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Pretreatment of cellulosic waste materials using Acid and Alkali to enhance bio-ethanol production

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Abstract

Bioconversion of lignocellulosic biomass to bioethanol has shown environmental, economic and energetic advantages in comparison to bioethanol produced from sugar or starch. However, the pretreatment process for increasing the enzymatic accessibility and improving the digestibility of cellulose is hindered by many physical-chemical, structural and compositional factors, which make these materials difficult to be used as feed stocks for ethanol production. Lignocellulosic substrates such as, Sugarcane waste, mushroom bed waste and corn waste were pretreated by physical, chemical and biological methods. Pretreatment with NaOH (6%) released a maximum of 120% and 108% cellulose in both sugarcane waste and corn waste whereas H_2SO_4 (6%) released 84% and 84% cellulose in vegetable waste and mushroom bed waste. The pretreated substrates were analyzed by FTIR.

Keywords: FTIR, pretreatment, cellulose, agro waste

Introduction

Pretreatment is one of the most expensive processing steps within the conversion of biomass to fermentable sugar (Mosier *et al.* 2005) [9]. Costs are due to the use of steam and chemical products and the need for expensive corrosion resistant reactors. With the advancement of pretreatment technologies, it is also believed to have great potential for the improvement of efficiency and lowering the costs through research and development. The goal of all pretreatment is to break the lignin seal and to disrupt the crystalline structure of cellulose in order to make cellulose more accessible to enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier *et al.* 2005) [9]. An effective and economical pre-treatment would accomplish the following requirements: releasing of reactive cellulosic fiber for enzymatic attack, reduces the destruction of hemicellulose and cellulose, decreases the formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, minimization of energy demand, decline of costs of the feedstocks, reduction of costs of material for construction of pre-treatment reactors, production of less residues, consumption of little or no chemicals and utilisation of cheap chemicals (Taherzadeh and Karimi, 2007) [16].

Enzymatic hydrolysis is an important step in conversion of inexpensive lignocellulosic materials to ethanol production. However, the recalcitrant nature of the lignocelluloses limits the access of hydrolytic enzymes to cellulose and hemicellulose components (Sun, & Cheng, 2002, Yu *et al.* 2009) [14, 18]. Existing pretreatment methods have largely been developed on the basis of physicochemical technologies such as steam explosion, dilute acid, alkali, and oxidation or varied combinations (Mosier *et al*,.2005) [9] but these processes usually require high temperature and operating pressure (Taherzadeh, & Karimi, 2008) [17]. In contrast, microbial pretreatment utilizes microorganisms and their enzyme systems to degrade lignin and hemicelluloses present in the lignocellulosic biomass with comparatively low energy and mild environmental conditions (Sun, & Cheng, 2002) [14]. This environmentally friendly approach has recently received increasing attention (Amirta *et al.* 2006) [3].

The annual production of bagasses in the world exceeds 100 million tons, more than half of which is produced in the western hemisphere. (Pandey, 1992) [10] Rice straw is a by-product of rice production and great bio resource. It is also one of the abundant lignocellulosic waste materials in the world. It is annually produced about 731 million tons, whereas in Asia 667.6 million tons. (Roberto *et al.* 2003) [13].

The cost of ethanol produced from lignocellulosic materials with currently available technology and under the present economic conditions is not competitive with the cost of gasoline. To make the process economically feasible, comprehensive process development and optimization are still required. The low enzymatic accessibility of the native cellulose is a prime problem for biomass-to-ethanol processes because lignin forms a protective covering around cellulose and hemicellulose, safeguarding the polysaccharides from enzymatic degradation. To convert the biomass into ethanol, the cellulose must be readily accessible for cellulase enzymes. Thus, by abolishing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during fermentation. Pretreatment with dilute acids and bases not only debase the lignin, but also increases the surface area for enzymatic activity (Dawson, Boopathy, 2007) [4]. After pretreatment, the enzymatic hydrolysis of substrate is an auspicious way for obtaining cellulose sugars (mostly glucose) from lignocellulosic materials [because it has the advantages of reduced sugar loss through side-reactions, is milder and more specific] (Adsul *et al.* 2005) [2].

Pretreatment of the raw material is perhaps the single most crucial step as it has a large impact on all the other steps in the process, e.g. enzymatic hydrolysis, fermentation, downstream processing and wastewater handling, in terms of digestibility of the cellulose, fermentation toxicity, stirring power requirements, energy demand in the downstream processes and wastewater treatment demands. Pretreatment is necessary for the bioconversion of lignocellulosic materials to fuels and other chemicals. The primary purpose of pretreatment is to make the lignocellulosic biomass accessible and reactive to allow high rates and yields on enzymatic hydrolysis (McMillan, 1994) [8]

Materials and methods

Collection of Cellulosic agro-waste

Sugarcane waste was collected from sugar factories. Mushroom bed waste, and corn waste were collected from Agricultural fields in and around of Tiruchirappalli.

Physical Treatment

The collected lignocellulosic materials Sugarcane waste, mushroom bed waste and corn waste were powdered through a combination of chipping, grinding and milling (vibratory ball milling) to reduce cellulose crystallinity. The finely powdered samples of all the cellulosic wastes were washed with distilled water to remove all the soluble contents present in the samples. The rinsing with water was continued until the wash water was clear.

Chemical Treatments

The lignocellulosic substrates such as Sugarcane waste, mushroom bed waste and corn waste were chemically treated as described by (Abraham and Kurup, 1996) [1].

Sodium Hydroxide Pretreatment

Sodium hydroxide was used as the alkaline substance and various aqueous solution of NaOH differing in concentration in the range of 1-10% (W/V) were prepared. The powdered lignocellulosic substrates such as Sugarcane waste, mushroom bed waste, and corn waste of 22 mesh sizes were mixed with aqueous solution of NaOH at a liquid to solid ratio of 1:10 (w/v) to soak these substrates in aqueous solution of NaOH at room temperature (37^0C) for 4 hr and autoclaving at 121^0C for 30 min. The residues were collected and washed extensively with tap water until neutral pH was reached, filtered and dried at 65^0C for two days.

HCL Pretreatment

Acid pretreatment each of the cellulosic waste was carried out by using dilute hydrochloric acid. Ten grams each of cellulosic wastes was soaked in 100 ml of 3% HC1 separately and incubated at room temperature for 10 hr with an agitation of 150 rpm and autoclaving at 121 °C for 30 min. The residues were collected and washed extensively with tap water until neutral pH was reached, filtered and dried at 65 °C for two days.

Sulfuric acid Pretreatment

Acid pretreatment of substrates was carried out by using dilute sulphuric acid. Ten grams each of cellulosic wastes was soaked in 100 ml of different concentration of H_2SO_4 (2-8%) separately and incubated at room temperature for 10 hr with an agitation of 150rpm and autoclaving at 121^oC for 30 min. The residues were collected and washed extensively with tap water until neutral pH was reached, filtered and dried at 65^0C for two days.

Chloroform Pretreatment

Ten grams of each cellulosic waste was mixed with 100 ml of chloroform separately at room temperature (37^0C) for 10 hr with an agitation of 150rpm and autoclaving at 121^0C for 30 min. The residues were collected and washed extensively with tap water until neutral pH was reached, filtered and dried at 65°C for two days.

Estimation of cellulose content in substrate:

Three milliliter acetic/nitric reagent was added to 1g of substrate and mixed well. The tube was placed in water bath for 30mins. The contents were centrifuged for 15-20 mins. To the supernatant 10 ml of 67% sulphuric acid was added and allowed to stand for one hour. The solution is diluted to 100ml.From the diluted solution, 1ml was taken and 10 ml of enthrone reagent was added. Test tubes were kept in water bath for 10mins and measured at 630nm**.** After estimation of cellulose efficiently pretreated substrates containing higher amount of cellulose is observed under FTIR spectroscopy.

Result

Size reduction of cellulosic materials

The lignocellulosic substrates were powdered physically to reduce the crystalline cellulose structure. The size of the materials was 10-30 μm after chipping, Sugarcane waste, Mushroom bed waste, and Corn waste were powdered by ball mill and separated based on their mesh size. The 22 mesh size samples (1-2 μm) after milling were selected for pretreatment process.

Estimation of cellulose

NaOH Pretreatment

In alkali-treatment, NaOH treated sugarcane waste, mushroom bed waste and corn waste showed variations in the cellulose content at different concentrations. Among the treated substrates, 6%NaOH pretreated mushroom bed waste and corn released 69, 120 and 108 % of cellulose respectively.(Fig:1)

HCL Pretreatment

Acid pretreatment method was derived from the concentrated acid hydrolysis such as concentrated HCl hydrolysis, which had been a major technology for hydrolyzing lignocellulosic biomass for fermentable sugar production. The dilute acid pretreatment has received numerous research interests and it has been successfully developed for pretreatment of lignocellulosic biomass. The dilute acid solubilized hemicellulose and lignin remain. In acid pretreatment, 6 % HCl treated sugarcane waste, mushroom bed waste and corn waste released 54,70, 59 and 70% of cellulose respectively.(Fig:2)

H_2SO_4 and Chloroform Pretreatment

The combination of both acid treatments is another way to improve the pretreatment of lignocellulosic wastes. In acid pretreatment, 4 % H_2SO_4 treated sugarcane waste, mushroom bed waste and corn waste released 92,100, 69 and 74% of cellulose respectively.6% chloroform treated sugarcane waste, mushroom bed waste and corn waste released 64,55,56 and 67% cellulose respectively.(Fig:3 & 4)

FTIR analysis of substrate

The FTIR spectroscopy is an appropriate technique to establish the variations introduced by the different treatment of on the chemical structures of cellulose. All spectra were dominated by the peaks at 3501 cm^{-1} and 1484 cm^{-1} that correspond to the stretching vibrating of O-H in cellulose and C-O in hemicelluloses and cellulose, respectively. The peak at 1627cm^{-1} in all samples was indicative of the C=O bonds of hemicelluloses. Peaks observed at 1627cm^{-1} also showed the presence of hemicelluloses. Lignin peak values range from 1542 and 1484 cm^{-1} was absent in pure cellulose powder and pretreated cellulosic wastes. Pure cellulose powder was considered as the control for untreated and pretreated cellulosic wastes

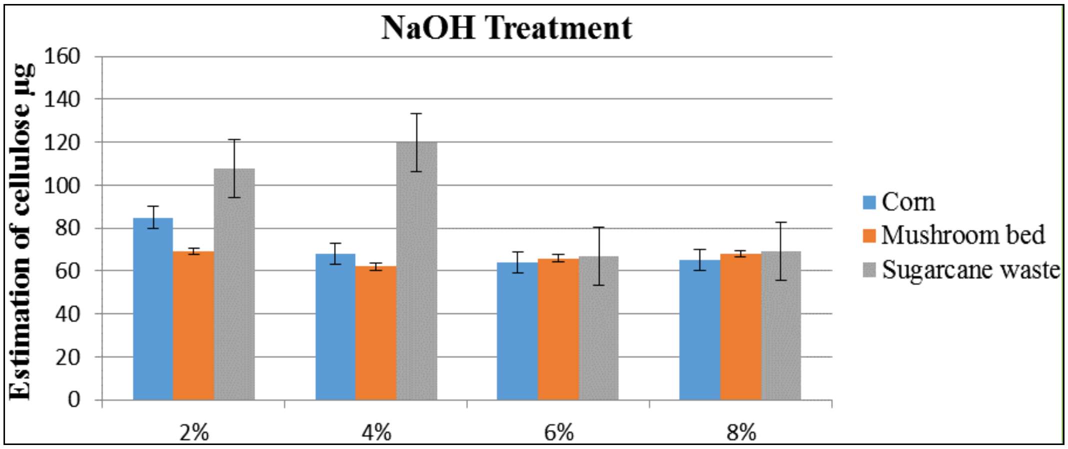

Fig 1: Estimation of cellulose in substrate in NaOH Treatment

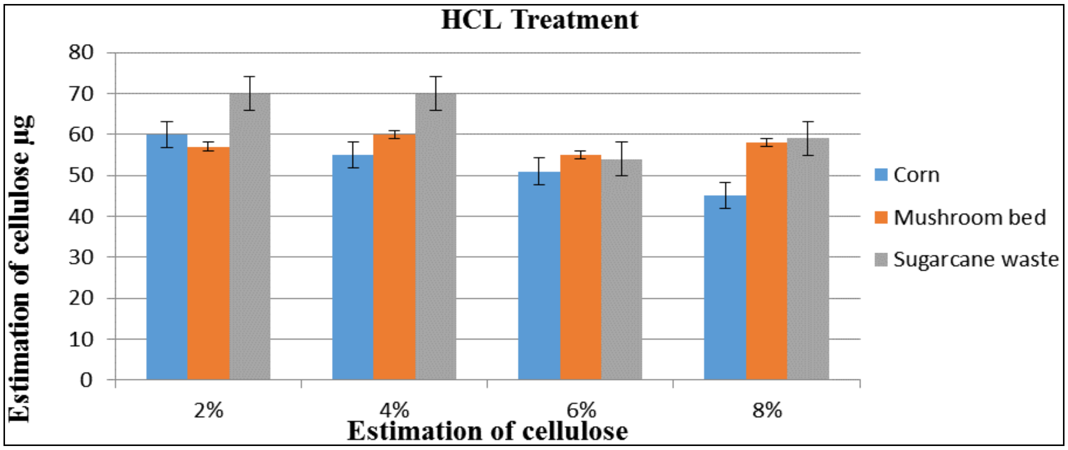

Fig 2: Estimation of cellulose in substrate in HCL Treatment

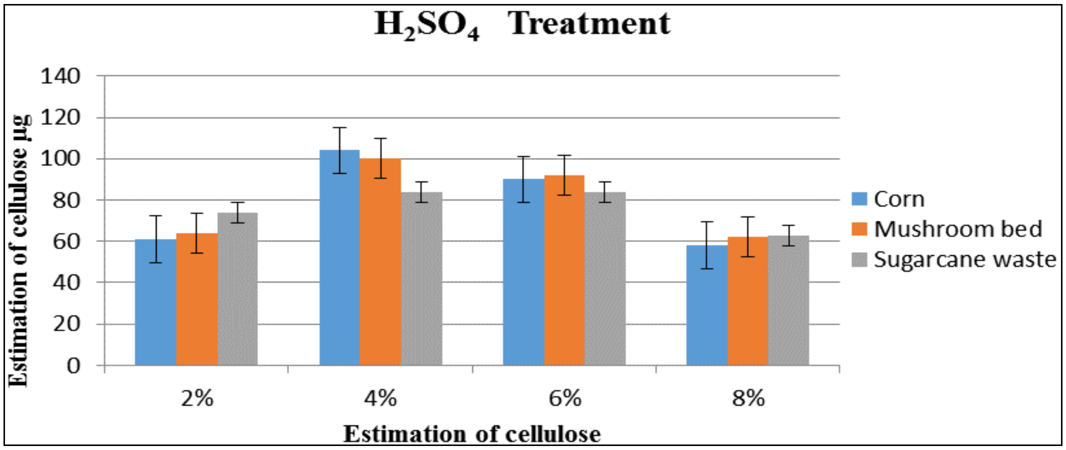

Fig 3: Estimation of cellulose in substrate in H_2SO_4 Treatment

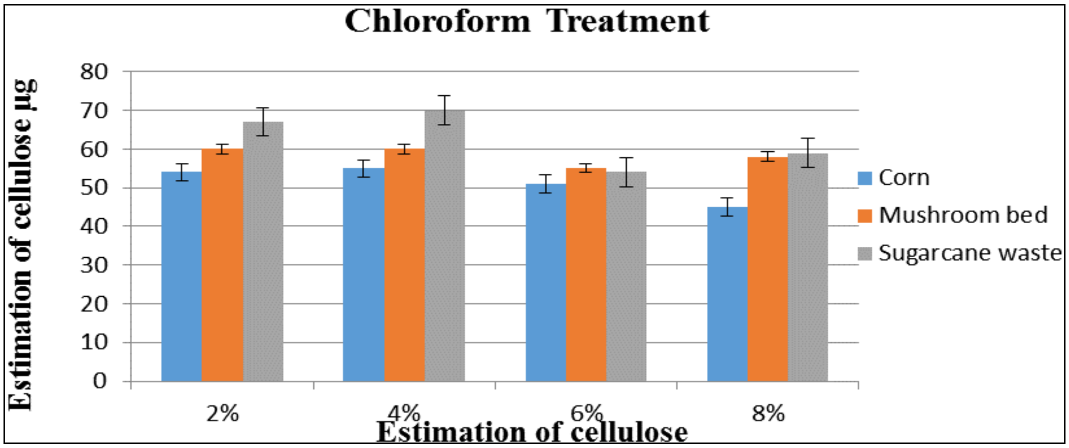

Fig 4: Estimation of cellulose in substrate in Chloroform Treatment

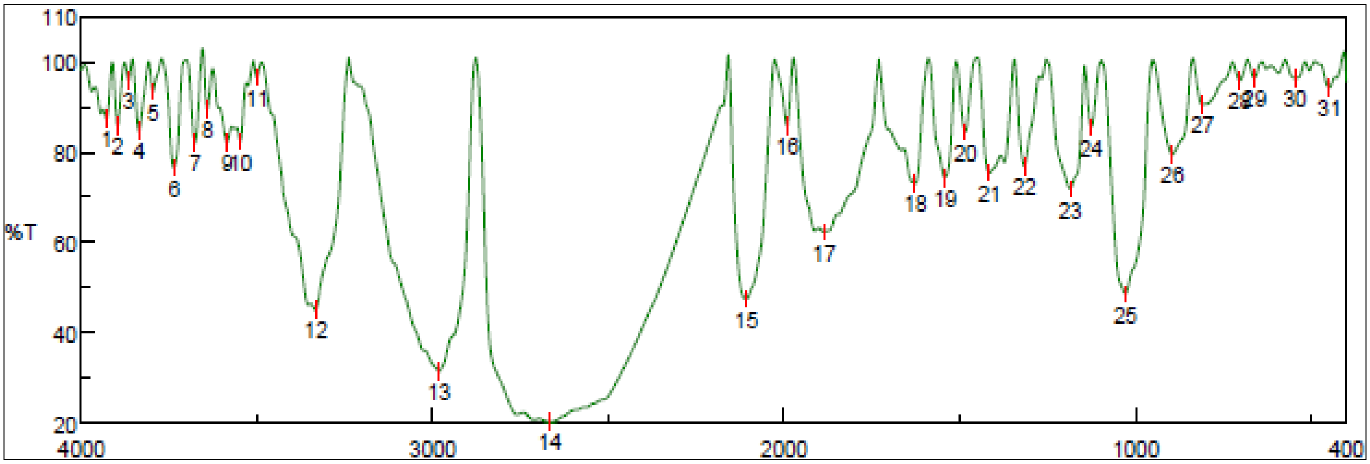

Fig 5: FTIR analysis of standard cellulose

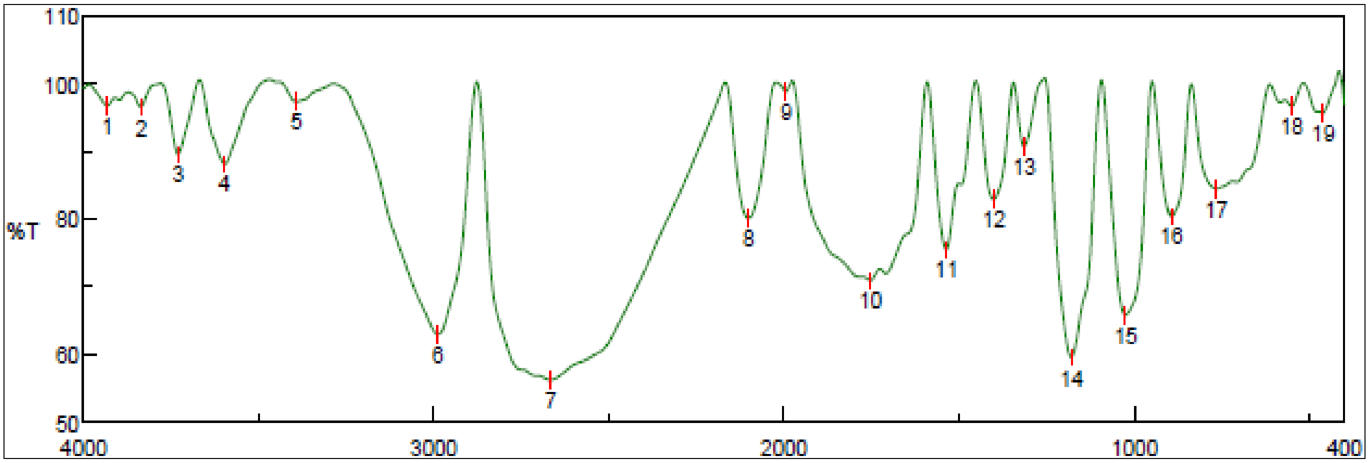

Fig 6: FTIR analysis of 4% NaOH pretreated Sugarcane

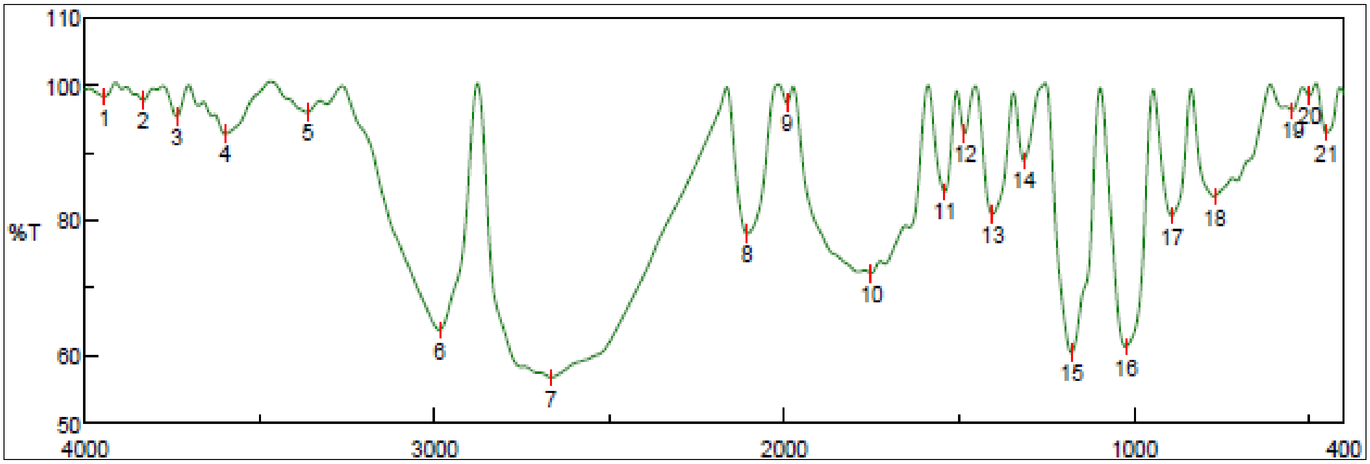

Fig 7: FTIR analysis of 6% NaOH pretreated Corn

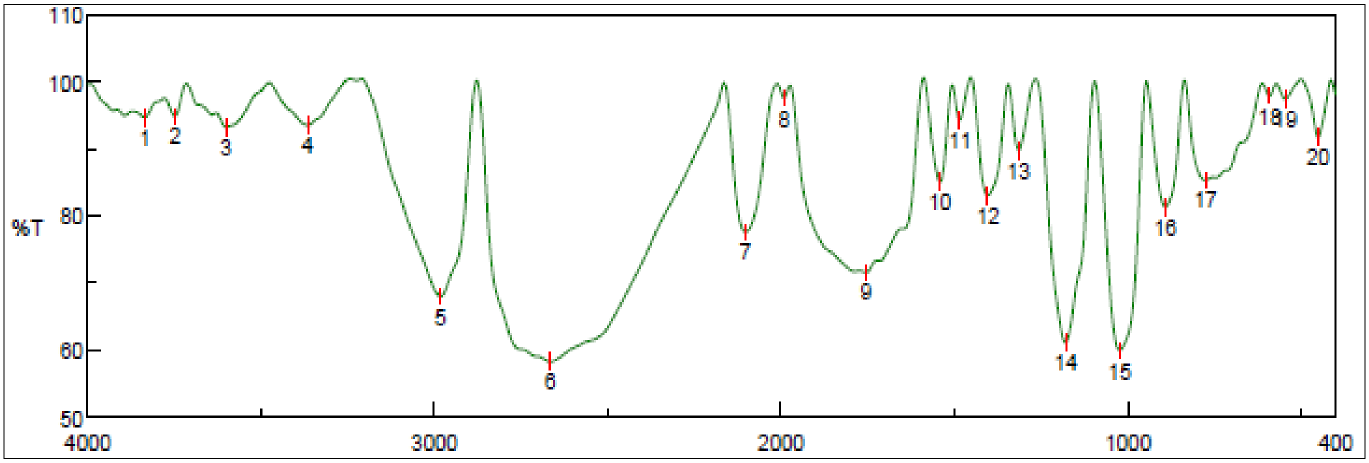

Fig 8: FTIR analysis of 6% NaOH pretreated Mushroom bed waste

Discussion

Biomass pretreatment, for the purpose of improving the biodegradability of biomass materials, have been extensively studied to process different biomass for cellulosic ethanol production. Numerous effective pretreatment techniques on various lignocellulosic biomass feedstocks were studied to increase the surface area which is one of the major approaches of a pretreatment by solubilization of the hemicellulose and/or lignin and/or altering the lignin.

The NaOH treatment was the best method for improving the use of rice straw and sugarcane waste. The effective pretreatment for individual substrates varies as it depends on the nature and condition of pretreatment. For corn waste, mushroom bed waste, and fruit waste 6% NaOH released high percentage of cellulose respectively. In the alkaline pretreatment the first reaction taking place are solvation and saponification. At strong alkali concentrations dissolution, peeling of end-groups, alkaline hydrolysis and degradation and decomposition of dissolved polysaccharides takes place. Loss of polysaccharides is mainly caused by peeling and hydrolytic reactions (Fengel and Wegener, 1984) [5]. Another important aspect of alkaline pretreatment is the change of the cellulose structure to a form that is denser and thermodynamically more stable than the native cellulose (Pettersen, 1984) [5, 11].

Treatment of lignocellulosic materials with diluted acids can efficiently improve the enzymatic hydrolysis. Dilute acid hydrolysis is probably the most commonly applied method among the chemical pretreatment methods. It can be used either as a pretreatment of lignocellulose for enzymatic hydrolysis, or as the actual method of hydrolyzing to fermentable sugars. The main reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose, especially xylan as glucomannan is relatively acid stable. Solubilized hemicellulose (oligomers) can be subjected to hydrolytic reactions producing monomers, furfural, hydroxymethylfurfural (HMF) and other (volatile) products in acidic environments (Fengel and Wegener, 1984 [5]; Ramos, 2003) [12]. During acid pretreatment solubilized lignin will quickly condensate and precipitate in acidic environments (Liu and Wyman, 2003; Shevchenko *et al.* 1999) [6, 15]. The 6% HCl pretreatment of sugarcane waste, corn waste, mushroom bed waste, and fruit waste gave better results of cellulose content compared to other pretreatment methods. The FTIR spectroscopy is an appropriate technique to establish the variations introduced by the different treatment of on the chemical structures of cellulose. Chemical treatments are effective for delignification and solubilization of hemicellulose (Sun *et al.* 2005) [9]

Hydrogen bonds prevent anaerobic microorganisms or degradation enzymes from reacting with cellulose. The absorption at 3430-3440 cm^{-1} represents the stretching of –OH groups, which was reduced after NaOH treatment. It specified that partial hydrogen bonds of cellulose were destroyed, leading to enhance accessibility of cellulose to reagents. The peak at 2920-2930 cm^{-1} represents the C-H stretching, the decrease of which content indicated that methyl and methylene of cellulose had some rupture.

The absorption at 1642 cm^{-1} is principally associated with deformation vibrations of H-OH in absorbed water. The contents of the functional groups mentioned above were all decreased after NaOH treatment. However, slight changes were found for some functional groups. For instance, the peak near 1373 cm^{-1} can be ascribed to C-H bending in cellulose and hemicellulose. The in-plane ring stretching gives a slight shoulder at 1103 cm^{-1}. The peak at 1058 cm^{-1} is indicative of C-O stretching at C-3, C-O stretching at C-6, and C-C stretching (Sun *et al.*, 2004).

According to (Mansfield *et al.* 1999) [7] the lignocellulosic materials in the original form are relatively resistant to microorganism attack but the hemicelluloses and lignin removal causes extensive changes in the structure and accessibility of cellulose that becomes more accessible and more open to swelling upon contact with microorganisms. Therefore, both intra- and intramolecular degradations of lignocelluloses were beneficial for bioethanol production.

Conclusion

The acid and alkali pretreatment of the substrate caused the crystallinity of cellulose to decrease. Absolute crystallinity could most impact cellulose production. However the overall complexity of the untreated might have actually induced greater enzyme production. Results comparable to conventional among the treated substrates, 6%NaOH pretreated fruit waste, mushroom bed waste and corn released 69, 120 and 108 % of cellulose respectively. In acid pretreatment, 6 % HCl treated sugarcane waste, mushroom bed waste, fruit waste and corn waste released 54, 70, 59 and 70% of cellulose respectively. In acid pretreatment, 4 % H_2SO_4 treated sugarcane waste, mushroom bed waste, fruit waste and corn waste released 92,100, 69 and 74% of cellulose respectively.6% chloroform treated sugarcane waste, mushroom bed waste, fruit waste and corn waste released 64,55,56 and 67% cellulose respectively.

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