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OPTIMISATION OF XYLOSE PRODUCTION USING XYLANASE

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ABSTRACT

Agricultural wastes like rice husk, rice bran, pineapple waste, sugar cane biogases, zuzubi oil cake, corn cobs, corn leaves, coconut fiber and wheat bran rich in xylose contents were hydrolyzed in pretreated form using xylanase produced by *Aspergillus sps* MR. Pretreatment was performed by using 0.1N NaOH at 100^0C for 60 minutes. Maximum xylose was produced from corn cobs at 50^0C, when incubated for 48 hours.

Key words: Xylose, Xylanase, *Aspergillus*, Agricultural wastes.

INTRODUCTION

Xylose commonly called wood sugar is a crystalline, natural 5-carbon aldose sugar (pentose) obtained from the xylan rich portion of hemi cellulose from plants (cell walls and fiber)[1]. Xylose is absorbed from the jejunum area of the small intestine. Once xylose enters the bloodstream, it is quickly distributed to the liver and gets metabolized. Xylose cannot be digested to produce energy. Therefore, it can satisfy the needs of those, who are fond of sweet and conscious of fat. Rice husk, rice bran, pineapple waste, sugar cane biogases, zuzubi oil cake, corn cobs, corn leaves, coconut fiber are some of the lignocelluloses materials with high xylose contents. Xylanase belongs to glucanase enzyme family, name given to a class of enzymes, which degrade the linear polysaccharide beta-1,4-xylan into xylose; thus, breaking down hemi cellulose, which is a major component of the cell wall of plants[2,3]. The objective of this work is to study the xylose production from various agricultural wastes by the hydrolytic method using xylanase at a temperature range of 35^0 to 60^0C and for a duration of 12 to72 hours to obtain solutions with a maximum xylose concentration. In the present work, alkali (NaOH) of 0.1N concentration is used in the

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pretreatment of raw material. The enzyme xylanase is isolated from *Aspergillus sps* MR.

EXPERIMENTAL

Materials and methods

Materials

Various agricultural and industrial materials are procured from the local market and used for the present study. All the materials were washed 4 to 5 times with tap water to remove extraneous matter, and then dried in oven at 70^{o}C.

Microorganism

An *Aspergillus* sp MR, used in the present study, was isolated from soil taken from the garden in the Malla Reddy Engineering College, Secunderabad, and A.P. The organism was maintained at 4^{o}C on slants of potato dextrose agar with regular sub-culturing every two weeks.

Pretreatment of agro-industrial material

Pre-processed agro-industrial materials were pretreated by alkali before enzymatic hydrolysis. A total of 10 g material was suspended in 1000 mL solution of 0.1N NaOH in a flask at 100^{0}C for 60 minutes, which maintain the ratio of solid to liquid as 1 : 10. Then the solid residue was collected by filtration and washed extensively with distilled water until neutral pH. Subsequently, this pretreated material was dried in the oven at 70^{0}C to maintain a constant weight to be used as the substrate for enzymatic hydrolysis.

Enzymatic hydrolysis

The pretreated fiber was hydrolyzed using xylanase in Erlenmeyer flasks. The hydrolysis was performed in 50 mM acetate buffer (pH 5.5) at 120 rpm at 50^{0}C for seventy two hours under shaking. To determine the effectiveness of different factors, the effect of temperature (35^{0}C to 60^{o}C) and time (12 to 72 hours) on enzymatic hydrolysis of agro-industrial material was studied.

Analytical methods

Estimation of xylanase activity

Xylanase activity was assayed according to the Suvarna et al.[4] Incubation of 0.1 mL of enzymatic extract with 1.9 mL of a solution of 1% oat spelt xylan (Sigma, USA) in 50 mM acetate buffer pH 4.8 was prepared. This mixture was left to react for 30 minutes.

Reducing sugars liberated by hydrolysis of this substrate were quantified by the dinitrossalicylic acid method. Results were expressed as the mean of triplicate measurements.

Protein determination

Protein concentration was determined according to the Lowry assay[5] against a standard curve of bovine serum albumin, fraction V (Sigma, USA).

RESULTS AND DISCUSSION

Various agro-industrial materials like rice husk, rice bran, pineapple waste, sugar cane bagasse, zuzubi oil cake, corn cobs, corn leaves, coconut fiber and wheat bran were obtained from the local market and pre-processed for enzymatic hydrolysis. The enzymatic hydrolysis was carried out by xylanase produced by the isolated *Aspergillus* sp MR. It can be observed from the results shown in Figure 1 that corn cobs release the highest sugar (580 mg), when compared to all other materials. Sugar cane bagasse and pineapple waste are next to the corn cobs. The zuzubi oil cake, rice husk and rice bran released least sugars. Wheat bran has released the moderate amount of sugars (350 mg). The further optimization experiments were carried out with corn cobs in order to improve the sugars release.

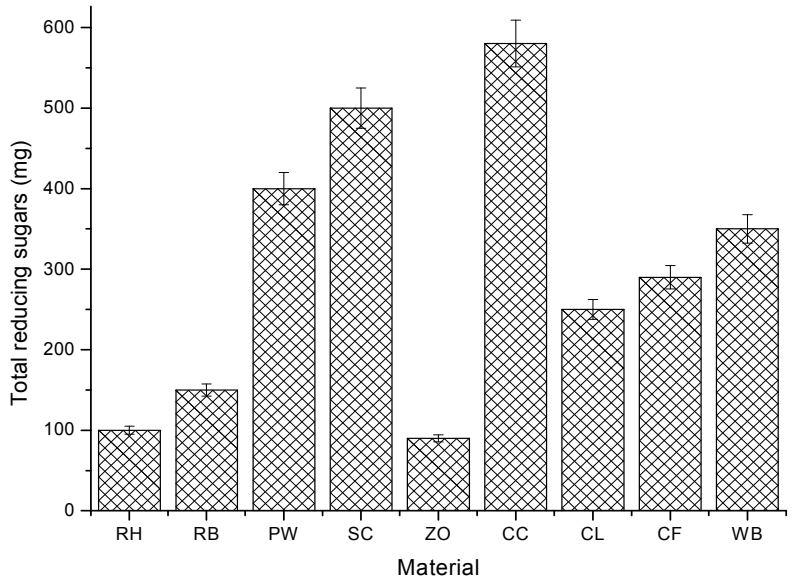

Fig 1: Hydrolysis of various agro-industrial materials. RH = Rice husk, RB = Rice bran, PW = Pineapple waste, SC = Sugar cane bagasse, ZO = zuzubi oil cake, CC = Corn cobs, CL = Corn leaves, CF = Coconut fiber and WB = Wheat bran

Figure 2 depicts the effect of temperature and incubation time on sugars release from the corn cobs. The temperature optimization studies were conducted at temperature ranging from 35^0 to 65^0C. From the Figure 2, it is observed that the initial hydrolysis rate was increased with enhancing temperature, and maximum hydrolysis rate was observed at 50^0C. Then hydrolysis rate decreased, when temperatures exceeded 50^0C. This result could be attributed to the thermal inactivation of xylanase at higher temperatures. The temperature of 50^0C was also found to be optimum for enzymatic hydrolysis of different lignocellulosic materials[6,7]. The hydrolysis of pretreated corncobs was carried out at a range of 12 to 72 hours and the results are presented in Figure 2. The hydrolysis of 10 g of corncobs after 48 h yielded 740 mg reducing sugars at 50^0C. The reducing sugars as well as percent hydrolysis rate decreased as soon as the time was prolonged after 72 hrs. This behavior might due to the inhibition of the enzyme action by the accumulated hydrolysis products.

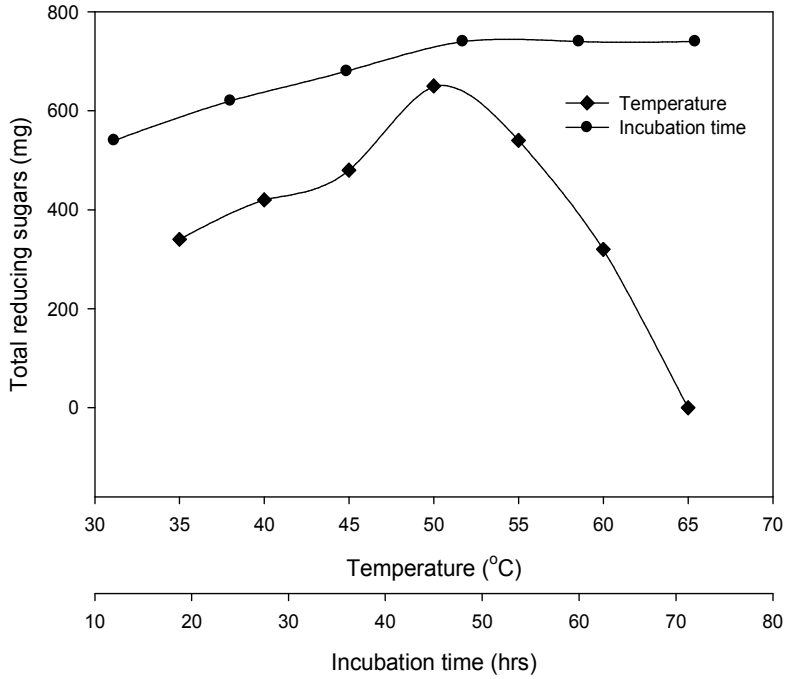

Fig 2: Effect of temperature and incubation time on sugars release from corncobs

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REFERENCES

1. E. Sanjust, A. Salis, A. Rescigno, N. Curreli and A. Rinaldi, Food Science Technol. International, **10**, 11-14 (2004).
2. G. Suvarna Laxmi, T. Sathish, Ch. Subba Rao, P. Brahmaiah, M. Hymavathi and R. S. Prakasham. Current Trends Biotechnol. Pharmacy**, 2 (3**), 447-455 (2008).
3. J. A. Ramírez, R. Aguilar and M. Vázquez, Annual Meeting and Food Expo- Anaheim, California (2002).
4. D. C. La Grange, I. S. Pretorius, M. Claeyssens, and W. H. Van Zyl, Appl. Environ. Microbiol., **67**, 5512-5519 (2001).
5. O. H. Lowry N. J. Rosebrough, A. L. Farr and R. L. Rondal. J. Biol. Chem., **73**, 193-265, (1951).
6. P. Sá-Pereira, A. S. L. Carvalho, M. Costa- Ferreira and M. R. Aires-Barros, Enzyme Microbial Technol., **34**, 278-282 (2004).
7. A. Cobos and P. Estrada, Enzyme Microbial Technol., **33,** 810-818 (2003).

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