The Effect of Oxygen Concentration on the Growth and Metabolism of *Saccharomyces cerevisiae* Grown with Excess of Potassium or in Potassium-Deficient Media

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(Received 16 June 1972)

1. *Saccharomyces cerevisiae* cells grown in limiting K^+ concentration have their growth inhibited by O_2 concentrations above 40%. With these conditions the cells grow very large and are unable to maintain ionic gradients when washed with water. 2. Cells grown in excess of K^+ showed the same pattern of change in cell size with change in O_2 concentration, but the magnitude of the changes was much less. Cells grown in excess of K^+ were not leaky. 3. Cell death, growth and development of 'leakiness' were not correlated in the cells grown in limiting K^+ concentration. 4. The activities of both alcohol dehydrogenase and cytochrome oxidase were higher in K^+ -deficient cells than in the cells grown with excess of K^+ . The differences were much larger when the measurements were made on a cellular basis than when made on a protein basis. 5. In 100% O_2 3 mM- K^+ in the medium was sufficient to produce normal yeast cells.

Bartley & Broomhead (1971) described the effect of limiting K^+ concentration in anaerobically grown *S. cerevisiae*. They showed that enzyme synthesis was limited by the K^+ concentration of the medium and that the adaptation of cells from anaerobic to aerobic conditions did not occur unless K^+ was freely available in the adaptation media. To study the aerobic-anaerobic transition in a way analogous to that of Chapman & Bartley (1968), an attempt was made to grow highly aerobic cells in a medium of limiting K^+ concentration under an atmosphere of O_2 . Unexpectedly, the cells failed to grow, whereas growth proceeded normally under these conditions when K^+ was in excess. In the present paper we describe the various effects O_2 concentration has on the growth of the cells, both when K^+ is in excess and when K^+ is at a minimal concentration.

Materials and Methods

Yeast strain

Saccharomyces cerevisiae strain no. 77 of the National Collection of Yeast Cultures (Brewing Industry Research Foundation, Nuffield, Surrey, U.K.) was used. It was subcultured monthly on agar-slope medium containing (per litre): Difco yeast extract, 5g; malt extract, 22.5g; glucose, 5g; ammonium tartrate, 10g; NH_4NO_3 , 2g; KH_2PO_4 , 2g; $MgSO_4 \cdot 7H_2O$, 1g; NaCl, 0.2g; $CaCl_2$, 0.2g; ferrous citrate, 10ml of 4mM soln.; agar, 20g (Polakis *et al.*, 1964).

The ion content of these cells, making the correction for interstitial water as done by Conway & Downey (1950), was: K^+ 156mM, Na^+ 7.0mM and Mg^{2+} 23mM.

Growth of cells

The media used were the same as those described by Bartley & Broomhead (1971) but with sodium lactate omitted. The inoculum was 0.07–0.09g dry wt. of cells/litre of medium. During incubation at 30°C cultures were gassed with either sterile O_2 -free N_2 , O_2+N_2 (1:4), O_2+N_2 (2:3), O_2+N_2 (3:2), O_2+N_2 (4:1) or 100% O_2 . When all the glucose is consumed there is a comparatively long (several hours) period where the dry wt. of the cells remains virtually constant. For the purposes of this paper the stationary phase has been defined as the earliest point in time when growth on glucose ceases. After this stationary phase growth recommences with ethanol and acetate. The time to reach this stage was determined in preliminary experiments and measurements were made only of cells that had just finished growing. Cells were harvested and disrupted in ice-cold water as described by Bartley & Broomhead (1971).

Analytical methods and measurement of enzyme activities

RNA was measured by the orcinol method of Schneider (1945) and DNA by the diphenylamine method of Burton (1956) on a hot 5% trichloroacetic

acid extract. All other analytical methods and enzyme measurements were done as described by Bartley & Broomhead (1971).

Results

Concentration of O₂

Effect on cell yield and wet weight per unit DNA. Table 1 shows the effect of O₂ concentration on cells grown with excess of K⁺ in the medium. The results are presented in absolute terms and also as percentages of the values obtained with 20% O₂. For the purposes of this paper such cells are regarded as 'normal'. The assumption has been made that the DNA/cell remains constant and therefore the ratio wet wt. of cells/DNA is a measure of cell size. This was confirmed visually in the light-microscope but no absolute measurements of size were made. In 20% O₂ the cells were much smaller than in N₂, but at higher concentrations of O₂ the cells once again became larger. The number of cell divisions was minimal

in N₂ and maximal in 20% O₂, and the cell yield roughly followed the number of cell divisions.

The cells whose growth was limited by K⁺ concentration (Table 2) showed the same pattern of change with O₂ concentration as cells with excess of K⁺, but at all O₂ concentrations they were very much larger than their counterparts.

Effect on the macromolecular composition of cells. Table 3 shows the effect of O₂ concentration on the protein, RNA and DNA. With excess of K⁺ the protein and RNA were minimal at 40% O₂, but the DNA content was maximal at 20% O₂, reflecting the small size of the cells. When concentration of K⁺ is limiting there is less protein in cells grown anaerobically and at 20% O₂ and presumably the cell contains a higher proportion of fat and carbohydrate. At 40% O₂ the RNA content of the low-K⁺ cells was minimal, and throughout the amount of DNA was much lower than in the counterpart with adequate K⁺. This difference reflects the large size of the cell grown in low concentrations of K⁺.

Table 1. *Effect of O₂ concentration on the cell size, number of generations and cell yield of S. cerevisiae grown with excess of K⁺*

Glucose (300 μmol) was added to 1 litre of medium and this was all consumed when growth ceased. The results are the means of two experiments. Columns labelled '% of normal cells' give percentages of the appropriate value compared with those from cells grown in 20% O₂ (i.e. 'normal cells').

Percentage of O ₂	Cell size		No. of cell divisions at end of growth		Yield of cells			
	(g wet wt./mg of DNA)	% of normal cells		% of normal cells	Wet wt.		Dry wt.	
					(g/litre)	% of normal cells	(g/litre)	% of normal cells
0	2.7	168	5.6	80	23	77	4.3	78
20	1.6	100	7.0	100	30	100	5.5	100
40	1.9	119	6.4	92	30	100	5.4	98
60	1.9	119	6.5	93	30	100	5.7	104
80	2.4	150	6.2	88	29	96	5.4	98
100	2.4	150	6.0	86	30	100	5.7	104

Table 2. *Effect of O₂ concentration on the growth of S. cerevisiae in media with low K⁺ (0.83 mM)*

At the higher O₂ concentrations some glucose remained at the end of growth (60% O₂, 78 μmol; 80% O₂, 100 μmol; 100% O₂, 149 μmol). The results are the means of two experiments and are given as the percentage of the value found for 'normal cells' (see Table 1).

Percentage of O ₂	Cell size	No. of cell divisions to end of growth	Yield of cells	
			Wet wt.	Dry wt.
0	251	48	23	25
20	175	60	28	33
40	202	50	20	23
60	470	45	17	19
80	510	37	13	16
100	600	25	10	9

Table 3. Comparison of the effect of O_2 concentration on the macromolecular composition of *S. cerevisiae* grown either in excess of K^+ (90 mM) or limiting concentration (0.8 mM) of K^+

Absolute values are given for the cells with excess of K^+ together with the percentage difference from 'normal cells' (a) and the percentage difference for the depleted cells in comparison with 'normal cells' (b). Absolute values are in mg/g dry wt.

Percentage of O_2	Protein content			RNA content			DNA content		
	(mg)	Percentage		(mg)	Percentage		(mg)	Percentage	
		(a)	(b)		(a)	(b)		(a)	(b)
0	475	110	75	83	83	94	2.0	48	33
20	430	100	82	100	100	105	4.2	100	52
40	364	85	91	54	54	39	3.0	71	40
60	367	85	90	84	84	86	2.9	69	41
80	386	90	99	80	80	94	2.4	57	28
100	447	104	114	81	81	95	2.2	52	28

Table 4. Effect of O_2 concentration on Na^+ and K^+ content resistant to water washing in *S. cerevisiae* grown in excess of K^+

The cells harvested at 0°C were washed twice in a large volume of ice-cold water by resuspension and resedimentation. After washing it was assumed that all the ions retained were intracellular. The cell water is calculated as described by Bartley & Broomhead (1971). The ion gradients (int./ext.) are calculated as concn. of retained intracellular ions/concn. of ions in the suspension medium. When the cells are suspended in water the gradients are, of course, much larger.

Percentage of O_2	Concn. of ions in medium (mM)			Concn. of ions in cell water (mM)			Ion gradients (int./ext.)		
	Na^+	K^+	$Na^+ + K^+$	Na^+	K^+	$Na^+ + K^+$	Na^+	K^+	$Na^+ + K^+$
0	43	72	115	14	137	151	0.3	1.9	1.3
20	42	73	115	8	224	232	0.2	3.1	2.0
40	48	80	128	25	210	235	0.5	2.6	1.8
60	46	78	124	11	205	216	0.2	2.6	1.7
80	50	78	128	7	200	207	0.1	2.6	1.6
100	48	72	120	14	183	197	0.3	2.5	1.6

Table 5. Effect of O_2 concentration on Na^+ and K^+ content resistant to water washing in *S. cerevisiae* grown in limiting concentration of K^+

Details are as given in Table 4.

Percentage of O_2	Concn. of ions in medium (mM)			Concn. of ions in cell water (mM)			Ion gradients (int./ext.)		
	Na^+	K^+	$Na^+ + K^+$	Na^+	K^+	$Na^+ + K^+$	Na^+	K^+	$Na^+ + K^+$
0	136	0.6	137	142	23	165	1.04	38	1.2
20	129	0.6	130	158	21	179	1.2	34	1.4
40	140	0.6	141	158	19	177	1.1	32	1.4
60	136	0.6	137	45	6	51	0.3	10	0.4
80	130	0.6	131	14	4	18	0.1	7	0.1
100	129	0.8	130	26	5	31	0.2	6	0.2

Effect on the Na^+ and K^+ content of the cells. Table 4 (excess of K^+ in the medium) and Table 5 (limiting K^+ concentration in the medium) show the amounts

of Na^+ and K^+ that are retained in the cells at the end of growth after twice washing with water. With excess of K^+ (about 75 mM) in the medium and O_2 absent the

cells contained 137mm-K⁺. In air the concentration of K⁺ was much greater (224mm) and this was approximately maintained at other O₂ concentrations, except at 100% O₂, where the concentration fell to 183mm. The Na⁺ content was always low (between 8 and 25mm) and the total Na⁺+K⁺ showed the same pattern as K⁺ alone. With minimal K⁺ in the medium (0.83mm) the intracellular K⁺ concentration was a maximum (22.5mm) under anaerobic conditions (Table 5). At 60% O₂ and above there was an abrupt decrease in the concentration of K⁺ that remained after washing. Under anaerobic conditions the Na⁺ content (142mm) was roughly the same as the K⁺ content of the cells with excess of K⁺. At 20% O₂ there was an increase in the Na⁺ content to 158mm, but above 40% O₂ there was a very large decrease in the concentration of Na⁺ retained after washing. At all concentrations of O₂ there was at least 50mm extra cation in the cells with excess of K⁺ compared with the cells in which K⁺ was limited. Above 40% O₂ cells grown to the stationary phase under limiting K⁺ conditions were therefore unable to retain either Na⁺ or K⁺ when washed with water. However, Mg²⁺ (28mm) was retained in these cells.

Maintenance of ionic gradients

The results with the higher O₂ concentrations suggest that the cells are unable to maintain an ionic gradient when the K⁺ concentration is very low in the medium. Further experiments were therefore made with cells grown under N₂ and in 20%, 80% and 100% O₂. The ion contents of the unwashed pellet sedimented directly from the growth medium and the loss of ions by subsequently washing in water were measured. Table 6 shows that the cells with excess of K⁺ maintained a gradient, ([ion] in cell water)/([ion] in medium), of about 2 in the presence of the medium and that after washing with water the ions remaining in the cell would have given, except in anaerobic conditions, a gradient greater than 1.5. At all concentrations of O₂ only about 20% of the intracellular ions were labile to washing with water. Under anaerobic conditions about 33% was labile. With limiting K⁺ concentration anaerobically and in air the cells could maintain a gradient of more than 2 and washing removed about 40% of the cations. With 80 or 100% O₂ the gradient maintained in the presence of the medium was about 1.5, but washing with water removed about 80–90% of the ions. These results support the idea that in limiting K⁺ concentration and high O₂ concentration the cells became leaky, but they are still able to maintain a concentration difference against the leak. Presumably the demand for energy to maintain the gradient is a partial explanation of the poor growth in higher oxygen concentrations.

Table 6. *Effect of O₂ concentration on the ion content of S. cerevisiae grown in excess of K⁺ and in limiting concentration of K⁺*

The concentration of ions in the unwashed cells is calculated from the total measured cations of the unwashed cell pellet and the calculated content of cations in the intercellular fluid.

Percentage of O ₂	Concn. of ion in medium (mm)		Concn. of ion in unwashed cells (mm)		Concn. of ion in twice-washed cells (mm)		Ratio $\frac{[Na^{+} + K^{+}]_{\text{internal}}}{[Na^{+} + K^{+}]_{\text{external}}}$		Total ions removed by washing (mm)	Total ions removed by washing (%)
	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	Unwashed cells	Washed cells		
0	72	43	205	17	134	14	1.93	1.29	74	33
	0.6	136	27	266	21	151	2.12	1.27	121	41
20	73	42	262	14	220	8	2.4	1.98	48	17
	0.6	129	27	276	22	165	2.3	1.44	116	38
80	78	50	231	10	192	7	1.88	1.55	42	17
	0.6	130	21	192	4	15	1.63	0.14	194	91
100	72	48	210	32	178	14	2.01	1.60	50	20
	0.8	129	20	171	8	26	1.47	0.26	157	82

The concentration of ions in the unwashed cells is calculated from the total measured cations of the unwashed cell pellet and the calculated content of cations in the intercellular fluid.

Time-course of development of cell 'leakiness'

Cells were harvested during growth in limiting K^+ concentration and with either 80 or 100% O_2 as gas phase. The cells were washed and the 'bound' ions measured. The results in Table 7 show that up to about 30h the labile ions were a normal proportion of the total ion content (compare with 0 and 20% O_2 in Table 6). At this time in 100% O_2 the cells showed approx. 90% viability when planted on a nutrient-agar containing excess of K^+ . Leakiness developed while the cells were still growing and there seemed no correlation between the percentage of 'labile' ions

and the viability of the cells. If the leakiness is a reflection of cell death the relationship is not simple and is not reflected in a parallel change in enzyme activities. It appears that the ion-transport system pumping ions into the cell is able to function at relatively high salt concentrations against a steadily developing leak.

Effect of K^+ concentration on the growth of cells in 100% O_2

Table 8 shows that above about 2.5mM- K^+ in the growth medium the cells showed little or no difference

Table 7. *Time-course of development of 'leakiness' in S. cerevisiae grown in limiting concentration of K^+*

The cells were grown with 80 and 100% O_2 as gas phase and were sampled at different times during growth. The $[Na^+]$ and $[K^+]$ of the medium and cells were measured and also the amount that was removed by washing in water.

Time of growth (h)	$[Na^+]$ of washed cells (mM)	$[K^+]$ of washed cells (mM)	Total $[Na^+ + K^+]$ of washed cells (mM)	Loss of $[Na^+ + K^+]$ by washing (mM)	Loss of $Na^+ + K^+$ by washing (%)
(a) Gas phase 80% O_2					
24	138	36	174	67	28
31	134	30	168	73	30
48	79	20	99	142	58
52	Growth ceased				
58*	40	11	51	190	79
(b) Gas phase 100% O_2					
24	134	40	174	17	9
30†	100	30	130	61	32
50	62	19	81	110	58
53‡	51	16	67	124	65

* 31% of the cells were viable at this time on agar with K^+ .

† 90% of the cells were viable at this time on agar with K^+ .

‡ Growth ceased at this time (10% of the cells were viable on agar with K^+).

Table 8. *Effect of K^+ concentration on the yield and macromolecular composition of S. cerevisiae grown in 100% O_2*

At the lowest concentration of K^+ less than one-third of the glucose was consumed before growth ceased, but by 2.2mM- K^+ virtually all the glucose was used up.

$[K^+]$ (mM)	Cell size (g wet wt./mg of DNA)	No. of cell divisions to end of growth	Yield of cells (g/litre)		Protein content (mg/g dry wt.)	RNA content (mg/g dry wt.)	DNA content (mg/g dry wt.)
			Wet wt.	Dry wt.			
0.83	3.6	1.8	2.5	0.5	436	141	1.4
1.58	3.7	4.1	12.5	2.0	478	163	1.7
2.2	3.0	5.0	20	3.8	502	178	1.8
3.4	2.1	5.7	23.2	4.1	548	149	2.7
4.3	2.3	5.7	23.7	3.8	514	156	2.7
6.3	2.1	6.0	28.2	5.2	565	184	2.6
8.5	2.3	5.9	28.2	5.0	548	175	2.5
10.4	2.1	6.0	28.2	4.9	518	153	2.7

from the cells grown in excess of K^+ . However, maximum yields of cells were not obtained until the concentration of K^+ in the medium had reached about 6.0mM. By 2.2mM- K^+ in the growth medium the cells were able to retain their ions against water washing and ion gradients between medium and cells were normal.

Effect of O_2 concentration on the activities of some enzymes in the cells under conditions of excess and limiting K^+ concentration

The results are presented as the activity/unit wt. of protein and activity/unit wt. of DNA. The second method of presentation takes into account the change in size that occurs under the different gas conditions. The absolute values are given for the cells with K^+ in excess and these results and those obtained from the cells grown with limiting K^+ are also given as % of the standard cell conditions (excess of K^+ and in air). Potassium phosphate buffer was present in all the enzyme assay mixtures except that used for alcohol dehydrogenase. Consequently the lowered activity found with pyruvate kinase and NADH oxidase cannot be due to lack of K^+ in the assay medium.

Enzyme activities

Pyruvate kinase [ATP-pyruvate phosphotransferase (EC 2.7.1.40)]. At all O_2 concentrations the specific activity of the enzyme was lower in the K^+ -depleted cells than in cells with excess of K^+ . However, Table 9 shows that on a cellular basis the total enzyme available was about the same or greater than the amount available to the cells with unlimited K^+ . In both types of cell the minimum activity was found with 40% O_2 .

Alcohol dehydrogenase [alcohol-NAD⁺ oxidoreductase (EC 1.1.1.1)]. However calculated, the alcohol dehydrogenase activity of the K^+ -depleted cells is always substantially greater than that of the cells with excess of K^+ (Table 10).

Cytochrome oxidase [cytochrome c-oxygen oxidoreductase (EC 1.9.3.1)]. With excess of K^+ (Table 11) the cytochrome oxidase activity up to 60% O_2 was as might be expected in yeast grown in a high concentration of glucose at a time when the glucose has just been consumed. Above 60% O_2 the inductive effect of the high O_2 concentration appears to overcome the repressive effect of the glucose and there is a substantial increase in the cytochrome oxidase activity. When K^+ concentration was limiting the repressive effect of glucose was not apparent, nor was the inductive effect of O_2 . The specific activity of the enzyme was about twice that of the standard cells at all O_2 concentrations, but the size differences in the two sorts of cell resulted in the K^+ -deficient cells having cytochrome oxidase activities that were up to tenfold those of their counterpart at the same O_2 concentration, and the activities were throughout three- to eight-fold those of the standard cells.

NADH-cytochrome c oxidoreductase (EC 1.6.2.1). Oxygen concentration had little effect on the activity of this enzyme in the presence of excess of K^+ . With limiting K^+ concentration there was a slight decline in the activity at O_2 concentrations above 60%. The larger size of the K^+ -deficient cells resulted in them having up to about twice the total enzyme as in the normal cells.

NADH oxidase [NADH-(acceptor) oxidoreductase (EC 1.6.99.3)]. The K^+ -replete cells had twice the specific activity when grown in 20% O_2 as those grown in N_2 and there was a further 70% increase in specific activity above 60% O_2 . At all concentrations of O_2 the K^+ -deficient cells had only 50–60% of the activity

Table 9. *Effect of O_2 concentration on the activity of pyruvate kinase in *S. cerevisiae* grown in excess of K^+ or limiting concentration of K^+*

The 'normal cells' were grown in 20% O_2 and with excess of K^+ in the medium.

Percentage of O_2	Activity			Activity		
	Cells with excess of K^+ (μ mol/min per g of protein)	% of normal cells		Cells with excess of K^+ (μ mol/min per mg of DNA)	% of normal cells	
		Excess of K^+	Limiting K^+		Excess of K^+	Limiting K^+
0	2340	84	73	620	177	147
20	2780	100	86	350	100	145
40	2180	79	36	212	60	67
60	3390	122	107	580	165	228
80	3640	131	65	865	240	202
100	2480	89	87	591	168	350

Table 10. *Effect of O₂ concentration on the activity of alcohol dehydrogenase in S. cerevisiae grown in excess of K⁺ or in limiting concentration of K⁺*

For details see the text.

Percentage of O ₂	Activity			Activity		
	Cells with excess of K ⁺ (μmol/min per g of protein)	% of normal cells		Cells with excess of K ⁺ (μmol/min per mg of DNA)	% of normal cells	
		Excess of K ⁺	Limiting K ⁺		Excess of K ⁺	Limiting K ⁺
0	825	114	262	312	300	520
20	722	100	250	105	100	380
40	994	138	304	119	113	490
60	1023	144	202	188	179	430
80	894	118	148	224	230	530
100	608	84	135	151	144	530

Table 11. *Effect of O₂ concentration on the activity of cytochrome oxidase in S. cerevisiae grown in excess of K⁺ or limiting concentration of K⁺*

Percentage of O ₂	Activity			Activity		
	Cells with excess of K ⁺ (μmol/min per g of protein)	% of normal cells		Cells with excess of K ⁺ (μmol/min per mg of DNA)	% of normal cells	
		Excess of K ⁺	Limiting K ⁺		Excess of K ⁺	Limiting K ⁺
0	6.7	63	200	1.7	128	384
20	10.7	100	340	1.3	100	560
40	5.6	52	172	0.6	48	330
60	4.6	43	236	0.7	51	540
80	30.0	280	224	5.8	450	810
100	32.1	301	254	6.6	510	810

of their counterparts. The size differential resulted in both sets of cells having very similar enzyme activities at the same O₂ concentration.

Effect of K⁺ concentration on the enzymic make-up of the cells grown in 100% O₂

Above about 2.5 mM-K⁺ in the growth medium the enzymes showed activities little different from those found when K⁺ was in excess.

Discussion

Hyperbaric oxygen is an inhibitor of the growth of micro-organisms (Hughes & Wimpenny, 1969), but *S. cerevisiae* has not previously been shown to be sensitive to the lower O₂ concentrations used in the experiments described in the present paper. Stuart *et al.* (1962) showed that exposure to very high concentrations of O₂ (several atmospheres) results in the loss of some cellular K⁺ from commercial baker's yeast.

Oxygen toxicity has been attributed, amongst other things, to the oxidation of SH groups in proteins and cofactors (Haugaard, 1968). Of particular interest is the involvement of SH groups in the 'sodium/potassium activated adenosine triphosphatase' (Wheeler & Whittam, 1964). More rapid oxidation of these groups might be expected to interfere with the 'ion pump'. However, it is clear that O₂ has little effect when K⁺ is freely available, but under circumstances where K⁺ and hence protein synthesis (Tempest, 1969; Lubin & Ennis, 1964) is limited the destructive processes could exceed the synthetic ones. Lack of K⁺ does not limit the synthesis of protein equally (Bartley & Broomhead, 1971); for example, the synthesis of alcohol dehydrogenase is a greater proportion of the newly synthesized proteins at all O₂ concentrations, whereas pyruvate kinase forms a lower proportion. Also, except at 80 and 100% O₂, cytochrome oxidase is preferentially synthesized when K⁺ is limiting.

Bartley & Broomhead (1971) showed that *S. cerevisiae* grown anaerobically was normal when the concentration of K⁺ in the medium was greater than

about 3 mM. The same concentration of K^+ restores normality in the presence of 100% O_2 .

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