European Journal of Biotechnology and Bioscience

www.biosciencejournals.com

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European Journal of Biotechnology and Bioscience

ISSN: 2321-9122
www.biosciencejournals.com
EJBB 2014; 2 (4): 04-13
Received: 04-09-2014
Accepted: 17-09-2014

Haripriya. R
PG and Research Department of Biotechnology, Srimad Andavan arts and Science College (Autonomous), Trichy.

Selvaraj. C
PG and Research Department of Biotechnology, Srimad Andavan arts and Science College (Autonomous), Trichy.

Naveenraj. D
PG and Research Department of Biotechnology, Srimad Andavan arts and Science College (Autonomous), Trichy.

Kirubakaran. S.A
PG and Research Department of Biotechnology, Srimad Andavan arts and Science College (Autonomous), Trichy.

Muthukumar. V
PG and Research Department of Biotechnology, Srimad Andavan arts and Science College (Autonomous), Trichy.

P. Thirumalaivasan
PG and Research Department of Biotechnology, Srimad Andavan arts and Science College (Autonomous), Trichy.

Correspondence:
P. Thirumalaivasan
Head, Department of Biotechnology, Srimad Andavan Arts and Science College, Trichy, Tamilnadu.

Pretreatment of cellulosic waste materials

Haripriya. R, Selvaraj. C, Naveenraj. D, Kirubakaran. S.A, Muthukumar. V, P. Thirumalaivasan

Abstract
Lignocellulosic substrates such as, rice straw, sugarcane bagasse and corpith were pretreated following physical, chemical and biological methods. Pretreatment with NaOH (4 and 6%) released a maximum of 78% and 80% cellulose in both sugarcane baggase and rice straw whereas HCl (3%) released 70% cellulose in coir pith. The pretreated substrates were analyzed by FTIR.

Keywords: Pretreatment, sugarcane bagasse, rice straw, coir pith.

1. Introduction
Lignocellulosic biomass has potential as a key element in further increasing the amount of bioenergy generation. Its global availability in large amounts lignocellulosic biomass is considered as one of the most promising resources for future bioenergy generation. Because of lignocelluloses are mainly comprised of cellulose, a polymer of six-carbon sugar, glucose; hemicellulose, a branched polymer comprised of xylose and other five-carbon sugars and lignin consisting of phenyl propane units (Zaldivar *et al.,* 2001) [23]. Sugarcane bagasse commonly known as sugar cane bagasse (SCB) is the major by-product of the sugar industry. It is a fibrous residue of cane stalks left over after the crushing and extracton of the juice from the sugar cane is one of the largest cellulosic agro-industrial by-products (Pandey, 1992) [13]. The annual production of bagasse in the world exceeds 100 million tons, more than half of which is produced in the western hemisphere. Bagasse composition consists of approximately 50% cellulose and 25% each of lignin and hemicellulose. Chemically, bagasse contains about 50% cellulose, 30% pentosans and 2.4% ash. Bagasse offer numerous advantages in comparison to other crop residues because of its low ash content such as rice straw and wheat straw, which have 17.5 and 11.0%, respectively, ash contents, for usage in bioconversion processes using microbial cultures. A bagasse has a great potential for the production of biofuels and chemicals due to its considerable amount of cellulose and hemicellulose (Pandey, 1992) [13].
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. Rice straw can potentially produce 205 billion liters of bioethanol per year, which is about 5% of total consumption. It is the largest amount from as single biomass feedstock. Rice straw predominantly contains cellulose 32-47%, hemicelluloses 19-27%, lignin 5-24% and ashes 18.8% (Roberto *et al.,* 2003) [16].
The other major agricultural residue in South India is coir pith .It represents ~50% of the waste from the coir industries. The coir pith contains high lignin (36%) and cellulose (44%). India is reported to produce 0.77 million metric tons of coconut (*Cocos nucifera*) equivalent to the availability of ~0.35 million metric tons of fibrous husk. About 50% of the husk accounts for the waste of coir industries as coir pith because of its high lignin content (~48%) and amorphous powdery nature (Kjallstrand *et al*., 1998) [6].
There are major limitations for efficient ethanol production from agricultural residues which include the close physical and chemical associations between lignin and plant cell wall polysaccharides, together with cellulose crystallinity (Martin *et al.,* 2002) [9]. The presence of lignin limits the fullest usage of cellulolse and hemicellulose. Thus by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during fermentation (Wyman, 1996) [23].

Therefore a pretreatment must be applied to remove lignin in the bagasse, decrease cellulose crystallinity and increase the surface area for enzymatic activity. As with other biochemical conversion pathways, identification and optimisation of pre-treatment methods to make these carbohydrates available for microbial consumption is one of the key engineering challenges.

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) [10].

2. Materials and methods

2.1. Collection of Cellulosic agro-waste

Sugarcane bagasse was collected from sugar factories, rice straw and coirpith were collected from Agricultural fields in and around of Tiruchirappalli.

2.2. Physical Treatment

The collected lignocellulosic materials (Sugarcane bagasse, rice straw and coirpith) 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.

2.3. Chemical Treatments

The lignocellulosic substrates such as, bagasse, rice straw and coirpith were chemically treated as described by Abraham and Kurup (1996) [1].

2.4. 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 rice straw, coir pith and bagasse 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 °C) for 4 hr 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.

2.5. Sodium Hydroxide-Acetic Acid Pretreatment

Ten grams of powdered lignocellulosic substrates such as, rice straw, coir pith and

bagasse of 20 mesh size were mixed with a mixture of 100 ml of 1% NaOH and 2% acetic acid (1:1) separately to soak these substrates in the mixture at room temperature (37 °C) with an agitation of 150 rpm for 10hrs 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.

2.6. Chloroform Pretreatment

Ten grams of each cellulosic waste was mixed with 100 ml of chloroform separately at room temperature (37 ^{0}C) for 10 hr with an agitation of 150rpm and autoclaving at 121 ^{0}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.

2.7. 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.

2.8. Sodium sulphite pretreatment

Ten grams each of the cellulosic waste was soaked in 70 ml of sodium sulphite (13.7%W/V) solution and incubated at room temperature for 3 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.

2.9. Butanol pretreatment

Organosolvent pretreatment each of the cellulosic waste was carried out using butanol. An aqueous solution of butanol was prepared with water in 1:1(v/v) ratio. Ten grams each of the cellulosic wastes were soaked with 100 ml of butanol mixture and incubated at room temperature for 3hr with an agitation of 150rpm and autoclaving at 121°C for 30 min with 0.005% of aluminium chloride as a catalyst. The residues were collected and washed extensively with tap water until neutral pH was reached, filtered and dried at 65°C for two days. Autoclaving at 121 °C with 100 ml of the pretreating reagent separately in the presence of 0.005% aluminium chloride as catalyst for 2 hr.

2.11. Hydrogen Peroxide-Ferrous Salt Pretreatment

A solution of 100 ml of 1% hydrogen peroxide containing 100 mg of ferrous salt was prepared and treated with 10 g each of the cellulosic wastes. The substrates were immersed in the solution mixture for 10 hr in a shaker (150 rpm) at 37 °C and autoclaving at 121 °C for 30 min with 0.005% of aluminium chloride as a catalyst. The residues were collected and washed extensively with tap water until neutral pH was reached, filtered and dried at 65 °C for two days.

2.12. Hydrogen Peroxide-Manganous salt pretreatment

Ten grams each of the cellulosic waste was immersed in 100 ml of 1% hydrogen peroxide containing 100 mg of manganous salt and kept in a shaker with an agitation of 150 rpm for 10 hr at 37 °C and autoclaving at 121 °C for 30 min with 0.005% of aluminium chloride as a catalyst. The residues were collected and washed extensively with tap water until neutral pH was reached, filtered and dried at 65 °C for two days.

2.13. Acetic Acid-Hydrogen Peroxide pretreatment
Acetic Acid-Hydrogen Peroxide solution was prepared by mixing 1% hydrogen peroxide and 1% acetic acid (1:1; v/v). Ten grams each of the cellulosic wastes were treated separately with 100 ml of the reagent and kept at 37 °C for 10 hr in a shaker (150 rpm). The residues were collected and washed extensively with tap water until neutral pH was reached, filtered and dried at 65 °C for two days.

2.14. Estimation of cellulose
Cellulose content was estimated by the method of Uppdegraff (1969). Hundred milligram of pretreated of cellulosic wastes were added with 5ml of Nitric reagent and boiled and cooled. It was centrifuged at 5000 rpm for 5min. The pellet was washed with distilled water. 10 ml of 67% sulphuric acid was added. 1ml of the sample was diluted to 100ml. To 1ml of the each diluted solution, 10 ml of freshly prepared ice cold Anthrone reagent was added and boiled in a boiling water bath for 10 min at 100 ˚C. Absorbance was recorded at 600 nm.

2.15. FTIR Analysis
FTIR analyses were carried out at the central instrumentation facility of St. Joesph's College, Tiruchirappalli, Tamil Nadu. Two milligram of pretreated cellulosic wastes were prepared by mixing with 200 mg of spectroscopic grade KBr. FTIR spectra were recorded using a Nicolet 520P spectrometer with detector at 4 cm^{-1} resolution and 20 scans per sample.

3. Results
3.1 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 .Straw, bagasse and coir pith 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 (Table 1 to 3).

3.2 Alkali Pretreatment
In alkali-treatment, NaOH treated sugarcane bagasse, rice straw and coirpith showed variations in the cellulose content at different concentrations. Among the treated substrates 6% NaOH pretreated straw, 4%NaOH pretreated bagasse and 8% NaOH pretreated coir pith released 80, 78.8 and 46.53 % of cellulose respectively (Tables 1 to 3).

3.3 Acid 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, 3 % HCl treated sugarcane bagasse, rice straw and coirpith released 73.1, 75.4 and 70 % of cellulose respectively (Table 1 to 3).

3.4 Acid –Alkali Pretreatment
The combination of both acid-alkali treatments is another way to improve the pretreatment of lignocellulosic wastes**.** One percentage of NaOH –2% of acetic acid treated rice straw, bagasse and coir pith retained 56, 61 and 37% respectively (Table 1 to 3)**.**

3.5 Organosolvent pretreatment
The organosolvent process is a delignification process, with varying simultaneous hemicellulose solubilization. In this process, an organic or aqueous organic solvent mixture with or without an acid or alkali catalysts is used to break the internal lignin and hemicellulose bonds. The bagasse treated with sodium sulphite released maximum cellulose (54.1%) and H_2O_2 –Fe salt treated showed minimum of 41.5%. Sodium sulphate treated rice straw consisted a maximum of 62% of cellulose whereas chloroform pretreatment was minimum with 32%. H_2O_2 –Fe and chloroform treated coirpith released 55 and 45% of cellulose. The results of different pretreatments on the amount of cellulose released from each of the three agricultural wastes are tabulated (Table 1 to 3).

3.6 FTIR analysis
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 3356 and 1,058 cm^{-1} that correspond to the stretching vibrating of O-H in cellulose and C-O in hemicellulose and cellulose, respectively. The peak at 1,642 cm^{-1} in all samples was indicative of the C=O bonds of hemicellulose. Peaks observed at 1,162 cm^{-1} also showed the presence of hemicellulose. Lignin peak values range from 1500 to 1599 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 (Figs. 1 to 7).

Table 1: Comparison of Physical, chemical and biological pretreatment of bagasse (N=3; ±SD)

Sl.no	Types of pretreatment	Bagasse		% of cellulose released
		Before pretreatment cellulose content (%)	After pretreatment cellulose content (%)	
1.	Milling	41±0.2	13.01±0.1	31.7±0.1
2.	Steaming	41±0.2	17.09±0.4	41.7±0.4
3	2% Sodium hydroxide	41±0.2	29.5±0.1	72±0.2
4	4% Sodium hydroxide	41±0.2	32.3±0.2	78.8±0.3
5	6% Sodium hydroxide	41±0.2	29.7±0.12	72.4±0.4
6.	8% Sodium hydroxide	41±0.2	26.5±0.3	64.6±0.2
7.	10% Sodium hydroxide	41±0.2	23±0.5	57±0.1
8.	1% Sodium hydroxide	41±0.2	25±0.4	60.9±0.5

	+2% acetic acid (1:1)			
9.	Chloroform	41±0.2	21±0.11	51.2±0.22
10	3% Hydrochloric acid	41±0.2	30±0.4	73.2±0.43
11.	13.7% sodium sulphite	41±0.2	22.2±0.31	54.1±0.1
12.	1:1 v/v butanol :water	41±0.2	20.1±0.4	49.04±0.5
13.	1% hydrogen peroxide + 100 mg of ferrous salt	41±0.2	17.02±0.6	41.5±0.1
14.	1% hydrogen peroxide +100 mg manganous salt	41±0.2	19.03±0.23	46.4±0.53
15.	1% hydrogen peroxide + 1% acetic acid	41±0.2	21.12±0.26	51.5±0.43

Table 2: Comparison of Physical, chemical and biological pretreatment of straw (N=3; ±SD)

Sl. No	**Types of pretreatment**	**Straw**		**% of cellulose released**
		Before pretreatment cellulose content (%)	**After pretreatment cellulose content (%)**	
1.	Milling	35±0.31	11.31±0.2	32.3±0.41
2.	Steaming	35±0.31	12.1±0.1	34.6±0.15
3.	2% Sodium hydroxide	35±0.31	21±0.12	60±0.19
4.	4% Sodium hydroxide	35±0.31	24.3±0.15	69.4±0.15
5	6% Sodium hydroxide	35±0.31	28±0.21	80±0.53
6.	8% Sodium hydroxide	35±0.31	22±0.42	62.9±0.22
7.	10% Sodium hydroxide	35±0.31	20.5±0.56	58.6±0.21
8.	1% Sodium hydroxide +2% acetic acid (1:1)	35±0.31	19.8±0.25	56.6±0.47
9.	Chloroform	35±0.31	12±0.31	34.3±0.43
10	3% Hydrochloric acid	35±0.31	26.4±0.67	75.4±0.91
11.	13.7% sodium sulphite	35±0.31	32.7±0.32	93.4±0.65
12.	1:1 v/v butanol :water	35±0.31	17.3±0.34	49.4±0.87
13.	1% hydrogen peroxide + 100 mg of ferrous salt	35±0.31	13.12±0.59	37.5±0.32
14.	1% hydrogen peroxide +100 mg manganous salt	35±0.31	13.7±0.48	39.1±0.23
15.	1% hydrogen peroxide + 1% acetic acid	35±0.31	14.01±0.22	40±0.56

Table 3: Comparison of Physical, chemical and biological pretreatment of coirpith (N=3; ±SD)

Sl.no	Types of pretreatment	Coir pith		% of cellulose released
		Before pretreatment cellulose content (%)	After pretreatment cellulose content (%)	
1.	Milling	26±0.11	12±0.32	46.2±0.12
2.	Steaming	26±0.11	14.1±0.34	54.2±0.42
3	2% Sodium hydroxide	26±0.11	12.43±0.65	47.8±0.54
4	4% Sodium hydroxide	26±0.11	15.9±0.31	61.2±0.67
5	6% Sodium hydroxide	26±0.11	14.4±0.43	55.4±0.71
6.	8% Sodium hydroxide	26±0.11	12.1±0.65	46.5±0.41
7.	10% Sodium hydroxide	26±0.11	11.4±0.43	43.8±0.76
8.	1% Sodium hydroxide +2% acetic acid (1:1)	26±0.11	9.7±0.11	37.3±0.91
9.	Chloroform	26±0.11	11.8±0.48	45.48±0.62
10	3% Hydrochloric acid	26±0.11	18.2±0.56	70±0.92
11.	13.7% sodium sulphite	26±0.11	11.8±0.26	45.4±0.14
12.	1:1 v/v butanol :water	26±0.11	11.7±0.72	45±0.22
13.	1% hydrogen peroxide + 100 mg of ferrous salt	26±0.11	14.5±0.13	55.8±0.18
14.	1% hydrogen peroxide +100 mg manganous salt	26±0.11	14.3±0.18	55±0.34
15.	1% hydrogen peroxide + 1% acetic acid	26±0.11	13.4±0.36	51.5±0.75

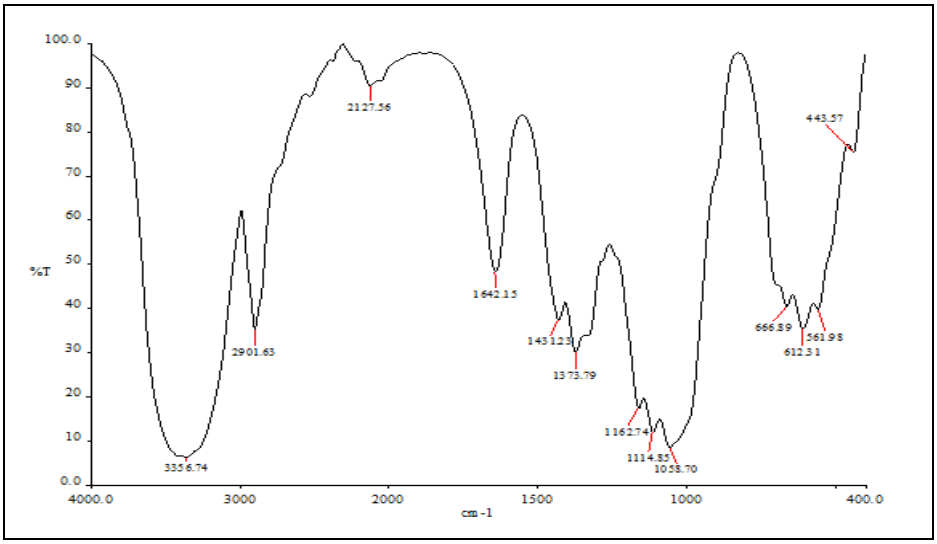

Fig 1: FTIR analysis of standard cellulose

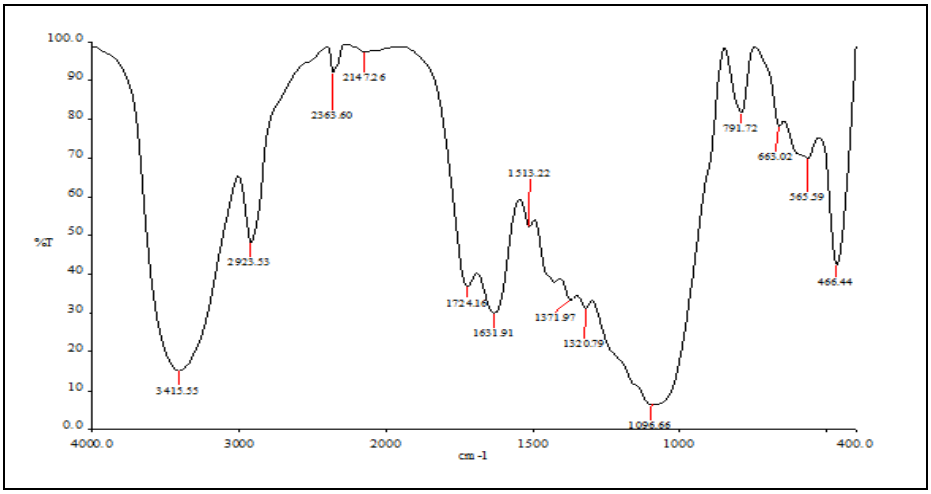

Fig 2: FTIR analysis of untreated straw powder (22µm)

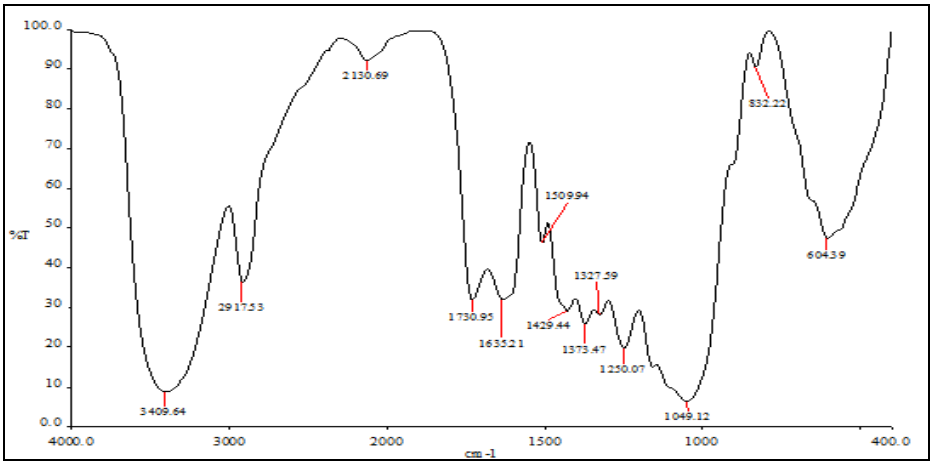

Fig 3: FTIR analysis for untreated bagasse powder (22 µm)

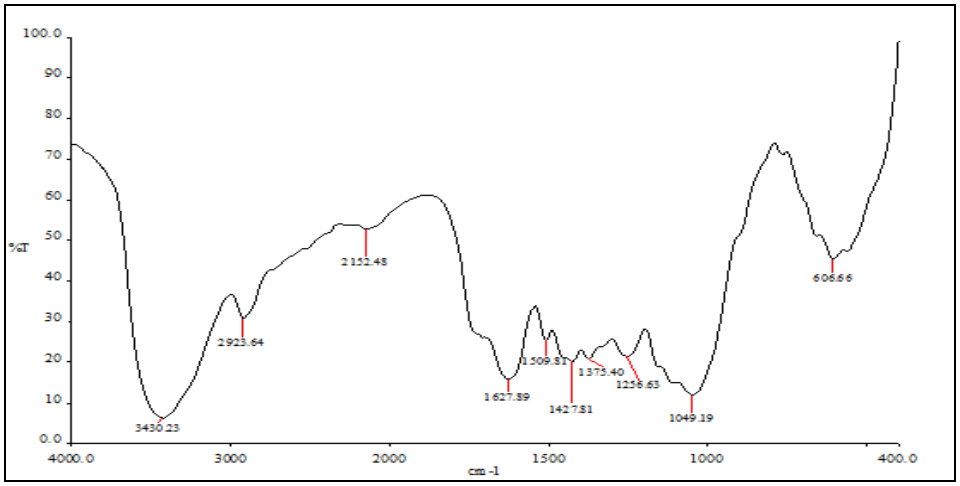

Fig 4: FTIR analysis of untreated coir pith powder (22 µm)

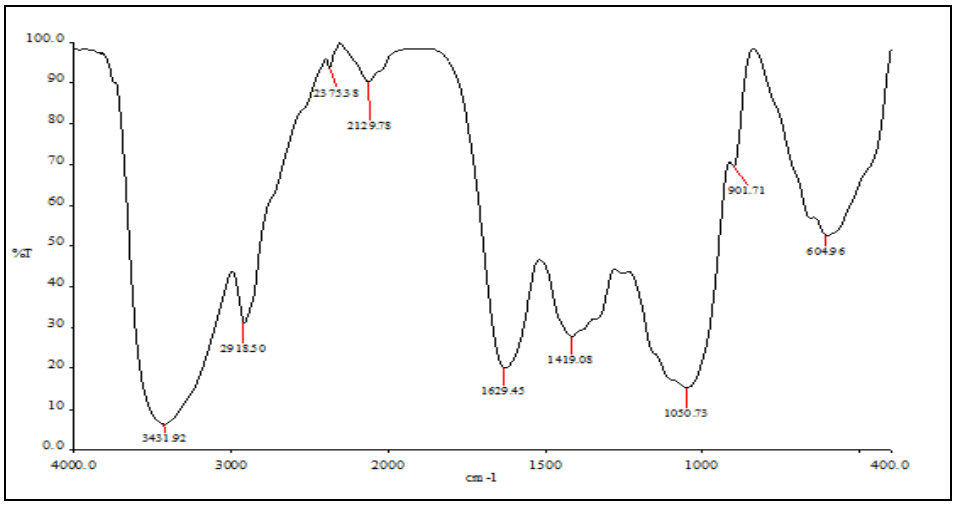

Fig 5: FTIR analysis of 6% NaOH pretreated straw powder (22 µm)

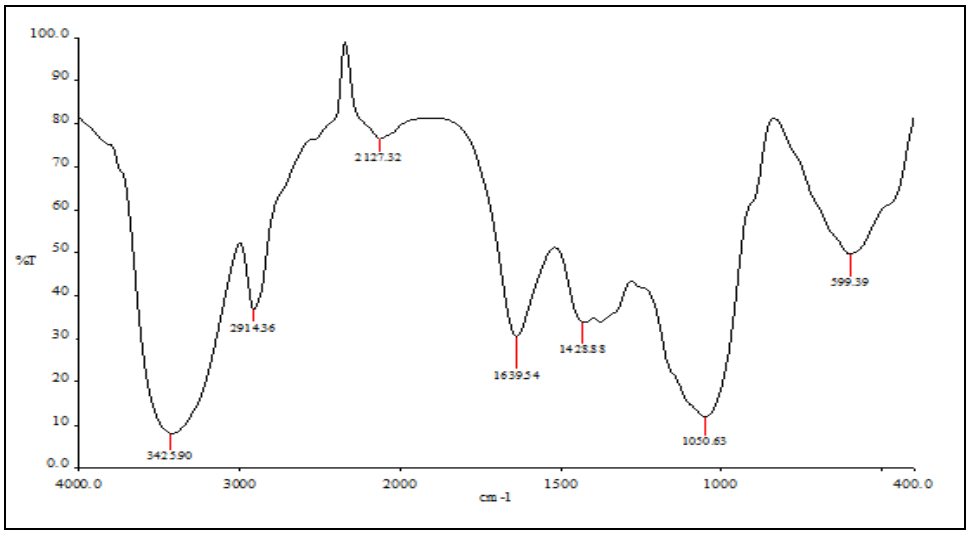

Fig 6: FTIR analysis of 4% NaOH pretreated bagasse powder (22µm)

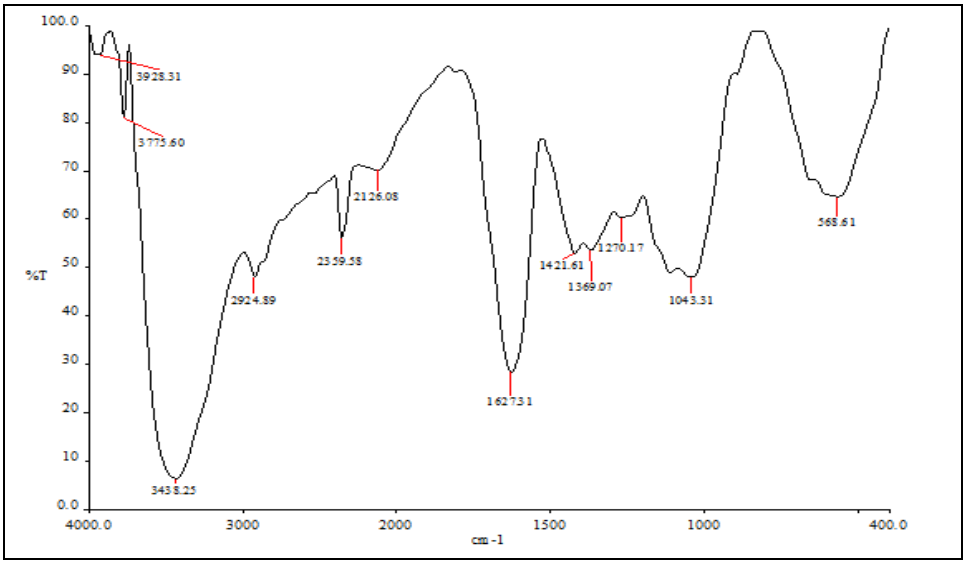

Fig 7: FTIR analysis of 3% HCl pretreated coir pith powder (22 µm)

5. Discussion

Biomass pretreatment, for the purpose of improving the bio-degradability 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 bagasse. The effective pretreatment for individual substrates varies as it depends on the nature and condition of pretreatment. For rice straw and baggasse, 6% NaOH and 4% NaOH released high percentage of cellulose respectively. In the alkaline pretreatment the first reaction taking place are solvation and saponification. This causes a swollen state of the biomass and makes it more accessible for enzymes and bacteria. 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) [3]. 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) [14].

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; Ramos, 2003) [3]. During acid pretreatment solubilized lignin will quickly condensate and precipitate in acidic environments (Liu and Wyman, 2003; Shevchenko *et al.,* 1999). The 3% HCl pretreatment of coir pith gave better results of cellulose content compared to other pretreatment methods.

Organosolvent can be used to provide treated cellulose suitable for enzymatic hydrolysis, using an organic or aqueous organic solvent to remove or decompose the network of lignin and possibly a part of the hemicellulose (Curreli *et al.,* 1997; Pan *et al.,* 2006) [2]. In this process, lignocellulose is mixed with organic liquid and water and heated to dissolve the lignin and part of the hemicellulose, leaving reactive cellulose in the solid phase.Solvents such as methanol, ethanol, butanol, phenol and hexamethylenediamine, with water, have reportedly been used (Holzapple *et al.,* 1984). In each system, the solvent action is accompanied by partial prehydrolysis affected by the presence of water and organic acid catalysts (acetyl groups), producing hemicellulose sugars. Such solvent-delignified celluloses have proven susceptible to enzyme hydrolysis. The main advantage of using solvents over chemical pretreatment is that relatively pure, low molecular weight lignin can be recovered as a by-product. The C5 sugars solubilized during organic solvent delignification are relatively clean and could be fermented to ethanol with appropriate microorganisms, such as the recombinant bacteria developed by Ingram and co-workers at the University of Florida (Ingram *et al.,* 1991). The organosolvent treatment was carried out with Butanol, the amount of cellulose released in the three different cellulosic substrates (rice straw, sugarcane bagasse and coirpith) were closely related.

The steam treatments after the physical and chemical treatments were carried out to catalyze the hydrolysis of the soluble hemicellulose oligomers (Mok and Antal, 1992). The objective of a steam pretreatment/steam explosion is to solubalize the hemicellulose to make the cellulose better accessible for enzymatic hydrolysis and to avoid the formation of inhibitors.

During steam pretreatment parts of the hemicellulose hydrolyze and form acids, which could catalyze the further hydrolysis of the hemicellulose. This process, in which the formed acids catalyze the process itself, is called 'auto-cleave' steam pretreatment. On comparing all the pretreatment techniques, the lignin content was considerably

higher in acid-treated than in alkali-treated bagasse. This was probably due to the fact that the acid hydrolyzed the cellulose and the hemicellulose and left the lignin intact; the alkali treatment, which was milder than the acid treatment, did not hydrolyze either the cellulose or the lignin.

Pretreatment was performed at low temperatures but with a relatively long time and high concentration of the base. Zhao *et al*., (2007) reported that pretreatment with NaOH could obtain a higher enzymatic conversion ratio of cellulose compared with conc. H_2SO_4 pretreatment. Compared with acid or oxidative reagents, alkali treatment appears to be the most effective method in breaking the ester bonds between lignin, hemicellulose and cellulose, and avoiding fragmentation of the hemicellulose polymers.

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). The bonds form lignin-carbohydrate complexes (LCCs), which are obstacles for efficient bioconversion of straw into biogas. FTIR was used to analyze the spectra of lignin, cellulose and hemicelluloses extracted from untreated and pretreated lignocellulosics substrates to investigate the changes of lignocellulosic complexs (LCC). Lignin is a multifunctional natural polymer. It is built up by oxidative coupling of three major C6-C3 (phenylpropanoid) units, which form a randomized structure in a tridimensional network by certain inter unit linkages, such as *β*-O-4, *β* -5, and *β* - *β*. The important functional groups of lignin units include carbonyls, phenol hydroxyls, aromatic rings, and methoxyls.

Cellulose is a linear polymeric compound, which is built up by coupling β-D-glucose using 1, 4- glycosidic bonds. Hydrogen bonds, methyls, methylenes, and C-O-C are some important functional groups of cellulose units. This technique clearly showed the changes in functional groups of lignin, cellulose and hemicellulose before and after pretreatment.

Changes in the functional groups of lignin in rice straw, bagasse and coirpith were analysed. During NaOH pretreatment, the chemical changes could be classified into three types:

(1) Disappearance of peaks. The peak at 1724-1730 cm^{-1} is assigned to the carbonyl (CdO) stretching unconjugated ketones. Carbonyls mainly exist in the side chains of lignin structural units and are also an important functional group in the side chains, which either aldehyde groups are lying in C-*γ* or keto lying in C- *β*. The disappearance of such peaks indicated that the side chain of lignin was broken down during NaOH treatment. The peak at 1320-1375 cm^{-1} is attributed to phenol hydroxyl stretching. The band disappeared after NaOH treatment because of the reaction of phenol hydroxyl with NaOH (Sekiguchi *et al.,* 2001). Decrease of functional group contents. The prominent peak at 1250-1260 cm^{-1} corresponds to methoxyl stretching. It was observed that, as compared to the untreated coirpith and bagasse, the content of methoxyl in the lignin of the NaOH and HCl treated one was decreased. This was mainly attributed to the nucleophilic reaction of methoxyl with NaOH. The peak at 1427 cm^{-1} represents C-H deformations (asymmetric in methyl, methylene, and methoxyl groups). Aromatic skeletal vibrations are assigned at 1631, 1513, and 1427 cm^{-1}. The contents of such peaks all decreased after NaOH treatment (Sosnowski *et al.,* 2003). Some bands such as the peaks at 1036 cm^{-1} appeared after NaOH and HCl pretreatment. This band represents aromatic ring deformation in the C-H plane. Its appearance implied that the content of aromatic compounds was increased. It was also observed that the absorption at 1167 cm^{-1}, which is indicative of ester bond stretching, disappeared after NaOH treatment. Such a reaction damaged the ester bond linkage between lignin and carbohydrate and released cellulose from the encapsulation of lignin, making more cellulose exposed and available for anaerobic microorganisms. The results from FTIR spectroscopies showed that NaOH and HCl pretreatment could not only break down the inter unit linkages but also change the functional groups of lignin. The changes of lignin structure would have certain impacts on the biodegradability of rice straw, bagasse and coirpith.

Changes of cellulose structures during pretreatment showed that the spectra of celluloses extracted from the untreated and 6% NaOH treated rice straw, 4% NaOH treated bagasse and 3%

HCl treated coirpith had similar profiles but different intensities of the absorption bands. The difference indicated that the structure of cellulose was changed after NaOH treatment, and the changes were both intra- and intermolecular. The intramolecular changes were represented by the decreases of functional group contents. Each glucose group of cellulose has three alcoholic hydroxyl groups. Hydrogen atoms and nearby oxygen atoms can form hydrogen bonds if their distance is less than 0.28-0.30 nm. 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). The content was obviously reduced after NaOH treatment, indicating the breakage of the linkages. This proved that NaOH was capable of breaking some intermolecular hydrogen bonds through complex chemical reactions. This would lead to the degradation of cellulose and make cellulose easier to be attacked by microorganisms, thus improving the biodegradability.

Hemicellulose is made up of a relatively limited number of sugar residues. The general formulas of hemicellulose are (C5H8O4)n and (C6H10O5)n, which are called pentosans and hexosans, respectively. There are β glycosidic linkages between the sugar units. FTIR spectroscopy analyses showed that the spectral profiles of the peak s in hemicellulose of the untreated and NaOH-treated rice straw, bagasse and HCl

treated coirpith were rather similar but the relative intensities of the peaks. This indicated that the two hemicelluloses had similar structures but different content of functional groups and linkages. The differences were the results from the intra- and intermolecular degradation of hemicellulose during NaOH pretreatment. The intramolecular degradation of hemicellulose was represented by the decreased contents of functional groups and the disappearance of some bonds after NaOH pretreatment. A strong broadband at 3422 cm^{-1} (Tashiro and Kobayashi, 1991) was found, which is attributed to the hydroxyl groups in the hemicellulose from both the untreated and NaOH-treated rice straws.

The intensity of the peak decreased after NaOH and NaOH treatment, because of the disruption and breakage of hydrogen bonds. In the carbonyl stretching region, the absorption at 1631-1641 cm^{-1} is principally associated with absorbed water. The linear and branched (1 - 4)-*β*-xylans, such as glucuronoxylan and arabinoxylans, showed the main peak maximum at about 1044 cm^{-1}. A small peak at 1512 cm^{-1} was found in two spectra, which is mainly due to the presence of a small amount of associated lignin in hemicellulose (Sun *et al.,* 2005). The peak at 1082 cm^{-1} corresponds to the C-OH bonding, which is strongly influenced by the degree of branching. The contents of the functional groups mentioned above all decreased after alkali treatment. However, some bands totally disappeared after NaOH treatment. The peak at 1252 cm^{-1} is indicative of CO stretching of syringyl units. The peak at 1155 cm^{-1} is characterized by the C-O-C vibrations in the anomeric region of hemicellulose. The two bands were found disappeared, elucidating that the structure of hemicellulose was changed after NaOH treatment; the hemicellulose was thus degraded. Intramolecular degradation was represented by the changes of the functional groups occurring in the hemicellulose structure as mentioned above. In the anomeric region (950-700 cm^{-1}), a small sharp peak at 791, 832 and 901cm^{-1} was observed. This peak corresponds to the C1 group frequency or ring frequency and is indicative of *β*-glycosidic linkages between the sugarunits. It was obviously reduced after NaOH and HCl treatment, indicating that the linkages between the sugar units were changed and intermolecular degradation occurred in the hemicellulose structure. According to Mansfield *et al.,*(1999) the lignocellulosic materials in the original form are relatively resistant to microorganism attack but the hemicellulose 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 lignocellulose were beneficial for bioethanol production.

6. Acknowledgement

We thank Srimad Andavan Arts and Science College, Trichy for providing the financial support.

7. Reference

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