KINETICS OF INACTIVATION OF INVERTASE

By K. S. G. Doss,* F.A.Sc. and H. C. Saraswat §

(Indian Institute of Sugar Technology, Kanpur)

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Introduction

Many of the physico-chemical properties of invertase are found to be dependent on hydrogen-ion concentration. For instance the spreading power[1] of invertase on aqueous substratum is reduced by over 90% as the pH of the substratum is changed from 1·0 to 2·0. Similarly the rate of accumulation[2] of invertase at the surface of aqueous solutions comes down tremendously as the pH is raised from 1·0 to 3·0. Further the inversion activity of invertase is found to be a function of pH. The work of Euler and Laurin[3] has shown that inactivation of invertase is also a function of pH. The present paper gives an account of some detailed observations on inactivation.

Experimental

1. *Materials used.*—(*a*) The sample of invertase used was supplied by Messrs. Sugar Manufacturers' Supply Co. Ltd., 7–8, Idol Lane, London. The invertase concentrate is prepared from baker's yeast. The solution contains nearly 55% of purest glycerine for stabilization of the enzyme. The solution is clear and transparent and colourless. The activity is found to be constant for a long period. The solution does not contain any surface-active substance or nitrogenous compound. These details were kindly supplied by Messrs. Sugar Manufacturers' Supply Co., Ltd., London. The nitrogen content of the invertase concentrate is found to be $0{\cdot}65 \pm 0{\cdot}06$ mg./c.c.

Further, the spreading properties of this invertase concentrate are found to be practically the same as those of pure preparation used by Gorter and Dieu in their studies.[4] We have also prepared a purified product by adsorption of the commercial sample on alumina and elution by Na_2HPO_4.[5] The purified product also gave results not appreciably differing from that of the original product, whenever a check was made. There appears to be no

* Professor of Chemistry, Indian Institute of Sugar Technology, Kanpur.

§ Professor of Chemistry, Christ Church College, Kanpur.

doubt that all the main features of the results reported here will be reproduced by any similar purified product.

(*b*) The copper reagent was prepared and standardised according to the usual method.[6]

(*c*) The chemicals used were of Reagent quality of Merck.

2. *Method of finding out the activity of invertase.*—Our invertase preparation had a time value of 58 minutes per c.c. In the present work we have taken arbitrarily the activity of the invertase present in 1 c.c. of this preparation as 100 units. We have measured the activity of the other products dealt with in this paper in terms of this arbitrary standard. In comparing the activities of the various products with that of the original stock solution we have discarded the time value method since (*a*) the method takes a long time and is rather laborious; (*b*) it requires a large quantity of invertase preparation; and (*c*) the end point corresponds to an advanced stage of inversion of sugar in that it represents the stage at which 75·9% of sugar has been inverted. In view of this we have preferred the method of Gorter and Dieu[7] with some modifications.

The principle of the method consists in finding out the number of milligrams of invert sugar produced by different quanttiies of the stock invertase preparation (time value 58 minutes per c.c.; invertase content assumed arbitrarily as 100 units per c.c.) by following a special experimental procedure. A graph is then constructed by plotting the number of milligrams of invert sugar produced against the number of arbitrary units of invertase used. The activity of any other preparation can then be found out by determining the number of milligrams of invert sugar produced under the special experimental conditions and referring to graph (Fig. 1).

The following procedure is adopted for carrying out the inversions for determining the activity of invertase preparations. 10 c.c. of 20% sugar solution is taken in a large diameter tube which is kept in a thermostat at 25° C. To this are added 7 c.c. of acetate buffer solution pH 4·5 and $(8 - x)$ c.c. of distilled water and x c.c. of invertase preparation under test. The mixture is kept at 25° C. for 5 minutes and then the reaction is stopped by adding excess of M/4 caustic soda solution (final pH 11). The solution thus obtained is made up to 250 c.c. in a measuring flask. 5 c.c. of this dilute solution are withdrawn and added to 5 c.c. of copper reagent or a multiple of this volume, contained in a large test-tube. After mixing the solutions the tube is placed in boiling water and heated for 15 minutes. The tube is cooled quickly by immersing in ice-cold water. The unreduced copper is determined without filtration by dissolving about 2 to 3 grams

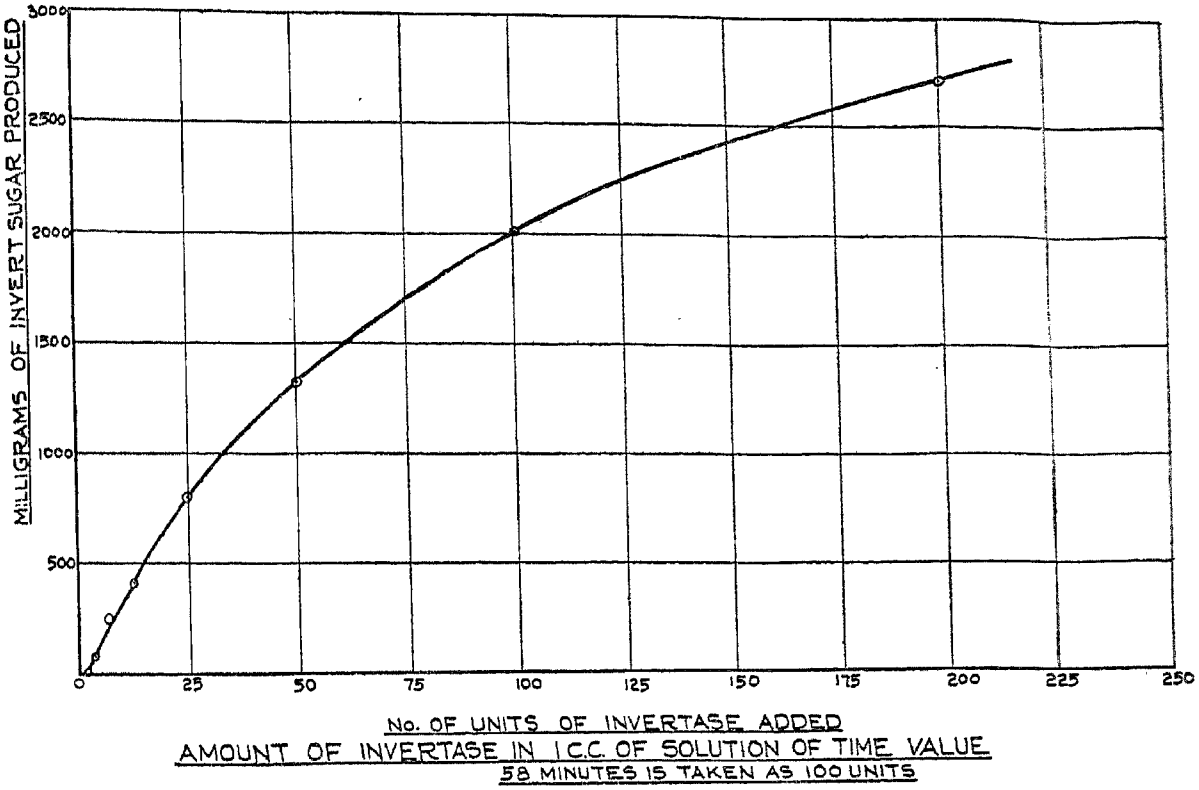

FIG. 1

of potassium iodide in it and acidifying it by 2M sulphuric acid. 5 c.c. of saturated potassium oxalate solution are added to stabilize the reaction.[8] After waiting for 5 minutes the liberated iodine is titrated against 0·1 N sodium thiosulphate using starch as indicator near the end point. The total copper in 5 c.c. of copper reagent is determined by a second titration with the copper reagent only. The difference between the two titration results is equivalent to the cuprous oxide precipitated by reducing sugar. Each c.c. of the difference indicates 6·36 milligrams of copper. The corresponding amount of reducing sugar is found from Munson and Walker Tables.[9]

To take into account any invert sugar that may be produced due to agencies other than invertase during the experimental determination of activity a blank is performed. This is done by taking 10 c.c. of 20% sugar solution without invertase and carrying out the whole experiment as before.

3. *Inactivation of invertase by changes in pH.*—For studying inactivation of invertase, the enzyme was introduced at a concentration of 2·5 units per c.c. in a medium of any required pH. 10 c.c. samples were drawn out at different intervals of time, the pH was quickly restored to 4·5 and their activity determined as described before. The results are given in Table I.

TABLE I

Inactivation of Invertase

Initial activity of invertase = 1/58 invertase units per c.c.

[Time value = 58 minutes per c.c.]

Activity of invertase at pH 4·5 after treatment, as a fraction of initial activity.

Time of treatment minutes	pH of the solution							
	1·29	1·34	1·38	1·58	1·78	2·3	3·02	4·5
5	0·05	0·2	0·55	0·625	0·875	0·95	0·925	1·05
10	0·025	0·03	0·425	0·55	0·775	0·85	0·95	1·05
20	0·025	0	0·225	0·525	0·775	0·85	0·95	..
30	0·02	0	0·15	0·475	0·775	0·85	0·95	1·05
40	0·016	0	0·05	0·4	0·775	0·85	0·95	..
50	0	0	0·03	..	..	..	..	..
60	0·01	0	0	..	..	0·85	0·95	1·03

DISCUSSION

Effect of pH on inactivation of invertase.—An examination of Table I shows that invertase is very stable at pH 4·5. As the pH is lowered to a critical value inactivation becomes exceedingly fast. This critical pH is found to be in the neighbourhood of about 1·8. At a pH of 1·3 and below there is a loss of activity of over 95% in 5 minutes time whereas at pH 1·8 the loss is only about 12% within the same time. The inactivation process has been shown to be not due to adsorption as vessels of different size did not effect the extent of inactivation. Similarly the transfer of solution from vessel to vessel, at low pH so as to come into contact with a large surface of glass did not affect the activity. The inactivation is not following the simple first order formula. There is a high initial rate of inactivation which is followed by a slow rate of inactivation.

There appear to be two processes occurring simultaneously though independently bringing about the inactivation of invertase. Only one of these processes comes into operation at comparatively high pH ($\geqslant 1 \cdot 78$) and is very fast. In the first 5 minutes the inactivation due to this cause would be practically over. The other process begins to be effective only at lower pH and has a measurable rate at a pH of 1·58. At still lower pH,

both the processes are occurring fast and the inactivation is extremely quick. Further work is needed to elucidate the nature of the two processes.

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SUMMARY

A detailed study has been made of the kinetics of inactivation of invertase at 25° C. in acid media. The results indicate the existence of two processes, one of them being very fast and coming up at comparatively high pH values and the other coming up at lower pH.

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