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Influence of *p*H and Aeration on Bacterial Formation of Amylase

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Amylase production by a *Bacillus subtilis* strain can occur without aeration after reaching the stationary phase of growth, provided the *p*H is controlled.

Bacillus subtilis H-1521, which was used in this study, was obtained from the University of Tokyo, Japan. It was grown in an extract of Proflo, a partially cooked cottonseed flour (Traders' Oil Mill Co., Forth Worth, Tex.), to which 4 g of Na_2HPO_4 per liter was added. The Proflo extract was obtained by boiling 100 g of this material in 1,000 ml of water at *p*H 2.0 (HCl) for 2 hr, allowed to stand overnight at room temperature, and centrifuged. The clear extract was autoclaved for 20 min at 20 lb of pressure, adjusted to *p*H 8.5 with NaOH solution, and centrifuged again. The final *p*H of the medium after sterilization was 7.2. This medium, with no solids in suspension, proved to be adequate for amylase formation either in batch or in continuous-culture experiments (J. E. Donato, Ph.D. Thesis, Univ. of LaPlata, LaPlata, Argentina, 1965).

Inoculum was grown in Proflo medium in shaken flasks at 30 C in a rotary shaker for 17 hr. Bacterial growth was followed by dry weight determinations. The official method recommended by the Association of Official Agricultural Chemists (1) was used for the estimation of enzyme activity. Larger-volume experiments were carried out in a New Brunswick 7-liter fermentor (model FS-307) at 30 C and 695 rev/min. At the beginning of every experiment, the air flow rate was 0.3 volumes/min. In some experiments, the air flow was cut off when the growth curve indicated the end of the exponential phase of growth (10 to 12 hr). When a *p*H control was required, a *p*H meter (model 23A, EIL Ltd., England) with the electrodes immersed into the culture was used. To maintain the *p*H within the required ranges, an NaOH solution (5%, w/v) was added.

Figure 1 shows the typical results obtained. Up to 12 hr (when the air supply was stopped), yields were quite similar in all of the experiments. From that moment on, the enzyme activity remained constant in the experiment conducted without aeration and with no *p*H control. In the other experiment, carried out under the same conditions but with a controlled *p*H, the amylase activity increased with time, attaining values similar to those observed in the aerated experiment. Hence, if the *p*H is controlled, aeration of the culture can be discontinued at the same time as the growth of the microorganism stops.

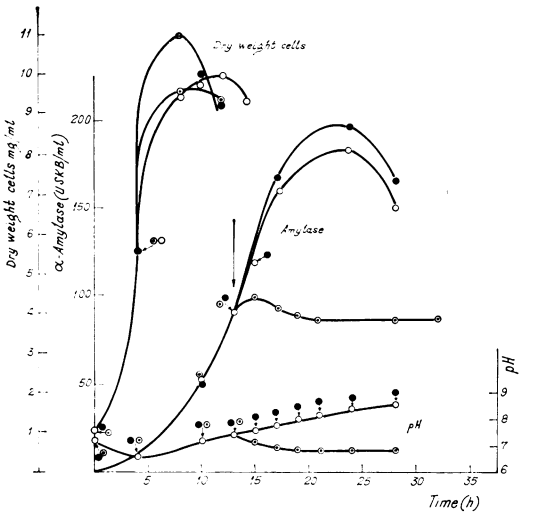

FIG. 1. *Influence of pH on enzyme formation after stopping the air supply. Aerated experiment* (○); *nonaerated experiment without controlled pH* (⊙); *nonaerated experiment with pH adjusted to values similar to those observed in the aerated experiment* (●). *The long arrow indicates the moment at which the aeration was discontinued.*

To the best of our knowledge this finding has not been reported previously. The explanation for this phenomenon is not clear, and further investigation is required to elucidate the influence of *p*H on enzyme formation, or on the release of enzyme from cells, and to consider strain differences as well as the influence of the components of the medium.

LITERATURE CITED

1. AOAC. 1960. Ninth ed., p. 129.