ENZYMATIC HYDROLYSIS OF CELLULOSE

Theory and Applications

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Foreword

This book reviews the theory and applications of enzymatic hydrolysis of cellulosic biomass, a renewable raw material for chemicals and energy. The subject has been of great interest during recent energy crises; but it may possibly be of greater interest as genetic engineering techniques are applied to the conversion of cellulose to sugars.

In either case, this excellent and thorough study fills a gap in the available literature on the subject. The state of the art and potential industrial processes are detailed. A section is also included on high productivity fermentation systems for the production of ethanol.

The information in the book is from *Enzymatic Hydrolysis of Cellulose: Theory and Applications* (LBL Report 13669), prepared by C.R. Wilke, B. Maiorella, A. Sciamanna, K. Tangnu, D. Wiley, and H. Wong of Lawrence Berkeley Laboratory and the University of California Department of Chemical Engineering, for the U.S. Department of Energy, June 1980.

The table of contents is organized in such a way as to serve as a subject index and provides easy access to the information contained in the book.

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Contents and Subject Index

Introduction

As a renewable raw material source for chemicals and energy, biomass has much promise. Grains and sugar, for example, can readily be converted to ethanol for use as a chemical intermediate or motor fuel. But longer range world needs will impose a limitation on the use of such crops for purposes other than food.

The cellulosic portion of biomass, however, represents an immense source of sugars which awaits only the development of the technology necessary for its economical utilization. Typically about 40% of the content of plant tissue is comprised of cellulose, a polymer of hexose sugars, and about 20% consists of hemicellulose, largely a polymer of pentose sugars. The monomer sugars can be produced by hydrolysis of the cellulosic fractions employing as catalysts either acids or enzymes. In light of an annual world production of biomass on the order of 10^{11} metric tons, the tremendous potential for a sugar based chemical and energy industry is apparent.

Enzymatic hydrolysis of cellulose is attractive because of its specificity and absence of the competitive degradation which normally accompanies acid hydrolysis. There has been a growing emphasis in research on enzymatic hydrolysis in recent years, stemming importantly from the pioneering work of Reese and Mandels at the U.S. Army Natick Laboratories. Over the last decade research in this field has been stimulated in the U.S. by major support from the National Science Foundation, the Department of Energy and its predecessor organizations, and most recently by the Solar Energy Research Institute. Research and development is actively underway in many other countries as well.

A large amount of research has been published on the theory of enzymatic hydrolysis and the various microbial, and other, sources of the enzymes. The

present report endeavors to supplement this information by emphasizing insofar as possible the status of the technology and of potential industrial processes for production of sugars from cellulose. A substantial research effort on cellulose conversion has been underway in the authors' laboratories at the University of California at Berkeley over the past ten years. This report is based in part upon this background of experience, and experimental data from relatively recent studies are presented in certain sections to make the information as timely and useful as possible.

Because of current interest in production of ethanol a section is included which summarizes various methods for high productivity fermentation systems.

I. Theory of Enzymatic Hydrolysis

A. Sources of Cellulases

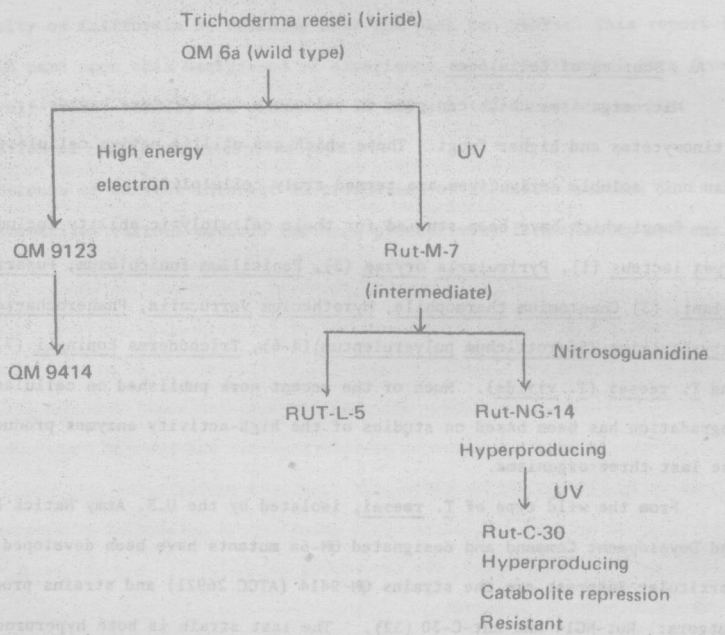
Microorganisms which can grow on cellulose include true bacteria, actinomycetes and higher fungi. Those which can utilize native cellulose rather than only soluble derivatives are termed truly cellulolytic.

Fungi which have been studied for their cellulolytic ability include Irpex lacteus (1), Pyricularia oryzae (2), Penicillium funiculosum, Fusarium solani, (3) Chaetomium thermophile, Myrothecium verrucaria, Phanerochaete chrysosporium (Sporotrichum pulverulentum)(4-6), Trichoderma koningii (7,8), and T. reesei (T. viride). Much of the recent work published on cellulose degradation has been based on studies of the high-activity enzymes produced by the last three organisms.

From the wild type of T. reesei, isolated by the U.S. Army Natick Research and Development Command and designated QM-6a mutants have been developed. Of particular interest are the strains QM-9414 (ATCC 26921) and strains produced at Rutgers: Rut-NG14 and Rut-C-30 (32). The last strain is both hyperproducing and catabolite repression resistant and has high xylanase activity. The geneology of these strains is shown in Figure I-1.Additional strains are being investigated for β-glucosidase resistant to end product inhibition.

B. Cellulolytic Enzymes

The first postulate concerning the nature of the mechanism of enzymatic hydrolysis was advanced by Reese, et al. (9). This was based on the fact that some organisms could hydrolyze native cellulose, while others degraded only soluble derivatives. A two-step process was envisioned. A component termed C_1 was thought to initiate hydrolysis by a preliminary activation or disaggregation of the cellulose chains. Subsequently, C_x, the second component, was responsible for the depolymerization to soluble cello-oligosaccharides.



Geneology of High Yielding Cellulase Mutants

Figure I-1

Supposedly, truly cellulolytic organisms possessed the C_1 enzyme, which other cellulose degrading organisms lacked.

Later work has shown, in contrast, that C_1 is a β-1,4-glucan cellobiohydrolase (10,11), and thus acts on the chains formed by C_x action, contrary to previous conjecture. Fractionation resulting in purification of these enzymes have been performed by numerous investigators (12-17). Purified components retain both C_1 and C_x activities, although relative values of each activity differ.

Enzymes designated C_1 and C_x show a large synergistic effect, the exact nature of which has not been ascertained. After purification, activity towards native cellulose is decreased. Subsequent combination of the purified enzymes in their original proportions results in the recovery of the original high activity.

C. Composition of the Cellulase System

The cellulase system has been shown to exhibit three distinct types of cellulolytic activity which bear the descriptive names: β-1,4-glucan glucanohydrolase (EC 3.2.1.4) an endo-enzyme; and β-1,4-glucan cellobiohydrolase (EC 3.2.1.91), an exo-enzyme; and β-glucosidase (EC3.2.1.21). These more exact names for the endo- and exoglucanases should replace the trival names C_1 and C_x.

The first enzyme is a randomly-acting endoglucanase, i.e. it acts on the interior of the polymer to generate new chain ends. This activity is assayed by reactivity towards soluble cellulose as shown by increases in reducing sugars or by decreases in viscosity.

The second enzyme acts on the nonreducing ends of the polymer chain to release cellobiose. This activity is determined by incubation with crystalline cellulose. When highly purified, it shows only slight activity due to the synergism of the enzyme system.

β-glucosidases hydrolyze cellobiose, and less actively short-chain cello-oligosaccharides, and are necessary for removal of product which would otherwise inhibit the progress of the hydrolysis. Recent investigation has suggested that β-glucosidase activity as determined by reaction with p-nitrophenyl-β-glucoside must be distinguished from activity towards cellobiose. The mode of action of the separate activities of the combined system is shown in Figures I-2 and -3, respectively.

D. Fractionation of Cellulases

Cellulases have been fractionated into their constituent components by a variety of biochemical procedures including ultrafiltration, gel filtration, ion-exchange chromatography, adsorption chromatography and iso-electric focusing Endoglucanases and exoglucanases have been found to be glucoproteins. Isozymes have been reported for each component.

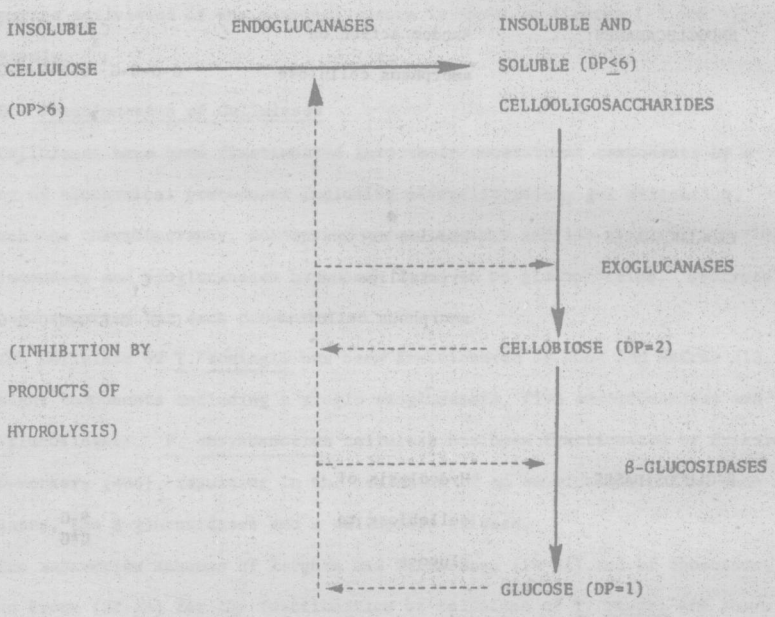
The cellulase of T. koningii has been fractionated by Wood and McCrae (12,18) into eight components including a single exoglucanase, five endoglucanases and two β-glucosidases. P. chrysosporium cellulase has been fractionated by Eriksson and co-workers (406), resulting in the isolation of an exoglucanase, five endo glucanases, two β-glucosidases and a cellobiose oxidase.

The separation schemes of Berghem and Pettersson (19-21) and of Shoemaker, Gum and Brown (22-24) for the fractionation of cellulase of T. reesei are shown in Figures I-4 and -5, respectively. From industrial preparations of cellulases, Berghem and Pettersson have reported isolation of two endoglucanases and a single exoglucanase and β-glucosidase, while Shoemaker, et al. have reported four endoglucanases, one exoglucanase and one β-glucosidase. The latter report a separation suitable for a large-scale purification procedure.

Physical properties of cellulase constituents prepared by various investigators are shown in Table I-1.

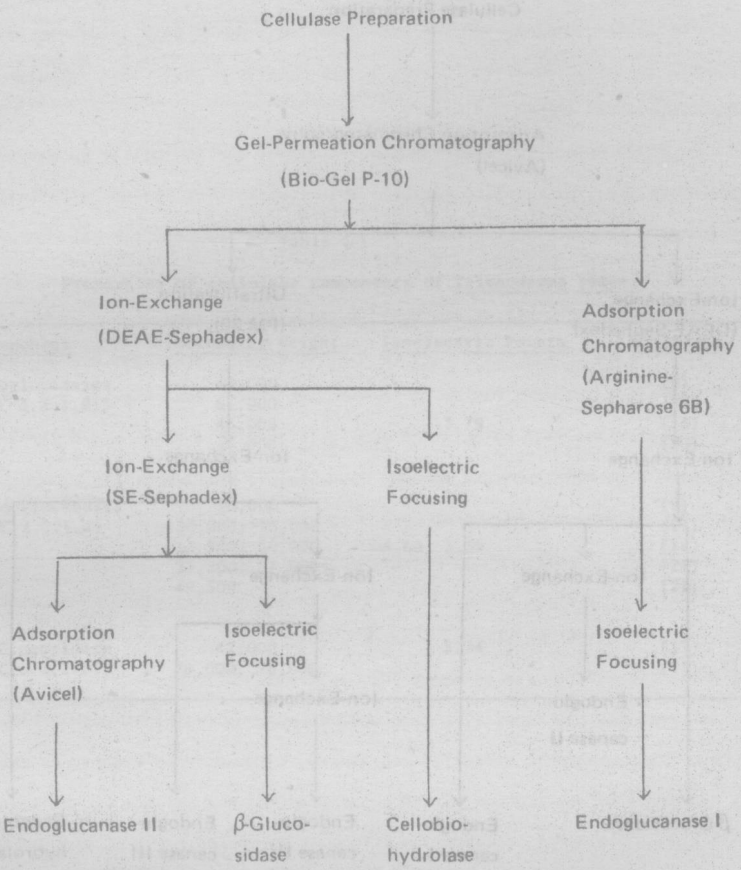
ENDOGLUCANASES:	Random action on amorphous cellulose	C_x ↓ G-G-G-G-G-G-G-G-G-G
EXOGLUCANASES:	Endwise action on crystalline and amorphous cellulose	C_1 ↓ G-G-G-G-G-G-G-G-G-G
β-GLUCOSIDASES:	Hydrolysis of cellobiose to glucose	β_1 G G≠G

Figure I-2. Separate Activities of the Cellulase Complex



MODE OF ACTION OF CELLULASE

Figure I-3.



Cellulase Fractionation (Berghem, Pettersson)

Figure I-4.