

BIOCHEMICAL CONVERSION PROGRAM  
ANNUAL REVIEW MEETING

at the

*SOLAR ENERGY RESEARCH INSTITUTE*

October 13-15, 1987

## ENZYMOLGY RESEARCH

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### PREAMBLE

Enzymology research at SERI in FY 1986-87 has focused on the problem of enhancing the effective lifetime of the key fungal cellulase enzymes under process conditions (i.e., those of SSF or SHF), since the cost of producing these enzymes has been identified as a sensitive issue in the overall bioconversion economics. The effective utilization of cellulase hydrolysis potential in these process schemes requires access to enzymes of high stability and a scheme for recovery of this activity before disposal of spent feedstock. The issue of adsorptive loss of cellulase activities was studied in FY 1987 and is reported here. Also reported in this document are studies designed to quantitatively describe the instability of the native fungal beta-glucosidase and to then investigate chemical crosslinking technology as a tool for achieving greater stability. In the process of performing this work a new affinity chromatography method was developed and is also described herein. Progress was also made in FY 1987 on the characterization of the highly thermostable cellulase enzymes secreted by the new thermophile Acidothermus cellulolyticus and these studies are reported as well.

The enzymatic degradation of cellulose to small reducing sugars is generally accomplished by the synergistic action of three classes of enzymes: first, the "endo-1,4-beta-cellulases" or 1,4-beta-D-glucan 4-glucanohydrolases (EC 3.2.1.4) which act at random on soluble and insoluble 1,4-beta-glucan substrates and are commonly measured by the detection of reducing groups released from carboxymethylcellulose (CMC); second, the "exo-1,4-beta-glucosidases" which include both 1,4-beta-D-glucan glucohydrolases (EC 3.2.1.74) which liberate D-glucose from 1,4-beta-glucans and hydrolyze D-cellobiose slowly, and 1,4-D-glucan cellobiohydrolase (EC 3.2.1.91) which liberates D-cellobiose from 1,4-beta-glucans; and third, the "beta-D-glucosidases" or beta-D-glucoside glucohydrolases (EC 3.2.1.21), which act to release D-glucose units from soluble celloextrins and an array of glycosides.