A SIMPLE TECHNIQUE FOR DETECTION OF CELLULASES*

SHEPLEY S. C. CHEN**

The classical source of cellulases has been the snail gut and the culture filtrate of the fungus *Myrothecium verrucaria.* In the higher plants, cellulases were reported to occur in the germinating cereals. The present paper will describe a simple technique by means of which the author has detected a cellulase (or cellulases) in the seeds of *Phacelia tanacetifolia* (honey bee plant).

Lots of 500 mg of the seeds were surface-sterilized in 75% ethanol, washed in distilled water, and imbibed for a desired period of time. Each lot was homogenized in mortar and pestle, with purified sand, in 6 ml of water or an appropriate medium. The slurry was then centrifuged at 1000×g for 20 min. The supernatant was collected and used as an enzyme source. These procedures were carried out in a cold room at 4°C.

The enzyme activity was followed by the release of the reducing groups from carboxymethylcellulose (CMC). To 1 ml of the enzyme preparation was added 1 ml of the substrate (0.2% CMC in 0.04 M acetate buffer, pH 5.0, containing 2×10^{-4} M penicillin G). The mixture was incubated at 37°C in a constant temperature water bath for O, 1, 2 and 3 hours, with occasional shaking. For controls, the enzyme extract and the substrate were incubated separately, then were combined at the end of the incubation period, 2 ml of dinitrosalicylic acid reagent was added to stop the reaction. The mixture was heated in boiling water for 5 min., cooled in running water, and 20 ml of distilled water was added. The absorbance (O.D.) was taken in a spectrophotometer at 500 millimicrons or in a colorimeter equipped with a green filter. The experiments were run in duplicates and the controls were set up individually. The O. D. of the control at zero time was set as nil.

Figure 1 shows the result of an experiment, in which the enzyme was extracted from the dry seeds of *Phacelia tanacetifolia.* One sees a steady increase tn reducing capacity in both the controls and the test as time goes on. The change observed in the controls must be due to the hydrolysis of the endogenous substrates (probably carbohydrates) present in the enzyme extract. The difference between the control and the test at each incubation period is ascribed to the enzymic hydrolysis of the added substrate, carboxymethylcellulose.

This result was cross-checked by the Ostwald's viscometric technique and

* This is a part of work done in 1965 at the Biological Laboratories, Harvard University Cambridge, Massachusetts, U. S. A.

** Present address: MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48823, U. S. A.

found to be valid. The next attempt would be to determine if the factors which affect germination of this seed, such as light and gibberellins, will influence the activity of cellulases.

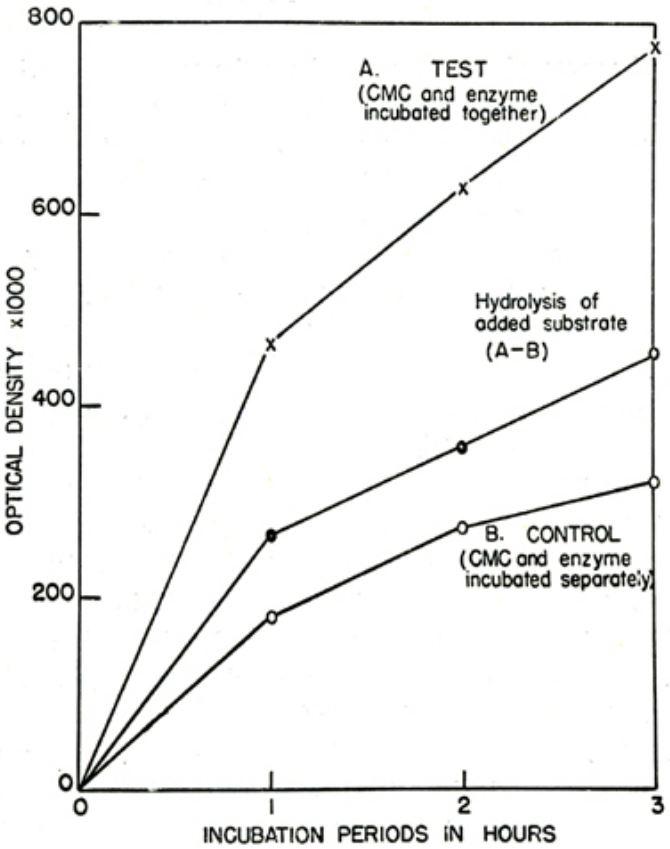

Figure 1. Time course of Hydrolysis of Carboxy methyl cellulose by Extract from *Phacelia* seeds