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Research Article

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Effect of different pretreatment methods on production of reducing sugars from tamarind kernel powder

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ABSTRACT

Bioethanol can be used as a second generation advanced biofuels. Currently it is mainly produced from starch but bioethanol production from starch leads to competition for food, land and price. Therefore, ligno-cellulosic agricultural residues are potentially used for bioethanol production to solve such challenges. The efficiency of the fermentation process mainly depends on the amount of reducing sugars which is further enhanced by selecting an efficient pretreatment process. In the present work Tamarind seeds have been chosen as the substrate. The yield of bioethanol mainly depends on the yield of reducing sugars which is again dependent on the various pretreatment methods used. So, the proposed work aims to carry out different pretreatment methods to identify the best pretreatment method for enhancing the yield of reducing sugars. The tamarind kernel powder will be extracted from tamarind seeds and the extracted tamarind kernel powder is subjected to various pretreatment methods like acid pretreatment, alkaline pretreatment and steam explosion. The amount of reducing sugars obtained, were then determined by di-nitro salicylic acid method. It was found that acid pretreatment with 0.3N HCl and 0.3N H_2SO_4 is the best pretreatment method among the selected pretreatment methods.

Keywords: *Tamarind kernel powder, Pretreatment, Ligno-cellulosic agricultural residues*

INTRODUCTION

Due to the increasing population there is an increasing demand for energy which has led to over exploitation of non-renewable energy resources. Therefore, there is a need towards the utilization of all the renewable sources of energy available. Currently bioethanol is mainly produced from starch but its production from starch leads to competition for food, land and price. Therefore, ligno-cellulosic agricultural residues are being potentially used for bioethanol production as they are natural, renewable, economicaland sustainable. Theprocess of biofuel production from lignocellulosic waste comprisesofpre-treatment, hydrolysis and fermentation. The importance of pre-treatmentprocess is that it enables efficient hydroxylation of the cellulose with solubilisation of hemicellulose by breaking the lignin, covering the cellulose and hemicellulose, which hinders the hydrolysis and fermentation. The fermentation of lignocellulosic biomass yields bioethanol and it has been found that the yield of bioethanol directly depends on the amount of reducing sugars present in the sample[1]. The main objective of pre-treatment is to have high yield of reducing sugar at low cost. Different pre-treatment methods available are acid pre-treatment, basic pre-treatment, steam pre-treatment, ammonia fibre explosion and ionic liquid pre-treatment. In the present work Tamarind Kernel Powder (TKP) has been selected as a ligno cellulosic substrate. The TKP is widely produced in India and its production was found to be about 20,000 tonnes[1].The seed kernel comprises of carbohydrates (73.68%), proteins (14.38%), ash (3.28%) and moisture (8.67%). Xyloglucan a major polysaccharide found in the tamarind seed is a heteropolymer made up of galactose, xylose and glucosein the proportion of12.18%,27.40% and 34.10% respectively. Its major portion contains carbohydrates which can be broken down into -reducing sugars. Reducing sugars then directly can be fermented to obtain bioethanol.Different pre-treatments were conducted with stipulated amount of TKP. Tamarind kernel powder solution was prepared then it was subjected to pre-treatment. In the current work TKP solution was acid pre-treated, base pre-treated and steam pre-treated. Pre-treated TKP solution

was heatedand centrifuged. The supernatant was removed after centrifuging and was subjected to DNSA (Di-Nitro Salicylic Acid) test. Optical density for different solutions were calculated and concentration of reducing sugars was determined for each of the solution and yield of reducing sugars obtained from different pre-treatment methods were compared.

EXPERIMENTAL SECTION

The Tamarind Kernel Powder was collected from Sri Balasanka Mill, Theni, Tamil Nadu, and India. In our work the following pre-treatment methods were chosen namely: acid pre-treatment with 0.5N HCl and 0.5N H_2SO_4, acid pre-treatment with 0.3N HCl and 0.3N H_2SO_4, basic pre-treatment with 0.5N NaOH and steam pre-treatment at 108^0C.
The general procedure formulated for all the pre-treatment methods is as follows: Firstly 10% TKP solution was prepared and then subjected to the selected pre-treatment method. The pre-treated solution was heated to 80^0C in a water bath as shown in Figure 1. The solution was then centrifuged at 10000 rpm for 30 minutes and then the supernatant was removed. The supernatant obtained was subjected to DNSA (di-nitrosalicylic acid) test as shown in the Figure 1. The optical density of the samples was recorded from UV-VIS spectrometer at 540 nm.

Figure 1:TKP solution kept in water bath at 80^0C ,DNSA testof the sample solution

Acid Pretreatment 0.5N HCl and 0.5 N H_2SO_4

10% TKP solution was prepared.0.5 N HCl and 0.5 N H_2SO_4 solution was prepared.50 ml each of 0.5 N HCl and 0.5 N H_2SO_4 was added to the 10 % TKP solution. The pre-treated solution was heated to 80^0C in a water bath. The solution was then centrifuged at 10000 rpm for 30 minutes and then the supernatant was removed. The supernatant obtained was subjected to DNSA (di-nitrosalicylic acid) test. The optical density of the samples was recorded from UV-VIS spectrometer at 540 nm.

Estimation of reducing sugars using Di-nitrosalicylic Acid (DNSA) method

Using a micro pipette 0.1ml, 0.5ml and 1 ml of hydrolysate obtained after pretreatment from each tubes were pipetted into test tubes and where made up to 3 ml by adding distill water. The test tubes were heated for 3 minutes and 3ml of DNSA reagent was added and where further heated in a water bath for 10 minutes. A glucose standard curve was plotted by taking a 0.2g/L solution of glucose. Glucose solution and water were added to 3ml of DNSA solution. The solutions were heated in a water bath and once there was a color change to dark red, 1ml of 40% Rochelle salt solution was added and after 5 minutes their optical density values were measured at 540 nm as shown in Table 1.

Table 1: Optical density values for standard glucose curve

Sl.No	Standard solution (ml)	Distilled water (ml)	Concentration (microgram/ml)	Optical density
Blank	0	3	0	-
1	0.5	2.5	100	0.039
2	1.0	2.0	200	0.091
3	1.5	1.5	300	0.196
4	2.0	1.0	400	0.303
5	2.5	0.5	500	0.484

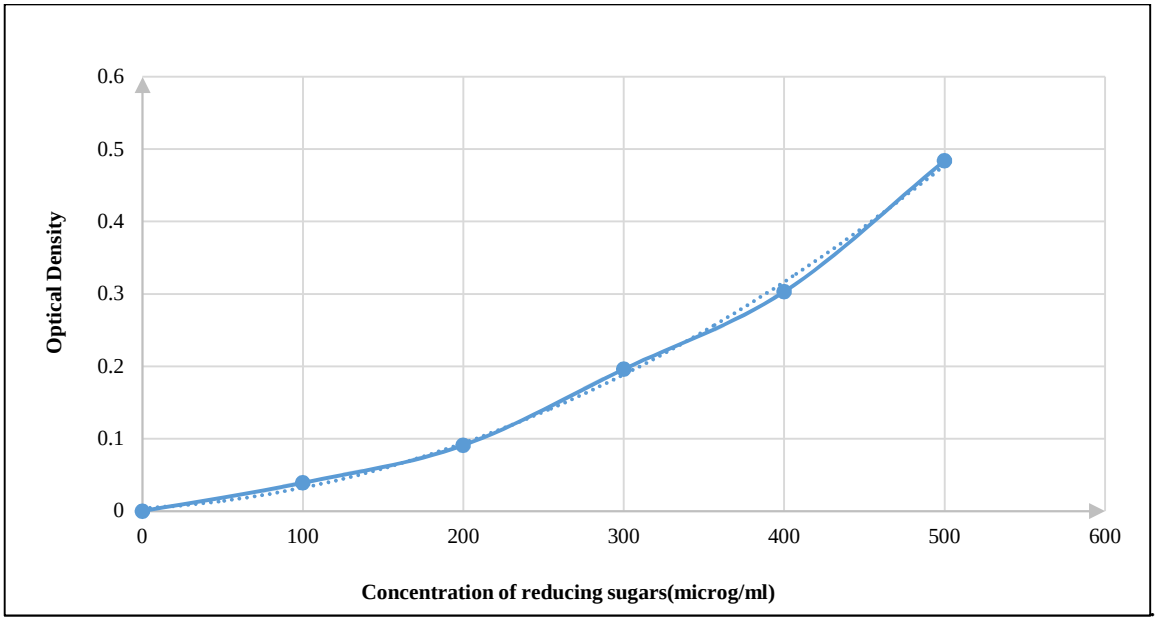

Figure 3: Standard glucose curve used to find out the reducing sugar concentration

RESULTS AND DISCUSSION

From the optical density values obtained by using UV-VIS spectrophotometer the corresponding reducing sugar values were estimated from the calibration graph as shown in Figure 3. From the obtained reducing sugar values the corresponding yield values have been calculated as follows:

Table 2: Yield values for different pre-treatment methods

Pretreatment method	Yield obtained (%)
Acid pretreatment by 0.5N HCl and 0.5N H_2SO_4	21.88
Acid pretreatment by 0.3N HCl and 0.3N H_2SO_4	25.52
Basic pretreatment by 0.5N NaOH	21.35
Steam pretreatment	27.97

From the above table it can be clearly concluded that the yield obtained is maximum for steam pre-treatment process followed by acid pre-treatment of 0.3N HCl and 0.3N H2SO4, followed by acid pre-treatment of 0.5N HCl and 0.5N H2SO4, followed by basic pre-treatment of 0.5N NaOH.

CONCLUSION

Tamarind seeds can possibly act as a biomass for bioethanol production due to their high carbohydrates content. Three kinds of pre-treatment were carried out in order to break the xyloglucan. Maximum amount of reducing sugars was obtained in case of steam pre-treatment at 108^0C.But since the steam pre-treatment requires higher energy, acid pre-treatment of 0.3N is considered more efficient and economical.

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