YEAST GROWTH IN RELATION TO THE DISSOLVED OXYGEN AND STEROL CONTENT OF WORT

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The extent of the requirement for oxygen in cells of brewing yeast is determined by the availability of oxygen during propagation. Cells with no oxygen requirement ferment satisfactorily when added to either air-saturated or de-aerated wort. Cells produced during fermentation develop an oxygen-requirement and ferment poorly when added to de-aerated wort because of restriction of both rate and extent of exponential growth. The quantity of dissolved oxygen needed to ensure satisfactory growth varies greatly with yeast strain. In all cases examined, the oxygen requirement can be eliminated by addition to the growth medium of ergosterol and Tween 80 However Tween 80 alone is without effect. It seems likely that oxygen is required because it is essential for biosynthesis of sterols.

Key words: *fermentation, growth, oxygen, sterol, wort, yeast.*

Introduction

The cultivation of yeast in aerobic rather than anaerobic conditions affects many aspects of the metabolic activity, chemical composition and structure of the cell. Although these differences have been widely studied and although the mechanisms which underlie the changes are understood in many cases, relatively little attention has been paid to the situation which holds in brewery fermentations. Here yeast is added to a medium which initially contains dissolved oxygen but which rapidly becomes and remains anaerobic. There is general agreement that it is desirable that the wort should contain some dissolved oxygen, but little quantitative information about the amounts which are required or about the effects of a deficiency. Accordingly, a study of the effects of oxygen on the performance of brewing strains of *Saccharomyces* has been carried out; the starting point for the study was the earlier recognition that the method of yeast propagation may alter the response to dissolved oxygen.[5]

Results and Discussion

Saccharomyces cerevisiae (NCYC 1236) was grown in shaken culture in Roux bottles and the yeast produced was then used to ferment aerated and de-aerated wort in stirred conditions. Nitrogen was passed through the head-space of the fermentors to prevent chance access of oxygen to the wort during fermentation. It was found that the pattern of growth and fermentation was similar in each wort (Table I), indicating that the cells grown in Roux bottles were without requirement for oxygen. However, when the yeast produced during the subsequent period of fermentation in either wort was examined in similar tests the extent of growth and the rapidity of fermentation were much less in the de-aerated wort (Table I, Fig. 1). The cells had thus developed an oxygen requirement during fermentation. When yeast was grown under fermentation conditions but with air in the headspace of the fermentor,

TABLE I

Change in Specific Gravity During Fermentation of Aerated and De-aerated Wort by *Saccharomyces cerevisiae* (NCYC 1236) Propagated in Different Ways

Time of fermentation (h)	Conditions of yeast production and specific gravity during fermentation			
	Shaken Roux bottles		Fermentation	
	Aerated wort	De-aerated wort	Aerated wort	De-aerated wort
0	1·040	1·040	1·040	1·040
8	1·038	1·038	1·038	1·037
16	1·032	1·032	1·030	1·029
24	1·021	1·022	1·019	1·024
32	1·012	1·012	1·010	1·018
40	1·008	1·007	1·006	1·015
48	1·007	1·007	1·006	1·014

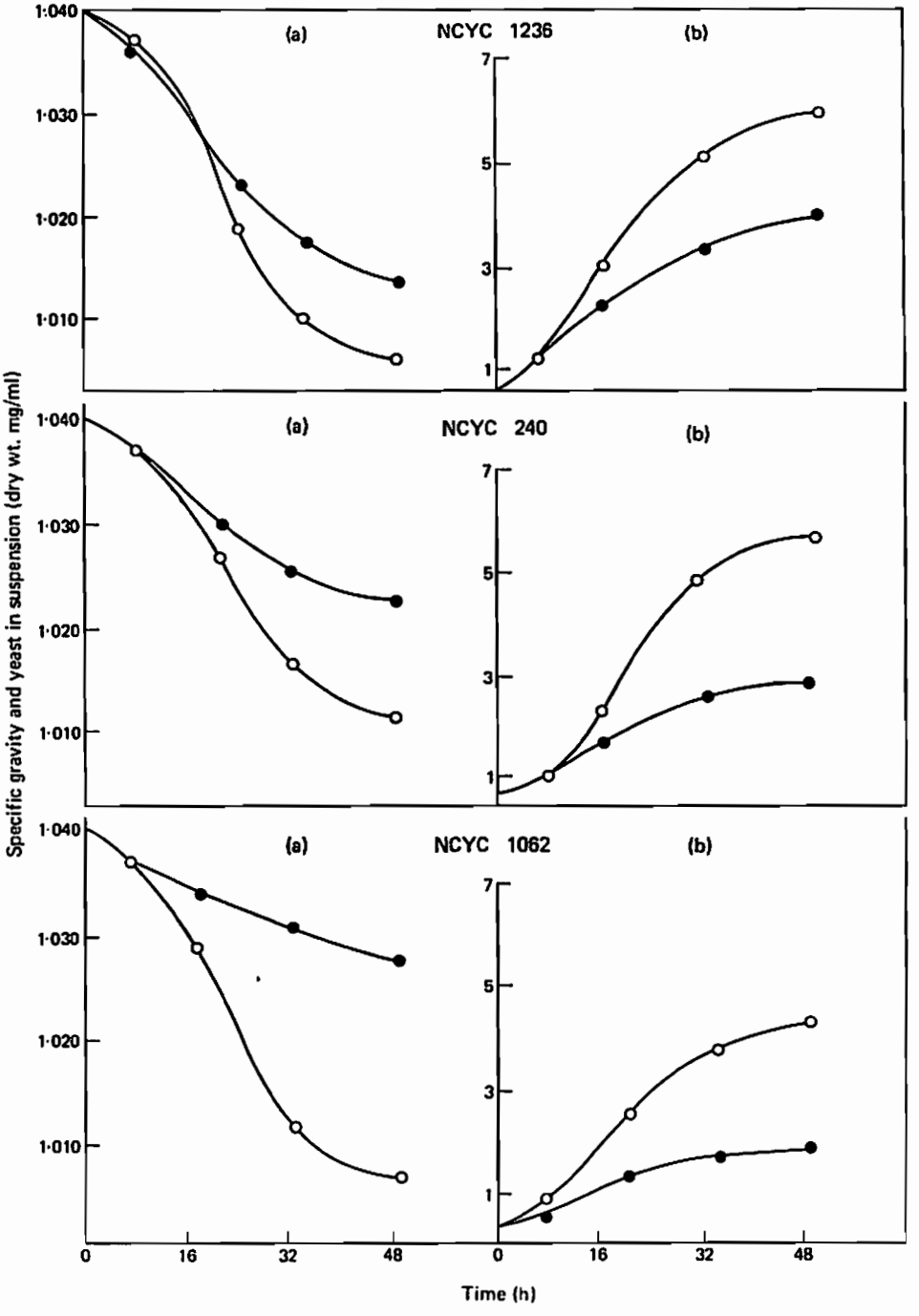

Fig. 1.—Changes during fermentation in (a) specific gravity and (b) yeast dry weight when de-aerated wort is pitched with cells with (●) and without (○) requirement for oxygen.
The results for the other yeast strains examined follow the pattern shown for strain 240.

the yeast produced did not require oxygen. The conditions of yeast growth thus influenced the requirement for oxygen of the harvested cells, and it is clearly the availability of air during the growth of the yeast which results in the production of cells with no oxygen requirement. Incidentally, it was confirmed that the diffusion of air through the cotton wool plug of shaken Roux bottles allows oxygen to be present in the gas phase in contact with the medium, and that in the stirred fermentations, oxygen diffuses through cotton wool plugs in the fermentor lid unless nitrogen is continually passed through the head-space (Table II).

TABLE II

OXYGEN CONTENT OF WORT AND OF THE HEAD SPACE GAS DURING FERMENTATION BY *SACCHAROMYCES CEREVISIAE* (NCYC 1236)

Time of fermentation (h) and specific gravity		Oxygen meter reading (arbitrary units).	
		Wort	Head space
Before pitching		140	150
0	1·040	110	150
0·5	1·040	1	100
1	1·040	0	100
1·75	1·040	0	130
3	1·040	0	90
22	1·020	0	0
25	1·016	0	1
27·5	—	0	3·5
29	—	0	6·5
30	1·011	0	11·5
46	1·006	0	50

Other strains of brewing yeast (NCYC 240, 1026, 1062, 1073, 1134, and 1307) were examined in similar tests and it was confirmed for each strain (*a*) that cells produced aerobically had no oxygen requirement, growing and fermenting similarly in air-saturated and de-aerated wort and (*b*) that cells grown in conditions which were anaerobic, apart from the oxygen present in solution in the air-saturated wort used as the growth medium, had a marked requirement for oxygen. Thus these oxygen-requiring cells fermented relatively slowly when added to de-aerated wort (*eg* Fig. 1).

When oxygen-requiring cells were added to wort containing no dissolved oxygen, they commenced to grow and, for a limited period, reproduced at an exponential rate (Fig. 1). The rate of reproduction in this period was somewhat reduced as compared with that of cells with no oxygen requirement. Moreover, with each strain examined, the extent of exponential growth was much restricted by the deficiency of oxygen. In consequence the rate of fermentation was much reduced (Fig. 1). Even though growth was restricted the cells continued to absorb amino nitrogen (Table III) and other assimilable nitrogenous compounds, albeit at a reduced rate. The total quantity of nitrogen present in the yeast population in the fermentor was only slightly reduced when growth was inhibited by oxygen deficiency. In consequence the nitrogen content of the yeast present was increased (Table III). The generalization that the fermentative value of yeast is proportional to its nitrogen content[12] obviously does not hold in these special circumstances.

It was apparent that some yeasts are more severely retarded in their activity by lack of oxygen than others, suggesting that strains might differ with regard to their oxygen requirement. Thus fermentation by oxygen-requiring cells of strain 1062 was much slower than that of cells of strain 1236 when the cells were added to de-aerated wort (Fig. 1). In order to confirm that variation in oxygen requirement did occur from strain to strain, cells of oxygen-requiring yeast were added to wort containing 0, 6 (air saturated) or 12 ppm of dissolved oxygen. In addition yeast from aerated culture was added to aerated wort to provide comparative results for yeast which did not require oxygen. The results showed first the general effect that cells with no oxygen requirement had a longer lag phase than the oxygen-requiring cells. The lag period was increased by between two and six hours in different strains when the yeast was propagated in aerated conditions. It was also clear that the quantity of dissolved oxygen necessary to bring the performance of oxygen-requiring cells to a level equivalent to that of the aerated cells varied substantially with strain of yeast. The lowest requirement shown was by strain 1236, in which the presence of 2 ppm of dissolved oxygen was adequate to meet the requirement (Table IV). Indeed, with this strain the increase of oxygen concentration to 12 ppm led to some retardation of growth and of fermentation (Table IV). Strains 1026 and 1073 required approximately 6 ppm of dissolved oxygen while the requirement of strain 240 was

TABLE III

AMINO NITROGEN CONTENT OF WORT AND TOTAL NITROGEN CONTENT OF OXYGEN-REQUIRING *SACCHAROMYCES CEREVISIAE* (N.C.Y.C. 1236) DURING FERMENTATION WHEN ADDED TO AIR-SATURATED WORT (A) AND DE-AERATED WORT (B)

Time (h)	Specific gravity		Amino nitrogen content of wort (mg/100 ml)		Total yeast N (mg/ml of suspension)		N in yeast (%)	
	A	B	A	B	A	B	A	B
0	1·040	1·040	19·0	19·0	0·07	0·06	10·4	10·1
8	1·038	1·038	17·3	16·8	0·11	0·10	8·3	8·5
16	1·032	1·033	11·1	11·8	0·21	0·17	8·6	7·8
28	1·018	1·024	5·2	6·4	0·34	0·29	7·5	9·3
32	1·015	1·021	5·0	5·8	0·33	0·29	6·9	8·8
40	1·011	1·019	4·7	5·6	0·35	0·29	5·8	7·1
44	1·010	1·018	4·7	5·5	0·35	0·29	4·8	6·8

almost met by the presence of 12 ppm (Table IV). Strains 1073 and 1026 thus behave similarly to the yeast used in an earlier study.[7] In three further strains, 1134, 1307 and 1062 the requirement was greater than 12 ppm (Table IV).

It is well known that yeast grown in a synthetic medium in rigidly anaerobic conditions requires the addition to the medium of ergosterol and unsaturated fatty acids if growth is to occur satisfactorily.[1,2] It has also been shown that low viability of yeast after brewery fermentation can be due to a deficiency of unsaturated fatty acids.[11] Cells of *Saccharomyces cerevisiae* are unable to synthesize both ergosterol and unsaturated fatty acids in the absence of molecular oxygen.[1,2] It seemed probable that the oxygen requirement of cells grown in non-aerated conditions reflected the need for oxygen for such synthetic reactions and accordingly the effect of adding ergosterol, dispersed in Tween 80, as a source of unsaturated fatty acid, on the behaviour of oxygen-

TABLE IV

CHANGE IN SPECIFIC GRAVITY DURING FERMENTATION OF WORT BY *SACCHAROMYCES CEREVISIAE* WITH (+) OR WITHOUT (−) REQUIREMENT FOR OXYGEN IN RELATION TO OXYGEN AND STEROL CONTENT OF WORT

Initial oxygen content of wort (ppm) and duration of fermentation (h)		Specific gravity									
		Yeast strain and requirement for oxygen*									
		1236		1062		1134		1307		240	
		−	+	−	+	−	+	−	+	−	+
0	24	1·022	1·024	1·020	1·033	1·021	1·029	1·020	1·028	1·028	1·028
	48	1·007	1·014	1·008	1·028	1·007	1·025	1·008	1·018	1·011	1·023
2	24		1·018								
	48		1·006								
6	24	1·021	1·019	1·021	1·028	1·020	1·024	1·021	1·024	1·023	1·026
	48	1·007	1·006	1·008	1·020	1·008	1·013	1·008	1·011	1·011	1·017
12	24		1·020		1·026		1·023		1·023		1·024
	48		1·008		1·016		1·011		1·010		1·012
0 + 10 ppm ergosterol	24		1·019		1·018		1·020		1·020		1·020
	48		1·007		1·006		1·007		1·005		1·011

* − = Propagation in aerated culture to give cells with no oxygen requirement.
+ = Propagation in non-aerated culture to give oxygen-requiring cells.

requiring cells was examined. It was found that the addition of ergosterol and Tween 80 removed any requirement for oxygen in all strains that were examined. Tests using N.C.Y.C. 1236 showed that as little as 10 ppm of ergosterol, added to de-aerated wort, is sufficient to produce this effect (Fig. 2) and

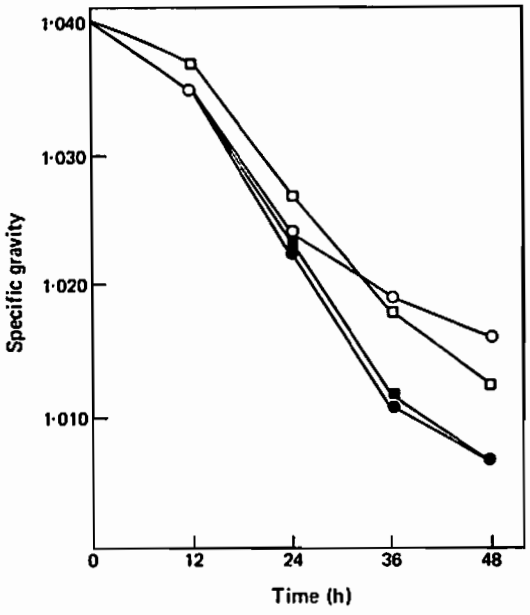

Fig. 2.—Change in specific gravity during fermentation of wort by oxygen-requiring *Saccharomyces cerevisiae* (NCYC 1236) in relation to the content of ergosterol: no ergosterol (○), 1 ppm (□), 10 ppm (●), 18, 70, 140 ppm (■).

tests with other strains confirmed that treatment at this level was effective (Table IV). Indeed, in the majority of cases the magnitude of exponential growth and thus the overall rate of fermentation was greater when wort was supplemented with ergosterol than when aerated cells were used without this addition.

Tween 80 alone was without effect in stimulating the performance of oxygen-requiring yeast. It has not proved possible, so far, to disperse ergosterol in wort other than in solution in Tween 80. Accordingly the influence of ergosterol alone has not been investigated. The results demonstrate that the requirement for oxygen is a requirement for ergosterol and possibly also for Tween 80. The effects reported here are thus distinct from those affecting viability reported earlier.[11] In view of the known presence of unsaturated fatty acids in wort[13] it may be that these substances are not limiting.

As ergosterol removed the oxygen requirement and as aerobically-grown yeast has no requirement for oxygen it seemed that differences in oxygen requirement should parallel differences in sterol content. Examination of strains of yeast grown with and without access to air confirmed that this was the case (Table V). It has also been observed that there are

TABLE V

ERGOSTEROL CONTENT OF *SACCHAROMYCES CEREVISIAE* WITH (+) AND WITHOUT (−) REQUIREMENT FOR OXYGEN

Yeast strain	Requirement for oxygen	Ergosterol content of yeast (%)
1236	−	0·85
	+	0·61
240	−	1·2
	+	0·14
1307	−	1·2
	+	0·25
1062	−	1·1
	+	0·12

qualitative differences between the sterols present in individual yeast strains grown in either aerated or non-aerated conditions and it seems possible that the difference between strains with regard to their oxygen requirement may reflect differences of this nature rather than in the absolute amount of sterol which is present.

Clearly the method of production of yeast prior to fermentation influences the subsequent requirement of the yeast for dissolved oxygen and may, in some circumstances, eliminate it. Furthermore, the extent of the requirement for dissolved oxygen in cells produced in a standard way varies with the strains of yeast used. For both reasons it is not possible to generalize about the concentration of dissolved oxygen which is required in wort prior to fermentation. It is apparent that the growth of yeast, which is controlled in air-saturated wort by the content of assimilable nitrogen, is also subject to control by the concentration of dissolved oxygen. As the growth of yeast and the flavour of the beer produced are inter-related,

it is not surprising that reports in the literature about the effects of wort aeration on fermentation refer to effects on beer flavour.[5,6] In view of the growth-regulating effect of the concentration of dissolved oxygen in wort it may be feasible to regulate fermentation by control of oxygen level, and so to eliminate some of the variations in growth which accompany seasonal changes in raw material. Indeed, as the oxygen requirements of a substantial proportion of the yeasts examined in this study exceed that given by air-saturated wort, it seems likely that such a control or restriction effectively exists in some breweries today.

It is important to realize also that yeast may derive oxygen from exposure to air during normal brewery operations other than at the stage at which it receives dissolved oxygen from the wort used for fermentation. This is especially so when yeast collects at the surface of the fermentation, or when a recirculation system is used in the fermenting vessel, and also in the handling of the yeast after fermentation is concluded. When a fermentation system is changed to one in which yeast is pumped from a sedimentation zone in one fermentor directly to another fermentor, it might be found that the requirement for oxygen by the yeast was increased as a result of the elimination of the chance contact with air. There would clearly be advantages if the requirement for oxygen could be eliminated by an alternative treatment. The recognition that the requirement is eliminated by the presence in wort of relatively low concentrations of sterols and unsaturated fatty acids may offer prospects of control in this direction in the future. Such treatment would be especially useful where strains such as 1062, which have a particularly marked requirement for oxygen, are involved.

Experimental

The strains of yeast used were obtained from the National Collection of Yeast Cultures (Brewing Industry Research Foundation, Nutfield, Redhill, England) and are referred to by their strain numbers in that collection. 1073 was *Saccharomyces carlsbergensis*; other strains were *S. cerevisiae*. All fermentations were carried out in stirred conditions using 2-litre vessels containing 1·5 litres of wort, at 20° C, and were sampled automatically as described previously.[4] Nitrogen gas was passed through the head-space of the fermentors throughout the period of fermentation. To remove oxygen from wort, nitrogen gas was passed through the wort for 3–4 h; wort was saturated with air by passing air through the wort for 3 hours, or by stirring the wort vigorously in an atmosphere of air for this time. In both cases oxygen meters[3] were used to confirm that these procedures were effective. The oxygen content of air-saturated wort was taken as 6 ppm. A higher concentration of dissolved oxygen was obtained by passing oxygen through the wort until the desired level was obtained, as measured by an oxygen meter. Amino nitrogen was determined by an automatic procedure[8] and the total nitrogen of yeast cells by a Kjeldahl method. The yeast present in suspension was measured as described earlier.[4] Ergosterol (B.D.H. Ltd., Poole, England) was recrystallized three times from ethanol before use. It was dispersed in Tween 80 (Koch-Light Laboratories, Colnbrook, England) as described by Andreasen & Stier.[1] Sterols were extracted from yeast cells by the method of Shaw & Jefferies[10] and the content of ergosterol in the extracts was measured spectrophotometrically.[10]

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