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EFFECT OF ADDITIVES ON XYLANASES PRODUCTION BY *BACILLUS CEREUS*

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ABSTRACT

The enzymatic cycles have gone through an insurgency since the improvement of the immobilization procedure. To help the adequacy of xylanase, various examination groups have immobilized it in different ways. Most of lignocellulose materials are comprised of generally 10% to 25% lignin, 20% to 30% hemicellulose, and 40% to half cellulose. These are the most abundant, recyclable materials, representing roughly 50% of all biomass. The tremendous majority of these lignocelluloses are gotten from rural and backwoods squander. Hemicelluloses are the most widely recognized polysaccharide segment in these lignocellulosic squanders after celluloses. These hemicelluloses make up most of the plant cell wall and are tracked down for the most part in the essential and auxiliary layers, where they connect with cellulose and lignin to give solidness to the plant fiber. Xylose, mannose, galactose, arabinose, and different sugars, as well as their uronic acids, make up hemicelluloses synthetically. Xylan is the most bountiful of the 5-C sugars found in hemicellulosic edifices, and it is generally present in the optional cell walls of angiosperms and gymnosperms. Xylan is situated in the lignin sheath, and it is artificially associated and ensnared with this sheath at numerous areas. The tremendous biotechnological uses of microbial xylanases, especially in the food, creature feed, paper, and mash businesses, as well concerning the bioconversion of lignocellulosic squander into esteem added items, have brought their importance up in the ongoing climate (Collins et al., 2005). Moreover, xylanases have exhibited an enormous commitment for cost-really extending the creation of various significant merchandise. Creation of SCPs, catalysts, fluid or vaporous fills, solvents, and sugar syrups, which can be utilized in that capacity or as a feed stock for other microbial cycles, are the fundamental open doors.

KEY WORDS: Xylanases, Production, *Bacillus Cereus,* Cellulose, Lignin, Enzyme.

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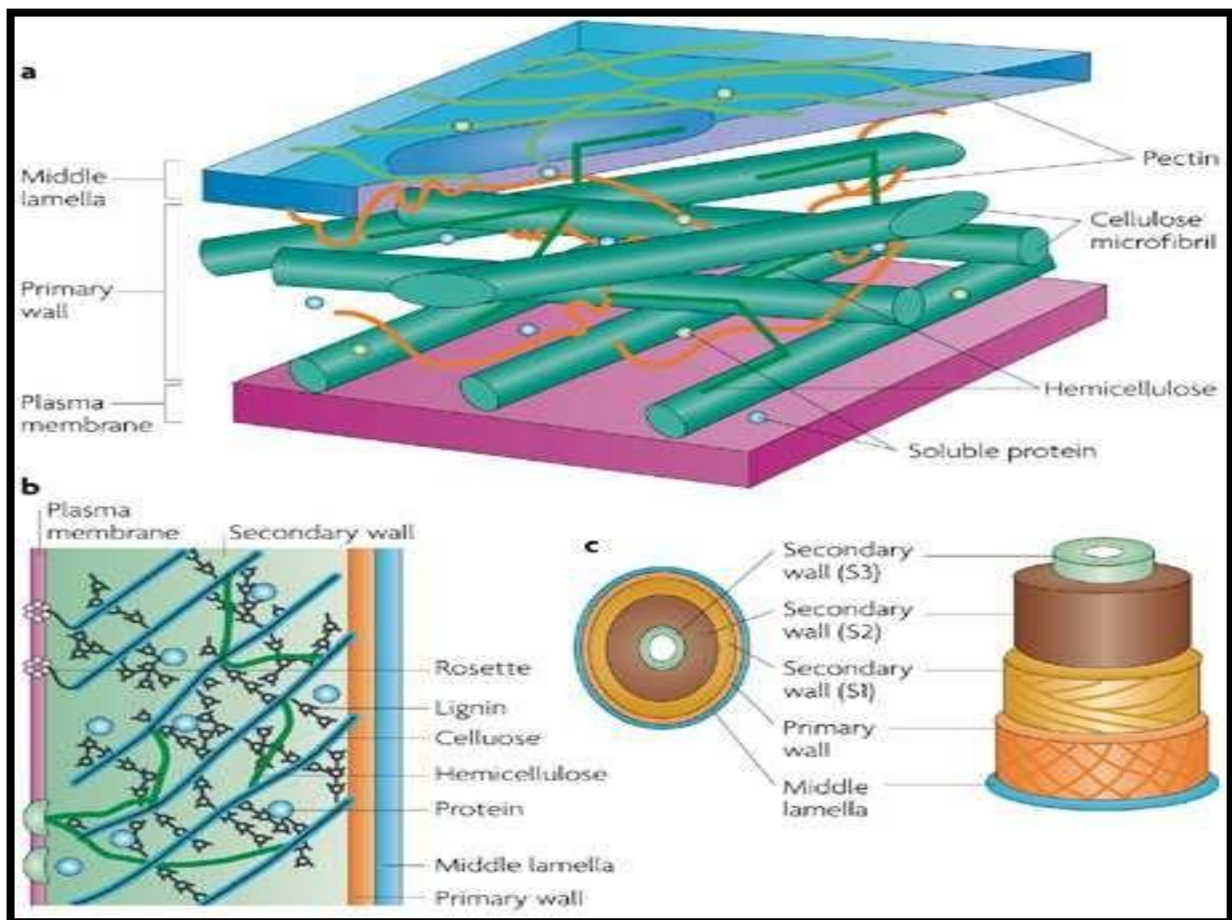
INTRODUCTION

Cellulose (35-half), hemicellulose (20-35%), and lignin (10-25%) make up most of lignocellulosic biomass (Sun and Cheng, 2002). In 1891, Schulze authored the expression "hemicellulose" to depict the divisions that could be detached or separated from plant materials utilizing weakened soluble base. After cellulose, hemicellulose is the second most common polymer on the planet (Saha, 2003). The fundamental part of hemicellulose, xylan, has a high potential for breakdown to create useful side-effects. It saves the primary uprightness of the plant cell wall by shaping covalent linkages with lignin and non-covalent linkages with cellulose (Collins et al., 2005; Polizeli et al., 2005). The xylans have all the earmarks of being sprinkled, interlaced, and covalently connected with the overlying lignin "sheath" at different focuses while making a coat around the hidden strands of cellulose



through hydrogen holding (Biely, 1985), which is steady with their underlying science and side-bunch replacements (Joseleau et al., 1992). Glucuronopyranosyl, 4-O-methyl-D-glucuronopyranosyl, - L-arabinofuranosyl, acetyl, feruloyl, and - coumaroyl side gatherings

are subbed to variable degrees on the hompolymeric spine chain of endo-1,4-xylopyranosyl buildups in the principal chain of xylan (Li et al., 2000).

Fig. 1.1 Structure of arrangements of cellulose, hemicellulose and lignin in the cellwalls of lignocellulosic biomass (a) crossection of cell wall, (b) vertical section, (c) schematic arrangments of cell wall in plant cells

Dependent upon their area, Xylan has moving hidden and compound characteristics. It shows fluctuating development and is generally present in different plant species, in many showcases moving plan and is comprehensively present in different plant species, in a large number of kinds of tissues, and in cells. The fundamental hemicellulose parts of discretionary cell walls, which make up 20-30% of the biomass of hardwoods and herbaceous plants, are xylans. There are contrasts among hardwood and softwood xylans concerning side social affair substitution and polymerization force. Hard wood xylan has a more critical degree of polymerization (150-200) than sensitive wood xylan (70-130). (Sunna and Antranikian, 1997; Kulkarni et al., 1999). Hard wood

contains the best O-acetyl-4-O-methyl glucuronoxylan of any hemicellulose. It has 10-20 xylopyranosyl stores in the chief chain for every 1 - 1,2-associated 4-O-methyl glucuronic destructive substituent. At C-2 or C-3, acidic destructive is esterified with around 60-70% of xylose units (Beg et al., 2001). When xylan is honored to get solvent base extraction, these acetyl bundles are easily taken out, which addresses a piece of xylan's most of the way dissolvability in water (Sunna and Antranikian, 1997). The fundamental piece of xylan in sensitive wood plants is arabino-4-O-methyl glucuronoxylan. Despite 4-O-methyl glucuronic destructive, - arabinofuranoside units are moreover displaced (Wong et al., 1988). Softwood xylans are included arabino-4-O-methylglucuroxylans and contain more 4-O-methylglucuronic destructive than their hardwood accomplices. The C-2 position is where the 4-O-methylglucuronic destructive stores are joined. Softwood xylans come up short on acetyl pack and have - L-arabinofuranose units joined by - 1,3-glycosidic bonds at the C-3 spot of the xylose rather than the acetyl bundle. In softwoods, the extent of 4-O-methyl-D-glucuronic destructive, - D-xylopyranose, and L-arabinofuranose is 100:20:13. (Puls and Schuseil, 1993). The fundamental hemicellulosic parts of the discretionary mass of softwoods are mannan-type hemicelluloses such glucomannans and galactoglucomannans, but they are simply found in follow aggregates in hardwoods (Girio et al., 2010).

RESEARCH METHODOLOGY

Xylanase activity estimation

The release of reducing sugars during the enzyme substrate reaction was used to measure xylanase activity. Miller's approach was used to calculate the release of reducing sugars (Miller, 1959). By adding 1.5 mL dinitrosalicylic acid (DNSA) reagent and boiling the reaction mixture for 15 minutes in a boiling water bath, the reduced sugars released could be determined and calculated. After cooling to room temperature, the color of the resulting combination was measured at 540 nm in comparison to enzyme and substrate controls. Under normal assay conditions, one unit (U) of enzyme activity is defined as the amount of enzyme that catalyzes the release of one gram of reducing sugars from the substrate in one minute. The activity of xylanase was measured in units per milliliter (U/ml). The amount of reducing sugar xylose was calculated using a standard curve plotted with xylose concentrations ranging from 50 to 500 g/ml. All of the experiments were done in triplicate and the findings reported are the average of the three results.

DNSA reagent composition

Components	Quantity/500 ml
Sodium hydroxide	5g
Phenol	1 ml
Sodium potassium tartarate	100 g
Sodium bisulphate	250 g
DNSA	5 g

Final volume of 500 ml was made up with distilled water

Estimation of cellulase activity

The crude extract's cellulase activity was determined using the same procedure that was used to determine xylanase activity. The carboxymethyl cellulose activity was measured at several pH levels ranging from 6 to 12 for the cellulase assay.

The amount of enzyme necessary to release 1 g of reducing sugar (as glucose)/ml/min under standard assay conditions was defined as one unit of cellulase activity, which was expressed as U/ml.

Media composition for xylanase production

Enrichment culture was used to isolate an extracellular xylanase-producing bacterium. Microorganisms were screened and isolated from soil samples gathered from various places. For bacterium isolation, 50 mL of nutritional broth was mixed with 5 gm of soil sample, and 0.25 percent Xylan was added as a carbon source. Enriched cultures were refreshed three times over the course of five days by transferring 1 ml inoculums into new media. Following that, 100 l of inoculum was collected from the enriched cultures and screened using the Spread plate technique, followed by incubation at 45 oC for 14 - 48 hours. Microbial colonies that had grown on nutritional media were picked up, streaked, and kept on separate plates. The xylanase output of maintained cultures was then assessed qualitatively, and all positive cultures were then screened quantitatively.

Application of xylanase in the paper industry

It is generally known that using chlorine compounds in the bleaching process produces harmful and long-lasting chlorinated organic byproducts, which eventually damage water bodies. Xylanase-mediated pre-bleaching has been proved in multiple published publications to be an environmentally benign and economically attractive method that can reduce the amount of bleaching chemicals necessary to achieve a given brightness in subsequent chemical bleaching stages (Zhao et al., 2006; Sacitha et al., 2007). During the current experiment, the physical and chemical parameters of pulp and paper were determined using the standard methods of the Technical Association of Pulp and Paper Industry (TAPPI), Atlanta, GA (Anonymous. 1991).

RESULTS AND DISCUSSION

Effect of additives on xylanases production by *Bacillus cereus*

The effect of several additives on xylanase production was studied, including metalloenzyme inhibitors, surfactants, and fatty acids. Olive oil (2670 U/ml) was shown to be a stimulator, but SDS (370 U/ml), EDTA (420 U/ml), and Glycerol (470 U/ml) were discovered to be strong inhibitors for xylanase synthesis (Table-1).

Surfactants and fatty acids have been shown to positively regulate enzyme synthesis in numerous studies (Singh et. al., 1991a; Battan et. al., 2007). Surfactants have also been

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shown to have a positive effect on xylanase activity (Xin et. al., 2017). The addition of surfactants and fatty acids increases the permeability of cell membranes, causing rapid secretion of enzymes from the cell. Metallo–enzyme inhibitors like EDTA and certain surfactants like SDS, on the other hand, have been extensively established for inhibiting the synthesis of xylanase and other hydrolytic enzymes (Kuhad et. al., 2006; Battan et. al., 2007; Ahlawat et. al., 2007). However, Xin et al., 2017 found that tween series had a favorable influence on xylanase secretion for bioethanol synthesis.

Table-1: Effect of additives on xylanase production by *Bacillus cereus*

Additives (0.2% w/v)	Activity (U/ml)
Control	2380
Tween 20	1620
Tween 80	510
Olive Oil	2660
SDS	320
Glycerol	460
EDTA	410
Triton X	520

Xylanase production under optimized conditions

After optimizing the various medium elements, a special medium called "Xylanase Production Medium" was created and used for further research. The following is the composition of the xylanase manufacturing medium: (See Table-2)

Table 4.7: Composition of the xylanase production medium

Xylanases Production Medium	**Quantity (%w/v)**
Wheat bran	1%
Yeast Extract	1.5%
Sodium nitrate	0.5%
Olive oil	0.2
MgSO4	10mM
pH	7.5

Temperature	40oC
Incubation Period	30h
Inoculam Age	24 h
Inoculam Size	3%

As the different production parameters were optimized, the xylanase yield rose up to 7.10 times when compared to the un-optimized medium (Table-3). As a result, a higher level of xylanase was produced in optimized media under optimal circumstances for industrial use.

In their investigation of xylanase production from pseudomonas sp. XPB-16 IN submerzed fermentation, Dhiman and Mukherjee (2017) found a 5.586-fold increase.

Table-3: Comparison of xylanase production under unoptimized and optimized conditions.

Xylanases activity under unoptimized conditions	**Xylanases activity under optimized conditions**	**Increase**
375 U/ml	2680 U/ml	7.10fold

Purification of xylanase

Ammonium sulphate fractionation and then ion-exchange chromatography methods were used to purify the isolated enzyme xylanase from Bacillus cereus to homogeneity. After mixing with ammonium sulphate, the precipitates were collected, and four cut-offs were created based on the amount of salt applied, namely 0-20 percent, 20-50 percent, 50-70 percent, and 70-90 percent; finally, dialyzed (cut-off 19kDa) against 0.1 M Glycine-NaoH. (pH 9.5). The proportion of these cut-offs with the highest xylanase activity was kept for further investigation. Using a quantitative estimation assay and protein estimation, it was discovered that the 50-70 percent fraction had the highest enzyme activity and protein concentration. Salt precipitation resulted in a 36 percent recovery of xylanase, resulting in a specific activity increase from 88.12 to 218 Ug-1. The cut-off proportion was treated to the CM-Sephadex column (bead size 40-125) after the salt precipitation process. After gradient elution of the enzyme from the column containing xylanoytic activity, just a single peak was identified after collecting different fractions of unbound proteins. After examining the

absorbance of the bound fractions, the apparent pure fraction number 43-55 showed the highest absorbance, and these fractions with the highest enzyme enzymatic activity were pooled. The enzymes had a purification fold of 11.35 and a specific activity of 218 U/ml. The steps of purification are summarized in (Table-4).

Yadav et al., 2018 found similar results for protein purification from A. kamchatkensis NASTPD13 utilizing sephadex G100 gel-permeation column chromatography. Archana and Satyanarayana (2003) reported a double stage xylanase purification from B. licheniformis utilizing salt precipitation, ion-exchange chromatography, and DEAE- Sephadex A-50, which yielded 38 percent xylanase with a 14.4-protein fold. Other researchers used CM-Sephadex for xylanase purification as well (Panbangred et. al., 1983; Battan and Rodrigues, 1993). Single-step purification of crude xylanase was also reported in some circumstances, where crude xylanase was purified directly using chromatographic separation techniques. On a Sephadex G-100 column, two xylanases, xyl – I and xyl – II, were isolated to homogeneity from Aspergillus caespitoses using the technique, with 23.8 and 2.4 recovery folds, respectively (Sandrim et. al., 2004).

Table- 4: Summary of purification of xylanase from *Bacillus cereus*

Purification steps	Total enzyme ($U\ min^{-1}$)	Total protein (mg)	Specific activity ($U\ mg^{-1}$)	Purification fold
Crude extract	918320	21865	42	1
$(NH_4)_2SO_4$ saturation (50-70%)	283216	3212	88.14	3.0
CM- Sephadex	24934	115	216	11.36

In the paper and pulp industries, xylanase is used

The xylanase's broad range of operational values at high pH and temperature ranges makes it suitable for a wide range of industrial applications. At the commercial level, the most crucial factor is the procedure's economic viability. To achieve this, we endeavored to optimize the technique for high yield manufacture of the enzyme in order to reduce the cost of the

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xylanase and ensure its commercial success. The enzyme's cellulase-free nature makes it acceptable for the bio-bleaching stage in the pulp and paper industry, and its low Mw makes it more suitable for pulp fiber.

CONCLUSION

It was explored if extracted xylanase could be used in the bio–bleaching of eucalyptus kraft pulp. The extracted enzyme is ideal for use in the paper and pulp sector in place of the kraft (sulphate) process due to its free activity, broad operational pH, and high stability, as well as good activity even at 70oC. The enzyme treatment was done in a'single lay out' method. After 120 minutes of retention time, 10 U g-1 of xylanase was shown to be optimal. After adjusting the various bio-bleaching reaction conditions, it was discovered that pH 10.0 and 65oC produced the greatest drop in Kappa no. and the greatest gain in whiteness (CIE WL). There was an 18% reduction in chlorine consumption. Following chlorination,'single lay out' resulted in a 48 percent increase in brightness. K.No and P.No both showed a drop of 12.9 percent and 4.5 percent, respectively. Enzymatic treatment improved various pulp qualities such as whitness (CIE Wl), yellowness (b*), and ClO2 content, as well as lowering the amount of ClO2. In comparison to standard chemical bleaching, there was a noticeable reduction in COD (Chemical Oxygen Demand) and a considerable improvement in many pulp qualities such as tensile strength, breaking length, burst factor, burstness, tear factor, and tearness. Based on these findings, it was determined that the enzyme was suitable for use in bio-bleaching in the paper and pulp industries.

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