August-October, 2012, Vol. 2, No. 4, 2135-2141 e- ISSN: 2249 –1929

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

Available online at www.jcbsc.org

Section D: Environmental Sciences

CODEN (USA): JCBPAT

Research Article

Valorization of Jute Caddies for production of extra cellular endoxylanase by *Penicillium janthinellum*

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Received: 22 August 2012; **Revised** 18 September 2012; **Accepted:** 20 September 2012

ABSTRACT

Jute caddies is one of the important waste products generated from the processing of jute fibres in jute mills, which cause serious pollution problem in and around the mill locality. These waste caddies were collected and utilized in the fermentation medium for submerged cultivation of a strain of Penicillium janthinellum as sole carbon source for the production of extra cellular endo xylanase. The strain showed best enzyme production at pH 8.0 and at a temperature of 28°C and at a concentration of 2% (w/v) jute caddies. Gelatin and Mn2+ could increase the enzyme production up to certain extent. The kinetics of the enzyme production and substrate utilization showed that highest endoxylanase production could be achieved at 48 hour of growth and jute caddy was degraded as sole carbon source very slowly up to 72 hours, after which the rate of utilization increased.

Keywords: Endoxylanase, Jute caddies**,** *Penicillium janthinellum*,, submerged fermentation, optimization.

INTRODUCTION

Jute textile manufacturing is the most prominent industry in West Bengal due to availability of raw jute in the state. A number of jute mills (63 composite mills) are present along the banks of river Hooghly in the State of West Bengal, India and is a major contributor to the pollution. Jute caddies is the important ligno cellulosic waste produced in these factories as the unspinnable short fibers generated in the jute mill looms during jute processing. India produces 0.28 million quintals of jute caddies a year, which is mainly

used as boiler fuel or is simply swept away that causes pollution problem in and around the jute mills[1]. A few works have been done on the utilization of jute wastes by mixing it with cow dung to produce biogas [2, 3, 4]. Jute caddies were used as a potential raw material for hand-made paper [5]. Being a potential source of ligno cellulose, the jute caddies can be regarded as a promising source for the production of several value added materials like enzymes sugar and alcohol. But, so far the literature survey is concerned; no report is available on utilization of these caddies as the substrate for fermentation for enzyme production. Therefore attempts have been made to utilize these jute caddies in the fermentation media as the sole carbon source for the production of endoxylanase (EC 3.2.1.8) an enzyme of immense industrial importance.

The present paper deals with the optimization of parameters for the production of extra cellular endoxylanase by a strain of *Penicillium janthinellum* utilizing jute caddies as sole carbon source.

MATERIALS AND METHODS

Microorganism and Cultivation of the strain: *Penicillium janthinellum* MTCC 10889, the working strain in this study was grown in 1% PDA plates for 48 h at 28°C and for endoxylanase production the strain was cultivated in liquid state fermentation (LSF) 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).

Chemicals: All chemicals used were of analytical grade.

Preparation of substrates: Various ligno cellulosic effluents were collected from jute mills and were dried, pulverized and sieved as 40 mesh particle size before using in fermentation media in place of pure xylan.

Cultivation in solid state fermentation medium: *P. janthinellum* was cultured in 100 ml Erlenmeyer flasks containing totally dried substrates and salts (based on 10ml medium) moistened with 0.5 ml of distilled water at 28°C. Cultures were picked up at different time intervals; sterile water was added to make up its final volume equivalent to that of 10 ml LSF media, followed by a thorough cyclomixing.

Enzyme extraction and assay: The culture broth was filtered in Whatman filter paper and centrifuged at 3,500 rpm for 3 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) beechwood xylan (Sigma) dissolved in 0.1 (M) phosphate buffers (pH-6) was incubated at 55°C for 10 min. The reducing sugar released was measured by the dinitrosalicylic acid method [6] taking xylose as standard. Blanks were prepared with inactivated enzymes. One unit of exoxylanase was defined as that amount of enzyme that liberated 1 milli mole of xylose per milliliter per minute of reaction.

Optimization of different parameters for enzyme production: With a view to replace beechwood xylan (Sigma), a costly substrate for endo xylanase production, various cheap and abundantly available agro wastes, namely jute caddies and other textile mill wastes were supplemented as carbon source. The optimum concentration of the best inducer was detected by varying its concentration (0.5-3 % w/v) in the cultivation medium. The best temperature for endoxylanase production by *Penicillium janthinellum* was detected by cultivating the strain at various temperatures (7°C-47°C) at pH 8.0. On the other hand the pH optima was detected by varying the initial pH (4-11) of the cultivation medium for enzyme production with the help of acetate buffer for pH 4-6, phosphate buffer for pH 6-8 and tris-HCl buffer for 8-11.Similarly, the effect of nitrogen source and metal ions were studied by supplementing various organic and inorganic nitrogen sources (0.09% w/v) and different salts of metal ions (10 mg) in the culture medium. The effect of cultivation time was determined by picking up the culture containing flasks with

optimized media at various time intervals (24-120 hours) , followed by an assay of the enzyme activity. The rate of utilization of the jute caddies was measured by taking the dry weight of the same, after 0-120 days of cultivation. Each experiment was done in triplicate and their values were averaged.

RESULTS AND DISCUSSION

Effect of substrate and fermentation type: *Penicillium janthinellum* MTCC 10889 was found to degrade various wastes generated in jute and cotton mills of which jute caddies showed the best result (**Fig. 1**) and the submerged fermentation was found to be better than solid state fermentation (SSF). Hence for subsequent experiments, the submerged fermentation of jute caddies was used.

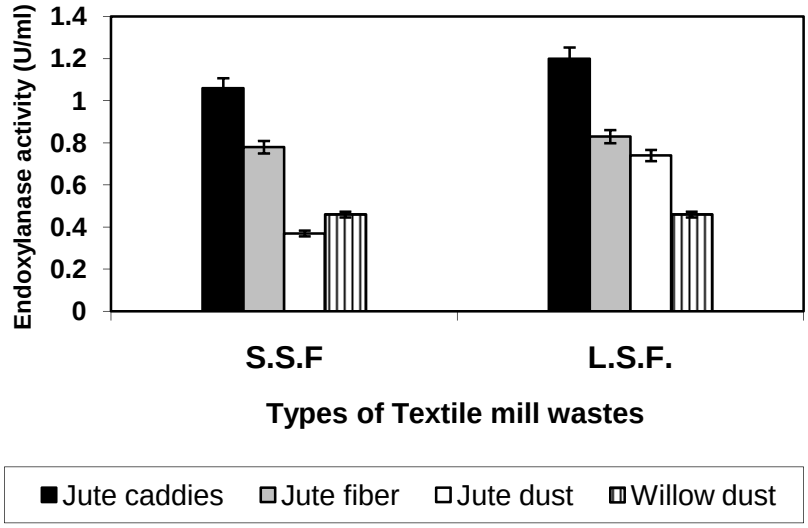

Fig.1: Inducing effect of various textile wastes for endoxylanase production by *Penicillium janthinellum* MTCC 10889.

Effect of substrate concentration on endoxylanase production: The optimum concentration of jute caddies for endoxylanase production was found to 2% (w/v), further increase of which could not further increase enzyme production (**Fig. 2**) probably due to enzyme limitation.

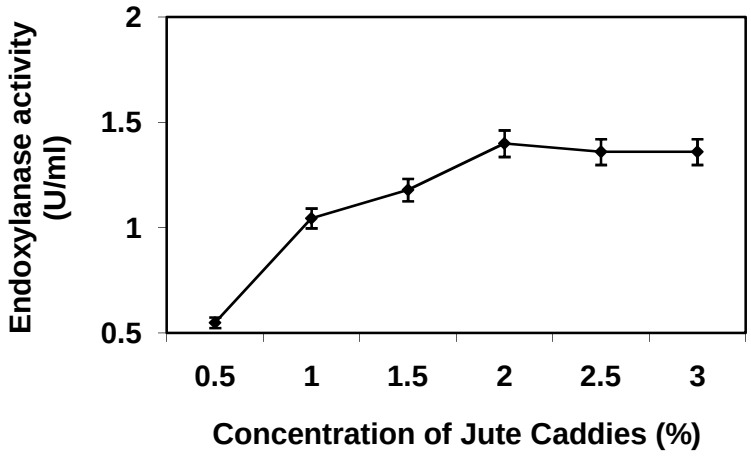

Fig.2: Effect of Jute caddies concentration for production of endoxylanase by *Penicillium janthinellum* MTCC 10889

Effect of pH on endoxylanase production from jute caddies: The LSF was carried out at various pH ranging from 4.0 to 9.0 and the optimum pH for enzyme production was found to be at 8.0 (**Fig. 3**). But the pH optima were found to be 6.0 and 7.0 when grown in dried grass and rice husk respectively[7]. This variation of pH requirement for enzyme production might be due to their respective lignin content.

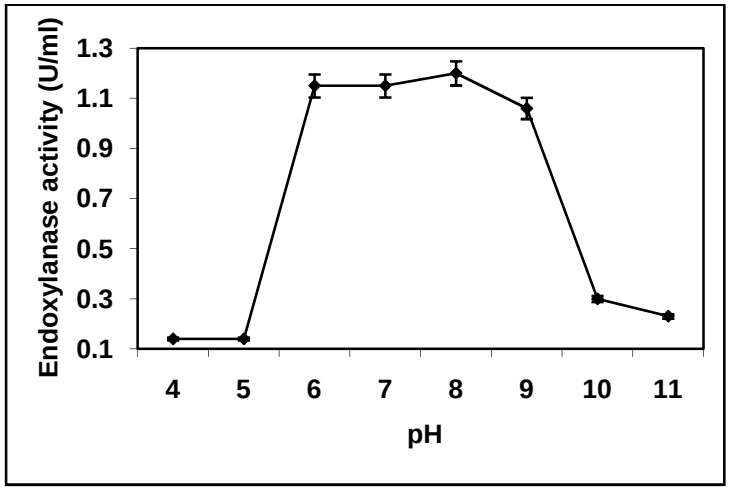

Fig.3: Effect of pH on endoxylanase production by *Penicillium janthinellum* MTCC 10889

Effect on temperature on endoxylanase production from jute caddies: The maximum enzyme production from submerged state of Jute caddies was observed at 28°C (**Fig. 4**) which was similar to the temperature optima showed by other strains of *Penicillium janthinellum* [8,9].

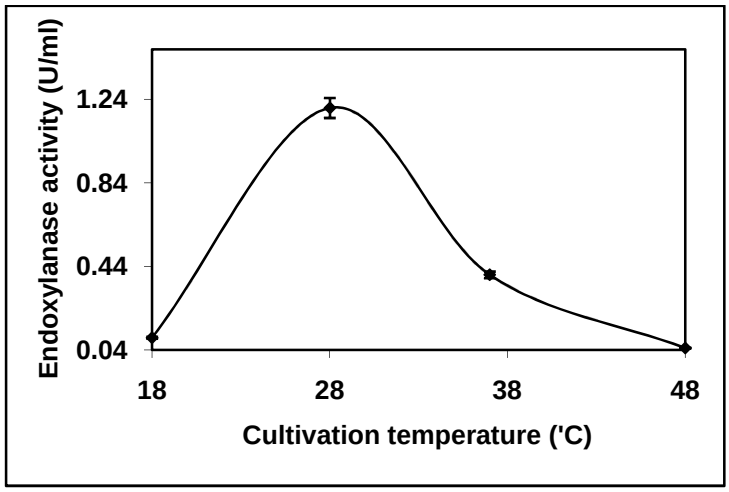

Fig.4: Effect of temperature on endoxylanase production by *Penicillium janthinellum* MTCC 10889

Effect of nitrogen source on endoxylanase production from jute caddies: Among the nitrogen sources tested, gelatin followed by ammonium sulfate was proved to be the best nitrogen source for enzyme production (**Table 1**). But enzyme production was remarkably decreased in presence of yeast extract, a report contrary to that reported from *Penicillium canescens* 10-10c [10].

Effect of metal ions on endo xylanase production from jute caddies: Except Mn^{2+}, no other metal ions could significantly increase the enzyme production, (**Table 2**) which goes in agreement with the already reported nature of the strain[7]. The enhancing effect of Mn^{2+} might be due to its requirement as a cofactor of the enzyme. On the other hand heavy metals like Hg^{2+} and Cu^{2+} resulted in about 50-67% deactivation of the enzyme.

Table-1: Effect of nitrogen source on endoxylanase production from Jute caddies

Nitrogen source	Endoxylanase activity (U/ml)
Peptone	1.20
Tryptone	1.10
Gelatin	1.30
Urea	1.02
Yeast Extract	0.98
Ammonium sulfate	1.22

Table-2: Effect of metal ions on endoxylanase production from Jute caddies

Metal ions	Endoxylanase activity (U/ml)
None	1.2
Na^{+}	1.2
K^{+}	1.2
Mg^{2+}	1.1
Mn^{2+}	1.7
Ca^{2+}	1.2
Fe^{2+}	0.9
Sr^{2+}	1.2
Cu^{2+}	0.8
Hg^{2+}	0.6
EDTA	0.8

Effect of cultivation time on endo xylanase production from jute caddies: Highest endo xylanase production could be achieved within 48 hours of cultivation with jute caddies (**Fig. 5)** after that it gradually decreased, probably due to the depletion of nutrients in the medium which stressed the fungal physiology resulting in the inactivation of secretary machinery of the enzymes [11].This was a relatively rapid production than already reported endo xylanase producing strains of *Penicillium janthinellum* taking 120 hrs [9, 12]and other filamentous fungi like *Aspergillus carneus* [13] and *Aspergillus niger* [14] taking 144 hrs and 96 hours respectively.

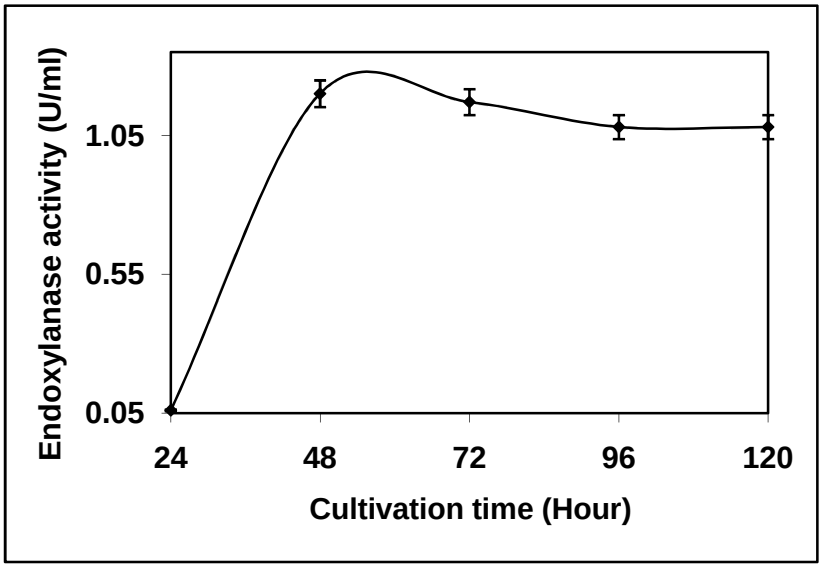

Fig.5: Effect of cultivation time on endoxylanase production by *Penicillium janthinellum* MTCC 10889

Kinetics of jute caddies utilization for endo xylanase production: Jute caddies supplemented in the cultivation medium was found to be utilized by the fungus as sole carbon source very slowly up to 72 hours (**Fig. 6**) , after which the rate of utilization increased, although no significant increase in enzyme synthesis was noted. The carbon source was continued to be extracted from jute caddies thereafter was probably for the maintenance of growth and spore formation of the fungus.

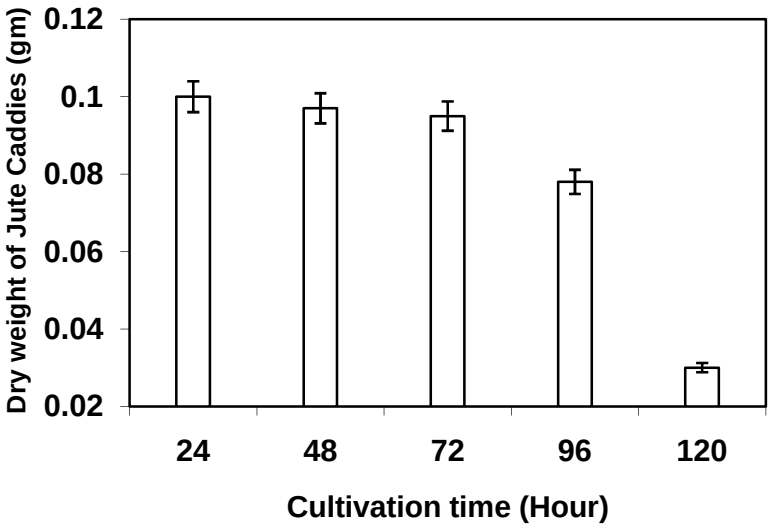

Fig.6: Utilization of Jute caddies by *Penicillium janthinellum* MTCC 10889

CONCLUSIONS

Microbial xylanases represent one of the largest groups of industrial enzymes and they have attracted a great deal of attention during the past few decades [15]. Their potential biotechnological applications in various industries include the food, feed, fuel, textile, detergents, paper and pulp industries and in waste treatment [16]. The cost of carbon source plays a major role in the economics of xylanase production [17] and hence, to reduce the cost of xylanase production various agro wastes or industrial wastes enriched with lignocellulosic materials as substrates rather than using the expensive pure xylans would be a better

choice. Jute caddies, so far reported to be a potent left over material from jute mills, contributing pollution could be effectively utilized for the production of endo xylanase. The ability of the present fungal strain to degrade jute caddies and production of quite a high amount of extra cellular endo xylanase within a relatively short period of time made this strain useful from industrial point of view.

ACKNOWLEDGEMENT

The authors are thankful to Life Science Research Board, Defence Research and Development Organization (DRDO), New Delhi, India for the financial assistance. The authors like to thank Mr. Partha Sarathi Roychowdhuri, Jute and Textile Consultant for kindly providing the lignocellulosic effluents of textile mills especially the jute caddies.

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