Research Article

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DEGRADATION OF TAMARIND SEED XYLOGLUCAN FOR THE PRODUCTION OF BIOETHANOL

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Abstract: Ethanol serves as the best alternative energy resource. Xyloglucan, a major polysaccharide in tamarind seed had to be broken down for the release of monomers which could be used as substrate for ethanol production. Tamarind Kernel Powder (TKP) was subjected to various pretreatment methods like acid treatment and physiochemical treatment for the breakdown of xyloglucan where 31.9g/l and 41.7g/l of reducing sugars were released respectively. The acid treated hydrolysate was detoxified to remove the inhibitory compounds and then subjected to fermentation by *Pachysolen tannophilus*. It was found that 11% of ethanol was produced from acid pretreated TKP.

Keywords: TKP, Pachysolen tannophilus, Xyloglucan

INTRODUCTION

The increased emission of greenhouse gases like CO2 into the atmosphere due to the utilization of energy obtained from fossil fuel is the main reason for the rapid climatic change. As per the report published in 2003 by the European Union, by 2030 the amount of CO2 emitted will be double the level that was emitted during 1990 [1]. The increase in population and industrialization has led to an increase in demand for energy resources such as oil and petroleum. The report from International Energy Agency (IEA) states that there will be an increase in oil demand of about 1.3% per annum between 2005 and 2030 [2]. These lead to an urgent need for the development of alternative energy source that is obtained from non-fossil origin.

Ethanol can serve as the best alternative resource [3]. Maize, corn, sugarcane, tapioca starch are the most commonly used raw materials for the production of bioethanol [4]. During 2004 nearly 32 million tons of maize cultivated in U.S was utilized for the production of bioethanol which accounts for 12% of its total maize production [2]. On using such crops for the production of ethanol, limits the usage of agricultural land for the production of food crops to meet the food demand and also increases the cost of ethanol production. The substrate used for the ethanol production determines its cost. To overcome these problems the waste from the agro industries such as crop residues, seeds, grasses, saw dust, wood chips can be used as raw materials for the production of ethanol [3].

Tamarindus indicus is a widely grown tree in the tropical regions. Nearly 2, 30,000 tonnes of tamarind fruits are harvested annually and are mainly composed of 55% pulp, 33.9% seeds, 11.1% shells and fiber [5]. The tamarind kernel powder production in India is about 20,000 tonnes [7]. The seed kernel comprises of carbohydrates (73.68%), proteins (14.38%), ash (3.28%) and moisture (8.67%) [7]. Xyloglucan is the major polysaccharide found in the tamarind seed. It is a heteropolymer made up of galactose, xylose and glucose in the ratio of 1:2.25:2.8. The backbone of xyloglucan is made up of β-(1,4)-glucan in which at certain positions it is substituted with α-D-xylopyranose and β-D-galactopyranosyl-(1,2)-α-D-xylopyranose [9]. The production of bioethanol from any hemicellulosic wastes involves three main steps namely pretreatment, hydrolysis and fermentation [7].

The main objective of pretreatment is to have high yield of sugar at cheaper cost [2]. So pretreatment is the most essential step in ethanol production. Pretreatment techniques include physical or mechanical, chemical and enzymatic methods. Combination of treatment can also be adopted. The selection of pretreatment method is mainly based on the nature of the feedstock used and the interested fraction of the feedstock [6]. By adopting an appropriate pretreatment method xyloglucan can be degraded to give xylose, galactose and glucose. This could be further subjected to fermentation for ethanol production. Of various chemical pretreatment methods, acid pretreatment is the conventional method for ethanol production from wood and agricultural wastes [7]. Mineral acids like HCl and H_2SO_4 are used frequently for the acid hydrolysis. The key factors responsible for the formation of toxic compounds are the temperature and the acid concentration used. These toxic compounds hinder the ethanol production. Detoxification plays crucial role in removing those compounds before using the hydrolysate for fermentation [10].

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Thermal treatment includes steam explosion and liquid hot water treatment. In case of steam explosion, at high temperature the pressure is built up and then drastically reduced which may result in the hydrolysis of polysaccharide and also the formation of acetic acid [2]. The pre impregnation of substrate with acids may increase the efficiency of hydrolysis. This kind of pretreatment may decrease the time and temperature required for the hydrolysis [1].

There are various methods available for detoxification among which, over liming and neutralization proves to be the best alkaline detoxification method. Over liming with Ca $(OH)_2$ is more efficient than using NaOH [11].

The final step in ethanol production is fermentation by using the pretreated and detoxified hydrolysate as the substrate. Fermentation is carried out using *Pachysolen tannophilus* MTCC (1077) which uses xylose as the major carbon source for its growth and production of ethanol [12].

The present study deals with the various pretreatment methods for the breakdown of xyloglucan and further fermentation of hydrolysate for the production of ethanol.

MATERIALS AND METHODS

The Tamarind seeds were collected from the market place at Pollachi, Tamilnadu, India.

Microorganism Used:

Yeast species was obtained from IMTECH, Chandigarh (*Pachysolen tannophilus* MTCC 1077).

Decortication of tamarind seed:

Tamarind seeds were decorticated based on the procedure as per Panigrahi *et al.*, [13]. Tamarind seeds were washed and kept in water bath maintained at 90°C for 2 h. The seeds were then repeatedly washed to remove the seed coat and dried in hot air oven at 80°C for 4 h. Dried seeds were grinded well and sieved to get Tamarind Kernel Powder (TKP).

Hydrolysis of TKP:

Acid treatment: To 20 ml of 10% (w/v) Tamarind Kernel Powder, acids at varying concentration from 4% to 7% HCl and 3% H_2SO_4 were added and kept in the water bath maintained at 80°C. The acid treated samples were withdrawn at varying time intervals (30, 60, 90 and 120 min) and centrifuged at 10,000 rpm for 10 min. The supernatant was subjected to DNS test to estimate the amount of reducing sugars released from the acid hydrolyzed TKP.

Thermal treatment: Tamarind Kernel Powder of about 10% (w/v) was subjected to steam treatment at varying temperature (120°C, 160°C, 200°C, 270°C). The samples were withdrawn at varying time intervals (10, 20, 30min) at each temperature. The steam treated samples were centrifuged at 10,000 rpm for 10min. The supernatant was subjected to DNS test to estimate the amount of reducing sugar released from the steam treated TKP.

Physiochemical treatment: To 10% (w/v) of TKP 0.5% and 0.9% H_2SO_4 were added and kept undisturbed for 16 h at room temperature. The above samples were steam treated at 270°C for varying time intervals (10, 20, 30min). The treated samples were centrifuged at 10,000rpm for 10 min. The supernatant was subjected to DNS test to estimate the amount of reducing sugar in hydrolyzed sample. The same procedure was carried out for HCl at varying concentration (0.5% to 4 %).

Estimation of reducing sugar in the hydrolysate:

The amount of reducing sugar in the hydrolyzed TKP was estimated by 3, 5-Dinitrosalicylic acid method (3, 5 DNS) as reported by [14].

Detoxification of hydrolysate:

The hydrolyzed TKP was centrifuged at 10,000 rpm for 15 min to obtain the acid hydrolysate. Detoxification procedure to remove inhibitory compounds was carried out based on the procedure followed by Palmqvist *et al.*, [15]. The pH of the hydrolysate was adjusted to 9.5±0.5 by adding 1M Ca $(OH)_2$ (over liming) and then centrifuged to remove the precipitate containing the toxic compounds. The pH was then readjusted to 5.5 by adding 1M H_2SO_4.

Fermentation:

The culture was grown in glucose yeast extract media. 4% of one day old culture was then inoculated into fermentation media and incubated at 30°C (16). The fermentation media contains yeast extract 5(g/l), peptone 5(g/l), ammonium phosphate 5(g/l), magnesium sulphate 0.2(g/l) dissolved in detoxified hydrolysate. The sample was withdrawn for every 4 h interval and subjected to ethanol estimation. The production of ethanol was found to be between 16-32 h of incubation.

Estimation of ethanol:

The amount of ethanol in the fermented TKP solution was estimated by using acid dichromate method as reported by Pramanik *et al.*, [17].

RESULTS AND DISCUSSION

Acid treatment:

The amount of glucose, xylose and galactose released from the pretreated TKP was estimated from the respective standard curves and it was found that the maximum sugars were released when TKP was treated with 4% (0.48N) HCl at 80°C for 120min

liberating 31.9 g/l of reducing sugars (Figure.1). In order to increase the rate of hydrolysis, TKP was subjected to physicochemical treatment.

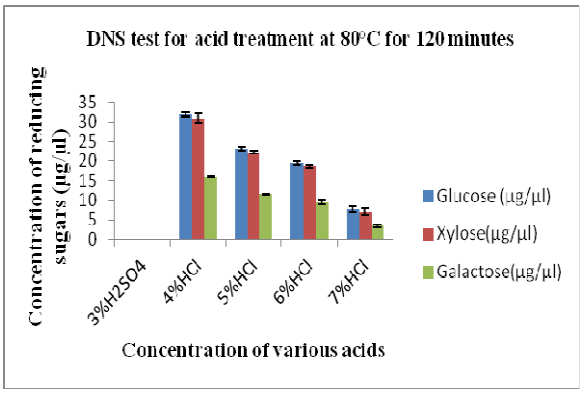

Fig.1: Concentration of reducing sugars (Glucose, Xylose, Galactose) at 80°C for 120 min.

Physiochemical treatment:

On treating TKP with 4% (0.48 N) HCl overnight along with steam treatment at 270°C for 20 min releasing 41.7 g/l of reducing sugars which was better than acid treatment (Figure 2). Chen *et al.,* [6] reported that 45 g/l of reducing sugars was released on treating TKP with 2N H_2SO_4 at 120°C for 30 min.

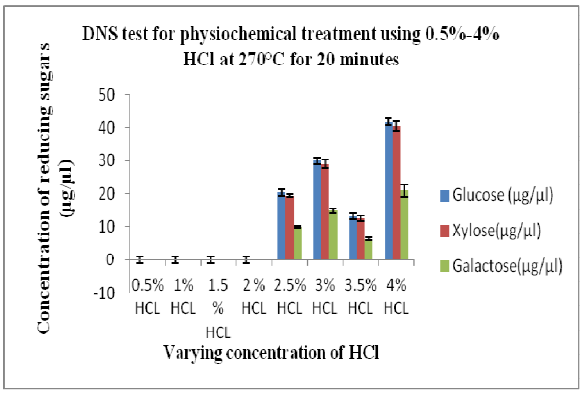

Fig. 2: Concentration of reducing sugars (Glucose, Xylose, and Galactose) soaked in 0.5% to 4% HCl overnight and steam treatment at 270°C for 20 min

Ethanol production:

Detoxified acid hydrolysate was subjected to ethanol production using *Pachysolen tannophilus*. The samples were withdrawn for every 4h interval, centrifuged and the supernatant was subjected to ethanol estimation by acid dichromate method. Palmqvist *et al.,* [15] reported that ethanol production started from 12th h of fermentation. The peak in 4th h may be due to interference from the components present in the media during the estimation of ethanol. The amount of ethanol produced was estimated from the respective standard curves and it was found that maximum volume of 11% of ethanol was produced at the 20h (Figure.3). Menon *et al.,* (2010) reported that 14 g/l of ethanol was produced using TKP as the major carbon source by *Debaromyces hanseii* [7].

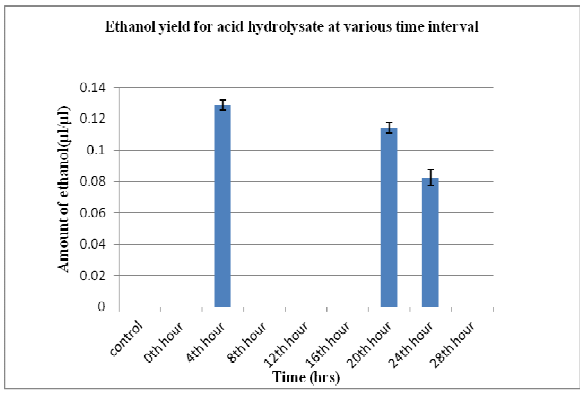

Fig. 3: Amount of ethanol produced from the acid hydrolysate

CONCLUSIONS

Three kinds of treatments were carried out in order to break the xyloglucan and the hydrolysate was detoxified and used for ethanol production. No sugars were released during thermal treatment. In case of acid treatment, the maximum hydrolysis of TKP occurred when it was treated with 4% HCl at 80°C for 120 min which resulted in the release of 31.9 g/l of reducing sugars. After detoxification, the acid treated sample was subjected to ethanol production where 11% (v/v) of ethanol was produced at 20th h of fermentation by *Pachysolen tannophilus.* Physiochemical treatment by soaking TKP in 4% HCl overnight and then treating the same at 270°C for 20 min released 41.7 g/l of reducing sugars. There was no production of ethanol in physiochemically treated TKP. This may be due to the accumulation of inhibitory and toxic compounds.

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