Comparative Studies on the Dissimilation of Reserve Carbohydrate in Four Strains of *Saccharomyces cerevisiae*

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It has been shown that a brewing strain of *Saccharomyces cerevisiae* accumulated large quantities of reserve carbohydrate during anaerobic growth (Chester, 1963*a*). During harvesting, contact with oxygen initiated the breakdown of a large proportion of these reserves. Evidence was also presented that the yeast reserves provided a sufficient energy source for the synthesis of respiratory enzymes during the aeration of anaerobically grown resting cells.

Chester (1961) compared baking and brewing strains of *Saccharomyces cerevisiae*, and reported that in anaerobically grown baking yeast the carbohydrate breakdown after harvesting was relatively much less than in the brewing yeast grown under the same conditions, and that the breakdown was not stimulated by oxygen.

The present paper compares the ability of a number of strains of yeast to accumulate reserve carbohydrate during anaerobic growth and describes the effect of partial pressure of oxygen on the breakdown of these reserves in washed-cell suspensions. The yeasts are also compared with respect to respiratory adaptation and the effect of partial pressure of oxygen on this adaptation.

METHODS

Yeast culture and preparation of suspensions. The baking strain of *Saccharomyces cerevisiae* used in this work was no. 77 from the National Collection of Yeast Cultures. Cultures of this yeast were very powdery in appearance when shaken and microscopic examination showed that there was no tendency for the cells to flocculate at any stage in the fermentation.

Three brewing strains of *Saccharomyces cerevisiae* were examined. All were single-cell cultures obtained from strains originally isolated from the Park Royal Brewery. Strain 4236 has been described previously (Chester, 1959, 1961, 1963*a*). This strain, when grown in the medium described below, did not flocculate, although in some media such as beer wort there was a tendency for the cells to aggregate and form small flocs towards the end of the fermentation.

Strain 7000 was a very flocculent yeast. This grew initially as dispersed cells which clumped together to form aggregates of thousands of cells when about half the glucose in the medium had been fermented. Although derived from a single cell, this strain showed a gradual decrease in

degree of flocculence while being subcultured during the course of this work. A study of the changes in flocculence in this yeast has been described (Chester, 1963*b*).

Strain 7001 was a flocculent yeast in which the daughter cells did not separate from the parent during growth, resulting in the formation of branching chains. The cells making up these chains appeared to be only loosely connected since shaking of the cultures caused most of the cells to separate.

The yeast was grown at 25° in stationary 250 ml. flasks containing 75 ml. of the medium described by Spiegelman & Nozawa (1945). This had the following composition: yeast extract, 2 g.; peptone, 5 g.; (NH₄)₂SO₄, 1 g.; KH₂PO₄, 2 g.; MgSO₄·7H₂O, 0.25 g.; CaCl₂·6H₂O, 0.13 g.; sodium lactate (50%), 7 ml.; glucose, 80 g.; distilled water to 1 l.

Conditions of growth, harvesting and preparation of suspensions were as described by Chester (1963*a*). All four strains had similar rates of fermentation and all were harvested 48 hr. after inoculation, by which time their total carbohydrate contents had reached a maximum. The concentration of glucose left in the media was less than 0.5% in all cases.

The washed suspensions of strains 77 and 4236 in 67 mM-KH₂PO₄ consisted of single cells. Although most of the cell aggregates of strain 7000 were broken up during the washing procedure, flocs were rapidly reformed in the final suspension. The branching chains of strain 7001 were largely broken during the washing procedure and the cells did not aggregate again in the final suspension, which consisted mainly of single cells together with a few flocs.

Analytical methods. The methods of analysis were as described by Chester (1963*a*).

RESULTS

Carbohydrate content of yeast. The total carbohydrate contents of yeasts harvested from 48 hr. cultures are compared in Table 1. There was a significant difference ($P < 0.01$) in the total carbohydrate content of strains 77 and 4236, both non-flocculent yeasts. The two flocculent brewing yeasts also had significantly lower carbohydrate contents than the brewing strain 4236 ($P < 0.01$).

The proportions of the main storage and structural carbohydrates contained in the yeast are shown in Table 2.

Variations within a single-cell culture in ability to store carbohydrate. During the period in which this work was carried out, strain 7000 became less

flocculent. Examination showed that this culture had become a mixture of variants differing in their degree of flocculence. These differences were in the size of the flocs and in the time taken for re-aggregation of cells to occur after dispersion by shaking (Chester, 1963*b*). The carbohydrate content was determined of sub-strains selected at intervals in the range of flocculence (Table 1). The most flocculent sub-strain did not store as much carbohydrate as the less flocculent yeast. Sub-strain 1 had a significantly lower carbohydrate content than sub-strain 2 ($P < 0.01$), and that of sub-strain 2 was significantly lower than that of substrain 3 ($P < 0.01$).

Breakdown of reserve carbohydrate. When washed-cell suspensions were shaken either aerobically or anaerobically in manometer flasks at 28°, carbohydrate disappeared from all of the four strains of yeast.

To compare the relative tendencies of the four strains to degrade their carbohydrate reserves, the carbohydrate that disappeared during equilibration of the manometer flasks for 15 min. and during 5 hr. subsequent aerobic incubation may be expressed as a percentage of the carbohydrate present

in the freshly made suspension. From the results given in Table 3 it was calculated that about 26 % of the initial carbohydrate disappeared from suspensions of strain 77, whereas for the brewing strains the values were: strain 4236, 49 %; strain 7000, 50 %; strain 7001, 50 %.

In strain 77 the carbohydrate disappearance was the same under aerobic and anaerobic conditions, in contrast with the brewing strains in which carbohydrate breakdown was stimulated under aerobic conditions.

In strain 7001 the carbohydrate appears to be especially unstable. During equilibration 20 % of the initial carbohydrate disappeared in the anaerobic flasks and 25 % in the aerobic flasks. After 1 hr. the total breakdown was 35 % (anaerobic) and 43 % (aerobic).

Effect of partial pressure of oxygen on the breakdown of reserve carbohydrate. To investigate the effect of partial pressure of oxygen on the carbohydrate breakdown, the manometer flasks were gassed with nitrogen, 10 % oxygen in nitrogen, air and 100 % oxygen. In suspensions of strain 77, variation of the atmosphere in the flasks had very little effect on the carbohydrate breakdown (Table 4). In strains 4236 and 7000 the amount of carbohydrate that disappeared increased with increasing partial pressure of oxygen.

Rates of respiration during dissimulation of reserve carbohydrate. The initial rates of respiration of the two flocculent yeasts were significantly lower ($P < 0.05$) than those of the powdery yeast (Table 5). In addition, the four strains showed differences in the way their respiration rate changed while their reserves were metabolized. These differences are shown in Tables 3 and 5.

The rates of respiration of strains 77, 4236 and 7000 all increased for a time. The rate of respiration of strain 77 reached a maximum about 105 min. after the start of manometric readings and then decreased. Strains 4236 and 7000 developed maximum rates of respiration after longer periods

Table 1. *Total carbohydrate content of yeast*

Sub-strains 1, 2, 3 and 5 of strain 7000 were isolated by micro-manipulation from an old single-cell culture. The degree of flocculence in sub-strains is denoted by: + + + + +, most flocculent; +, least flocculent. Total carbohydrate is expressed as $\mu\text{g.}$ of glucose equiv./mg. dry wt. of yeast. Values are given as means $\pm$ s.d. of the numbers of analyses shown in parentheses.

Yeast strain	Carbohydrate content
77	356.5 $\pm$ 18.9 (10)
4236	460.0 $\pm$ 27.1 (10)
7000	382.3 $\pm$ 23.1 (10)
7001	304.0 $\pm$ 30.8 (10)
7000 sub-strain 1 (+ + + + +)	313.4 $\pm$ 18.2 (5)
7000 sub-strain 2 (+ + + +)	346.5 $\pm$ 9.2 (5)
7000 sub-strain 3 (+ + +)	371.6 $\pm$ 7.3 (5)
7000 sub-strain 5 (+)	381.2 $\pm$ 9.4 (5)

Table 2. *Carbohydrate composition of yeast*

Total carbohydrate determined on whole cells was calculated as follows: Total (as hexose) = total (as glucose) + mannan (as mannose) - mannan (as glucose).

Yeast strain	Percentage of total carbohydrate			
	77	4236	7000	7001
Carbohydrate				
Trehalose	5.3	7.6	5.2	5.8
Glycogen	62.3	75.6	70.5	51.5
Mannan	16.2	7.4	12.3	23.5
Glucan	16.2	9.4	12.0	19.2
Sum of fractions ($\mu\text{g.}$ of hexose equiv./mg. dry wt. of yeast)	366	487	377	290
Total carbohydrate ($\mu\text{g.}$ of hexose equiv./mg. dry wt. of yeast determined on whole cells)	346	505	405	306

in the manometer flasks (Tables 3 and 5). The rate of increase of Q_{O_2} of the flocculent strain 7000 was significantly lower than that of strain 4236 ($P < 0.01$).

Strain 7001 did not show any increase in the rate of respiration. The synthesis of respiratory enzymes is involved in the increase in rate of respiration with time (Chester, 1963*a*), and the energy for

Table 3. *Time relations of carbohydrate disappearing and gaseous exchanges in yeast*

0, Initial carbohydrate content of the yeast; 0', carbohydrate content after equilibration for 15 min.; x , the carbohydrate decrease (%) during equilibration; a , carbohydrate decrease (%); b , CO_2 release (μ moles/mg.); c , O_2 uptake (μ moles/mg.); d , R.Q.

		Initial wt. of yeast (mg.)	Initial carbo- hydrate content (μ mole)																	
Time (hr.) Yeast strain	...	0	0'	x	0'-1				1-2				2-3							
					a	b	c	d	a	b	c	d	a	b	c	d				
Aerobic results																				
77	6.28	11.60	10.82	6.7	11.5	0.73	0.71	1.03	4.3	0.96	1.03	0.93	3.8	0.47	0.47	1.00				
4236	5.80	15.92	13.29	16.5	17.5	1.21	0.66	1.84	5.2	0.84	0.92	0.91	5.6	0.73	1.09	0.67				
7000	5.89	11.78	10.60	10.0	19.9	0.93	0.42	2.22	14.6	0.60	0.53	1.13	0.9	0.42	0.60	0.70				
7001	5.47	9.89	7.45	24.7	18.3	0.90	0.32	2.81	3.3	0.46	0.35	1.31	1.7	0.30	0.31	0.97				
Anaerobic results																				
77	6.28	11.60	10.60	8.6	10.5	0.41	—	—	4.7	0.17	—	—	3.8	0.07	—	—				
4236	5.80	15.92	13.60	14.6	13.9	0.64	—	—	4.5	0.32	—	—	3.3	0.24	—	—				
7000	5.89	11.78	10.60	10.0	12.7	0.50	—	—	8.0	0.29	—	—	4.7	0.16	—	—				
7001	5.47	9.89	7.88	20.3	14.5	0.52	—	—	5.6	0.12	—	—	1.7	0.11	—	—				
		Initial wt. of yeast (mg.)	Initial carbo- hydrate content (μ mole)																	
Time (hr.) Yeast strain	...	0	0'	x	3-4				4-5				0'-5							
					a	b	c	d	a	b	c	d	a	b	c	d				
Aerobic results																				
77	6.28	11.60	10.82	6.7	0	0.34	0.33	1.03	0	0.24	0.23	1.04	19.6	2.74	2.76	0.99				
4236	5.80	15.92	13.29	16.5	2.4	1.00	0.98	1.02	1.4	0.49	0.49	1.00	32.1	4.27	4.14	1.03				
7000	5.89	11.78	10.60	10.0	3.7	0.36	0.67	0.54	1.3	0.54	0.70	0.77	40.5	2.85	2.92	0.98				
7001	5.47	9.89	7.45	24.7	1.1	0.28	0.31	0.90	0.5	0.29	0.32	0.91	24.9	2.22	1.61	1.38				
Anaerobic results																				
77	6.28	11.60	10.60	8.6	1.5	0.05	—	—	0	0.04	—	—	20.5	0.74	—	—				
4236	5.80	15.92	13.60	14.6	2.9	0.19	—	—	1.4	0.11	—	—	26.0	1.50	—	—				
7000	5.89	11.78	10.60	10.0	5.7	0.13	—	—	0.8	0.09	—	—	31.9	1.17	—	—				
7001	5.47	9.89	7.88	20.3	1.2	0.08	—	—	0.5	0.05	—	—	23.5	0.88	—	—				

Table 4. *Effect of partial pressure of oxygen on carbohydrate disappearance in yeast*

0, Initial carbohydrate content of the yeast; 0', carbohydrate content after equilibration for 15 min.; the total decrease in carbohydrate at the times indicated is expressed as a percentage of the initial content, 0.

Time (hr.) Yeast strain	...	Initial wt. of yeast (mg.)	Initial carbohydrate content (μ moles)		Gas in flasks	Total decrease in carbohydrate (%)							
			0	0'		2.0	3.0	3.5	4.0	4.5	5.0	5.5	7.0
77	8.34	16.10	14.70		N ₂	21.1	—	25.1	—	26.6	—	—	—
					Air	15.0	—	25.0	—	25.0	—	—	—
					O ₂	20.3	—	25.8	—	27.3	—	—	—
4236	6.97	17.30	15.60		10% O ₂	—	44.0	—	—	—	52.5	—	56.6
					Air	—	50.8	—	—	—	54.8	—	61.8
					O ₂	—	55.9	—	—	—	59.8	—	66.1
7000	6.00	12.80	11.66		10% O ₂	42.7	—	—	49.1	—	—	58.3	—
					Air	42.7	—	—	51.9	—	—	58.3	—
					O ₂	49.4	—	—	61.0	—	—	61.0	—

Table 5. Summary of rates of gaseous exchange and changes in rates of respiration in yeast

Values are given as the means $\pm$ S.D. of the numbers of analyses shown in parentheses.

Yeast strain	...	77	4236	7000	7001
Initial Q_{O_2}		12.0 $\pm$ 2.9 (4)	14.3 $\pm$ 1.7 (19)	8.7 $\pm$ 1.3 (8)	7.1 $\pm$ 2.1 (5)
Initial Q_{CO_2}		12.7 $\pm$ 2.8 (4)	29.9 $\pm$ 2.2 (19)	22.6 $\pm$ 4.1 (7)	18.7 $\pm$ 1.3 (4)
Initial R.Q.		1.06 $\pm$ 0.02 (4)	2.13 $\pm$ 0.28 (19)	2.65 $\pm$ 0.36 (7)	2.69 $\pm$ 0.47 (4)
Maximum Q_{O_2}		18.5 $\pm$ 3.6 (4)	26.7 $\pm$ 3.0 (19)	19.7 $\pm$ 4.0 (8)	—
Time to reach maximum Q_{O_2} (min.)		105 $\pm$ 52 (4)	229 $\pm$ 53 (19)	326 $\pm$ 47 (8)	—
Rate of increase of Q_{O_2} /min.		0.071 $\pm$ 0.026 (4)	0.056 $\pm$ 0.0168 (19)	0.035 $\pm$ 0.0156 (8)	—

Fig. 1. Respiratory adaptation of yeast strain 7001 in the presence of glucose. Each manometer flask contained 4.5 mg. of anaerobically grown yeast, suspended in 67 mM- KH_2PO_4 . Glucose (10 μ moles), dissolved in 67 mM- KH_2PO_4 , was placed in the side arm. The final volume in each flask was 3 ml. ■, Endogenous; □, glucose added to main compartment; ○, glucose added 30 min. after equilibration; ●, glucose added after 90 min.; ◐, glucose added after 210 min.

this synthesis is probably derived from the carbohydrate reserves. In strain 7001 the carbohydrate reserves were largely broken down (and the released energy possibly dissipated) during equilibration of the manometer flasks and the first hour of manometric measurements (Table 3). The experiments were repeated in the presence of glucose to supply an alternative source of energy. Under these conditions there was an increase in rate of respiration similar to those in strains 77 and 4236 (Fig. 1).

Effect of partial pressure of oxygen on rates of respiration. The respiratory adaptations of strains 77, 4236 and 7000 in manometer flasks filled with either 100 % oxygen or air are compared in Fig. 2. The only effect of 100 % oxygen on strain 77 was

a small overall increase in the rate of respiration, whereas in strains 4236 and 7000 100 % oxygen caused a considerable decrease in the rate of increase in respiration.

Respiratory quotients. The initial R.Q. (respiratory quotient) of the baking yeast strain 77 measured over 1 hr. was about 1.06, whereas the R.Q. values of the three brewing strains were high, their values ranging from about 1.7 to 3.1 (Table 5).

DISCUSSION

Spiegelman, Reiner & Cohnberg (1947) demonstrated that the endogenous carbohydrate reserves may be used as a source of energy for the adaptation of yeast to ferment galactose. They also showed that there was a general inverse relation between adaptation time and the amount of the endogenous reserves. In the present work, the quantity of reserve carbohydrate in the brewing strains seems to affect the rate of respiratory adaptation. Strain 4236 possessed the largest carbohydrate reserves and its respiration increased at the fastest rate. Strain 7000 with a lower content adapted more slowly, and strain 7001 with the lowest carbohydrate content showed no increase in respiration. The slower rate of adaptation shown by strain 7000 could not be accounted for by the aggregation of the yeast cells hindering diffusion of oxygen, since when oxygen instead of air was used in the manometer flasks the carbohydrate breakdown was increased but the rate of adaptation was made slower still (Fig. 2 and Table 4). A more rapid breakdown of carbohydrate in the presence of oxygen than in air was also observed in the non-flocculent strain 4236, and again there was a slower rate of respiratory adaptation. This slower rate may be due to the rapid carbohydrate breakdown in oxygen, releasing energy at a faster rate than it can be used to synthesize enzymes, resulting in the wastage of a proportion of the reserves. Support for this suggestion is given by the experiments on strain 7001, in which even in air the reserves were degraded extremely rapidly and no increase in respiration occurred (Fig. 1 and Table 3).

The experiments in which glucose was supplied to strain 7001 showed that this strain could readily

Fig. 2. Effect of oxygen on endogenous respiration. (a) Yeast strain 77; (b) yeast strain 4236; (c) yeast strain 7000. The manometer flasks were gassed for 10 min. followed by a 5 min. equilibration period. $\circ$, Air; $\bullet$, oxygen.

adapt when an energy source was available. The results shown in Fig. 1 also indicate that aeration of the yeast in the presence of the degradation products of the reserve carbohydrate (e.g. ethanol) does not result in increased oxidation of the glucose added to the suspensions after different time-intervals. This suggests that the cell under these particular conditions can best utilize the energy released by glycolysis for the synthesis of respiratory enzymes. This observation agrees with that of Slonimski (1955), who found that ethanol was much less effective than glucose as an exogenous source of energy for respiratory adaptation.

The presence of oxygen greatly stimulates the adaptive synthesis of a number of enzymes (Leibowitz & Hestrin, 1942; Schultz, Atkin & Frey, 1940; Spiegelman, 1945). This requirement for oxygen is confined to the initial period of adaptation (Spiegelman, 1945). This oxygen effect on adaptation presumably operates on the source of energy supply, which suggests that the mechanism may be the same as the effect of oxygen in initiating and stimulating the breakdown of carbohydrate reserves in the brewing yeast. If the mechanism is the same, it would appear from the present results that the oxygen-sensitive system is developed to a much greater extent in the brewing strains.

Endogenous fermentation has been induced in baking yeast by the action of sodium azide or 2,4-dinitrophenol (Rothstein & Berke, 1952). Brady, Duggan, McGann & Tully (1961) have shown that the fermentation induced by azide, 2,4-dinitrophenol or arsenite was stimulated by

oxygen. This stimulation by oxygen was greatest for the azide-induced fermentation. These observations would suggest that the oxygen-sensitive endogenous fermentation in the brewing yeast may be due to the possession of a less effective 'azide-sensitive' control system.

Masschelein, Jeunehomme-Ramos, Castiau & Devreux (1963) examined the effect of 2,4-dinitrophenol on flocculent and non-flocculent brewing yeasts and found that a greater endogenous fermentation was induced in the flocculent yeast. Their results, together with those reported above on the different degrees of stability of the reserve carbohydrate in the four strains of yeast, lend support to the theory that the inhibitors are acting on a control system that is deficient in some yeasts to a greater or less extent.

The properties of the brewing strains may be in the nature of an adaptation to anaerobic growth. In the natural state, a yeast growing on a fermentable substrate will, when all the fermentable material has been used, require enzymes for assimilating and obtaining energy from the products of the fermentation. With the cessation in the production of carbon dioxide from fermentation, the environment is likely to become more aerobic. It would be expected that a mechanism, initiated by the presence of oxygen, for the rapid utilization of the reserves to provide energy for enzyme synthesis would be a considerable advantage to the yeast.

Little comparative work between strains has been published on the accumulation and dissimulation of

yeast carbohydrate. Spiegelman & Nozawa (1945) compared the dissimilation of carbohydrate in six strains, including yeasts other than *Saccharomyces cerevisiae*, by measurement of the endogenous gaseous exchanges. They concluded from the vanishingly small anaerobic evolution of carbon dioxide and R.Q. about 1.0 that dissimilation of reserve carbohydrate is not by a fermentative mechanism and is purely an aerobic process. Consideration of R.Q. values suggests that strain 77, with R.Q. 1.06, may be similar to the strains described by Spiegelman & Nozawa (1945), but even this yeast shows significant anaerobic breakdown of carbohydrate (Table 3). However, differences in the method of growth or harvesting of the cultures may account for this difference.

Comparison of the carbohydrate contents of the four strains of yeast used in the present work suggests that the amount of carbohydrate accumulated is characteristic of each strain. The two flocculent brewing strains both have much lower carbohydrate contents than has strain 4236, and it is possible that a lower carbohydrate content may be associated with flocculent growth. This suggestion is supported by examination of the sub-strains of 7000 which showed that the most flocculent have significantly lower carbohydrate contents. However, these results may be due to chance selection and further work is necessary to investigate the possible link between carbohydrate content and flocculence.

SUMMARY

1. There were wide differences in the amount of carbohydrate accumulated under anaerobic conditions by each strain of yeast.
2. The breakdown of reserve carbohydrate in washed suspensions of the baking yeast was relatively much less than in the brewing strains. Aerobic conditions increased the breakdown of reserve carbohydrate in the three brewing yeasts, but in the baking yeast there was no difference in the carbohydrate breakdown under aerobic and anaerobic conditions.

3. Increased partial pressure of oxygen increased the carbohydrate breakdown in the brewing yeasts but had no effect on that in the baking yeast.

4. Increased partial pressure of oxygen decreased the rate of respiratory adaptation in the brewing yeast but had no effect on the adaptation in the baking yeast.

5. In the three brewing strains there was an inverse relation between the amount of stored carbohydrate and the rate of respiratory adaptation.

6. The relation between the carbohydrate reserves, respiratory adaptation and oxygen is discussed. It is suggested that the properties of the brewing yeast may be due to the partial loss of a system that controls the utilization of reserve carbohydrate.

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