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Bio-Ethanol Production from Textile Cotton Waste via Dilute Acid Hydrolysis and Fermentation by *Saccharomyces cerevisiae*

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Abstract

The present work deals with the process bioconversion of cellulose from textile cotton waste, obtained from a textile industry, into ethanol by using the methods of acid pretreatment, hydrolysis and fermentation by *Saccharomyces cerevisiae*. The process includes the pretreatment method of the cotton waste fibers with dilute sulphuric acid and heating the mixture at a high temperature to break the crystalline structure of the cellulose to facilitate the hydrolysis of cellulosic component by dilute acids. The hydrolysis of the cellulose content into sugars is done by dilute acid hydrolysis that involves incubation period at a high temperature for 72 hours. Finally, the fermentation of the hydrolyzed waste is done using *Saccharomyces cerevisiae* under proper incubation conditions to produce ethanol.

Key Words: Textile cotton waste, Cellulose, Bio-ethanol, Pretreatment, Acid hydrolysis, Fermentation

Introduction

Ethanol is a renewable substitute of petroleum fuels such as petrol and gasoline. Presently, major portion of the ethanol produced worldwide is produced by the fermentation of sugars obtained from molasses, cereals, and fruits. The process of obtaining ethanol from various cellulosic wastes has been worked out in the past (Stephen L. Rush et al; Clifford Bradley et al) [1, 2]. Pilanee Vaithanomsat et al [3] have utilized steam explosion method for the pretreatment of the sunflower stalks for bio-ethanol production. Addition of catalyst such as sulfuric acid has been found to improve the pretreatment (K. Pakhala, et al) [4]. However, the steam explosion method is expensive and requires more capital, so chemical methods of pretreatment have been developed as alternative. Chemical pretreatment of cellulosic materials is done by using chemicals such as dilute acid, alkali, organic solvent, ammonia, sulfur dioxide, carbon dioxide or other chemicals to make the biomass more digestible by the enzymes. Keikhosro Karimi et al [5] have used chemical pretreatment method by using dilute H₂SO₄ for ethanol production from rice straw. The pretreatment can be done by using sodium hydroxide after it is finely ground (Fatma, H. et al) [6]. Cellulose, the most abundant polysaccharide on earth, is a highly ordered polymer of cellobiose representing over 50% of the wood mass (Elba P.S. Bon et al) [7]. Hydrolysis is the process of breakdown of cellulose into cellobiose and glucose, which can be accomplished either by enzymes or by acid (Qian Xiang et al) [8]. The enzymatic hydrolysis is accomplished by mixing the pretreated material with enzymes such as cellulase and beta-glucanase produced by microorganisms. Fatma H. et al [6] have utilized *Trichoderma Reesei* for enzymatic hydrolysis. The dilute acid process involves a solution of about 1-2 percent

sulfuric acid concentration at a high temperature. Nutawan Yoswathana et al [9] have utilized dilute sulfuric acid (1-9%) for the hydrolysis of rice straw for ethanol production. The major advantage of dilute acid hydrolysis is that it is quicker than concentrated acid hydrolysis and hence can be used as a continuous process. *The disadvantage of this method is that the sugar conversion efficiency is only about 50%. Also due to high temperature and pressure, large portion of the sugars is degraded which is not fermentable.*

H. D. Zakpaa et al [10] have utilized the SSF method for the production of bio-ethanol from corncobs. Also different types of microorganisms have been used. Many genetically engineered microorganisms are developed that can utilize all five of the major biomass sugars – glucose, xylose, mannose, galactose and arabinose. *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Aspergillus niger* are some of the widely used microorganisms for ethanol fermentation.

Any material having cellulose as one of its constituents may be used to produce bio gas. India is one of the countries having a large number of textile. The country consumes cotton fibers approximately 26 million tons per year, of which approximately 0.21 million tons of cotton waste is generated during yarn manufacture. (C. Sundar Raj et al) [12]. In India, where adequate disposing technology is not available, the cotton waste generated is mostly disposed off by burning. This may increase the CO₂ level in the atmosphere and also pollutes the surrounding areas. This powder waste also leads to the growth of harmful bacteria, which induce allergic reaction in human. Cotton waste is of high solid powder content and is rich in cellulose. The present paper describe a method for production of ethanol from textile cotton waste by the

conversion of cellulose into reducing sugars and fermenting the sugars into ethanol.

Materials and Methods

The textile cotton waste was collected from Woolworth Limited, Raipur. The length of the fibers was initially reduced to 0.5-0.6 inches and soaked in water for overnight to remove dirt and other particulates.

Saccharomyces cerevisiae was isolated from rotten grapes using techniques such as serial dilution and spread plate method using Potato-Dextrose Agar Medium and identified by observing the cells under a compound microscope. Pure culture of the yeast was prepared on Saubourd's Agar Media by keeping the plate at 37°C for at least 4 days and was maintained on YPD (yeast extract/peptone/dextrose) agar slants sealed with sterile mineral oil at room temperature (~25 °C). The pretreated waste cotton was hydrolyzed by dilute acid hydrolysis method. Anaerobic batch fermentation of 200 ml broth media consisting of pretreated and hydrolyzed textile cotton waste was carried out in order to convert the released sugars into ethanol, the conversion process being accomplished by the enzymes released by *Saccharomyces cerevisiae*. The pH of the solution was brought to 4.2 by adding required amount of 4 M NaOH to accommodate yeast growth. Triplicate fermentation broths of same composition were prepared and incubated in the same conditions. The fermentation was continued for 9 days and samples were taken from each of the three broths on each alternate day for analysis to get triplicate results.

Cellulose estimation was done by Anthrone method as explained by Sadasivam and Manickam in Biochemical Methods^[13]. Instead of just taking 1ml of the diluted solution, a series of volumes (say 0.4 to 2ml corresponding to 40- 200 µg of the cellulose) was taken and developed the color. The sugar estimation was done by was done by Dinitrosalisyllic acid (DNS) method (Biochemical methods)^[13].

Rate of hydrolysis and Cellulose Conversion (C.C) as a result of hydrolysis were calculated using the estimated data. The following formulae by Arthe R. et al ^[14] were used for calculating these two parameters:

Rate of hydrolysis:

$$V = \frac{ds}{dt} = \frac{Glucose_t - Glucose_{t_0}}{t - t_0}$$

Where, *Glucose_t* is the concentration of glucose after time *t*, and *Glucose_{t₀}* is the concentration of glucose before hydrolysis. *t* and *t₀* are the final and the initial time in hours respectively.

The cellulose conversion (C.C) percentage:

$$C.C = \frac{(Glucose_t - Glucose_{t_0}) \cdot 100}{C}$$

The ethanol estimation was done by a modified form of potassium dichromate method as described by Caputi et al ^[15] while glucose estimation was done by DNS method.

Results

The concentration of cellulose in the textile cotton waste after pretreatment step was estimated to be 147.70 mg/g of

sample. The remaining amount was impurities and other synthetic fibers present in the textile cotton waste. The amount of glucose in the unhydrolyzed cotton waste was estimated to be 0.01 mg/g of sample which is negligible. After hydrolysis with 2% sulfuric acid, the amount of glucose in the material increased to 2.60 mg/g of sample, after 3% sulfuric acid, the amount of glucose in the material increased to 9.50 mg/g of sample, and after hydrolysis with 5% sulfuric acid the amount of glucose increased to 12.45 mg/g of sample (Figure-1). The rate of hydrolysis (Figure-2) is calculated. The estimation of glucose and ethanol concentration in the broth was done by drawing standard graphs with the help of a UV-spectrophotometer as explained earlier.(Fig. 3 and 4). The fermentation broth contained about 5.85 mg/ml of glucose in the first day. Glucose was estimated in the fermentation broth on alternate days. After 9 days of fermentation the amount of glucose remained was about 2.3 mg/ml as shown in Table 1. The day wise consumption of glucose in the fermentation broth is shown in Fig. 5. Similarly, the estimation of ethanol in the broth after 1 day showed a concentration of 0.016 ml/ml which gradually increased to 0.079 ml/ml in 9 days, as shown in Table 2. The day wise increase of ethanol in the fermentation broth is shown in Fig 6. Distillation of the broth was done by simple distillation method and at the end of the process about 14.50 ml of ethanol was recovered from 250 ml fermentation broth. The ethanol recovered can be purified by further techniques.

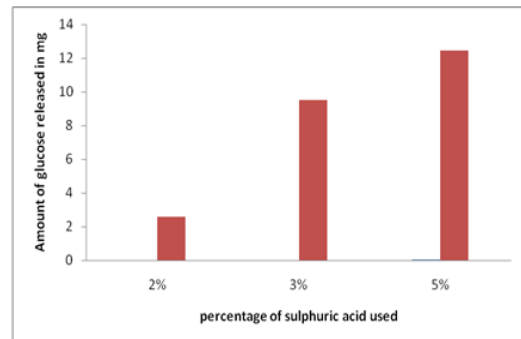


Figure 1 Shows the amount of glucose released as a result of hydrolysis after 3 days with 2%, 3% and 5% H₂SO₄

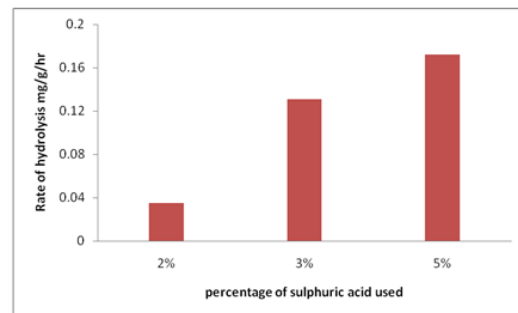


Figure 2 Comparison of the rates of hydrolysis with 2% , 3% and 5% H₂SO₄ . Clearly rate of hydrolysis is more when hydrolysis is done with 5% H₂SO₄

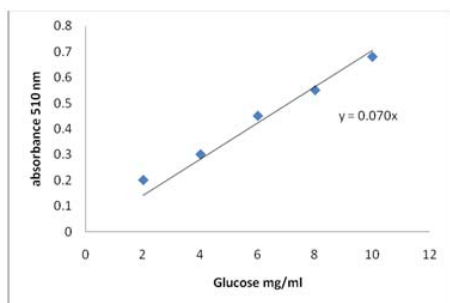


Figure 3 Standard graph for Glucose estimation by DNS method

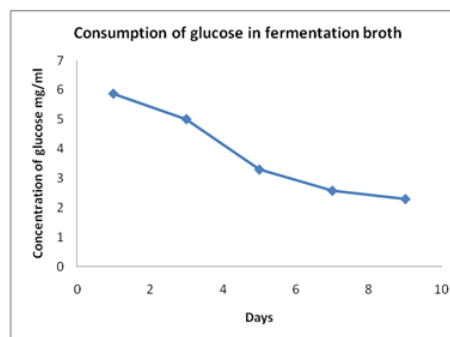


Figure 5 Day wise estimation of glucose in the fermentation broth

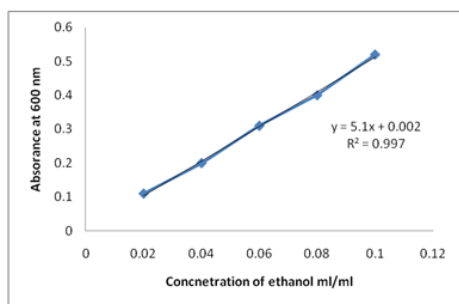


Figure 4 Standard graph for ethanol estimation by Potassium Dichromate method

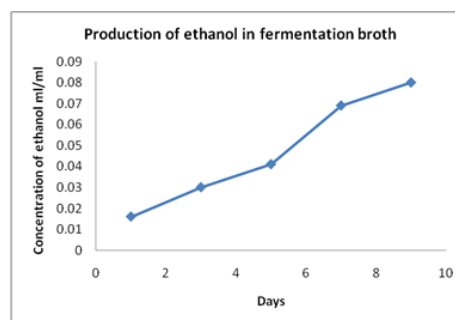


Figure 6 Day wise estimation of ethanol in the fermentation broth

Table 1 Day wise estimation of glucose in the fermentation broth in triplicates

Sample collected on		Optical Density			Conc ⁿ of glucose, mg/ml		
		1 st Flask	2 nd Flask	3 rd flask	1 st flask	2 nd flask	3 rd flask
Day 1	Glucose estimation by DNS Method	0.42	0.41	0.39	6.00	5.86	5.57
Day 3		0.37	0.35	0.32	5.29	5.00	4.57
Day 5		0.24	0.23	0.22	3.43	3.29	3.14
Day 7		0.17	0.18	0.19	2.43	2.57	2.71
Day 9		0.15	0.16	0.17	2.14	2.29	2.43

Table 2 Day wise estimation of ethanol in the fermentation broth in triplicates

Sample collected on		Optical Density			Conc ⁿ of ethanol, ml/ml		
		1 st flask	2 nd flask	3 rd flask	1 st flask	2 nd flask	3 rd flask
Day 1	Ethanol estimation by Dichromate method	0.078	0.080	0.081	0.015	0.016	0.016
Day 3		0.153	0.154	0.156	0.030	0.030	0.031
Day 5		0.190	0.210	0.225	0.037	0.041	0.044
Day 7		0.345	0.350	0.350	0.068	0.069	0.069
Day 9		0.380	0.410	0.425	0.075	0.080	0.083

Discussion

Textile cotton waste has been found to be a good raw material for cellulosic ethanol production. The pretreatment and acid hydrolysis of the cotton waste to convert the cellulose into reducing sugars has shown positive results. The test for glucose in hydrolyzed and unhydrolyzed sample was carried out and the results suggest a positive outcome for cellulose hydrolysis. It is also confirmed that, the amount of released sugars upon hydrolysis increase with the concentration of acids used in the acid hydrolysis. The rate of hydrolysis of cellulose in the cotton waste was found to be 0.172 and 0.035

mg/ml per hr when hydrolyzed with 5% and 2% H₂SO₄. So, by using 5% H₂SO₄ we can achieve better rate of hydrolysis as well as cellulose conversion without seriously affecting the pH. Hydrolysis with 5% H₂SO₄ shows 8.4% conversion of cellulose and the estimation of glucose in the unhydrolyzed samples shows that the cotton waste does not have reducing sugars in it. So, hydrolysis produces enough amount of reducing sugars upon acid hydrolysis by dilute acids. The released sugars were fermented for 9 days and the estimation of ethanol after each alternate day in the cotton waste fermentation broth shows that the amount of ethanol increase each day, along with a regular

decrease in the sugar content of the broth showing that large part of glucose was utilized by *Saccharomyces cerevisiae* and converted to ethanol. It was estimated that at the end of 9th day of fermentation, atleast 7-8 % ethanol was produced in the fermentation broth approximately.

The concentration of glucose broth was estimated to be around 5.85 mg/ml in the hydrolyzed textile cotton waste which was reduced to 2.30 mg/ml at the end of fermentation. The rate of consumption of glucose is lesser in the first 2 days of fermentation, but increased during the next 4 days. Although the production of ethanol is slow, sugars released due to hydrolysis of the cotton waste can be easily fermented by *S. cerevisiae* into ethanol. About 14.5 ml ethanol was successfully recovered from the broth by simple distillation method which shows that the broth can be easily distilled to recover and purify ethanol.

Conclusion

It can be concluded that textile cotton waste has a capability to undergo acid hydrolysis and fermentation for production of bio-ethanol. Cotton waste is a good source of cellulose and can be utilized for cellulosic ethanol production. In the future the textile cotton waste, which is a waste byproduct of the textile industry can be used as a good raw material for ethanol production and also solve the problem of safe disposal of the byproduct. In the future further improvements in the process can be carried out to enhance the productivity.

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