**Mandels M, Andreotti R & Roche C.** Measurement of saccharifying cellulase. *Biotechnol. Bioeng. Symp.* 6:21-33, 1976.
[US Army Natick Development Center, Natick, MA]

A method for measuring cellulase activity is described. This assay is easy to perform, quantitative, and reproducible and can be used to predict the quantity of enzyme required for large-scale hydrolysis. [The *SCI*® indicates that this paper has been cited in over 330 publications, making it this journal's most-cited paper.]

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In the early 1970s the oil crisis generated interest in using cellulose as a chemical and energy resource. One promising approach was to hydrolyze the cellulose to glucose with fungal enzymes and then to ferment the glucose to ethanol, which could be used as a liquid fuel.[1] The Energy Research and Development Administration and other agencies began to sponsor such research and were eager to set up demonstration projects. A major economic limitation was the cost of the cellulase enzymes. The biotechnologist needed an enzyme unit to measure productivity in a fermentor and to compare strains and mutants. The bioengineer needed an enzyme unit on which he could put a dollar value and from which he could predict sugar output in his reactor.

Most enzyme assays involve a single enzyme acting on a soluble substrate of fixed composition. Cellulase is a mixture of enzymes that act synergistically, i.e., individual purified enzymes have little or no action on insoluble cellulose but the appropriate mixture can totally convert it to glucose. Cellulose is an insoluble, recalcitrant, and variable substrate. The rate and extent of enzyme reaction are affected by chain length and crystallinity of the cellulose, as well as by any physical or chemical pretreatments. The inevitable result of the complexity and variability of enzyme and substrate was a bewildering variety of cellulase assays developed and used in different laboratories. Results from these assays were not comparable.

Our paper proposed a simple, useful unit of cellulase activity. We selected Whatman No. 1 filter paper as a substrate because it is widely available and because it is similar to a "real" process substrate—not too resistant, not too susceptible. For cellulase assays, initial rates of reaction are not meaningful because the most susceptible portions of the substrate are rapidly hydrolyzed but the rate quickly declines as the increasingly resistant residues are attacked. If the cellulase preparation lacks some of the essential components (i.e., is incomplete), hydrolysis soon ceases. Our major contribution was the recognition that for quantitative results the enzyme unit must be based on a fixed percent conversion and that to be meaningful (i.e., to eliminate incomplete cellulases), this conversion must be great enough to include some of the crystalline cellulose. We found that a 4 percent conversion of 50 mg of paper gave quantitative, reproducible values and that the unit can be used to predict conversion in a saccharification reactor, taking into consideration that the efficiency of the unit in extensive conversion will be only 5-10 percent.[2]

The unit we proposed has been widely accepted and used. In 1976 the Commission on Biotechnology of the International Union of Pure and Applied Chemistry decided to evaluate cellulase assays and to recommend standard procedures. A series of meetings was held, including a workshop at the Massachusetts Institute of Technology in 1982. The commission recommended the filter paper assay as described in our paper as the standard cellulase assay.[3]

1. **Mandels M, Hontz L & Nystrom J.** Enzymatic hydrolysis of waste cellulose. *Biotechnol. Bioeng.* 16:1471-93, 1974. (Cited 195 times.)
2. **Mandels M, Medeiros J E, Andreotti R E & Bissett F H.** Enzymatic hydrolysis of cellulose: evaluation of culture filtrates under use conditions. *Biotechnol. Bioeng.* 23:2009-26, 1981. (Cited 45 times.)
3. **Ghose T K.** Measurement of cellulase activities. *Pure Appl. Chem.* 59:257-68, 1987.



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