Preface

Significant interest in cellulose degradation started in the 1950s when cotton-based uniforms of the U.S. military were being destroyed by microorganisms during the Korean War. At that time, the major focus for cellulase research was the inhibition of these enzymes to prevent the degradation of the uniforms. In the 1970s, which ushered in the first oil crisis due to instability in the Middle East, the interest in cellulases switched to the use of these enzymes to explore the potential of cellulosic biomass as a renewable, and thus sustainable, replacement of fossil-based sources of oil and its associated products. The use of cellulases in second-generation biofuels is now a major driver for the substantial worldwide interest in these enzymes. Over the past 60 years, cellulase research has evolved as new methods became available. Until the early 1980s, cellulases were purified from their endogenous host and then subjected to enzyme characterization. With the advent of gene cloning and gene sequencing, enzyme characterization from the endogenous host very rapidly became redundant, and for the following 10 years, cellulases were characterized from noncellulolytic hosts such as *Escherichia coli*. The sequence information of these enzymes led to their assignment to 12 discrete families providing evidence for convergent evolution. In 1983, Bayer and Lamed discovered that cellulase enzymes expressed by anaerobic organisms associate to form multienzyme complexes known as cellulosomes. In the late 1980s, it became evident that cellulases often displayed modular architectures in which the catalytic domain is linked to noncatalytic cellulose binding domains (now referred to as carbohydrate binding modules). Finally, in the early 1990s, and for 10 years thereafter, the crystal structure of a large number of cellulases were determined, which provided substantial insight into how the topology of the active site and distal substrate binding regions contribute to the mode of enzyme action. The new millennium brought additional advances in cellulase methodologies. These include the use of cellulases as biosynthetic enzymes through their conversion to glycosynthases; small angle X-ray scattering is now routinely used to study the topology of multimodular cellulases; it is now possible to visualize, in real time, the movement of cellulases across cellulose fibers using high-speed atomic force microscopy; cellulosomes assemblies can be visualized using X-ray crystallography and a suite of high-throughput technologies ranging from the rapid analysis of complex plant cell wall structures to the saccharification of plant biomass by cellulase cocktails have recently been developed.

This volume of *Methods in Enzymology* has attempted to inform readers of all the methods currently deployed in cellulase research. The volume is loosely grouped into six sections. Chapters 1–7 focus on cellulase assays that deal with activity measurements and product analysis; Chapters 8–10 describe methods used to characterize the structure of the enzymes and their interaction with substrate; Chapters 11–13 provide methods used to characterize cellulose-specific carbohydrate binding modules; Chapters 14–16 describe methods used to increase the industrial utility of enzymes and microbes; Chapters 17–20 target the methodology used to explore and isolate cellulolytic microorganisms, while the final four chapters, 21–24, describe methods used to study dockerin–cohesin interactions that lead to cellulosome assemblies. Each chapter provides a background to the topic described placing the enzymes, microorganisms, or analytical method in the context of cellulase research.

When working on cellulases that are attacking polysaccharides, particularly insoluble forms of cellulose, one must recognize that these enzymes depart from classical enzyme-catalyzed reactions. Thus, because of the polymeric nature of the substrate, target sites are not uniformly distributed in solution and thus it is not always easy to apply typical Michaelis–Menton kinetics. Maybe more significant are the problems associated with studying the action of cellulases on crystalline substrates. The reaction is slow and the substrate has several different substructures that vary in access to enzyme attack. Thus, simple kinetics (product release versus time) is rarely linear as substrate access will alter as the reaction proceeds. Typically, in the cellulase field, rather crude measurements of enzyme activity are used such as the time it takes for 80% of substrate to be degraded. Thus readers who come from a typical enzyme field should be aware of the problems presented with studying the action of cellulases against, particularly, insoluble substrates; there is no continuous assay, the initial rate has no relevance, and the degree of enzyme processivity (Chapter 5) needs to be carefully considered. I hope this volume will be particularly useful to scientists new to the cellulase field, while also providing an important methodological reference for multidisciplinary groups already working in the cellulase field.

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