Enrich, XI(II): 32 - 35, Jan – June, 2020

Optimization of Growth Time for Endoxylanase Production from *Bacillus* sp Using Coir Pith as Substrate

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Abstract

Xylan is the second most abundant naturally occurring renewable polysaccharide available on earth. Xylanase, the enzyme which degrades xylan finds application in several industries like pulp and paper bleaching, food, feed, textile, pharmaceuticals, and lignocellulosic biorefinery which has led to an increase in its demand globally. The aim of this study is to optimize the xylanase production from *Bacillus* sp by providing various growth time in constant pH (7.0) and temperature (37^0C) using the agricultural residue, coir pith as substrate. It was observed that 168hrs promoted the maximum production of enzyme with the final pH 6.5 and the temperature 37^0C respectively.

Keywords: Xylanase, *Bacillus* sp, coir pith.

Introduction

Xylan constitutes the major non cellulosic polysaccharides of the primary cell wall of grasses and secondary wall of all angiosperms (Diaz *et al*., 2004). It is readily available in nature, followed by cellulose, the second most abundant polysaccharide which covers 33% of total lignocellulosic biomass found on the globe (Collins *et al*., 2005; Polizeli *et al*., 2005). Xylanases are produced by different living organisms such as microorganisms, protozoans, molluscs, and also found in the rumen of higher animals (Beg *et al*., 2001). The xylanases are mainly produced by microorganisms, e.g., bacteria, fungi, and actinomycetes at industrial scale (Motta *et al*., 2013). The utilization of lignocellulosic biomass (LCB) for the production of different biochemicals such as bioethanol, enzymes, and value-added compounds has tremendously improved in recent years. Xylanase is required in a huge amount for industrial level applications with characteristic properties to survive the harsh industrial level processing (Qiu *et al*., 2010). Therefore, there is a need to select potent microorganisms for enhanced xylanase production, followed by optimization of media components. Strains of *Bacillus* are known to produce xylanase on various lignocellulosic substrates. The main objective of the present study is to optimize the production of xylanase during its growth on the coir pith substrate as a carbon source.

Materials and methods

Isolation of *Bacillus* sp

Isolation of *Bacillus* sp from coirpith soil sample was made on the selective media Bacillus Isolation Agar. The well isolated colonies were subjected to the following tests adopting the scheme recommended by Bergey's Manual, 1998.

Culture media

The agricultural residue, coir pith was used as a substrate for xylanase enzyme production. Xylan extract was prepared by following the method of Panbangred *et al*., 1987. This carbon source was uniformly mixed with a mineral salt solution (100ml). After sterilization, the flasks were cooled and inoculated with 1ml of bacterial culture and incubated at 37^0 C for about 7days.

Enzyme Extraction

The inoculated samples were centrifuged at 10,000 rpm for 20minutes. The cell free supernatant phase was collected and used as a stock crude enzyme solution. The amount of xylanase released was measured by DNS method(Sadasivam and Manickam,1992).

Result and Discussion

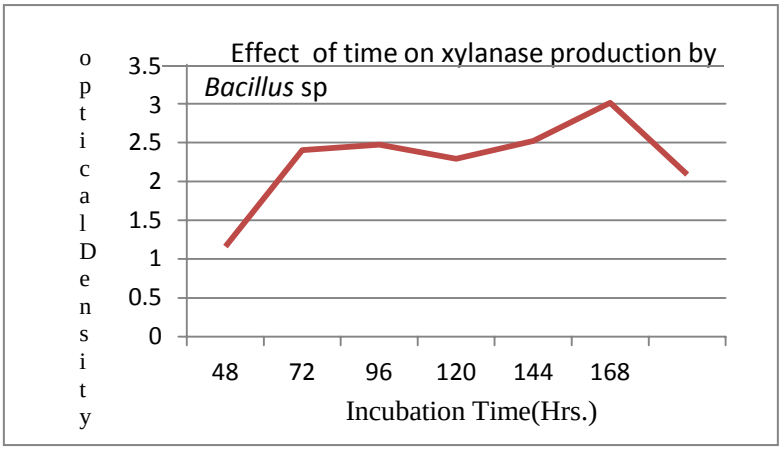
Several workers investigated the effect of various substrates on xylanase production. In this study, coir pith, which is considered to be the not easily degradable agricultural residue, was used as the substrate for xylanase production. The maximum enzyme production was observed on168 hrs incubation time. These results were close to the findings of Virupakshi *et al.,* 2005 in *Bacillus* sp. Similarly, Senthilkumar *et al*., 2005 revealed that the enzyme production was more from *Aspergillus fischeri* when wheat bran used as solid substrates.

Table 1. Effect of time on xylanase production by *Bacillus* sp

Incubation (h)	Xylanase production (IU/ml)
48	1.16
72	2.40
96	2.47
120	2.29
144	2.52
168	3.01
192	2.09

The time of xylanase production varies from organism to organism. In *A.niger* maximum production occurred after 72hrs (Haq *et al*., 2002). The concentration of reducing sugar showed a significant increase in enzyme production with an increase in time, which was presumed to be rapid hydrolysis of xylan in the medium (Table 1). In the present study, maximum xylanase production noticed at 168h. After that xylanase production was declined. The production of enzyme xylanase is greatly influenced by different environmental factors.

Fig 1. Effect of time on xylanase production by *Bacillus* sp



Acknowledgment: I would like to thank the Chairman, Director and Principal of APC Mahalaxmi College for women for providing facilities and their constant encouragement to carry out this research study.

Funding: None

Data availability: All datasets and statistical report analyses during this study are included in the manuscript.

Ethics Statement: This article does not contain any studies with human participants or animals.

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