the manual injection port of HPLC equipped with Refractive index (RI) detector(Shimadzu LC20-A, Japan), degassing unit and Hi-plex H column (Agilent, India). Sulphuric acid (5m mol l^{-1}) was used as mobile phase with a flow rate of 0.7ml/min. The column temperatures were maintained at 60 °C. The assay of various compounds was performed using standard of glucose, xylose, arabinose, mannose, galactose, acetic acid, and ethanol. HPLC separates the various components in the sample based on the retention time since each component has different retention times.

3.8. Physical Analysis (FTIR, XRD)

The crystallinity index (CrI) of raw materials and pretreated materials were determined by X-ray diffractometer (Panalytical3040/00, Netherlands) using Cu K-alpha radiation (k = 0.15418 nm); The operating current and operating voltage were maintained at 20mA and 30kV, respectively. The range of 2θ is maintained from 5° to 30°at a scan speed of 10° per minute. The extent of crystallinity in the biomass was defined as biomass crystallinity index and was calculated by using the following equation.

$$\mathrm{CrI}\ (\%) = \left[\left(I_{002} - I_{am} \right) / I_{002} \right] 100 \qquad (1)$$

Where CrI is the crystallinity index, I002 is the maximum intensity of the 002 peak at 2θ = 20.43°, and Iam is the intensity at 2θ =18.48°.

Also, the chemical nature of untreated and pretreated biomass samples was examined using FTIR spectrometer (Spectrum Two, PerkinElmer) in the wave number range of 400-4000 cm^{-1}. The KBr method was used by mixing 1.5mg of biomass thoroughly with 200mg KBr powder and pressed into 13mm discs using a hydraulic press[13].

4. RESULTS AND DISCUSSIONS

The composition of waste paper was obtained as moisture content 7.8%, ash content 13.2%, cellulose 92%, hemicelluloses 4%, and lignin 8% respectively. The experimental designs for $H_2SO_4$and NaOH pretreatment with glucose yield after saccharificationare shown in Table 1. A maximum yield of 87.28% glucose obtained after saccharificationfrom biomass pretreated at 80°C, 24 h, and 2.5% of sulfuric acid. For base pretreatment, the maximum yield of glucose was 58.14% obtained after saccharification from biomass pretreated at 57.5°C, 24 h, and 0.5 %w/v of NaOH.

4.1. Fermentation of saccharified waste paper

After 40 h of incubation in an orbital shaker, the optimum ethanol produced for the sample pretreated with H_2SO_4 is 6.61 g/L, pretreated with NaOH is 4.37 g/L and that of untreated paper is 3.42

g/L.Theethanol yields were enhanced up to 73 % and 22 % with H_2SO_4 and NaOH pretreatments when compared with untreated paper. The corresponding area under the curves at retention of 22 min are 470836, 311185, 271307 for H_2SO_4 pretreated sample, NaOH pretreated sample and untreated sample respectively.

4.2. XRD study of waste paper pretreated with H_2SO_4 and NaOH

The crystallinity of cellulose is one of the main factors influencing enzymatic hydrolysis. The texture of untreated and pretreated samples of waste paper was investigated by XRD and has been shown in Fig.7. The cellulose crystallinity value of an untreated sample of waste paper was 11.50 % while that of NaOHpretreated sample was 19.79 % and the H_2SO_4pretreated sample was 26.97 %. For lignocellulosic biomass, crystallinity measures the relative amount of crystalline cellulose in the total solid. The crystallinity of the pretreated sample was increased due to the removal of lignin and hemicellulose (both of which are amorphous).

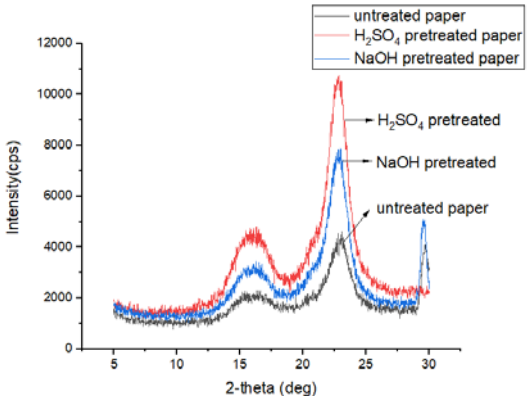

Fig.7.X-ray diffraction diagram of untreated and H_2SO_4 and NaOH Pretreated water paper

4.3. FTIR study of waste paper pretreated with H_2SO_4 and NaOH

FTIR spectral profile of untreated and pretreated samples of waste paperhas been shown in Fig.8. It can be observed that bands at 3394 cm^{-1} (O–H stretching in hydroxyl group), 2924 cm^{-1} (C–H stretching), 1648 cm^{-1} (conjugated C=O stretch), 1257 cm^{-1} (C-O stretching or OH deformation) and 1025 cm^{-1} (structural and nonstructural carbohydrate band) were decreasedafter pretreatment compared to untreated sample of waste paper. Cellulose content increaseddue to a decrease in amorphous components (lignin and hemicelluloses).

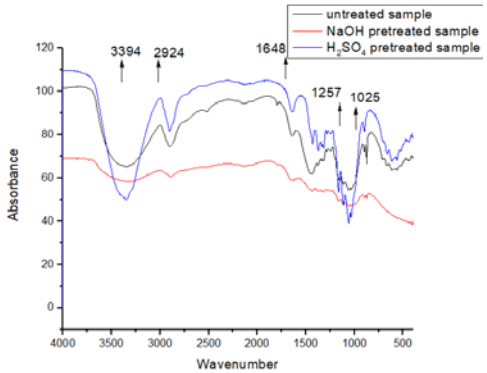

Fig.8. Fourier transform infrared spectra of untreated and H_2SO_4 and NaOH pretreated paper

Table 1: Experimental design for pretreatment of paper with H_2SO_4 in terms of affecting factors and glucose response obtained

Run No.	Temperature, A (°C)	Residence Time, B (h)	Concentration, C (%v/v)	Glucose yield %
1	57.5	6	1	16.12
2	57.5	15	2.5	58.67
3	80	6	2.5	85.14
4	80	15	4	81.25
5	57.5	15	2.5	60.35
6	35	15	1	50.14
7	57.5	24	4	53.27
8	57.5	6	4	87.18
9	57.5	15	2.5	63.16
10	80	24	2.5	87.28
11	57.5	15	2.5	61.41
12	57.5	15	2.5	54.13
13	35	6	2.5	72.52
14	35	24	2.5	86.16
15	80	15	1	73.11
16	57.5	24	1	59.21
17	35	15	4	83.34

Table 2 Experimental design for pretreatment of paper with NaOH in terms of affecting factors with glucose response

Run No.	Temperature, A (°C)	Residence Time, B (h)	Concentration, C (%w/v)	Response: Glucose yield %
1	35	15	2	30.53
2	35	15	0.5	40.14
3	57.5	24	2	52.31
4	57.5	15	1.25	34.54
5	57.5	6	2	46.52
6	80	24	1.25	23.18
7	57.5	15	1.25	34.57
8	57.5	15	1.25	35.42
9	80	15	2	42.31
10	80	6	1.25	26.57
11	57.5	15	1.25	35.23
12	57.5	24	0.5	58.14
13	80	15	0.5	23.56
14	35	24	1.25	51.52
15	35	6	1.25	18.47
16	57.5	6	0.5	31.69
17	57.5	15	1.25	34.99

5. CONCLUSION

Waste paper is found to be favorable source to produce ethanol since it containshigh fraction of cellulose. Waste paper was pretreated with NaOH and H_2SO_4 at different conditions of time and temperature. The cellulases were used to hydrolyze pretreated paper and glucose was produced. These values were statistically analyzed and polynomial equations consisting significant factors were derived. Fermentation process was done using *Pichia stipitis*. The ethanol yields were enhanced up to 73% and 24% with H_2SO_4pretreatment and NaOH pretreatment on comparison with untreated paper. The pretreated and untreated paper were also characterized using Fourier transformed infrared spectroscopy (FTIR) and X-ray diffraction (XRD).

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