XIV.—*Yeast Crops and the Factors which Determine them.*

By Arthur Slator.

Many investigations have been carried out to determine the factors which influence the amount of yeast produced by growth in a suitable medium, and much of the important earlier work on the subject is published in these *Transactions*. It has been shown in recent years that the growth of micro-organisms under simple conditions follows the exponential law of increase, and equations expressing growth and fermentation have been worked out and experimentally verified. In this communication an attempt has been made to express yeast crops in terms of generation-times and fermentative activity of the yeast and to use such equations to test some of the generally accepted views on the subject. The reproduction of yeast in a medium such as malt wort takes place in definite phases. A period of quiescence (lag phase in growth) is followed by unrestricted growth (logarithmic phase in growth), which continues until retarding influences, such as lack of necessary food or the production of toxins, come into play. Growth finally ceases when these retarding influences become sufficiently great. Under the usual conditions of yeast growth and alcoholic fermentation several factors play a part in limiting growth, but a simple case where one factor only determines the total growth will be first discussed.

Conditions can be so arranged that the sugar in the medium can be made this limiting factor. All other foods must be in large excess, and substances toxic to growth must not be allowed to accumulate. The crop then admits of calculation in the following manner.

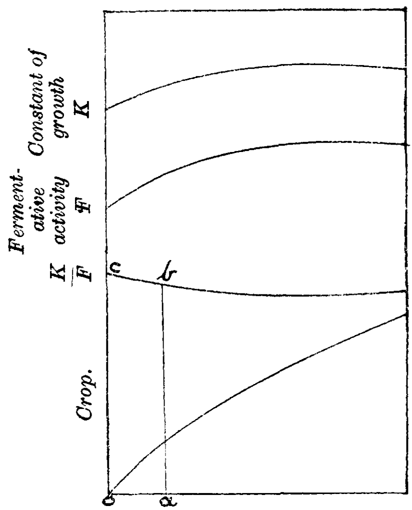
It has been shown (Slator, *Bio-Chem. J.*, 1913, **7**, 198) that during the logarithmic period of growth the ratio of the increase in the number of cells n to the amount of sugar s fermented is a constant, and that this ratio $\frac{n}{s} = \frac{K}{F}$, where K is the constant of growth and F is the fermentative activity of the yeast. During later stages in growth, K and F may not remain constant, but at any given time they have definite values, and the small increments in the yeast and sugar fermented are determined by the equation $\frac{dn}{ds} = \frac{K}{F}$.

We have therefore $n = \int \frac{K}{F} ds$ + constant. Yeast crops (from a small seeding) are therefore determined by the way this ratio K/F varies with different concentrations of sugar. A graphical method of calculation of such crops is shown in Fig. 1. The values of K and F are obtained experimentally and plotted against the corresponding concentration of sugar. The K/F curve is then constructed. The crop obtained during the complete fermentation of "a" grams of sugar is represented by the area $oabc$. A curve can now be constructed showing yeast crops with various initial concentrations of sugar.

This theoretical curve should coincide with the experimental one as long as the sugar is the limiting factor determining the final crop. In the case described in the experimental part of this paper the ratio K/F is almost constant, and the crop is approximately proportional to the initial concentration of the sugar.

This method of calculating yeast crops is of general application, and a similar diagram can be constructed when other limiting factors are predominant. The other factors discussed in this paper are those relating to the seeding, oxygen, carbon dioxide, and temperature. Only a brief reference is made to the important influence which nitrogenous food can exert on yeast crops.

FIG. 1.



The influence of the amount of seeding admits of simple calculation if no change in the limiting factor takes place owing to change in the amount of seeding. The ratio K/F is independent of the number of yeast cells: the growth under definite conditions should therefore be a constant. The yeast crop is the sum of the seeding and the growth, the latter being constant. That this relationship holds good was first shown by A. L. Stern (T., 1901, **79**, 943; *J. Inst. Brewing*, 1902, **8**, 690). A. J. Brown had previously drawn the conclusion that the final yeast crop is almost independent of the amount of the seeding, and deduced the idea of a non-multiplying limit to yeast growth (T., 1892, **61**, 369). H. T. Brown considers the cell increase of most importance (*Ann. Botany*, 1914,

28, 197). There would probably be general acceptance of the idea of a constant cell increase under constant conditions if it were not for the fact that large seedings of yeast refuse to bud or show only a small increase (A. J. Brown, *loc. cit.*). A satisfactory explanation of this abnormality is given here. It was found that if actively growing yeast cells are used for seeding, the normal increase takes place even though the seeding is very large. Quiescent yeast cells pass through a lag phase before active growth occurs, and if retarding influences accumulate quickly the yeast never passes out of its quiescent state. Abnormally low crops are sometimes observed if very small seedings are used. Many investigators have noticed this effect (compare Lampitt, *Bio-Chem. J.*, 1919, **13**, 461). If actively growing yeast is used for seeding, this abnormality partly disappears, and the effect is probably connected with the lag phase in growth.

Much of the interest in yeast growth centres round the favourable influence which atmospheric oxygen has on the growth of the organism. Although it is more than 40 years since Pasteur's work on the subject appeared, there is still much diversity of opinion as to the method by which air acts. A. J. Brown (T., 1905, **87**, 1395) drew the conclusion that the development of yeast in malt wort is determined by the oxygen initially dissolved in the wort. H. T. Brown (*Ann. Botany*, 1914, **28**, 197) considers that the growth can be attributed to oxygen absorbed by the yeast used for seeding. It is possible, however, that these experimental results can be interpreted in other ways. Carbon dioxide retards yeast growth to a much greater extent than is recognised in these experiments. Moreover, yeast growth takes place under anaerobic conditions, although the cells have lost all their "oxygen charge" (see table IX). Euler and Lindner ("Chemie der Hefe," Leipzig, 1915) summarise the work of Pasteur, Chudiakow, Buchner and Rapp, Delbrück and others on the subject. They recognise the retarding influence of carbon dioxide on growth, and conclude that oxygen accelerates the growth of the yeast cells. One of the arguments against this view is the fact that growth in its simplest form is not accelerated by air or oxygen. Unrestricted growth is appreciably retarded by oxygen (see table V). In a short summary of the factors which influence growth (*J. Soc. Chem. Ind.*, 1919, **38**, 391 R), the view (previously discussed by other authors) is taken that malt wort contains a substance which easily gives up its oxygen to the yeast and that this substance accounts for growth under anaerobic conditions. Attempts were made to verify this assumption by estimating yeast crops under conditions that the unknown substance would become the limiting factor determining the crop. The experiments were unsuccessful and this explanation appears to be incorrect. The matter has been reconsidered in the light of these experiments and the following interpretation is advanced. It is considered that there are two different modes* of growth of yeast, each involving a different set of chemical reactions. The initial stages of growth in malt wort illustrate the one mode of growth. Oxygen, either free or combined, or absorbed by the yeast, plays no part in the process. Free oxygen slightly retards this growth. The energy necessary for growth is obtained from the fermentation of the sugar. Carbon dioxide retards the growth, and if air is passed through the fermenting wort, yeast growth is increased owing to the displacement of carbon dioxide. Growth in lactose yeast water illustrates the other mode of growth. This growth is unaccompanied by alcoholic fermentation, but zymase is present in the cells. Free oxygen is here essential to growth, and if absent hardly any growth occurs. The necessary energy is obtained through some oxidation reaction carried out by the yeast. Carbon dioxide in large amounts also retards this growth. The factors determining which mode of growth takes place when both are possible have not yet been completely investigated. The available nitrogenous food is probably of importance, for whilst yeast growth in wort is not influenced by air at the beginning of the reaction, yeast growth in a medium prepared from half-fermented wort is favourably influenced by air (Slator, *Bio-Chem. J.*, 1918, **12**, 254).

Whilst the influence of large amounts of air on yeast growth admits of explanation on these lines, the influence of small amounts is less easy to understand. A wort saturated with air produces a larger yeast crop than one which contains no air.

The increase is out of all proportion to what would be expected from the amount of air available.† A number of experiments were carried out to obtain further information on the subject. It was found that if fermentation and yeast growth occur under conditions that supersaturation of the wort with carbon dioxide takes place the effect is readily verified. If, however, care is taken to control the concentration of the carbon dioxide the

* Pasteur considered yeast to be an organism endowed with two modes of life, and the theory advanced here agrees in some respects with that of Pasteur. The aerobic growth in lactose yeast water was investigated by Pasteur, and the view that this growth depends on air has been confirmed. The very slight growth under anaerobic conditions is probably due to a trace of fermentable sugar.

† According to H. T. Brown's figures, 1 c.c. of oxygen dissolved in wort causes the growth of 6000×10^6 yeast cells. Experiments in sealed tubes showed less than 100×10^6 cell increase for each c.c. of oxygen.

effect disappears. The conclusion is drawn that the influence of dissolved air is only indirect, and doubts are cast on the usually accepted explanation of the matter, namely, that the effect is due to the favouring influence of the dissolved oxygen. It is possible that supersaturation by carbon dioxide is lessened by the presence of dissolved air and that larger yeast crops are a consequence of this influence. The work of Findlay and King (T., 1913, **103**, 1170) on the rate of evolution of gases from supersaturated solutions is of much interest in this connexion.

Many of the arguments advanced in this publication depend on the assumption that carbon dioxide retards yeast growth. That the gas has this effect was considered to be the case by Delbrück (1886) and Foth (1887). (For a summary of the work see Delbrück and Hayduck, "Die Gärungsfuhrung," 1911, Berlin.) The correctness of these conclusions is accepted in this paper, but it is considered that the effect is more far-reaching than either these investigators or others have realised. A. J. Brown (T., 1905, **87**, 1406) concluded that carbon dioxide had no such effect, and that the retarding influence attributed to the gas was due to exclusion of oxygen. The experiments, however, are not conclusive, for no precautions are taken to remove the carbon dioxide formed during fermentation. Measurements of rates of growth in wort and in wort saturated with carbon dioxide show a very marked retarding influence of the gas. Some measurements of yeast crops grown under conditions that carbon dioxide becomes the limiting factor confirm these results in an interesting manner (see table IV). The influence of temperature on yeast crops is small (compare Stern, *J. Inst. Brewing*, 1902, **8**, 694). Both K and F have temperature-coefficients not greatly different from each other. If, therefore, the sugar is the factor controlling growth, the crops which depend on the ratio K/F will be almost independent of temperature (see table XI). This has been experimentally verified.

A publication by Carlson (*Biochem. Zeitsch.*, 1913, **57**, 513) is of much interest. Measurements of yeast growths and yeast crops in wort are made. The wort is kept continually stirred and the amount of yeast in suspension estimated by centrifuging, a method adopted here. If allowance is made for the fact that Carlson's wort is saturated with carbon dioxide, the conclusions regarding rates of growth are in general agreement with those given here and in previous papers. His method of treating yeast crops is less easy to follow, for no precaution is taken to make a single factor the limiting one which determines the crop; moreover, it is unlikely that the relationship between concentration of food supply and growth is as simple as is assumed.

The question whether any food accessory substance (vitamin) is necessary to yeast growth has been considered in so far as it affects the experiments described here. No evidence of the necessity of such a substance was forthcoming, for heating the wort to a high temperature and filtering through Fuller's earth caused no decrease in the value of the wort as a medium for growing yeast.

EXPERIMENTAL.

Methods of Estimating Concentrations of Yeast.

Two methods were employed in this investigation to estimate the amount of yeast suspended in a liquid. When the amount was greater than a few million per c.c. it was estimated by centrifuging and measuring the quantity of deposit. The straight 5 cm. graduated capilliary tube used for blood analysis is not sensitive enough to estimate any but large amounts of yeast. If, however, tubes with a bulb at the end are used, the volume is increased and smaller concentrations can be conveniently estimated. Three tubes were used which had capacities given in table I.

TABLE I.

Tube.	Volume.	Equivalent of 1 scale division.
1	0·011 c.c.	$44·5 \times 10^6$ cells per c.c.
2	0·064 ,,	$7·65 \times 10^6$,, ,,
3	0·447 ,,	$1·11 \times 10^6$,, ,,

A suspension of yeast containing 216×10^6 cells per c.c. by counting under the microscope gave by centrifuging in tube 2 a deposit showing 27·7, 27·6, 27·5 scale divisions (0·5 mm. in length), on diluting to twice the volume the deposit was 14·1, 14·2, 13·9, and on diluting again to twice the volume the readings were 7·0, 7·4, 7·1. The averages are 27·6, $\frac{1}{2} \times 28·2$, $\frac{1}{4} \times 28·8$. One scale division on tube 2 is therefore equivalent to $216 \times 10^6/28·2 = 7·65 \times 10^6$ cells per c.c. The other tubes were similarly calibrated and checked one against the other. As tube 1 is a straight capilliary tube the yeast when it has settled down to a constant volume has a consistency of 4450 million per c.c. This corresponds approximately with the consistency of pressed barm. Yeast cells vary in size, but the figures given in table I have been used throughout the paper to convert scale divisions into cells per c.c.

Carlson used a method of this kind, the deposit from about 15 c.c. being determined. Paine (*Proc. Roy. Soc.*, 1911, [*B*], **84**, 289) also employed straight capillary tubes for estimating the volume of yeast cells suspended in a liquid.

When the number of yeast cells is below a few million per c.c., yeast counts under the microscope were made to determine the concentration. The usual hæmacytometer was employed, but the average number of cells appearing per field was estimated and the concentration obtained by dividing this average by the volume of liquid appearing in the field of the microscope.

Methods of Growing the Yeast.

The medium used in most of these experiments was lightly hopped malt wort. Yeast grows readily in this medium and large crops are produced. To eliminate the influence of carbon dioxide small quantities of wort were placed in large test-tubes to which had been sealed a piece of glass tubing. The tube is then exhausted or filled with any desired gas, sealed up, and rotated in a thermostat. Most of the carbon dioxide passes from the liquid into the space above. In some experiments accumulation of carbon dioxide is required. The fermentations were then carried out in straight tubes in which the liquid is sealed. High pressures of gas are obtained in this way. The yeast used in most cases was a Burton yeast (*S. cerevisiae*). In some experiments a pure culture of actively growing yeast was used, and in others quiescent yeast (pressed barm).

The Influence of Seeding on the Crop.

Tests were first made to determine whether large seedings of yeast would grow. The results, which are summarised in table II (p. 123), show what conditions must be fulfilled for growth to take place.

If actively growing yeast cells are used for seeding, normal growth takes place even though the seeding is very large (370×10^6 cells per c.c.). Large seedings of quiescent yeast cells show only a small growth (Expt. *h*). When the carbon dioxide cannot escape, moderately large seedings show no growth at all (Expt. *i*).

Under conditions of continual agitation larger crops were obtained, and the cell increase was found to be approximately constant, although the seeding varied greatly. In the following experiments about 3 c.c. of wort (D 1·050) were placed in a tube of capacity about 100 c.c., the tube being afterwards exhausted and sealed. The time of growth was three days at 20°.

TABLE II.

Wort, D 1·050. *Yeast concentrations, unit* $= 7 \cdot 65 \times 10^6$ *cells per c.c.*

Expt.	Temp.	Time.	Yeast.	Vessel.	Agitation.	Seeding.	Crop.	Cell increase.
a	25°	2 days	actively growing	large tubes exhausted	occasional shaking	49·3	62·5	13·2
b	25	2 ,,	,,	,,	,,	37·0	48·7	11·7
c	25	2 ,,	,,	,,	,,	24·7	37·0	13·7
d	25	3 hours	,,	flask	,,	25·2	32·5	7·3
e	25	1 day	,,	,,	,,	25·2	37·2	12·0
f	25	3 hours	quiescent	,,	,,	15·0	19·1	4·1
g	25	1 day	,,	,,	,,	15·0	28·0	13·0
h	25	1 ,,	,,	,,	,,	33·1	35·2	2·1
i	15	1 ,,	,,	sealed tube almost full	,,	18·1	18·1	0·0

TABLE III.

Yeast concentrations, unit = $7 \cdot 65 \times 10^6$ *cells per c.c.*

Expt.	Seeding.	Crop.	Cell increase.	Average.
a	37·1	56·0	18·9	18·8 = 143 × 10^6 cells per c.c.
	27·8	48·6	20·8	
	18·6	37·9	19·3	
	13·9	30·6	16·7	
	9·3	25·9	16·6	
	4·6	25·1	20·5	
b	19·3	37·8	18·5	17·2 = 132 × 10^6 cells per c.c.
	13·6	29·7	16·1	
	3·4	20·4	17·0	

The Influence of Carbon Dioxide on the Crop.

A wort (D 1·050) was seeded with yeast. A tube was filled almost completely, exhausted to remove dissolved air, and then sealed. Into a large tube was introduced a small quantity of the seeded wort, and this tube was exhausted and sealed. Both were rotated slowly at 20°. The yeast increase in the first case amounted to 30×10^6 cells per c.c., and in the second to 140×10^6 cells per c.c. This great difference is attributed to the carbon dioxide which remains in solution in the first case, and is removed in the second. If simultaneously an ordinary fermentation open to the atmosphere is carried out, the cell increase amounts to about 80×10^6 cells per c.c. This is a typical result obtained from a large number of experiments of this kind. It was considered probable that carbon dioxide could be made the limiting factor which determines the growth. The matter was tested by varying the exhausted space above the wort and determining the influence such variations had on the crop. If v_1 and v_2 are the volumes of liquid and space respectively, then the concentration of carbon dioxide in the liquid when a given amount of gas is formed is proportional to $\alpha = \frac{v_1 + v_2/s}{v_1}$, where s is coefficient of absorption of the gas in the liquid. A consideration of Fig. 1 shows that the cell increase should be proportional to α if the carbon dioxide is the limiting factor. This was found to be the case, as table IV (p. 125) shows.

If the tube is full ($\alpha = 1$) cell increase is $2 \cdot 9 = 22 \times 10^6$ cells per c.c. In Expt. g, where the space above is great, proportionality no longer exists. When more dilute worts were used, similar results were obtained, but as the space increased the deviation from proportionality occurred earlier. Experiments in which the space above was not exhausted, but filled with air, showed that the air

TABLE IV.

Wort, D 1·101. *Small seeding of yeast; exhausted tubes; temp.* = 25°; *tubes rotated three days*; $s = 0{\cdot}70$.

Expt.	v_1.	v_2.	a.	Crop (unit = $7{\cdot}65 \times 10^6$ cells per c.c.).	Crop/a.
a	23·3	1·2	1·08	3·0	2·8
b	23·0	1·3	1·08	3·0	2·8
c	14·5	4·8	1·46	4·1	2·8
d	11·8	4·0	1·48	4·5	3·1
e	10·3	8·3	2·13	6·1	2·9
f	12·3	11·7	2·33	6·5	2·8
g	7·4	17·2	4·26	9·9	2·3
				Average (excluding *g*) ...	2·9

increased the crop. One c.c. of air ($\frac{1}{5}$ c.c. oxygen) caused an increased growth averaging 15×10^6 cells.

The Influence of Air and Oxygen on Growth and Crop.

Small seedings of yeast in wort were allowed to grow one to two days at 20° in tubes which were continually rotated. Some of the tubes were exhausted, some filled with air, oxygen, and carbon dioxide. The constants of growth were calculated in the usual way, and the results are summarised in table V.

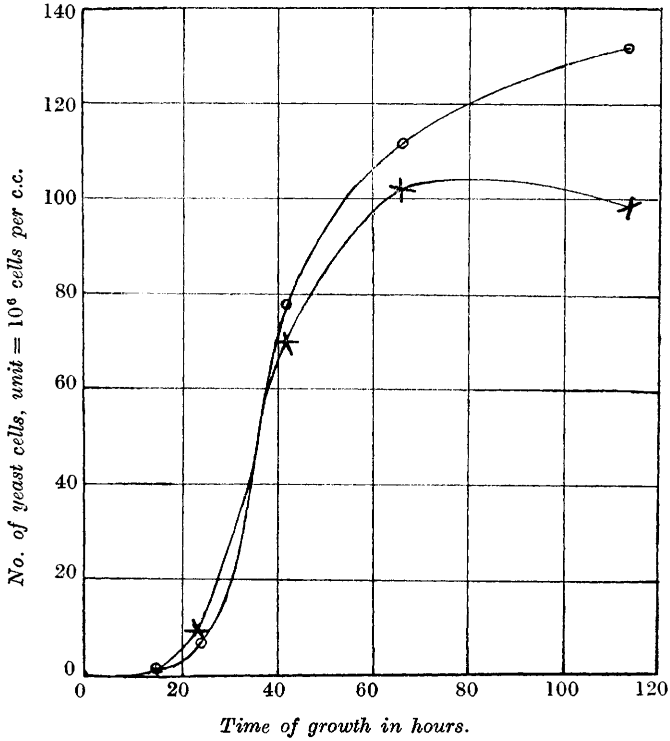
TABLE V.

	Wort, D 1·047.		Wort, D 1·040.	
	0·434K.	G.T.	0·434K.	G.T.
Exhausted	0·110	2·7 hours.	0·109	2·7 hours.
Air	0·107	2·8 ,,	0·092	3·3 ,,
60 per cent. oxygen + 40 per cent. nitrogen	0·096	3·1 ,,	—	—
100 per cent. oxygen	0·085	3·5 ,,	—	—
100 per cent. carbon dioxide	0·079	3·8 ,,	0·073	4·1 hours.

The influence of air on the whole range of growth was then investigated, large tubes being used to eliminate the influence of carbon dioxide. The curves in Fig. 2 show the retarding influence of air during the early stages of growth and the favouring effect during the latter stages. The final yeast crop is greater under aerobic conditions.

In tables VI and VII are summarised some experiments on yeast growth in worts saturated with air. Wort (D 1·040) was introduced into a pressure flask and seeded with yeast. All dissolved air was rapidly removed by exhausting the flask. Fifty c.c. of the seeded wort were then introduced into a large test-tube. A parallel experiment was made with wort saturated with air. Although in the first case the wort is exposed to the atmosphere and traces of air become dissolved, the amount dissolved in the second case is much greater and the effect on growth is very noticeable.

FIG. 2.



Anaerobic growth ×.
Aerobic growth ○.
Influence of CO_2 *eliminated.*

TABLE VI.

Temp. about 15°. *Unit* = 7.65×10^6 *cells per c.c.*

Yeast.	Seeding.	Time of growth.	Cell increase: Exhausted wort.	Cell increase: Aerated wort.	Ratio.
Quiescent	1·4	18 hours.	1·7	2·5	100 : 148
,, ...	1·4	24 ,,	1·5	3·4	100 : 227
,, ...	3·0	48 ,,	5·0	7·3	100 : 146
,, ...	3·0	18 ,,	2·8	4·0	100 : 143
Actively growing	0·2	48 ,,	2·3	5·5	100 : 239
,,	0·1	48 ,,	2·0	4·0	100 : 200

The experiment was repeated, but the wort, both free from air and saturated with air, was placed in bottles. The bottles were filled completely with the liquid and stoppered to prevent escape of gas. In this case no such differences were observed.

TABLE VII.

Temp. about 15°. *Unit* $=7{\cdot}65 \times 10^6$ *cells per c.c.*

Yeast.	Seeding.	Time of growth.	Cell increase: Exhausted wort.	Cell increase: Aerated wort.	Ratio.
Quiescent	1·0	18 hours.	1·8	1·8	100 : 100
,, ...	1·0	18 ,,	1·1	1·1	100 : 100
Actively growing	1·5	18 ,,	2·8	3·0	100 : 107
,,	0·2	40 ,,	2·5	2·8	100 : 111
,,	0·2	48 ,,	2·6	2·8	100 : 108

A number of experiments were then made in which yeast growth took place under anaerobic conditions and under conditions that a limited quantity of air was present. The tubes were rotated in a thermostat. Usually these small quantities of air had little or no influence on the yeast growth. In Expt. *e*, when large quantities of air are present, an appreciable retardation in growth is noticed.

TABLE VIII.

Temp. 20°. *Unit* $=7{\cdot}65 \times 10^6$ *cells per c.c.*

Expt.	Yeast.	Vol. liquid : vol. air.	Seeding.	Time of growth.	Cell increase: Anaerobic	Cell increase: aerobic.	Ratio.
a	Quiescent	5 : 1	0·2	48 hours.	4·7	4·8	100 : 102
b	,,	1 : 1	5·2	2 ,,	1·4	1·3	100 : 93
c	,,	1 : 1	5·2	6 ,,	5·7	5·1	100 : 90
d	,,	5 : 150	2·9	4½ ,,	1·1	1·0	100 : 90
e	,,	5 : 150	2·9	6 ,,	2·3	1·6	100 : 70
f	Actively growing.	1 : 1	1·7	8 ,,	5·7	6·4	100 : 113

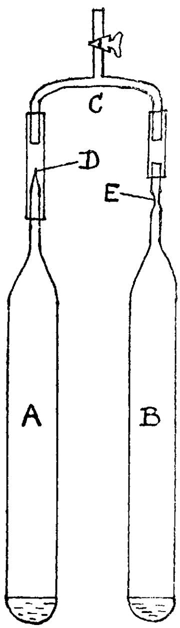
All these experiments show that small quantities of air have little influence so long as the concentration of the carbon dioxide is controlled. The results are in accordance with the idea that dissolved air aids yeast growth owing to the effect it has in lessening the supersaturation of the wort with carbon dioxide. The early stages of growth of quiescent yeast do not require air (table VIII, Expts. *a*, *b*, *c*, *d*). The following experiments were carried out to test H. T. Brown's suggestion that oxygen absorbed by the seeding yeast determines the subsequent growth. Yeast was grown in a small quantity of wort in an exhausted tube *A*. This yeast was used to seed fresh wort in another tube, the process being carried out under strictly anaerobic conditions in the manner shown in Fig. 3. The T-piece *C* is exhausted and the wort in *B* is boiled to free it from air. The point *D* is then broken off, some of the

TABLE IX.

Expt.	Density of wort.	Time of growth and temp.	Crop (unit=$7 \cdot 65 \times 10^6$ cells per c.c.).
1	1·040	2 days at 25°	8·0
2	1·052	,, 20	10·0
3	1·063	,, 20	15·1
3*a*.........	1·063	,, 20	15·0
4	1·040	,, 20	11·4
4*a*.........	1·040	,, 20	11·2

liquid from *A* passes into *B*, which is then sealed at *E*. The results which are given in the above table show that good crops were obtained in tube *B* in spite of all absence of air.

FIG. 3.



In Expts. 3*a* and 4*a* the seeded wort was allowed to come in contact with the air for some hours and the tube then exhausted. This contact with the air had, however, no influence on the subsequent crop. There seems therefore no necessity for oxygen to be at the disposal of the yeast used for seeding.

The Influence of Concentrations of Sugar.

Some difficulty was experienced in obtaining a suitable medium to show the effect of sugar on yeast crops when this constituent of the medium is made the limiting factor. The medium finally chosen was the residue of a fermented wort (D 1·080). This wort was fermented with yeast and the alcohol distilled off. The process was repeated in order to free the medium entirely from fermentable sugar. To the final residue (D 1·017) was added small amounts of glucose, and this proved a good medium for wild yeast growth, although unsuitable for the growth of *S. cerevisiae*. The following table summarises measurements of *K*, *F* and crops with different concentrations of sugar, the organism used being a wild yeast of the *S. ellipsoideus* type.

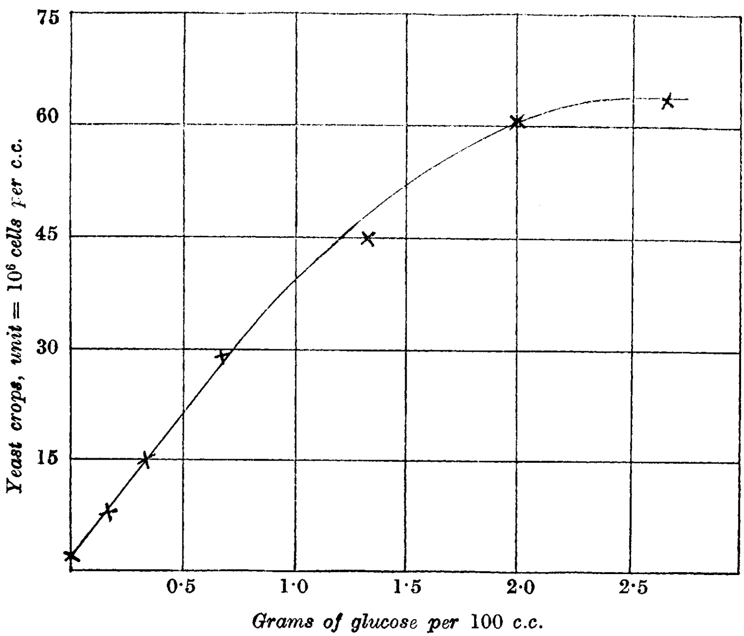
TABLE X.

Temp. = 20°. K = constant of growth in hours^{-1}.
F = grams of sugar fermented per hour per yeast cell.
K/F = yeast cells produced per gram fermented.

Grams of glucose per 100 c.c.	G.T.	K.	F.	K/F.
1·50	2·86 hours.	0·242	57×10^{-12}	4200×10^6
1·00	2·84 ,,	0·244	57	4300
0·50	2·84 ,,	0·244	56	4400
0·20	2·82 ,,	0·246	53	4600
			Average ...	4400×10^6

According to table X, when 1 gram of sugar is fermented, 4400×10^6 cells should be produced. Some rather indefinite cor-

FIG. 4.



rection has to be made for the amount of sugar used in building up the yeast. This correction would reduce the figure to approximately 3700×10^6. The curve, Fig. 4, shows proportionality between crop and initial glucose concentration up to about 1 per cent. of sugar. The slope of the curve corresponds with

3900×10^6 cells per gram, a figure in fair agreement with that calculated from K and F.

Influence on Temperature.

The following table summarises measurements of K and F of yeast (*S. cerevisiae*) in wort (D 1·040). The ratio K/F is approximately independent of temperature.

TABLE XI.

Temp.	G.T.	K.	F.	K/F.
10°	11·6 hours.	0·060	$12{\cdot}5 \times 10^{-12}$	4800×10^6
15	6·4 ,,	0·108	24·5	4400
20	2·95 ,,	0·235	47·0	5000
25	1·77 ,,	0·392	75·0	5200
			Average ...	4900×10^6

4900×10^6 cells are therefore produced when 1 gram of sugar is fermented. Assuming that this amount of yeast uses up 0·2 gram of sugar for its growth, 1·2 grams of sugar produce 4900×10^6 yeast cells (about 1·2 grams of pressed yeast). This represents the maximum possible crop.

In table XII is given the actual crops obtained at 15° and 25° under conditions that the sugar is the limiting factor (*a* and *b*), and under conditions that the carbon dioxide is the limiting factor (*c* and *d*). The medium in (*a*) and (*b*) is a mixture of 50 c.c. fermented wort residue and 5 c.c. of wort (D 1·040), and the experiment is carried out in large exhausted tubes. In (*c*) and (*d*) wort (D 1·101) is used in long tubes, half-filled in (*c*) and two-thirds filled in (*d*). They were exhausted and sealed. The cell increase was almost the same at 15° and 25°.

TABLE XII.

Unit $= 7{\cdot}65 \times 10^6$ *cells per c.c.*

	Seeding (actively growing).	15° Cell increase.	15° Time of growth.	25° Cell increase.	25° Time of growth.
a	4·8	2·3	2 days	2·3	1 day
b	0·7	2·2	2 ,,	2·5	1 ,,
c	1·8	7·1	2 ,,	7·1	1 ,,
d	0·5	3·4	3 ,,	3·8	2 ,,

Growth of Yeast in Malt Wort.

As a result of these experiments it is suggested that the generally accepted views on yeast growth should be considerably modified.

The influence of carbon dioxide is greater than has been realised, and great variations in the crop are obtained by altering the concentration of the carbon dioxide in the wort. Air (oxygen) has much less influence than is usually attributed to it. It plays no part in the initial budding of the yeast (the lag phase in growth) and has no direct accelerating influence during the first stages of growth. Later stages are favourable influenced by air; the aerobic vegetative growth here comes into consideration. Air dissolved in wort favours yeast growth. This is considered to be due to a decrease in the supersaturation of the wort with carbon dioxide and not to the yeast cell requiring free oxygen for its development.

Note on Spore Formation of Yeast Cells.

Little is known of the chemical reactions which accompany the formation of spores in yeast cells, but the belief is very generally held that oxygen plays an essential part in the process. This belief seems justified, but it was found that traces of air are sufficient to induce sporulation. The experiments were carried out in the following manner. Young and vigorous yeast cells of a wild yeast which readily formed spores were mixed with slightly acidified water (0·2 per cent. phosphoric acid neutralised with potassium hydroxide to give a P_H value 5—6) so that the solution was just turbid. Some of the liquid was placed in sterile conical flasks and some in tubes, which were then carefully exhausted. In two days at 23° abundant spore formation took place in the flasks, whilst no spores were formed under anaerobic conditions. In some cases a small quantity of air was allowed to enter the exhausted tube. One cm. pressure of air in the tube was found to cause the production of spores.

It is sometimes stated that the growth of yeast spores requires air. This proved incorrect, for spores were found to develop readily in wort free from air.

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