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Ultrasound Assisted Saccharification of Saw Dust for the Production of Bioethanol

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Abstract: Lignocellulosic wastes are used as feed stock since are abundantly available. The basic problems involved with bioethanol production from lignocellulosic wastes are high consumption of chemicals used for saccharification, delignification and glucose availability. These problems are considered as important themes of the present study. Ultrasound assisted saccharification was carried out using saw dust as a raw material. The sample was collected, washed, dried, ground, screened, segregated and delignified by acid hydrolysis. Further structural modifications were done using a sonochemical reactor. Saccharification of lignocellulosic wastes was carried out using acid hydrolysis with sulfuric acid. The saccharification experiments were carried out with and without sonication. The results showed that sonication improves the saccharification of cellulosic wastes. Higher yield of glucose was obtained from the medium saccharified using 5% sulfuric acid and sonication.
Keywords: Bioethanol, lignocellulosic wastes, saccharification, ultrasound treatment.

Introduction

Production of biofuels from biomass is receiving increased interest due to reduction in consumption of petroleum fuels; improving environmental conservation and recycling of wastes into useful products[3].

Ethanol produced from renewable biomass materials is known as bioethanol. The feed stock for bioethanol production[1] can be broadly classified into three main groups: (i) sucrose containing materials (e.g. sugar cane, sweet sorghum etc.,) (ii) starch containing feed stocks (e.g. corn, potatoes, rice, wheat, etc.,) (iii) lignocellulosic materials (e.g. rice straw, wheat straw, saw dust, bagasse, etc.,). Ethanol produced from sugar and starch containing materials are known as first generation bioethanol. The use of agricultural land and severe food shortages are the major drawbacks of first generation biofuels[6]. The second generation biofuels uses the lignocellulosic biomass and has the advantage that they do not compete with food production

The various resources for lignocellulosic biomass materials are: (i) industrial wastes like saw dust (ii) forest residues such as leaves, grasses (ii) agricultural wastes such as rice straw, wheat straw (iii) municipal solid wastes. Bioethanol produced from lignocellulosic biomass is of now increased interest due to their abundant availability and cheap.

Processing of lignocellulosics to bioethanol consists of four major operations: (1) Pretreatment (2) Hydrolysis (3) Fermentation and (4) Product separation/distillation[2]. Bioethanol production from lignoce-

llulosic materials is relatively a high cost process and produces low yield. The aim of the present study is to investigate the possibilities of improving glucose yield and ethanol productivity by applying an ultrasound pretreatment.

Materials and methods

Preparation of raw materials

Saw dust was collected from the nearby saw mill. The sample washed thoroughly with water; excess water drained and the sample dried in hot air oven, at 80^0C for overnight. The sample was ground well and screened. The fine particles having average size of 0.105 mm were collected.

Preprocessing

Preprocessing of the lignocellulosic biomass was carried out by treating with ultrasound. Sonochemical reactor of 24 kHz frequency waves input with a capacity of 1*l* was used. About 20 g of lignocellulosic sample was mixed well with 1*l* of distilled water. The mixture was sonicated for 1h. Then the mixture filtered using filter paper. The treated biomass sample dried in a hot air oven at 80^0C for 8h.

Delignification

Sample of preprocessed lignocellulosic biomass was sequentially delignified in acetic acid-water-HCl media of composition in the ratio 1:0.05:0.002 at a temperature of 100^0C; liquid-solid ratio of 100g/10g for the duration of 1h. The treated sample was kept for 5 days for good settling and then the sample was heated for 4 times and correspondingly washed well. This process was done for the removal of lignin and ash.

Estimation of cellulose

The estimation of cellulose content was done by anthrone method. The Acetic/HCl reagent was prepared by mixing 150 ml of 80% acetic acid and 15 ml of concentrated HCl acid.

Procedure

10 ml acetic/HCl reagent was added to a 0.5g of the sample in a test tube and mixed well. The tube was placed in a water-bath at 100°C for 30 min. The contents were cooled and centrifuged for 15–20 min. The supernatant was discarded and residue was washed with distilled water.10 ml of 67% sulphuric acid was added and allowed to stand for 1 h. 1 ml of the above solution was diluted to 100 ml. 1 ml of this diluted solution was added with 10 ml of anthrone reagent and the contents were mixed well. Then the test tubes were placed in a boiling water bath for 10min. They were cooled and the color was measured at 630 nm using UV-Vis spectrophotometer.

Estimation of Cellulose in CMC

0.25g of carboxymethylcellulose was taken and 10ml of 67% sulphuric acid was added and allowed to stand for 1h. The diluted solution was taken in various test tubes as 0.4ml, 0.8ml, 1.2ml up to 2.4ml. 100ml of anthrone reagent was added to each test tube. These test tubes were heated in water bath for 10mins and then cooled. Samples were analyzed in UV-visible spectrophotometer at 630 nm.

Estimation of glucose

0.5 to 3 ml of the extract was pipette out in test tubes and the volume is equalized to 3 ml with water in all the tubes. 3 ml of dinitrosalicylicacid *(*DNS) reagent was added and the mixture was heated in a boiling water bath for 5 min. 1 ml of 40% rochelle salt solution was added to this warm mixture, then cooled and the intensity of dark red color was read at 510 nm. A series of standards was run using glucose (0–500 µg) and a graph was plotted[7].

Saccharification

Acid hydrolysis of cellulose

Acid hydrolysis of cellulose was performed on preprocessed lignocellulosic waste samples using 20% H_2SO_4 at the temperature 100°C. About 5g of samples were added with 100 ml of acid solution and was heated in boiling water bath. The samples were shaken well during heating. This was continued for one hour. During the process, aliquots of samples were taken for every 10 min. The samples were analyzed for the concentrations of cellulose and glucose.

Ultrasound assisted acid hydrolysis of cellulose

Ultrasound assisted acid hydrolysis of cellulose was performed on preprocessed lignocellulosic waste samples using 5%H_2SO_4 in the sonochemical reactor (operating frequency: 24 kHz). About 5g of samples were added with 100 ml of acid solution and was heated in boiling water bath. The samples were shaken well during heating. This was continued for one hour. During the process, aliquots of samples were taken for every 10 min. The samples were analyzed for the concentrations of cellulose and glucose.

Results and discussions

Pretreatment and delignification of saw dust

The saw dust sample was preprocessed using sonication. The micro-structural changes produced by sonication, helps in the chemical reaction of acid with the cellulose[5]. It is the well known fact that the cellulose is highly hard structure. It cannot be degraded easily by ordinary chemical reactions. Though acidic conditions are highly reactive towards degrading cellulosic structures, the lignocellulosic wastes cannot be hydrolyzed easily. To facilitate the hydrolysis of cellulose, mechanical rupturing techniques were normally practiced. For further improvement in the mechanical rupturing process, sonochemical rupturing was also used[10].

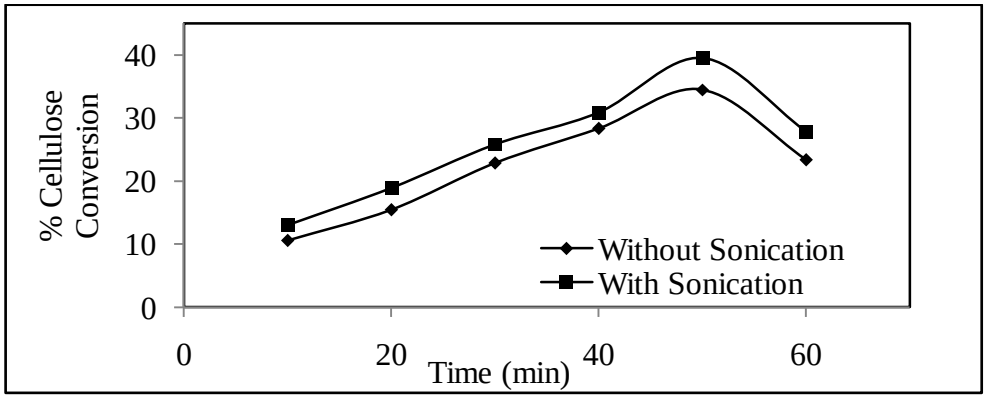
Sonochemical reactors normally use high frequency ultrasound to rupture the materials. These high vibrating waves can produce micro and even nanolevel particles easily. Also, several micro and nanopores can be created on the lignocellulosic materials so that the chemical agents can bind on the sites easily and react with the material effectively[4].

Delignification

Delignification of lignocellulosic wastes was carried out with acidic treatment of lignocellulosic wastes. Lignin is a hard polymer that coated most of the surface of wooden material. This provides strength to the wooden structure. The acetic acid dissolves the lignin present in the waste material and thereby removing the lignin. The supernatant obtained after the settling process was a dark brown solution which was predominantly lignin. The delignified material was filtered out and dried in hot air oven and stored for further use.

Saccharification process

Figure 1: Conversion of cellulose with and without sonication

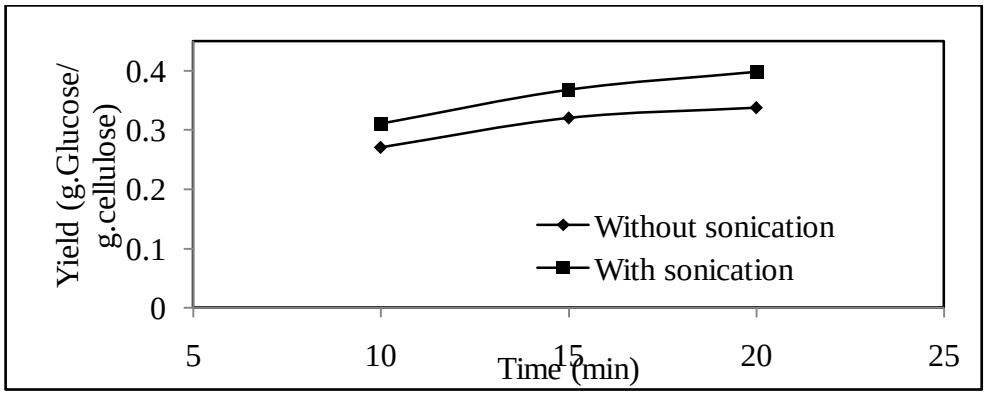


Acid hydrolysis of saw dust sample was performed with and without ultrasound using 5% and 20% sulfuric acid respectively. The results of cellulose conversion during hydrolysis with and without sonication are shown in (Figure- 1). The % cellulose conversion in both the cases increased with increase in time and there was a considerable increase in % cellulose conversion using sonication[8,9].

In both the cases the % cellulose conversion decreases during last 10 min of the process. Though the acid hydrolysis is efficient in conversion of cellulose into glucose, the percentage concentration of acid disables the solution to be further inoculated with yeast for ethanol fermentation. The pH of the solution decreases much such that the yeast could not survive in the medium. To neutralize pH conditions more amount of alkali should be added in the solution which increases the usage of chemicals in the industrial scale process. To overcome this problem, combined sonication procedure was implemented in this study. The main advantage of this system is that only 5% concentration of sulfuric acid is enough to get high glucose concentrations.

The amount of glucose yield during saccharification process with and without sonication is shown in (Figure- 2). Up to first 20 min the glucose yield increases linearly for both the processes. The highest glucose yield in the case of saccharification with sonication was 0.389g glucose / g cellulose. This is almost 25% increased yield compared to acid hydrolysis without sonication.This follows the trend obtained for the conversion of cellulose. All the cellulose converted is not converted in to glucose. There are the formations of some by-products, which account for the reduced conversion.

Figure 2: Glucose yield with and without sonication



Summary and conclusions

Fossil fuel crisis and environmental problems give rise to the idea of alternative fuels. Bioethanol is one of the important alternative fuels. Several feedstocks are used for bioethanol productions which include corn, molasses and starch containing agricultural products. To overcome the practical difficulties involved with the feed stock, lignocellulosic wastes are used as feed stock. These lignocellulosic wastes are abundantly available. A novel method of ultrasound assisted saccharification was carried out in a sonochemical reactor. Saw dust was used as a raw material. The experimental results show that the ultrasound assisted saccharification increases glucose conversion and thereby the ethanol yield and reduce the consumption of sulfuric acid for acid hydrolysis when compared to saccharification without sonication.

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