STUDIES ON THE MECHANISM OF HYDROLYSIS OF CELLULOSIC SUBSTANCES BY CELLULASE ENZYMES

BY

VIRENDRA SWARUP BISARIA

THESIS SUBMITTED TO THE INDIAN INSTITUTE OF TECHNOLOGY, DELHI
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY, DELHI
NEW DELHI - 110029

DECEMBER 1977

CERTIFICATE

This is to certify that the thesis entitled "Studies on the Mechanism of Hydrolysis of Cellulosic Substances by Cellulase Enzymes" submitted by Mr. Virendra Swarup Bisaria has been prepared under my supervision in conformity with the rules and regulations of the Indian Institute of Technology, Delhi. The research report and results presented in the thesis have not been submitted for any degree in any other University.

T.K. Ghose

(T.K.Ghose)

ACKNOWLEDGEMENT

I express my sincere gratitude to my research supervisor, Prof. Tarun K.Ghose, for his expert guidance, stimulating discussion, continued encouragement and keen interest throughout the period of this investigation.

I am grateful to Dr. P.Ghosh, Mr. Subhash Chand, Mr. C.P.Dwivedi, Mr. Vikram Sahai, Mr. R.K.Sachdev and other friends and colleagues for their valuable suggestions and enthusiastic cooperation.

Mr. S.Sen Ray and Mr. V.K.Ghosh are to be thanked for their skillful technical help. Appreciation is also extended to Mr. G.N.Sharma for his willing assistance. Finally, Mr. R.N.Shukla is thanked who patiently typed this manuscript.

V.S.Bisaria

(V.S.Bisaria)

5.1 Summary

Most cellulosic substances contain an appreciable amounts of cellulose and hemicellulose which on enzymatic hydrolysis yield simple sugars which could be converted to a variety of useful products such as food, feed and chemicals. Bagasse a complex cellulosic waste residue was chosen as a model substrate for determining the mechanism of its hydrolysis by the cellulase enzymes because of its abundant availability and being an energy crop. Since cellulases produced by T.viride (one of the most potent producers of cellulase enzyme) was not effective in hydrolyzing bagasse, a mixed enzyme derived from cellulases of T.viride and A.wenti was prepared. It was found that the mixed enzyme prepared from the culture filtrates of these two fungi on the basis of the same protein level in 1:1 proportion was most effective in solubilizing bagasse. The effectiveness of the mixed enzyme was found to be due to the presence of a definite combination of all the components taking part in the cellulolytic process.

The specific adsorption of cellulase components was found to be maximum at 50°C and pH 4.8, the conditions optimum for bagasse hydrolysis, suggesting a relatively higher adsorption of the active cellulase components compared to

the non-active proteins because of their higher affinity with the substrate.

Repeated adsorption of the cellulase enzymes on fresh bagasse and the repeated washing of the adsorbed enzyme-substrate complex revealed that there exist an equilibrium distribution of activities in the liquid and the solid phases. The adsorption of cellulase components on bagasse, therefore, followed the Freundlich adsorption isotherm, $Y = K.X^n$, in which the value of exponent, n, was unity. Continuous adsorption and desorption studies revealed that it was possible to desorb the cellulase components in a manner that the ratios of activities in desorbed phase are the same as that on the adsorbed phase.

Since the adsorption of cellulase components on cellulosic materials is a pre-requisite step for their hydrolysis, simultaneous adsorption of exo-glucanase, endoglucanase and xylanase on hydrolyzable cellulosics corroborates the mechanism of synergistic enzyme action postulated by Wood, Pettersson and Eriksson (16, 17, 76). Preferential adsorption of endoglucanase compared to that of exoglucanase suggests that these are the enzymes which go in first and cause disorganization in the cellulosic chains.

Since the interactions of the cellulase components present

in the mixed enzyme have not been identified in the studies mentioned above, the four major enzyme components viz. exoglucanase, endoglucanase, xylanase and β-glucosidase were purified using solvent purification, gel filtration and ion-exchange chromatographic procedures. The extent of adsorption of the cellulase components on bagasse was observed to be comparatively less when these purified components were present in mixture compared to their individual adsorption. It suggests that the bagasse surface contains some common sites on which atleast a part of these components could be adsorbed. The low activation energy values of the individual components indicate the physical nature of adsorption phenomenon.

Xylanase pretreatment has been shown to create more available cellulosic surface because of the higher hydrolysis rates observed with xylanase pretreated bagasse by exo- and endoglucanases. The xylanase action was, however, most effective when present in combination with the endo-and exo-glucanases suggesting that all the three components act synergistically for solubilization of bagasse. The production of glucose by the hydrolysis of β-glucosidase free enzyme solution suggest the presence of some additional route for its appearance in the hydrolysis products other than that of β-glucosidase.

CONTENTS

CHAPTER-I:

SECTION-I:	INTRODUCTION AND OBJECTIVE	... 1
SECTION-II:	LITERATURE SURVEY	... 10
CHAPTER-II:	MATERIALS AND METHODS	... 39
CHAPTER-III:	RESULTS	... 58
CHAPTER-IV:	DISCUSSION	... 150
CHAPTER-V:	SUMMARY AND CONCLUSION	... 169

NOMENCLATURE

REFERENCES