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Identification Of Degradation Products Of Cellulase Enzyme Activity In Various Conditions Of Fungicides

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Abstract

The inhibitory effects of two fungicides, m- dinitrobenzene and pentachloro phenol were studied on purified carboxymethyl cellulase enzymes, isolated from the fungus *Aspergillus niger*. The biodegradation products were identified by thin - layer chromatography (TLC), using the solvent system as Acetone : Water : Chloroform : Methanol (8 : 0.5 : 1 : 1, vvvv). Sugars were identified by spraying p - anisidine hydrochloric acid sol. or 20% sulfuric acid in methanol on three ascents developed. It was found that the degradation products of enzymatic hydrolysis of substrate are glucose n cellobiose, as indicated by the Rf values.

Keywords: Chromatography, Sugars, Degradation.

INTRODUCTION

Cellulose is most abundantly found in nature. It is very easily and very commonly attacked by various microorganisms, mainly fungi, which biodegrade it by converting it into a mixture of sugars. Fungi biodegrade the cellulosic materials by accumulating cellulase enzymes in their culture media. It, therefore, becomes necessary to curb the activities of these fungi by means of suitable fungicides. The mechanism of action of fungicides on cellulolytic fungi was studied for the protection of various cellulosic materials in stores, like timber, leather, textiles etc.

Cellulase enzymes complex consists of three components- endoglucanase, exoglucanases & β - glucosidase. Each component is complex in nature. Studies have established that when acting separately, these enzymes have little or no action on highly ordered cellulose. But when together, act synergistically to extensively solubilize the most refractory cellulosic materials. The conversation of cellulose into alcohol has attracted attention in view of the industrial production of alcohol as a fuel source. Wood hemicellulose hydrolysates were utilized by microorganisms, such as, Klabsiella, Pneumoniae and Clostridium acetobutylicum for production of liquid fuels

and chemicals, like, 2,3- butanediol and acetone - butanol (Yu, E.K.C. et al., 1984) [1]. Chaparro - Beltran, Manvel (1986) [2] described that the catalytic hydrogenation of cellulosic wastes at 320° with KCO_2H and a cobalt catalyst produced a fuel oil in 30.25% yield. A liquid fuel obtained by the same process at 330° from municipal wastes has a low oxygen content and higher calorific value than that of the cellulosic waste derived fuel.

MATERIALS AND METHODS

2.1 Chemicals

All the inorganic chemicals used were of analytical grade, obtained from BDH Laboratories Bombay, India. Carboxymethyl Cellulose was obtained from Loba Chemie Indo Austranal.

2.2 Microorganism

The cellulolytic fungus *Aspergillus niger* was obtained from National Sugar Institute, Kanpur. The culture was grown on PDA SLANTS containing filter paper strips as cellulosic substrate at 30 ± 2°C for ten days and maintained at 4°C by subculturing every month.

2.3 Elaboration of Enzymes

On 7th day of growth of the organism, the mycelia were harvested by filtration through four layers of cheese cloth. The culture filtrates were directly centrifuged at 5,000 rpm for 20 minutes at 4°C. Simultaneously, the mycelial mat was collected on preweighed Whatman filter paper (no. 1), washed with distilled water and dried at 70°C until constant weight was obtained. The supernatant obtained was used as a source of crude Extracellular Enzymes.

After removing the supernatant, the mycelial mat, thus obtained was dried between the folds of filter paper. The partially dried mycelia were crushed in a grinder with small amount of distilled water which was diluted to obtain 2.0% solution of mycelia. This suspension was used for the study of Intracellular Enzymes.

2.4 Identification of Biodegradation Products by Thin layer chromatography

TLC was done on silica gel plates (20 × 20 cm, I. Merck). The solvent system used was Acetone : Water : Chloroform : Methanol (8 : 0.5 : 1 :1 , vvvv). After development of three ascents with the solvent, sugars were detected by spraying with p- anisidine hydrochloric acid solution or 20% sulfuric acid in methanol and heating at 120°C for 5 minutes.

RESULTS AND DISCUSSION

The carboxymethyl cellulose solution was hydrolyzed in presence and absence of the two fungicides, m-dinitrobenzene and pentachloro phenol, separately by repeatedly adding carboxymethyl cellulase enzymes. Deproteinization of the hydrolysates was followed by the concentration of the mixture to a small volume. Using pyridine - water - butanol as the irrigation mixture, chromatograms were run separately and the spots were sprayed with aniline hydrogen phthalate. In case of hydrolysates with fungicides, the paper chromatograms revealed no spots because of complete inhibition of enzyme activity. On the other hand, two spots were obtained

with hydrolysates in absence of fungicides. The Rf values of the two spots and of various known sugars are presented in the Table.

Table

R_f values

Sugars	R_f values
Degradation products	0.44
	0.38
Xylose	0.52
Glucose	0.45
Galactose	0.41
Cellobiose	0.37
Cellotriose	0.23
Cellotetraose	0.18
Cellohexose	0.09

It is evident from the Table that the degradation products of enzymatic hydrolysis of substrate are glucose and cellobiose because the Rf values of the two spots, viz., 0.44 and 0.38 are in close agreement with those of glucose and cellobiose respectively. Lee and Fan, 1979 [3], have also reported glucose and cellobiose as the hydrolysis products, obtained by the action of cellulase enzymes on cellulose. Kinoshita et al. (1986) [4] found that glucose accounted for 55 - 60% of the product in the hydrolysis of cellulose by cellulases of *Sporotrichum cellulophilum*. An increase in glucose and cellobiose production by 10% and 48%, respectively, was noted by Bao and Renganathan (1992) [5] by the addition of 10 µg/ml cellobiose oxidase (CBO) to a reaction mixture containing *Trichoderma viride* cellulase and microcrystalline cellulose. Beguin and Aubert, 1994 [6], described that the induction of cellulases appears to be effective by soluble products generated from cellulose by cellulolytic enzymes synthesized constitutively at a low level. These products are presumably converted into true inducers by trans-glycosylation reactions.

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