

studies have revealed that the carbohydrate-nitrogen ratio appears to constitute an essential factor in the elaboration of the enzyme. It is generally recognised that starch stimulates the production of the enzyme, but no quantitative data is available in literature. The present study has been undertaken to determine the effect of the addition of varying amounts of starch to a given quantity of nitrogen, on the formation of diastase by *Aspergillus oryzae*.

The method of culturing the fungus, of making the enzyme extract and of determining the

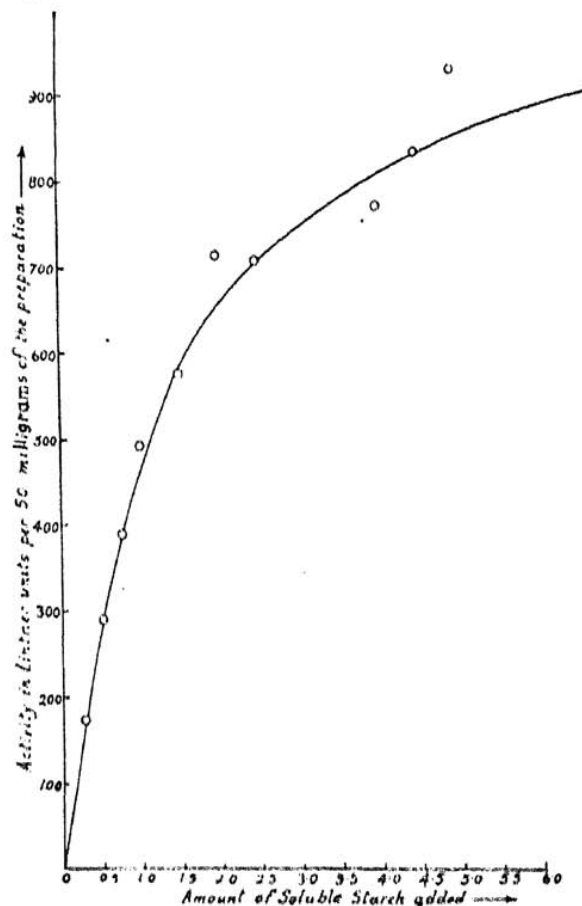


FIG. 1

diastatic activity, are similar to those described in our earlier communications. Finely shredded and acid-digested and purified asbestos was used to provide an inert mat for the growth of the fungus. The basal nutrient solution was composed of:—Peptone (Difco) 60 grs.; potassium dihydrogen phosphate 1 gm.; potassium citrate 1 gm.; magnesium sulphate, 7H<sub>2</sub>O 1 gm.; ferrous sulphate 0.01 gm.; zinc sulphate 0.01 gm.; calcium chloride 0.5 gm.; water 1 litre.

The medium was constituted as follows:—A given weight (5 gm.) of the asbestos was placed in 250 c.c. conical flasks, moistened with 12.5 c.c. of the nutrient solution (corresponding to 290 mgms. of nitrogen) and treated with graded amounts of soluble starch. The mass was intimately mixed, autoclaved at 20 lbs. for 30 minutes on two successive days. The flasks were then inoculated with a suspension of the spores of the fungus, incubated at 30° C. for three days, the resulting moldy

#### INFLUENCE OF CARBOHYDRATE TO NITROGEN RATIO ON THE FORMATION OF DIASTASE BY *ASPERGILLUS ORYZAE*

OUR previous work has shown that the formation of diastase by *Aspergillus oryzae* is influenced by the character and complexity of the nitrogen employed in the culture medium. Further

TABLE I

Reaction Mixture No.	1	2	3	4	5	6	7	8	9	10	11
Starch added in gms.	0.25	0.50	0.75	1.0	1.5	2.0	2.5	3.5	4.0	4.5	5.0
Activity per 50 mgms. of preparation in Lintner units.	173	291	308	402	574	711	704	747	760	824	924
Total number of units elaborated by the mixture (in thousands of Lintner units).	4.17	7.10	9.55	12.30	13.96	18.81	20.28	21.96	22.95	26.37	30.49

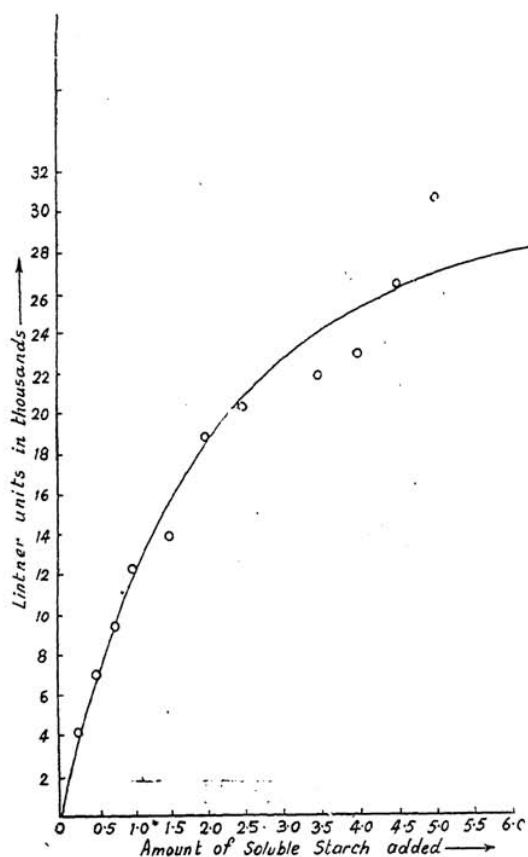


FIG. 2

mass extracted with 25 c.c. of water and the diastatic activity of the extract determined. The results are given in Table I and graphically represented in Figs. 1 and 2. Fig. 1 gives the activity of the preparations from fungi raised on different concentrations of starch; Fig. 2 gives the total quantity of diastase (in Lintner units) elaborated by the different experimental mixtures.

A close study of Table I and the Figures 1 and 2, reveals that with an increase in the proportion of starch, there is a steady increase not only in the purity of the diastase but also in the total quantity of the enzyme formed.

The total quantity of the diastase which is formed in proportion to added starch tends to fall off rapidly after the two per cent. concentration of starch (see Fig. 2). The economic optimum for the ratio of the soluble starch to nitrogen appears to lie somewhere about 7:1.

It has been found that starch is completely utilised in all the mixtures so far investigated and the reducing sugar content of the preparations has been found to be insignificant. Section of Fermentation Technology,

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