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Invertase Formation in *Saccharomyces fragilis*

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SUMMARY: The formation of invertase in *Saccharomyces fragilis* was studied by the continuous culture technique. Invertase formation was markedly inhibited by glucose in the culture medium at concentrations greater than 0.001 % (w/v), by galactose and lactose at all concentrations tested, and by several other carbon sources. The mean generation time of the organisms and the concentration of ammonia and growth factors had no significant effect on invertase formation. The pH value of the growth medium modified the amount of enzyme formed. The invertase activity, together with non-enzymic hydrolysis of sucrose by the acidic culture medium, was sufficient to account for the disappearance of the observed amount of sucrose from media containing sucrose.

During the growth of a micro-organism under ordinary culture conditions in a limited volume of medium the growth rate continually changes and the chemical composition of the medium varies as a result of the metabolism of the organism. In such circumstances it is difficult, if not impossible, to assess all the factors which control the enzymic activities of the cells. However, if a steady state could be achieved so that known environmental factors could be kept constant, it would be considerably easier to investigate the effect of each such factor. One way of achieving a culture in a steady state is by use of the continuous culture technique described by Monod (1950) and by Novick & Szilard (1950 *a, b*), in which the growth rate is determined by the concentration of one particular constituent of the growth medium. Under such conditions the effects of other constituents on enzymic activity can be studied, uncomplicated by changes in growth rate. Also, by altering the rate of addition of the medium, and hence of the limiting factor, the growth rate can be varied without appreciably affecting the steady state concentration of other substances in the growth medium. Therefore, it should be possible to determine whether growth rate *per se* has any effect on enzyme formation.

There have been many reports of variations of the enzymic activities of bacterial and yeast cells with growth time. Wooldridge, Knox & Glass (1936) found that the dehydrogenase activities of several bacterial species were low in 'young' cultures, increased with continued growth and then decreased again. Gale & Stephenson (1938) reported a similar effect with serine deaminase in *Escherichia coli*. Gale (1940) found that the activities of the bacterial amino-acid decarboxylases were highest when the culture density was maximal. Similar results were obtained with aspartase in *E. coli* (Gale, 1938) and were shown to be due to a change in the chemical composition of the medium, for when the medium in which a culture had grown was sterilized and re-inoculated, the aspartase was high at all stages of growth. Davies (1953) found that

when *Saccharomyces fragilis* was grown in a medium containing 2% (w/v) glucose, the invertase activity was low during the early phases of growth but later increased rapidly. When the glucose concentration was increased to 10% (w/v), invertase activity was low at all stages of growth. The author concluded from these and other experiments that the presence of glucose in the medium inhibited the formation of invertase. Invertase synthesis was demonstrated in suspensions of washed organisms and was inhibited by glucose and fructose.

The present work describes the effect on invertase formation in *Saccharomyces fragilis* of certain sugars and other carbon sources. The effects of the mean generation time of the organism, concentrations of ammonium and hydrogen ions and of growth factors have also been studied. Most of this work was carried out by means of the continuous culture technique.

METHODS

The chemostat. Fig. 1 shows the most satisfactory type of chemostat devised for this work. It resembles the chemostat of Novick & Szilard (1950*a*), the main difference being in the aerator and the medium feed. The aerator has the dual functions of aerating and stirring the medium. The flow of air through the aerator was sufficiently great to set up an atomizing action, sucking culture fluid in through the side opening and expelling it together with fresh medium at the base. Standard taper (B 24) ground glass joints at 'A' and 'B' enabled the cap and the aerator to be removed to facilitate cleaning; 'B' also served as the inoculation port. The medium was supplied by a Bayliss and Muller type peristaltic pump (Booth & Green, 1938) driven from a 0.125 h.p. constant speed induction motor through a train of gears. Alterations in the pumping rate are achieved by changing the gear ratio. The pump rollers and rubber tubing were lubricated with silicone grease. The variation in flow rate with a fixed gear ratio was $\pm 0.5\%$.

Fig. 1. Diagram of chemostat.

Organisms. *Saccharomyces fragilis* Jørgensen was obtained from the Centraalbureau voor Schimmelcultures, Delft, and *Torulopsis pulcherrima* from the National Collection of Type Cultures, Elstree, in 1946.

Media. The basal medium was that of Davies, Falkiner, Wilkinson & Peel (1951) with Difco yeast extract replaced by the following amounts of growth factors ($\mu\text{g./ml.}$): biotin, 0.01; pantothenic acid, nicotinic acid, pyridoxine, thiamine, each 1.0; inositol, 5.0. The basal medium (less thiamine) was

sterilized by autoclaving. The various carbon sources, glucose, galactose, glycerol, sodium acetate, sodium citrate, sodium lactate, and sodium succinate were autoclaved separately; sucrose, lactose and thiamine were sterilized by filtration; ethanol was added as absolute ethanol. The carbon source and thiamine were added aseptically to the basal medium and sterile water added to replace that lost by evaporation.

For chemostat experiments the basal medium was made up in graduated aspirator bottles. For static cultures the medium was dispensed into Roux bottles (150 ml./bottle) and for shaken cultures into 500 ml. Erlenmeyer flasks (150 ml./flask). The technique for studying variations in enzymic activity with time of growth was that described by Davies (1953). The inoculum was grown for 24 hr. in boiling tubes containing the basal medium + 2% (w/v) glucose, except for the experiments where growth took place on ethanol, glycerol, sodium acetate, sodium citrate, sodium lactate or sodium succinate, in which cases the glucose was replaced by the respective carbon source. The growth temperature was 30°.

Chemostat procedure. Monod (1950) showed that under steady state conditions of growth in a chemostat, the rate of addition of medium (or washing-out rate) determines the mean generation time of the organisms. The relationship can be expressed as follows: Mean generation time = $(\log_e 2)/\text{washing-out rate}$. The minimum value for the mean generation time is determined by the composition of the medium and the gas phase and increases in the washing-out rate beyond this point result in dilution out of the culture to vanishing point.

In practice the chemostat was connected aseptically to the medium reservoir, the air supply turned on, the chemostat filled with medium and inoculated. After 15–20 hr. the addition of medium was begun at the lowest practicable rate (10 ml./hr.). It was determined that after a further 24 hr., steady state conditions prevailed and the first sample was collected in a receiver cooled in ice. The flow rate was then increased to the value chosen for the next sample which was collected on the following day. This procedure was continued until the lowest practicable mean generation time of 1.5 hr. was attained.

Organism suspensions. The yeast was centrifuged from the growth medium, washed twice with distilled water and re-suspended in distilled water. Suspension density was determined by drying a sample at 105° to constant weight. This procedure was adopted because it was found that the ratio of turbidity to dry weight could vary over a twofold range depending on how the yeast was grown.

Invertase activity was estimated by the method of Davies (1953), which uses cetyltrimethylammonium bromide. The unit of activity has been defined as the amount of enzyme which hydrolyses 1 μ mole sucrose in 1 hr. at 25°. From six replicate samples collected from a chemostat running at a fixed flow rate it was found that the coefficient of variation was less than 5% when the invertase activity was greater than 100 units/mg. When the activity was low (< 2 units/mg.) the absolute variation between replicate samples was less than 0.5 unit/mg.

Analysis of growth media. Ammonia was determined by steam distillation in a micro-distillation apparatus (Markham, 1942) followed by nesslerization (Folin & Wu, 1919). Sugars were determined on 0.04–1.8 ml. samples by fermentation in Warburg manometers according to the method of Reithel (1951) modified by increasing the concentration of sodium azide to 1.3×10^{-3} M. Yeast was grown for 24 hr. in Roux bottles containing 150 ml. of the basal medium of Davies *et al.* (1951) and an appropriate sugar at a concentration of 2% (w/v). The yeast was harvested as described above and suspended in 0.17 M-potassium phosphate buffer (pH 6.0) to give a cell density of c. 100 mg./ml. Sodium azide was added to a concentration of 4×10^{-3} M and the preparation was shaken for 2 hr. at 25° to eliminate endogenous fermentation. Each manometer cup contained 1 ml. of this preparation in a final fluid volume of 3 ml. Carbon dioxide evolution at pH 6.0 was 95–98% of the theoretical 2 mole CO₂/mole hexose for glucose, sucrose and lactose, and 92–95% for galactose.

For determination of glucose and sucrose, *Saccharomyces fragilis* was grown on a medium containing glucose. For determination of galactose and lactose *S. fragilis* was grown on a medium containing galactose. For determination of invert sugar in cultures containing sucrose *Torulopsis pulcherrima* was grown on a medium containing glucose.

Chemicals. Kerfoot's Biochemical Reagent Sugars were used throughout; other chemicals were of analytical quality.

RESULTS

Invertase formation during growth in glucose media in shaken flasks

The change in invertase content of the cells with time of growth under various conditions is shown in Fig. 2*a, b*. Curves marked *A* show invertase formation and growth in a medium containing an initial concentration of 1.5% (w/v) glucose, 352 mg. ammonia-N/l. and 20% of the standard amount of growth factors. The invertase content of the cells remained low during the greater part of growth and then rose rapidly to high values. This rise in activity was concurrent with the disappearance of glucose from the medium. For curves marked *B* the medium initially contained 0.1% (w/v) glucose. Growth ceased earlier and at a lower cell density than in the previous case. The invertase activity was again low in the early phases of growth. Curves *C* and *D* (Fig. 2*b*) show the effect of decreasing the amount of nitrogen in the medium; in both cases growth ceased before glucose was fully utilized and the invertase activity was low throughout the experiment. Growth started at approximately the same rate in both cases, but the final yield of organisms was lower in the medium containing the smaller amount of nitrogen. Curves *D* and *E* show the effect of alterations in the amount of growth factors, while glucose and nitrogen are kept constant. The growth curves were almost identical, but whereas in *D* the invertase content remained low, with the smaller concentration of growth factors (curve *E*) glucose utilization continued after growth ceased and invertase activity increased concomitantly. The reason for this is not understood.

Invertase formation during growth in the chemostat

Effect of glucose concentration and mean generation time. When the yeast was grown in a chemostat supplied with medium initially containing 9.5 % (w/v) glucose, 105 $\mu\text{g.}$ ammonia-N/ml. and standard concentrations of growth factors, the glucose concentration in the culture became steady at approximately 8 % (w/v), the nitrogen concentration at approximately 20 $\mu\text{g./ml.}$ and

Fig. 2. The effect on invertase content of growth for different periods of time in media containing various amounts of glucose, nitrogen and growth factors. $\times$ — $\times$, invertase activity; $\circ$ — $\circ$, culture density. The glucose concentrations in the cultures at the different times are shown in the table. Curve *A*: 1.5 % (w/v) glucose, 352 mg. ammonia-N/l., 20 % of standard growth factors. Curve *B*: 0.1 % (w/v) glucose, otherwise as curve *A*. Curve *C*: 2.0 % (w/v) glucose, 352 mg. ammonia-N/l., standard amounts of growth factors. Curve *D*: 105 mg. ammonia-N/l., otherwise as curve *C*. Curve *E*: 20 % of standard growth factors, otherwise as curve *D*.

Medium	Residual glucose (% w/v) at various times (hr.)						
	6	9	12	15	18	24	36
<i>A</i>	1.2	1.0	0.64	0.24	0.03	Nil	Nil
<i>B</i>	0.05	0.01	Nil	Nil	Nil	Nil	Nil
<i>C</i>	1.8	1.6	1.3	0.55	0.26	0.15	0.14
<i>D</i>	1.8	1.7	1.5	1.4	1.3	1.2	1.2
<i>E</i>	1.8	1.7	1.6	1.4	1.2	0.57	Nil

the invertase activity of the resultant organisms was less than 0.6 unit/mg. at all values of the mean generation time. Low degrees of activity were also observed when the supplied medium contained 2 % (w/v) glucose; in this case the glucose concentration in the culture was always greater than 0.5 % (w/v); the nitrogen concentration was less than 3 $\mu\text{g./ml.}$ for all flow rates except that corresponding to a mean generation time of 1.6 hr. when it rose to 7 $\mu\text{g./ml.}$

Fig. 3 shows the invertase activities and culture glucose concentrations for growth in media initially containing 1.0, 0.5 and 0.25 % (w/v) glucose. In these cases the activity varied with the mean generation time, but could also be correlated with the glucose concentrations in the cultures. In Fig. 4, which is compiled from the results of a large number of experiments, the invertase activity is plotted against the logarithm of the glucose concentration in the culture for mean generation times of 3 and 10 hr. The two curves are substantially the same, showing that the rate of cell division has little effect on

the biosynthesis of invertase under these conditions. It would appear that invertase formation only occurs to a significant extent in media containing less than 0.01 % (w/v) glucose.

Effect of growth factors and nitrogen concentration. Biotin, nicotinic acid and pantothenic acid are essential for growth of this strain of *Saccharomyces fragilis*; inositol, pyridoxine and thiamin are stimulatory (Bigger, 1952). It is possible to devise conditions in which nitrogen and glucose are in excess; this

Fig. 3

Fig. 4

Fig. 5

Fig. 3. Variation of invertase activity with mean generation time. Initial glucose concentrations (% w/v) in media: A, 1.0; B, 0.5; C, 0.25.

Fig. 4. Effect of culture glucose concentration and mean generation time on invertase activity. x—x, cells grown with a mean generation time of 3 hr.; O—O, cells grown with a mean generation time of 10 hr. The limit of sensitivity of the sugar analysis procedure is 0.001 % glucose.

Fig. 5. Effect of culture glucose concentration and nitrogen concentration on invertase activity. x—x, culture containing $< 2 \mu\text{g. ammonia-N/ml.}$; O—O, culture containing $> 10 \mu\text{g. ammonia-N/ml.}$

can be done by making one or other growth factor limiting. Under these conditions it was found that neither variation in nitrogen concentration nor in the nature of the growth factor which was limiting, had any appreciable effect on the invertase activity. The overriding influence of the steady state glucose concentration in the culture is again shown by Fig. 5 in which the logarithm of the culture glucose concentration is plotted against the invertase activity for the two cases (a) where the nitrogen concentration is less than $2 \mu\text{g./ml.}$; (b) where it is greater than $10 \mu\text{g./ml.}$ The two curves are almost identical at glucose concentrations greater than 0.001 % (w/v), which is the limit of sensitivity of the sugar determination procedure. This demonstrates clearly the absence of any significant effect of nitrogen concentration, irrespective of the nature of the growth-limiting substance.

Effect of pH value of growth medium. The pH value of the culture in the chemostat varied between pH 2.6 and 3.4, depending on the concentration of sugar in the medium. By adding appropriate amounts of potassium hydroxide to the medium it was possible to grow the yeast over a wide range of pH values

in media of otherwise similar composition. When the basal medium contained 0.1 % (w/v) glucose and the mean generation time was 4 hr., the potential invertase activity (i.e. activity measured at the optimal pH value for invertase action) varied with pH value as shown in Fig. 6, curve *A*. Between pH 3.5 and 7.7 there was no detectable glucose in the culture; between pH 7.7 and 8.3 the glucose increased to 0.014 % (w/v). The effective activity (see Gale & Epps, 1942) is also plotted, and was found to decrease steadily as the pH value increased from 3.5 to 8.3 (curve *B*).

Fig. 6. Effect of culture pH value on invertase content. Curve *A*: potential activity of cells grown with a mean generation time of 4.0 hr. in a medium initially containing 0.1 % glucose. Curve *B*: effective activity of the aforementioned cells. Curve *C*: potential activity $\times 10$ of cells grown with a mean generation time of 3.5 hr. in a medium initially containing 2.0 % glucose. The potential activities were determined at the optimum pH value (3.7) and a correction made for acid hydrolysis in the test system.

When the yeast was grown in the basal medium plus 2 % (w/v) glucose at a mean generation time of 3.5 hr., the potential activity increased from 0.6 unit/mg. at pH 2.6 to 6 units/mg. at pH 7.6 (Fig. 6, curve *C*), while the effective activity was approximately constant over the whole range.

Media containing sugars other than glucose

Sucrose. Fig. 7 shows that when growth occurred in a medium containing an excess of sucrose (1 %, w/v, curve *A*) the invertase activity was low (0.5–2.0 units/mg.). Since the culture was found to contain both sucrose (0.3–0.6 %, w/v) and invert sugar (0.02–0.05 %, w/v), it was impossible to decide whether sucrose itself exerts any specific effect on the invertase activity. When the yeast was grown in a 0.2 % (w/v) sucrose medium (curve *B*) the invertase activity was high. Although in this case the invertase activity

varied with mean generation time, it could again be correlated with changes in the sugar (mostly invert sugar) concentration in the culture (curve *C*). The growth obtained in both concentrations of sucrose was approximately the same as that obtained in corresponding media containing glucose.

When the yeast is grown in an excess of sucrose it is possible to compare the disappearance of sucrose from the medium with the maximum possible disappearance calculated on the basis of the observed invertase activity. The results are given in Table 1. It is evident that the sucrose disappearance can

Fig. 7

Fig. 8

Fig. 7. Effect of growth at different mean generation times in media containing sucrose. Curve *A*, invertase activities $\times 10$ during growth in a medium initially containing 1% (w/v) sucrose. Curve *B*, invertase activities during growth in a medium initially containing 0.2% sucrose. Curve *C*, culture invert sugar concentrations corresponding to curve *B*.

Fig. 8. Curves *A* and *B*: effect of adding galactose (1%, w/v, curve *A*; 0.1%, w/v, curve *B*) to cultures growing on 0.1% (w/v) glucose medium with a mean generation time of 3.5 hr. Curve *C*: effect of adding glucose (1.0%, w/v) to a culture growing on 0.2% (w/v) galactose medium with a mean generation time of 3.7 hr., the activities are shown times ten for clarity.

Table 1. *Correlation between observed sucrose disappearance in media containing an excess of sucrose and that calculated from the invertase content*

Enzymic hydrolysis measured at pH value and temperature of culture and at optimal sucrose concentration. Resulting values were then corrected to allow for the fact that the culture sucrose concentration was suboptimal, the correction factor being 0.9.

	Expt.		
	I	II	III
pH value of culture	3.15	3.19	3.12
Sucrose disappearance due to spontaneous hydrolysis at culture pH value (mg./ml.)	0.2	0.2	0.2
Sucrose disappearance due to enzymic hydrolysis (mg./ml.)	1.3	1.7	1.2
Observed sucrose disappearance (mg./ml.)	1.5	1.9	1.3

be accounted for by the invertase content of the yeast provided due allowance is made for non-enzymic hydrolysis of sucrose under the acidic conditions of the medium.

Lactose or galactose. In Table 2, the invertase activity of yeast grown in various concentrations of lactose or galactose is compared with that for glucose media. It will be seen that the response is different in that high degrees of activity were not obtained in low concentrations of lactose or galactose, in fact, on increasing the galactose concentration to 2% (w/v) the invertase activity rose fivefold to tenfold. Similar results were obtained for mean generation times between 1.6 and 10 hr.

Table 2. *Invertase activities of Saccharomyces fragilis grown in media containing various sugars*

Yeast grown in chemostat at a mean generation time of 3.3 hr. Invertase activities expressed as units/mg. dry weight.

Concentration of sugar in growth medium (% w/v)	Invertase activities for growth on		
	Glucose	Galactose	Lactose
2.0	0.2-0.6	4.0	—
1.0	0.2-0.6	0.6	1.4
0.2	200	0.2	0.7

Mixtures of glucose and galactose. The effect of adding galactose to an exponentially growing culture which had a high invertase activity was demonstrated by the following experiments. From a culture growing with a mean generation time of 3.5 hr. in the basal medium + 0.1% (w/v) glucose a 10 ml. sample was collected and then galactose was added directly to the culture to give a final concentration of 1.0% in the first experiment and 0.1% (w/v) in the second experiment. Further samples were collected at intervals and their invertase contents determined. There was an immediate and rapid decrease in the invertase activity followed by a rise (Fig. 8, curves *A* and *B*). The decrease was greater with 1% (w/v) galactose and the activity was low for a longer time, but the rate of decline in activity was greater with 0.1% galactose.

The effect of adding glucose (final concentration 1.0% (w/v)) to a culture growing exponentially with a mean generation time of 3.7 hr. on a 0.2% (w/v) galactose medium is also shown in Fig. 8, curve *C*. A rise in activity to 6 units/mg. was observed 6 hr. after addition of glucose. Table 3 shows the

Table 3. *Effect on invertase content of growth in media containing various amounts of glucose and galactose*

Saccharomyces fragilis grown in chemostat at a mean generation time of 3.3 hr. In all cases the culture hexose concentration was less than 0.001% (w/v).

Initial glucose concentration (% w/v)	0.20	0.18	0.15	0.11
Initial galactose concentration (% w/v)	Nil	0.02	0.05	0.09
Invertase activity (units/mg. dry wt.)	190	127	36	6.5

effect on invertase formation of growth in a medium containing various amounts of glucose and galactose and demonstrates clearly the inhibitory effect of galactose under these conditions.

Growth in other carbon sources. Under aerobic conditions *Saccharomyces fragilis* used arabinose, fructose, glucose, galactose, lactose, mannose, sucrose, raffinose, xylose, ethanol, glycerol, acetate, citrate, lactate or succinate for growth. Ribose, sorbose, dulcitol, mannitol, sorbitol and sodium potassium tartrate were not utilized. Except for the sugars, and for lactate for which the minimum mean generation time was 3.5 hr., the minimum mean generation

Fig. 9. Effect of 'age of culture' on the invertase content of *S. fragilis* grown on basal medium containing non-carbohydrate carbon sources. ×, invertase activities; ○, culture density; —, basal medium plus 1% (w/v) sodium succinate; ---, basal medium plus 1.0% (w/v) ethanol; -.-., basal medium plus 1% (w/v) glycerol.

time exceeded 5 hr. and in some cases (acetate, citrate) was 8–12 hr. It was not practicable to use the chemostat for such low growth rates. Cultures were therefore grown in Roux bottles on basal medium containing 1% of various substances as carbon sources and the invertase activity determined after various periods of growth.

Acetate, citrate or succinate gave similar results up to the time when growth ceased. In the earlier stages of growth the invertase activity was about ten times that in a 1% (w/v) glucose medium, the activity then increased to a maximum towards the end of growth. With succinate, but not acetate or citrate, there was a fall in invertase activity shortly after growth had ceased (Fig. 9).

Ethanol at a concentration of 1% (v/v) gave an invertase activity of c. 15 units/mg. which showed little variation throughout growth.

With glycerol invertase activity was high (80 units/mg.) at the start of the exponential phase of growth but it rapidly fell to about 40 units/mg. at which

level it remained constant until after growth ceased when there was a further fall to 9 units/mg. (Fig. 9).

With lactate the invertase activity remained at a low level (0.1–1.0 unit/mg.) throughout the growth period.

DISCUSSION

The formation of many enzymes is suppressed by the presence of glucose in the growth medium (Gale, 1943). The effect on invertase formation, however, is wider than an inhibition by glucose, for if the invertase activity in the presence of low concentrations of glucose is taken as the uninhibited value, then all carbon sources tested are inhibitory, though with some, such as raffinose (Davies, 1953) and glycerol, the inhibition is small. The type of response shown by the yeast depends on both the nature and the concentration of the carbon source. For instance, the response to galactose differs from that to glucose; the highest activities (about 2 % of that observed in a medium containing low concentrations of glucose) are found in media initially containing 2 % (w/v) galactose, while lower invertase activities are observed when the galactose concentration is reduced. Yeast which has grown on a medium containing an excess of sucrose has a low invertase activity. At first sight this would seem to indicate that invertase formation is suppressed by sucrose. However, the low activity can be accounted for by the presence of low concentrations of invert sugar in the culture. Because of this, it is impossible under the conditions used to decide whether sucrose has any inhibitory or stimulatory action on invertase formation. The observed low concentrations of invert sugar in these cultures are in accordance with the hypothesis of Hestrin & Lindegren (1952), that biosynthesis of carbohydrases in yeast continues until the rate of production of hexose by hydrolysis of oligosaccharides slightly exceeds the rate of utilization of the hexose, the accumulating hexose then inhibiting further enzyme formation. The fact that, after allowance had been made for acid hydrolysis in the culture, sucrose disappearance in these cultures agreed closely with that calculated on the basis of the invertase activity of the organisms in washed suspension, would suggest that the sole pathway of sucrose metabolism is via hydrolysis to invert sugar.

Davies (1953) found that with sucrose as energy source synthesis of invertase by washed suspensions of *Saccharomyces fragilis* proceeded optimally between pH 4.0 and 5.0, the optimum pH value for enzyme action being approximately 4.0. However, the same relationship was observed when glucose was the energy source, in which case the energy supply is not dependent on the activity of the enzyme. In growing cultures the formation of invertase shows a different response to pH value, the potential activity of the yeast grown in a 0.1 % (w/v) glucose medium being at a minimum when the culture pH value is 5.4. The activity increases as the pH value is reduced to 3.5 or increased to 7.7. Between pH 7.7 and 8.3 the activity decreases again, probably due to an increase in the glucose concentration in the culture over this range. The optimal effective activity is obtained with a culture at pH 3.5. Since the invertase

activity of organisms grown in a 2% (w/v) glucose medium over a wide range of pH values is low, it would appear that the inhibitory effect of high concentrations of glucose occurs at all pH values.

In the introduction it was stated that by use of the continuous culture technique it should be possible to assess the part played by changes in the rate of division and in the concentration of various nutrients on the invertase content of the organisms. It has now been shown that the rate of division and the concentration of ammonium ions and growth factors in the culture have no significant effect on the invertase content of the organisms under the conditions employed. As the pH values of the cultures (2.6–3.4, depending on sugar concentration) normally shows little change, this factor also has little effect on the invertase content, though, as described above, large changes in pH value have a marked effect. The main factor which controls the invertase content of *Saccharomyces fragilis* in media containing glucose is the glucose concentration.

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