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Bioconversion of cotton waste from textile mills to bioethanol by microbial saccharification and fermentation

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ABSTRACT

Energy flows from many sources, exists in a variety of interchangeable forms, and drives all systems. New and renewable sources of energy can make significant contribution, but their role and potential in the short term should not be overstated. It has been estimated that new and renewable sources of energy at present meet only 5-10 per cent of global energy-requirements, which may hopefully go up to 30% by 2050 A.D. Renewable energy resources and technologies have the potential to provide long-lasting solutions to the problems faced by the economic and environmental sectors of a nation. In the present work, cotton waste from textile mills were collected, pooled, processed and used as source for the production of bio-ethanol by microbial saccharification and fermentation process. The cotton waste was subjected to acid and alkali pretreatment to expose the sugars for further enzymatic hydrolysis by the cellulase enzyme produced from Trichoderma reesei. The results of pretreatment showed that the acid pre-treated substrate enhanced enzyme action and released more amount of sugar compared to the alkali pre-treated substrates. The amount of sugar released was found to increase with the increasing concentration of acid (110 mg/ml) or alkali (70 mg/ml). The sugars were then fermented with Saccharomyces cerevisiae to obtain alcohol and the amount of alcohol produced in batch and fed batch fermentation were 90 and 86 ml/l respectively. Thus, the results of the present work clearly revealed that the cellulosic cotton wastes could be converted into bioethanol with enzymatic hydrolysis followed by fermentation.

Keywords: Cotton waste, Bioconversion, Pretreatment, Bioethanol.

INTRODUCTION

Bio-ethanol produced from renewable biomass has received considerable attention in current years. Ethanol can be used as a gasoline fuel additive and transportation fuel. This in turn helps to alleviate global warming and environmental pollution. The decreasing reserves and increasing value of petrochemicals have renewed the interest in the production of bioethanol and its use as fuel and chemical feedstock. Bio-ethanol used as a source of energy is a more welcoming alternative fuel. It would be more than just complementing for solar, wind and other intermittent renewable energy sources in the long run [10].

Cellulosic materials are renewable natural biological resources and generation of biobased products and bioenergy from such substances is important for the development of humans. Various industries across the world generate huge volumes of cellulosic waste, which have an immense potential to be utilized for the production of several bio-products. These dedicated substances provide a low-cost and uniquely sustainable resource for production of many organic fuels and chemicals that can reduce greenhouse gas emissions, enhance energy security, improve the economy, dispose of problematic solid wastes, and improve air quality.

Cotton is one of the important cash crops of India and the cotton based textile mills situated especially in southern region generate huge volumes of fibrous cotton waste rich in cellulose everyday. Waste management is one of the biggest problems faced by the cotton ginning industry. Ginning one bale (227 Kg) of spindle harvested seed cotton lint contributes between 37 and 147 Kg of waste [14]. The general makeup of cotton gin waste consists of sticks, leaves, burs, soil particles, other plant materials, mote and cotton lint [13]. Slight differences in the proportions of the components are usually found between varying mechanical harvest methods [14]. The stripper harvesting method generates more waste than the spindle harvesting method. These cotton wastes containing minute fibers when been suspended in air may cause serious manifestations affecting mainly lungs. They are often dumped as such or incinerated.

Cellulose (40-60% of the dry biomass) is a linear polymer of glucose, the orientation of the linkages and additional hydrogen bonding make the polymer rigid and difficult to break. In hydrolysis the polysaccharide is broken down to free sugar molecules by the addition of water. This is also called saccharification. The product, glucose is a six-carbon sugar or hexose. It provides the major source for hexose in woody biomass. Cellulose is believed to have a highly crystallized structure due to the existence of hydrogen bonds. In contrast to its amorphous region, the crystalline region of cellulose makes it hard to hydrolyze [11]. Some scientist also explored ethanol production from cotton gin waste [4]. Based on approximations of the composition of the cotton plant, he developed a general design for 2-4 million gallons per year ethanol production plant. The idealized design considered simultaneous methane production, as well as avenues for recycling energy.

The study was focused on the following main objectives to make use of the huge volume of waste cotton for the production of alcohol. The cotton wastes samples were subjected to pretreatment and then saccharification was carried out by microbial cellulases. The liberated sugars were then fermented microbially and produced bioethanol was estimated and quantified.

MATERIALS AND METHODS

Substrate

Different types of cotton wastes used for the study were directly procured from Sri Kannapiran Mills Pvt. Ltd., Coimbatore, India. All the wastes (sweeping waste, dropping waste, comber noil, gutter waste, gin waste, hard waste) were pooled together, processed mechanically to reduce the length of fibres and remove the debris material contained in it. The fibers were then boiled in water at 100°C for 30 minutes. After removal from the boiling water, the fibers were rinsed with deionized water and air-dried.

Compositional analyses of the cotton waste

The compositional analyses of the cotton wastes namely, moisture content, ethanol extractives content, acid insoluble residues, ash content and sugar content were performed using standard methods. The moisture content of the processed cotton waste was determined by the solid determination method of ASTM E 1754-95 (ASTM, 1995). Similarly, the ethanol extractives were determined as described by ASTM 1690-95 (ASTM, 1995). The acid insoluble residue and ash fractions were also determined following the ASTM E1721-95 procedure (ASTM, 1995). The carbohydrate fractions of cotton waste were analyzed by gas chromatography with Supelco SP-2380 capillary column.

Pretreatment of cotton waste

About 200 ml of dilute sulphuric acid and sodium hydroxide was prepared with a concentration range of 0%, 0.5%, 1.0% up to 5.0% in separate 500 ml Erlenmeyer flasks. The flasks were added with 3g of processed cotton waste and autoclaved at 121°C for 30 minutes. The flasks containing the pre-treated cotton waste were then neutralized by washing with distilled water. The acid pre-treated and alkali pre-treated samples were dried separately for further analysis.

Compositional analyses of pretreated cotton waste

The moisture, ethanol extractives, acid insoluble residue and ash of the pretreated cotton waste were determined following the same procedures as the analysis of the cotton waste before pretreatment.

Cellulase Enzyme Production and Extraction

*Trichoderma reesei (*MTCC # 164) was cultured on Potato Dextrose Agar Plates, incubated at 28 ºC for seven days for the development of spores. The basal medium used for growth of *Trichoderma reesei* and cellulase production was Reese and Mandels Mineral salts solution containing a pH of 4. The flasks containing 200 ml of sterile basal medium was seeded with a spore suspension of the fungus and incubated at 28 ºC for 5 days. After 5 days of incubation, the flasks were retrieved from shaker. Culture broth was transferred to sterile centrifuge tubes, centrifuged until mycelia settled and supernatant was filtered in a sterile nylon cloth. Extraction was done under sterile conditions to prevent any microbial contamination. The crude filtrate containing enzyme was assayed for its activity in terms of filter paper units by DNSA method. About 2.5g of the untreated, acid and alkali pre-treated cotton waste were taken, added 0.5ml of the microbial cellulase enzyme and the amount of glucose released up on enzymatic hydrolysis was estimated. An enzyme blank was also prepared without adding substrate and flasks were

incubated at 50°C for 24 hours. The flasks were estimated for the sugar content at 0th hour and after 24 hours by means of DNSA method.

Determination of kinetic parameters

The apparent kinetic parameters (Vmax and Km) of the cellulase enzyme produced from *Trichoderma reesei* were determined by varying the concentration of CMC from 0.0 to 0.7 mg / ml in 0.1 M sodium phosphate buffer at pH 7.0. The assays were performed with the enzyme, which had been diluted appropriately with 0.02 M sodium phosphate, pH 7.0. Similarly, the effects of pH, temperature, cations and EDTA on the cellulase production were analyzed by growing the fungus in Reese and Mandels mineral salts solution under varying culture conditions. The varying culture conditions studied were pH (5.0 and 9.0), temperature (30 - 60ºC), cations concentration (Na+ and Mg++ at concentrations of 0, 10, 20, 30, 40, 50, 60 and 70 mM) and EDTA (0, 10, 20, 30, 40, 50, 60 and 70 mM). The enzyme produced under these conditions was assayed by their ability to release the sugars from the processed cotton waste.

Fermentation of released sugars for bio-ethanol production

Saccharomyces cerevisiae was selected for the fermentation of released sugars. The *Saccharomyces cerevisiae* was enriched in Saboraud's Dextrose Broth and used in the fermentation of the released sugars to alcohol.

1. Batch fermentation

Initially 1 litre of the pretreated, hydrolyzed substrate was prepared in distilled water at pH 4.5. The mixture was transferred to a 2 l fermentor (MDL 200 B.E. Marubishi, Japan) and autoclaved at 121°C for 15 minutes. After sterilization, an inoculum containing *Saccharomyces cerevisiae* (10 g/l) was added for fermentation. The process was conducted at 35°C for 24 hours, with mild agitation (100 rpm). Nitrogen gas was bubbled into the reactor at flow rate 100 ml/min to assure on anaerobic environment. Sodium hydroxide (3M) was used to maintain the pH.

2. Fed – batch fermentation

Initially 1 l of the pretreated, hydrolyzed substrate was prepared in distilled water at pH 4.5. The mixture was transferred to a 2 l fermentor (MDL 200 B.E. Marubishi, Japan) and autoclaved at 121°C for 15 minutes. After sterilization, an inoculum of *Saccharomyces cerevisiae* (10 g/l) was added and the fermentation was conducted under the same conditions used for batch fermentation. After 6 hours of fermentation, 100 ml of fresh medium previously sterilized containing 50 g/l of glucose was supplemented to the fermentation mash and the process was conducted up to 24 hours. The alcohol content of all the flasks upon incubation were estimated at 24 hours time intervals as outlined by Caputi *et al.,* (1968).

RESULTS AND DISCUSSION

Compositional analysis of the cotton waste

The different cotton wastes used in the present study were collected, processed mechanically and the chemical composition of the pooled cotton waste was determined according to the standard methods and the results were compared to the contents released after acid and alkali pretreatment (table 1).

Table 1. Compositional Analysis of Untreated Cotton Waste and Pre-treated Cotton Waste

Compositional Analysis	Before Pretreatment (%)	After Pretreatment (%)	
		Acid	Alkali
Arabinan	2.3	2.0	1.21
Xylan	9.4	10.41	8.53
Mannan	1.1	3.22	1.60
Galactan	2.4	3.54	1.33
Glucan	37.1	37.14	36.42
Total Sugars	52.3	56.31	49.09
Acid Insoluble Residues	28.8	29.51	25.96
Ash	10.5	3.26	3.03
Ethanol Extractives	7.7	7.38	9.82

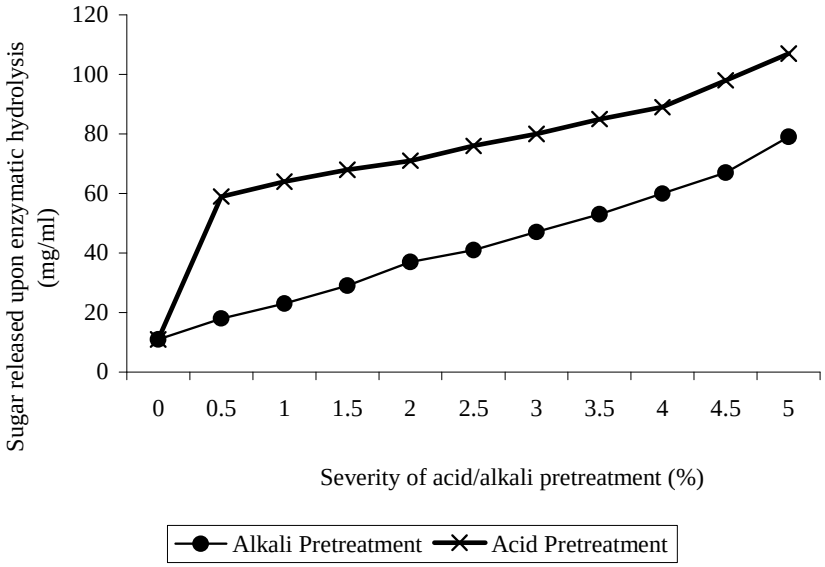
The result showed that acid pre-treated cotton waste was found to possess relatively higher percentage of total sugars (56.31%) compared to alkali pre-treated cotton wastes (49.09%). Similarly, the percentage concentration of glucan and acid insoluble residues was also found to be relatively higher for acid pre-treated (37.14% and 29.51%) than for alkali pre-treated cotton wastes (36.42% and 25.96%) respectively. The correlation coefficient between the acid and alkali pre-treated cotton waste was found to be 0.9944. It clearly shows the drastic decrease in xylan content of fibers with increasing steam explosion severity [8]. A gradual decrease in glucan content of fibers with increasing steam explosion severity can also be observed from the result. These observations agree with similar results obtained in previous works. It was also observed reported that a rapid decrease in xylan content of steam exploded yellow poplar was obtained with increasing treatment severity. Similar decreases in xylan content were seen for steam exploded wheat straw and steam exploded sugarcane bagasse. Characterization of substrate (cotton gin waste) has been done using standard methods. The main composition of cotton gin waste is as follows: 22.40% of acid soluble lignin, 26.80 % of cellulose, 32.10 % of Hemicellulose, 10.2 % of ash content & 9.9 % of moisture content [7].

Effect of pretreatment on the cotton waste

Acid and alkali pretreatment had great influence on the sugar release through enzymatic hydrolysis of the cotton waste. Figure 1 shows the amount of sugar released up on enzymatic hydrolysis of the acid / alkali pre-treated cotton waste.

From the figure, it was understood that the amount of sugar released upon enzymatic hydrolysis was greater for acid pre-treated cotton waste compared with alkali pre-treated cotton waste. As the percentage of acid or alkali used for pretreatment increased, the amount of sugar released also increased. Several pretreatment methods are reported in the literature such as physical chemical and enzymatic but sulphuric acid of about 0.5% is usually used at 150-185°C [6]. It was showed that the saccharification level has been increased to 33 in comparison to 4% saccharification of untreated wheat straw when the substrate was pretreated with 2% NaOH for 4 hours at room temperature [2].

Figure 1. Efficiency of Pretreatment on enzymatic hydrolysis of cotton waste



Determination of kinetic parameters

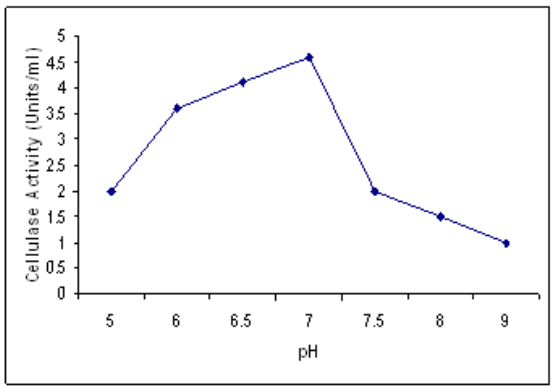
The effect of various kinetic parameters such as pH, temperature, cationic concentration and EDTA concentration on the activity of the purified cellulase enzyme was studied and the results are shown in figure 2.

From figure 2, it was clear that the maximum enzyme activity was observed at pH value of 7.0 and at temperature 35˚C. Similarly, maximum cellulase activity was found at 30 mM, 20 mM and 30 mM concentrations of sodium ions, magnesium ions and EDTA respectively. The work showed that the maximum glucose yield was at temperature 45°C and at pH 4.5 when *Saccharomyces cerevisiae* cellulase was used for saccharification and fermentation of distilled shochu mash [15]. The work showed that EDTA inhibited the activity of the cellulase enzyme. It was also observed that the cellulase activity decreases rapidly with increasing concentrations of EDTA i.e., as the concentration of EDTA increases, the cellulase activity decreases and eventually reaches zero at high EDTA concentrations [3].

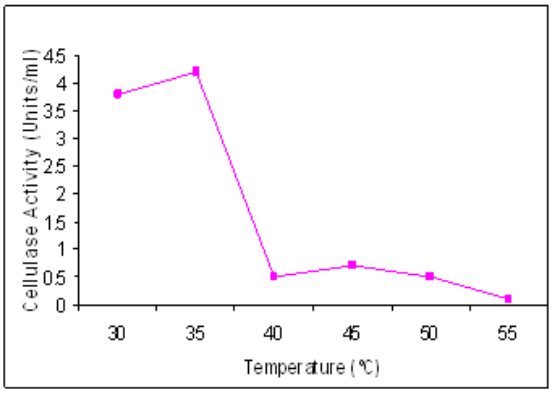
The purified cellulase from the studied yeast can be considered as unsusceptible to acidic and alkaline conditions because it showed activity in a broad range of pH between 3 and 9. The increase or decrease of pH values over or below the optimal value of 5.0 was not followed by a rapid loss of activity, because it kept more than 86 % of its activity at a pH value of 4. On the other hand, *Trichoderma reesei*, *Thermoascus aurantiacus* and *Bacillus circulans* were more on the acid side (pH 4.5). Data obtained from this study also revealed that EDTA as a chelating agent had no effect on the activity. This may rule out that cations are not involved in active catalytic site of the enzyme. The obtained results revealed that a stimulating effect on the cellulase activity was caused by Cu++ and Mn++ [9].

Figure 2. Determination of optimum kinetic parameters on the performance of the cellulase enzyme

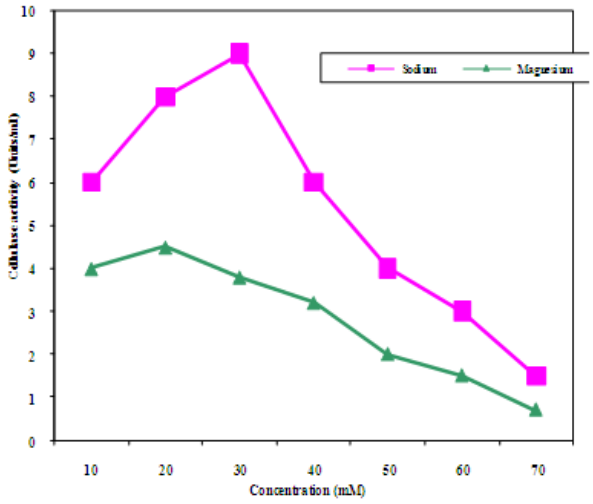
a. Effect of pH on Cellulase Activity



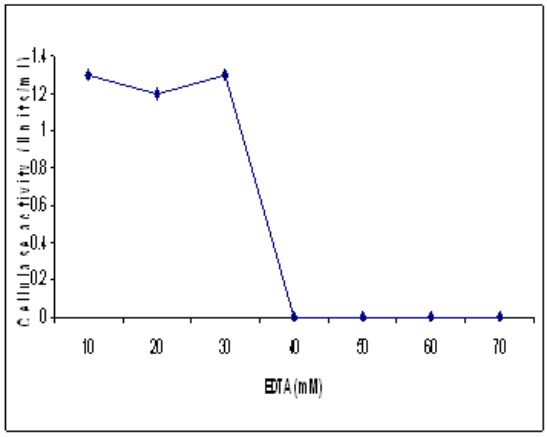
b. Effect of Temperature on Cellulase Activity



c. Effect of sodium and magnesium ions on cellulase activity



d. Effect of EDTA on cellulase activity



Fermentation of released sugar to bioethanol

The acid and alkali pre-treated substrates were then subjected to alcohol production to release fuel ethanol. The amount of alcohol produced upon action of *Saccharomyces cerevisiae* on the pre-treated and hydrolyzed cotton waste is presented in table 2.

From the table, it was clear that the amount of alcohol produced increases with increasing concentration of acid or alkali used for pretreatment and the was found that the acid pre-treated cotton waste was efficient in alcohol production compared to alkali pre-treated cotton waste. The correlation coefficient was found to be 0.8509 when the acid and alkali pre-treated samples were subjected to alcohol production. The *Saccharomyces* developed from toddy has shown substantial alcohol fermentation activity. it was established in the work that pH 4.25 and temperature 30˚C was the optimum values. The new yeast strain was able to ferment the sugar

solution containing at least 200 g/l sugar and 93 g/l ethanol was obtained with an ethanol yield based on sugar utilized of 0.47. Inhibition of product was observed above 200 g/l sugar solution.

Table 2. Alcohol Production from Pretreated and Hydrolyzed cotton waste

Acid / Alkali Pretreated samples (%)	Alcohol Produced (ml/l)	
	Acid Pre-treated samples	Alkali Pre-treated samples
0	1.9	1.9
0.5	11.5	3.3
1.0	12.1	4.1
1.5	13.5	5.1
2.0	13.9	6.8
2.5	14.2	7.3
3.0	14.3	7.4
3.5	14.6	7.9
4.0	15.0	8.1
4.5	15.6	8.4
5.0	16.0	8.7

High rate of fermentation time was reduced from 96 to 63 h to obtain optimum ethanol concentration of 48 g/l. The costly addition of yeast extract could be avoided and good growth and ethanol production were obtained using beef extract as medium component. The *S. cerevisiae* showed better results in terms of both ethanol concentration and ethanol yield compared to a bakers' yeast strain [12]. The alcohol production in batch and fed batch fermentation processes are given in table 3.

Table 3. Alcohol Production in Batch and Fed batch Fermentation process by *Saccharomyces cerevisiae*

Reactor (Volume)	Mode	Ethanol yield (ml/L)
Fermentor 2L	Batch	90
	Fed Batch	86

From the above results, it was understood that the ethanol yield was higher in batch fermentation compared to fed batch fermentation. Fermentation of the released sugars proved that ethanol could be produced from cellulosic cotton waste. From the 3.0 g processed cotton waste, about 100 mg/ml of glucose was released which, upon fermentation produces 16.0 ml/l ethanol. Pretreatment also had an influence on ethanol production. The acid pretreatment was found to be superior both directionally as well as statistically to that of alkali pretreatment in terms of ethanol production. Aeration is known to have a profound effect on yeast alcohol fermentation. Yeasts ferment sugars to ethanol primarily by way of the Embden-Meyerhof pathway. A small concentration of oxygen must be provided to the fermenting yeast, as it is necessary for the biosynthesis of certain polyunsaturated fats and lipids. Excess oxygen in the fermentation medium, on the other hand, will promote respiration and cell growth at the expense of ethanol productivity. Fermentation under aerobic conditions resulted in the least amount of ethanol production but gave the highest biomass yield compared with the other incubation conditions tried [1].

CONCLUSION

Energy security and climate change imperatives require large-scale replacement of petroleum-based fuels and improvement of vehicle efficiency. Most ethanol is currently produced by fermentation of either cornstarch or sucrose. Many avenues for the disposal or utilization of the wastes have been investigated throughout the years. The idea of recovering energy from cotton waste has been around for several decades. In the present study, different cotton wastes were pooled and their ability to release fermentable sugars for bio-ethanol production was studied. The cotton wastes were subjected to pretreatment process and the results indicate that the acid pre-treated cotton waste released more sugars upon enzymatic hydrolysis compared to the alkali treated cotton waste and the optimum condition for the production of cellulase from *Trichoderma reesei* was standardized. The 100 mg/ml reducing sugar released upon hydrolysis was subjected to alcohol production by fermentation with *Saccharomyces cerevisiae.* The alcohol production was studied both in batch and fed-batch fermentation and the results showed that the batch fermentation produced more alcohol compared to fed-batch process.

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