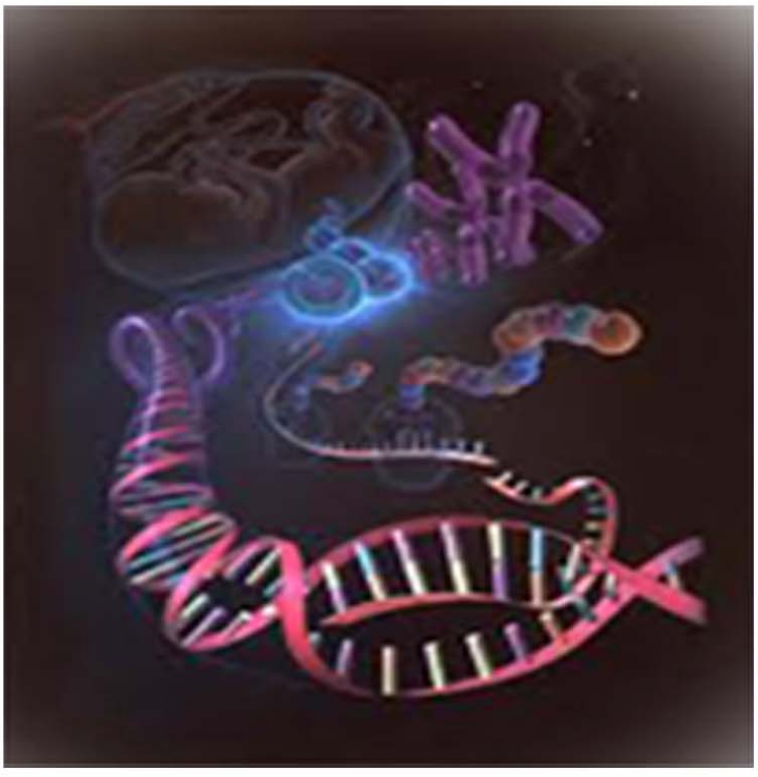
IJLBPR

ISSN 2250 - 3137
Vo1. 2, No. 2, April 2013

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 2, No. 2, April 2013

Research Paper

SACCHARIFICATION OF AGRO-WASTES BY ENDOXYLANASE FROM *STREPTOMYCES* SP OM 09

Rina Rani Ray[1]*

Corresponding Author:* ***Rina Rani Ray,* ✉ *raypumicro@gmail.com*

Extracellular endoxylanse produced by *Streptomyces* sp. OM 09 was used for the bioconversion of various hemicellulosic agro wastes of which dried grass was proved to be the best followed by sugarcane bagasse. Highest amount of sugar production was achieved in presence of 1.0% (w/v) of substrate using 180U of enzyme. The maximum amount of bioconversion was accomplished within 30 min of incubation. The optimum pH and temperature for such bio-conversion was found to be at 6.0 and 60 °C respectively. The amount of sugar production was enhanced in presence of Fe^{2+} and Sr^{2+}. Such bioconversion of dried grass, a potential yard waste into xylose and xylooligosaccharides may be commercially implemented for production of sugar and bio alcohol at a nominal cost.

Keywords: Dried grass, Endoxylanase, Saccharification, Streptomyces, Xylose

INTRODUCTION

Xylan is a complex branched polysaccharide found in plant cell wall and is the one of the most abundant biomass material on earth, which represents an enormous potential source of stored solar energy. Large quantities of ligno-cellulosic wastes are generated through forestry, agricultural practices and industrial processes, particularly from agro-allied industries (Okafor *et al*., 2007).These lignocellulosic wastes are usually left in unused or underutilised form, accumulate in the environment and thereby cause pollution problem (Abu *et al*., 2000).

But recently interests have been increased in the possible commercial utilization of waste biomass for both material (FitzPatrick *et al*., 2010) and energy recovery due to the steadily rising price of fossil fuels. Effective utilization of these wastes require bioconversion to sugar (xylose) followed by fermentation to bio alcohol. Xylan can be hydrolysed by endoxylanase (E.C.3.2.1.8) into xylose and xylo oligosaccharides (Rawasdeh *et al*., 2005), which can be further converted to xylitol or ethyl alcohol. As the cost of xylanase and substrate for the production of these sugar and bioalcohol plays a constraint in commercial production, usage of agricultural residues and microbial xylanase might be an easy solution not only for reducing the cost of sugar production but also for successful waste disposal. Dried grass constitutes a bulk of yard waste that often poses

[1] Microbiology Laboratory, Department of Zoology, Molecular Biology & Genetics, Presidency University, 86/1, College Street, Kolkata, India.

frequent disposal problem. Being a potential source of ligno cellulose, the dried grass can be regarded as a promising source for the production of several value added materials like sugar and alcohol. Though dried grass could be utilized as the sole carbon source for xylanase production (Ray, 2010; Kundu and Ray, 2011), almost no report is available on xylose production from dried grass. As actinomycetes have been explored to the lesser extent, especially regarding xylanase production (Ninawe *et al.*, 2006), a very few report are available on sugar production by xylanase of Actinomycete origin. In the present study, attempts have been made for bioconversion of dried grass to sugar with the help of microbial endoxylanase synthesised by a strain of *Streptomyces* sp .

MATERIALS AND METHODS

Microorganism and Cultivation of the Strain

Streptomyces sp., the working strain isolated from the forest buffer zone of Kanha National Park and Tiger Reserve, MP, India at an altitude of 600-900 m (Bhor and Ray, 2009), was cultivated at 28 °C in 100 mL. Erlenmeyer flasks each containing 10 mL Basal Medium (BM) composed of (g L-1): peptone 0.9; $(NH_4)_2HPO_4$ 0.4; KCl 0.1; $MgSO_4.7H_2O$ 0.1 and pure beechwood xylan (Sigma) 0.5 (pH: 7).

Enzyme Extraction and Assay

The culture broth was filtered in Whatman filter paper and centrifuged at 6,000 rpm for 5 min and the supernatant was used as the crude enzyme. To measure the activity of endoxylanase, the assay mixture (1 mL) containing an equal volume of enzyme and 1% (w/v) oat spelt xylan (Sigma) dissolved in 0.1 (M) phosphate buffer (pH-6) was incubated at 60 °C for 10 min. The reducing sugar released was measured by the dinitrosalicylic acid method (Bernfeld, 1955) taking xylose as standard. Blanks were prepared with inactivated enzymes. One unit of exoxylanase was defined as that amount of enzyme that liberated 1 micro mole of xylose per mL per minute of reaction.

Saccharification of Agro Waste Substrates

The agro wastes were collected from agricultural fields, washed thoroughly with water, air dried, pulverized and sieved to 40 mesh particle size, before using as substrate for saccharification.

A suspension of substrate (5 mg/mL) in 0.1 M phosphate buffer (pH 6) was incubated with endoxylanase in a screw capped tube for 30 min at 60 °C. The resultant supernatant was centrifuged at 5000 g for 5 min was analyzed by DNSA method (Bernfeld, 1951) using xylose as standard.

Optimization of Different Parameters for Sugar Production

To determine the most effective concentration of substrate, saccharification experiments were carried out with different concentration of dried grass keeping all other factors constant. Similarly, the most suitable enzyme concentration for bioconversion of dried grass was determined. To determine the effect of incubation time, saccarification was carried out under the optimum conditions. At specific time intervals aliquots (1 mL) were removed and the amount of reducing sugar was estimated (Bhat and Bhat, 1997). Effects of pH and temperature on saccharification were determined by the varying the pH of assay mixture from 4 to 9 and temperature from 20-70 °C. The role of metal ions on sugar production was checked by adding 10 mM of each one in saccharification mixture.

RESULTS AND DISCUSSION

Amongst the agro wastes tested, dried grass was proved to be the best followed by sugarcane bagasse (Table 1). The extent of bioconversion was dependent on the nature of hemicellulosic content in the substrate used. Hence for subsequent experiments of sugar production, dried grass was used as the substrate.

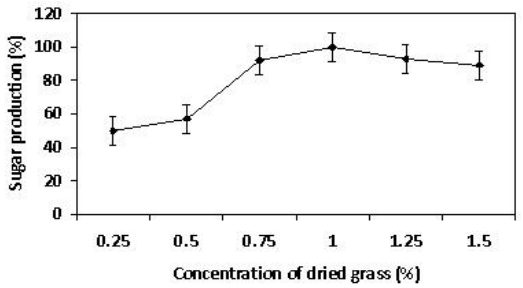
Table 1: Effect of Substrate Type on Sugar Production

Substrate (1 g)	Sugar (mg)
Dried grass	68
Sugarcane bagasse	62
Jute Fibre	44
Water hyacinth	37
Rice husk	24
Saw Dust	28

Note: 100% = 70 mg of sugar (xylose and xylo oligosaccharide).

Initially, the concentration of substrate (dried grass) was directly correlated with the extent of sugar production (Figure 1) as it showed a sharp increase in sugar production when the substrate concentration increased from 0.5% to 0.75%.

Figure 1: Effect of the Concentration of Dried Grass on Sugar Production by Endoxylanase of *Streptomyces* sp. OM 09

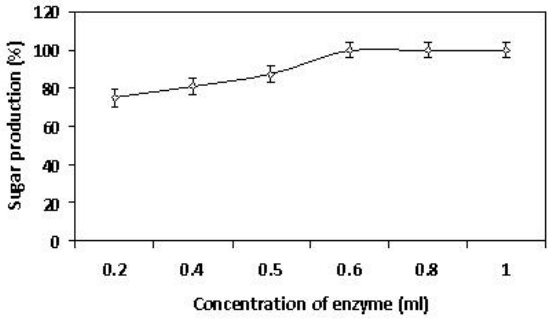


Note:100% = 70 mg of sugar (xylose and xylo oligosaccharide)

Highest rate of bioconversion was achieved in presence of 1.0% (w/v) of substrate. But the sugar production did not increase with further increase in substrate concentration. This could be due to the substrate saturation and enzyme limitation (Karmakar and Ray, 2011a).

The effect of enzyme concentration on sugar production (Figure 2) indicated that maximum bioconversion of dried grass was achieved in presence of 0.6 mL (180U) enzyme but due to enzyme limitation no further increase could be seen with increase in enzyme concentration.

Figure 2: Effect of Enzyme Concentration on Sugar Production by Endoxylanase of *Streptomyces* sp. OM 09

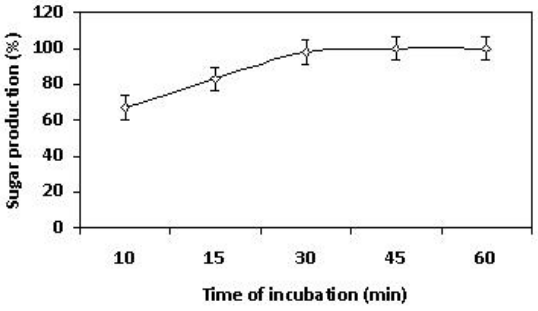


Note: 100% = 70 mg of sugar (xylose and xylo oligosaccharide).

The observation went in agreement with that found in the saccharification of lignocellulosics (Goyal *et al.*, 2008; Sallem Akhtar *et al.*, 2001).

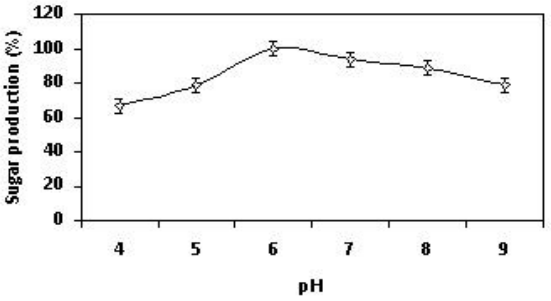
It was found that saccharifying potential of the enzyme was affected by different length of incubation and maximum bioconversion was accomplished within 30 min of incubation (Figure 3) followed by an equilibrium condition. This implied that maximum amount of substrate was hydrolyzed by the given amount of enzyme within this time, after which no further change took place. Similar result was found in saccharification of

Figure 3: Effect of Incubation Time on Sugar Production by Endoxylanase of *Streptomyces* sp. OM 09



Note: 100% = 70 mg of sugar (xylose and xylo oligosaccharide).

Figure 4: Effect of Incubation pH on Sugar Production by Endoxylanase of *Streptomyces* sp. OM 09

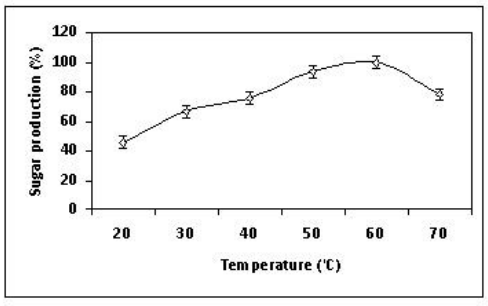


Note: 100% = 68 mg of sugar (xylose and xylo oligosaccharide).

cellulose by endoglucanase as reported by Karmakar and Ray (2011b).

The effect of pH and temperature on saccharification of dried grass indicated that the highest sugar production was achieved at pH 6 and at 60 ^{o}C (Figures 5 and 6) respectively. Almost similar pH and temperature optima were reported for the saccharification of sugar cane trash by xylanase of *Colletotrichum graminicola* (Zimbardi *et al.*, 2013) and saccharification of recycled paper sludge by *Paenibacillus campinasensis* xylanase gene expressed in *Bacillus megaterium* (Zheng *et al.,* 2012).

Figure 5: Effect of Incubation Temperature on Sugar Production by Endoxylanase of *Streptomyces* sp. OM 09



Note: 100% = 68 mg of sugar (xylose and xylo oligosaccharide).

Amongst the metal ions, Fe^{2+} followed by Sr^{2+} proved to be best for enhancing sugar production but was severely affected by Cu^{2+} (Table 2) which was almost similar to the effect of ions on saccharification of agro wastes by the endoglucanase of *Rhizopus oryzae* (Karmakar and Ray, 2011a).

Table : Effect of Additives on Sugar Production

Additive (10 mM)	Sugar Production (%)
None	100
K^{+}	96.7
Na^{+}	100
Sn^{2+}	77.4
Sr^{2+}	109.7
Mg^{2+}	93.5
Fe^{2+}	116.1
Cu^{2+}	32

Note: 100% = 70 mg of sugar (xylose and xylo oligosaccharide).

CONCLUSION

This study may be regarded as one of the few reported xylanase synthesised by *Streptomyces*

with saccharification potential and could open an avenue for effective utilization of yard wastes, the dried grass for production of xylose and xylo-oligosaccharides, a pre requisite for xylitol, the sugar alcohol of immense commercial significance.

REFERENCES

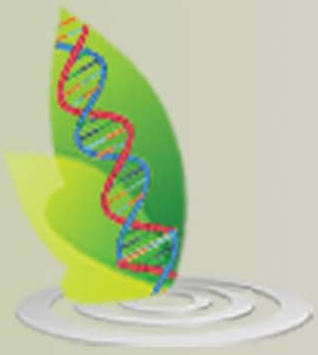
1. Abu E A, Onyenekwe P C, Ameh D A, Agbaji A S and Ado S A (2000), "Cellulase (E.C.3.21.3) production from sorghum bran by *Aspergillus niger* SL 1: An assessment of pretreatment methods." Proceedings of the International Conference on Biotechnology: Commercialization and Food security. Abuja, Nigeria, pp.153-159.
2. Bernfeld P (1955), "Amylases α and β", *Methods of Enzymol*., Vol.1, pp.149-150.
3. Bhat M K and Bhat S (1997), "Cellulose degrading enzymes and their potential industrial applications", *Biotechnol Adv.*, Vol.15, pp.583-620.
4. Bhor S and Ray R R (2009), "Production of endoxylanase by a strain of *Streptomyces* in Submerged Fermentation of Dried Grass", *The Bioscan.,* Vol. 4, pp. 675-678.
5. Fitzpatrick M C, Pressier E L, Porter A, Elkinton J, Waller L A, Carlin B P and Ellison A M (2010), "Ecological Boundary Detection using Bayesian Areal Wombling", *Ecol.*, Vol. 91, pp. 3448-3455.
6. Goyal M, Kalra K L, Sareen V K and Soni G (2008), "Xylanase Production with Xylan Rich Lignocellulosic Wastes by a Local Soil Isolate of *Trichoderma viride*" , *Braz J Microbiol.*, Vol. 39, pp. 535-541.
7. Karmakar M and Ray R R (2011a), "Saccharification of Agro Wastes by the Endoglucanase of *Rhizopus oryzae", Annals Biol. Res.*, Vol. 2, No. 1, pp. 201-208.
8. Karmakar M and Ray R R (2011b), "Optimization of Saccharification Conditions and Sugar Production by Endoglucanase from Rhizopus Oryzae PR7 MTCC 9642 using Response Surface Methodology", *Int. J. Pharma. Biomed Res.*, Vol. 2, No. 3, pp. 68-76.
9. Kundu A and Ray R R (2011), "Agrowaste Utilization and Production of Extra Cellular Endoxylanase by *Penicillium janthinellum* MTCC 10889 in Solid State Fermentation", *Int J Cur Res.,* Vol. 3, No. 7, pp. 120-124.
10. Ninawe S, Lal R and Kuhad R C (2006), "Isolation of Three Xylanase Producing Strains of Actinomycetes and their Identification Using Molecular Methods", *Curr. Microbiol.*, Vol. 53, pp. 178-182.
11. Okafor U A, Emezue T N, Okochi V I, Onyegeme-Okerenta B M and Nwodo-Chinedu S (2007), "Xylanase Production by *Penicillium chrysogenum* (PCL501) Fermented on Cellulosic Wastes", *African J. Biochem. Research*, Vol, 1, No. 4, pp. 048-053.
12. Rawashdeh R, Saadoun I and Mahasneh A (2005), "Effect of Cultural Conditions on Xylanase Production by *Streptomyces* sp. (strain Ib 24D) and its Potential to Utilize Tomato Pomace", *Afric. J. Biotechnol*, Vol. 4, pp. 251-255.
13. Ray R (2010), "Extra Cellular Endoxylanase Production from Solid State Fermentation

of Dried Grass by Streptomyces sp OM 09", *J. Exp. Sci.*, Vol. 1, pp. 33-36.

14. Saleem Akhtar M, Saleem M and Waheed Akhtar M (2001), "Saccharification of Lingocellulosic Materials by the Cellulases of *Bacillus subtilis", Int. J. Agric. Biol.,* Vol. 3, pp. 199-202.

15. Zheng H, Liu Y, Liu X, Han Y, Wang J and Lu F (2012), "Overexpression of a *Paenibacillus campinasensis* xylanase in *Bacillus megaterium* and its Applications to Biobleaching of Cotton Stalk Pulp and Saccharification of Recycled Paper Sludge" *Bioresour Technol.,* Vol. 125, pp. 182-187.

16. Zimbardi A L, Sehn C, Meleiro L P, Souza F H, Masui D C, Nozawa M S, Guimarães L H, Jorge J A and Furriel R P (2013), "Optimization of β-Glucosidase, β-Xylosidase and Xylanase Production by Colletotrichum graminicola under Solid-State Fermentation and Application in Raw Sugarcane Trash Saccharification", *Int. J. Mol. Sci.,* Vol. 14, No. 2, pp. 2875-2902.



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijlbpr@gmail.com or editor@ijlbpr.com
Website: www.ijlbpr.com

ISSN 2250-3137
02
9 772250 313001