formed, the assimilation of the fructose is initially rapid, presumably because it is in the furanose form, but becomes slow as the preferential absorption of glucose and the conversion of the fructofuranose to the equilibrium mixture of furanose and pyranose manifests itself. The behaviour of the yeasts in the mixture of sugars found in wort thus reveals itself as a resultant of effects which may be seen more clearly in simple mixtures.

We should like to take this opportunity to thank Miss June Sutton for her valuable assistance in this investigation. We are also indebted to the Directors of Whitbread & Co., Ltd., for giving permission for this work to be published.

INFLUENCE OF TEMPERATURE ON YEAST GROWTH AND FERMENTATION

By J. White, B.Sc., F.R.I.C., and D. J. Munns, B.Sc., A.R.I.C.

Received 1st *February*, 1951

The total amount of sugar used when yeast is growing in wort consists of the sum of the sugar used in production of new yeast ("assimilation") and that used in formation of alcohol and carbon dioxide ("fermentation"). The fundamentals of yeast growth in aerated wort are discussed in a mathematical treatment which shows that yeast yield and rate of sugar utilization depend upon the yeast growth coefficient (r) and the fermentation coefficient (s). Calculation of the growth and fermentation coefficients from various experimental data is illustrated. The value of each coefficient is strongly influenced by temperature, aeration, sugar concentration and other factors. The influence of temperature variations on each coefficient and on the total rate of sugar consumption ($s + 0{\cdot}476\, r$) over the range 20–43° C. is investigated, other conditions being standardized.

It is shown that the fermentation coefficient increases steadily up to 40° C. (104° F.), after which it falls away steeply. The growth coefficient increases steadily up to 30° C., after which it increases only slightly up to 36° C. and then falls off steeply. The yeast yield calculated on total sugar used diminishes with increasing temperatures of growth, particularly with temperatures in excess of 36° C. (96° F.).

Introduction

It has long been recognized that under certain conditions micro-organisms can develop exponentially; the term "logarithmic phase" of growth therefore needs little explanation. It may be recalled, however, that A. Slator (*Biochem. J.*, 1913, 7, 197) showed that there is a logarithmic phase in the growth of yeast, where if N is the number of cells seeded and n is the increase in cell number at the end of time t, then $n = N(e^{kt}-1)$, where k is a constant for the particular yeast used and the growth conditions employed. In brewing problems many investigations have shown that the logarithmic phase is often preceded by a lag phase and followed by an inhibitory phase, the whole course of growth being represented by the well-known S-shaped curve. R. S. W. Thorne (this *Journ.*, 1939, 472) has analysed the growth of brewery yeast; mathematical treatment has shown that the course of such growth could be represented by a logistic relationship. The amount of yeast Y present in a fermenting wort at the end of time t is given as

$$\log \frac{Y}{a-Y} = ct - b$$

where a, b and c are constants representing respectively, height, position on the time axis, and slope, of the logistic curve. A. Tait and L. Fletcher (this *Journ.*, 1923, 509) had previously shown that the duration of the lag phase was dependent on the age of the yeast, and that little or no lag was shown by vigorous seed yeasts. These authors further showed that progressive decrease in growth rate accompanied the accumulation in the medium of products of growth; vigorous yeast growth thus gives way to the inhibitory phase. Increasing carbon dioxide and ethyl alcohol concentrations were responsible for reductions in the growth rate, and Thorne later showed (this *Journ.*, 1939, 13) that several higher alcohols, some of which are produced during fermentation by breakdown of amino acids according to the Ehrlich mechanism, inhibit yeast growth.

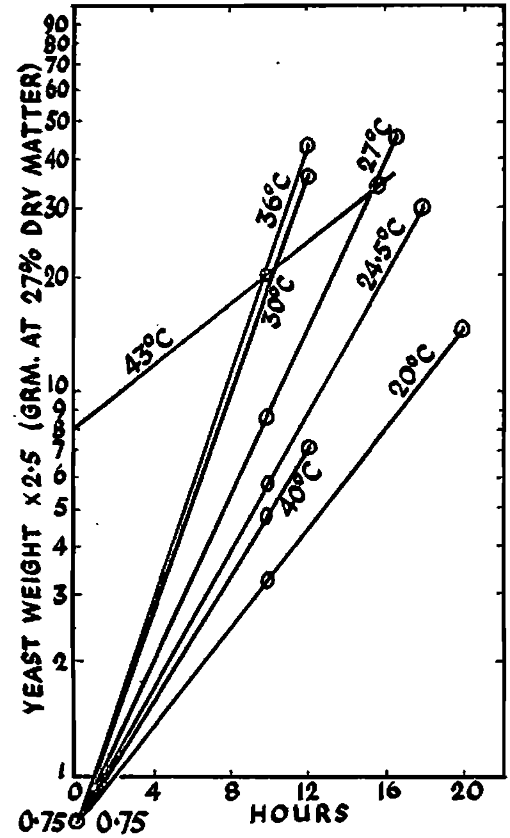

Fig. 1.—Growth of yeast at various temperatures. Yeast weight is plotted on a logarithmic scale.

The results of growth studies, in which bakers' yeast was propagated with vigorous aeration under the nutritional conditions stated below, suggest conclusions which differ considerably from those drawn from brewing studies. Employment of vigorous seed yeast has shown that a lag phase does not exist. Also, the conditions employed never permit the concentrations of ethyl alcohol and carbon dioxide to rise above very small figures, and other by-products of growth are in such small amount (traces only of glycerol and succinic acid) that it is improbable that growth is reduced by inhibitory action. The present studies are carried out in synthetic media and the question of amino acid degradation does not therefore arise. Even in large scale fermentations in a molasses medium, amino acid degradation products are produced only in small amount, and if the yeast is supplied with a sufficiency of assimilable carbohydrates, mineral salts and nutrilite factors, growth takes place in a logarithmic phase only. Typical growth/time curves, as plotted on logarithmic paper, are shown in Fig. 1. In such work it is useful to plot yeast quantity against time on semi-logarithmic-scale paper on which logarithmic growth appears as a straight line. This obviates the need for using tables of logarithms in reading the actual amounts of yeast present at any stage of the fermentation.

Previous work on yeast growth has usually been directed towards studying the rate of yeast growth without reference to both the rate of sugar usage and the rate of fermentation (*i.e.*, production of alcohol and carbon dioxide as opposed to assimilation of sugar to form new yeast). Mathematical treatment is used in the present communication in an attempt to correlate these variables. Two of the chief factors influencing growth and fermentation when yeast is propagated in wort are the concentration of sugar present and the degree of aeration to which the wort is subjected; the effects of these factors will be reported separately in due course. The present paper is confined to a discussion of the influence of temperature on the rates of yeast growth and fermentation in a synthetic wort of defined composition with standardized aeration and sugar concentration. The use of a synthetic medium obviates the necessity for taking into consideration the assimilation of organic non-sugar substances which are utilized by the yeast when molasses or malt worts are employed.

Mathematics of Yeast Growth

Growth coefficient (r).—Under constant conditions of temperature and aeration, and with adequate supplies of nutrients, yeast increases in quantity at a rate which at any given moment is directly proportional to the amount of yeast present at that moment.

If, therefore, A is the weight of yeast present at a given moment, the rate of yeast growth at that moment is rA where r is a constant factor for the particular conditions of growth. The coefficient (r) is expressed as grm. of yeast grown per hr. per grm. of yeast present. Such a system exemplifies the law of organic growth, being an exponential function similar to the law of true compound interest, and the amount of yeast which will be present after a period of time, t, may be calculated by the following fundamental equation:

$$A = A_o.e^{rt} \quad \ldots\ldots \quad (1)$$

Here A_o is the weight of seed yeast, A is the gross weight of yeast produced in the time t, and r is the growth coefficient. The rate of growth of the yeast can be obtained by differentiating the equation, thus:—

$$\frac{dA}{dt} = r.A_o.e^{rt} = rA.$$

The factor r defines the speed at which each normal yeast cell is growing. The influence on this factor of variations in, for example, temperature, is clearly of great interest.

Hourly modulus (H).—Another factor which is of practical importance in yeast studies is the hourly increase factor, H. If H equals 1·2, this means that one grm. of yeast grows to 1·2 grm. in one hr., to $1{\cdot}2^2$ in two hr., and to $1{\cdot}2^t$ in t hr. Therefore,

$$A = A_o.H^t \quad \ldots\ldots \quad (2)$$

Combining this with equation (1) it wil be seen that $H = e^r$, from which it follows that $r = \log_e H$.

Rate of assimilation of sugar.—Once the rate of growth of the yeast has been determined it is possible to calculate the rate at which sugar is being assimilated to form new yeast. It has been shown (White and Munns, this *Journ.*, 1950, 194) that the yeast used in these studies assimilates two-thirds of the carbon from each hexose molecule utilized for growth (as distinct from sugar which is fermented), the other one-third being eliminated as carbon dioxide. The yeast in question was found to contain an average of 12·7% of carbon, all yeast yields having been measured at 27% dry matter. 1 grm. of invert sugar contains 0·4 grm. of carbon and, if only two-thirds of this is used to form yeast it follows that 0·476 grm. of invert sugar are required to supply the 0·127 grm. of carbon in each grm. of yeast. Now, since the formation of 1 grm. of yeast means the assimilation of 0·476 grm. of hexose, a yeast growth rate of r (grm. per hr. per grm. of yeast present) means a sugar assimilation rate of 0·476 r. This factor is most interesting, both as a measure of the fraction of the sugar used which has been assimilated (as distinct from that fermented), and in calculating the important ratio between sugar usage and oxygen supplied.

The weight of sugar (y) assimilated in t hours is 0·476 times the net yield of yeast during this period, *i.e.*,

$$y = 0{\cdot}476\ (A - A_o).$$

$$\text{whence } y = 0{\cdot}476\, A_o\, (e^{rt} - 1) \ldots (3)$$

and the rate of assimilation of sugar at a given moment, t hr. from the start, is given by differentiating thus:—

$$\frac{dy}{dt} = 0{\cdot}476\ A_o.e^{rt}.$$

$$\text{whence } \frac{dy}{dt} = 0{\cdot}476\ rA \quad \ldots\ldots \quad (4)$$

Fermentation coefficient (s).—The other important characteristic of the yeast is the rate at which it is fermenting sugar to alcohol and carbon dioxide under the conditions of experiment. This is defined by the factor (s), which signifies the number of parts of hexose sugar fermented per hr. by each part of yeast. If the amount of yeast present remained constant, A_o grm. of yeast would ferment $sA_o.t$ grm. of sugar in t hr. However, the yeast is growing continuously during this period so that the amount of sugar (x) which is actually fermented in t hr. must be calculated by integration.

$$\frac{dx}{dt} = sA = sA_o.e^{rt};$$

$$\text{whence } x = sA_o \int_o^t e^{rt}.dt,$$

$$\text{whence } x = \frac{s}{r}.\ A_o\, (e^{rt} - 1) \quad \ldots\ldots \quad (5).$$

The experimental determination of s for yeast fermenting under given conditions of temperature is rather more involved than might at first be expected, which, no doubt, accounts for the lack of published figures for fermentation rates of yeasts under various conditions of temperature. The values which can be determined experimentally are the amount of yeast grown in a certain time and the total amount of sugar which has been used up during the experiment. As previously pointed out, some of the sugar has been assimilated and some fermented. Having measured the amount of yeast formed and calculated the growth rate, r, the weight of sugar assimilated (y) is obtained from equation (3). Hence, the amount of sugar fermented (x) can be arrived at, and the fermentation rate (s) calculated from equation (5). The two equations may be combined in the form:—

$$(x + y) = A_o\left(\frac{s}{r} + 0{\cdot}476\right)(e^{rt} - 1) \quad \ldots \; (6)$$

Here $(x + y)$ is the total weight of sugar consumed and, when r has been determined, s can be calculated.

The velocity of sugar consumption is found by differentiating this equation with respect to t.

$$\therefore \frac{d(x+y)}{dt} = rA_o\left(\frac{s}{r} + 0{\cdot}476\right)e^{rt}$$

$$\therefore \frac{d(x+y)}{dt} = rA\left(\frac{s}{r} + 0{\cdot}476\right) \quad \ldots . \; (7)$$

Efficiency (E).—In the production of yeast from sugar, the maximum efficiency is achieved when all the sugar used is assimilated to form new yeast, none being wasted by fermentation. In this case, 100 grm. of sugar yield 100/0·476 grm. of yeast, *i.e.*, 210 grm. of yeast. This represents 100% efficiency. When some fermentation also occurs, the efficiency (E), is determined by the ratio $\frac{s}{r}$, which measures the relative speeds at which sugar is being fermented and assimilated.

$$E = \left(\frac{100\,y}{x + y}\right)$$

Substituting values of y and $(x + y)$ from equations (3) and (6),

$$E = \left(\frac{47{\cdot}6}{\frac{s}{r} + 0{\cdot}476}\right) \quad \ldots\ldots . \; (8)$$

Yield of yeast calculated on the quantity of total sugar used is given %

$$\left(\frac{100\,r}{s + 0{\cdot}476\,r}\right) \quad \ldots\ldots \; (9)$$

Applicability of these equations.—It must be emphasized that the above equations are only strictly applicable to a system in which the yeast growth is truly exponential and the fermentation factor does not vary. If the amount of yeast present is estimated periodically during the course of the experiment and the figures are graphed on semi-logarithmic paper, true exponential growth is indicated by a straight line, the inclination of which is determined by the factor r (see Fig. 1).

Experimental

The aerated fermentations were carried out in litre scale experiments using 50 grm. of sucrose as source of sugar with 6 μgrm. of D-biotin, 500 μgrm. of calcium D-pantothenate and 50 mgrm. of meso-inositol as sources of essential nutrilites. The inorganic salts and other B-group vitamins used in the basal medium were precisely as described for the nutrilite assays (this *Journ.*, 1950,

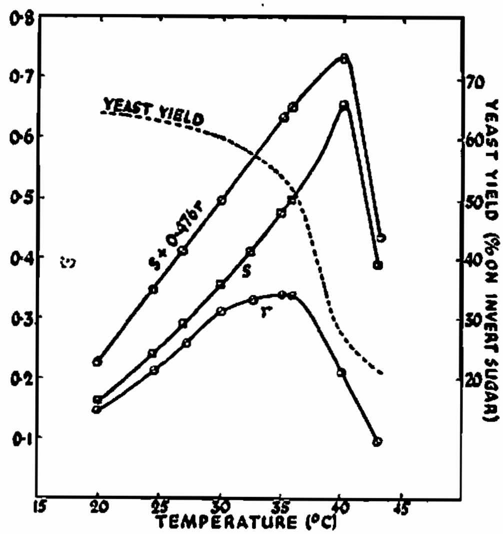

Fig. 2.—Variation of yeast yield, fermentation rate (s), assimilation rate (r), and total rate of sugar consumption (s + 0·476 r) with temperature.

141) and the aeration and general experimental techniques for yeast growth and for assay of the yeast crop produced are as described in that paper. For the present purpose, the growth of yeast after varying periods of time and the amounts of sugar used were carefully determined so that growth curves could be drawn for each temperature examined. Each growth curve shown in Fig. 1 represents several such determinations.

TABLE I

ILLUSTRATING THE CALCULATION OF GROWTH AND FERMENTATION COEFFICIENTS
(Initial sugar concentration = 50 grm. sucrose per litre.) (e = 2·718.)

Temperature °C.	Time (hr.) (t)	Sugar used (grm.) as invert)	Gross yeast (grm. A	Seed yeast (grm.) A_0	Net yeast (grm.) A-A_0	e^{rt} $\left(=\frac{A}{A_0}\right)$	r	s	Yield of yeast (% on invert sugar)	Rate of sugar assimilation $0·476r$	$\frac{100\ r}{s+0·476r}$ (=Yield)	H (=e^r)	Rate of total sugar usage ($s+0·476r$)
30	15	52·5	32·0	0·3	31·7	106·7	0·311	0·367	60·4	0·148	60·4	1·364	0·515
32·5	12	25·1	14·3	0·3	14·0	47·7	0·322	0·424	55·8	0·153	55·8	1·38	0·577
32·5	13	34·8	20·7	0·3	20·4	69·0	0·325	0·401	58·5	0·155	58·5	1·384	0·556
24·5	16	12·7	8·2	0·3	7·9	27·33	0·207	0·236	62·0 .	0·0985	62·0	1·230	0·3345
24·5	20	32·3	20·2	0·3	19·9	67·33	0·210	0·242	61·5	0.0996	61·5	1·234	0·3416
24·5	22·5	48·7	29·8	0·3	29·5	99·33	0·204	0·240	60·5	0·0971	60·5	1·226	0·3371
20	23	13·7	9·4	0·3	9·1	31·33	0·149	0·147	68·5	0·0709	68·5	1·161	0·2179
20	28	29·7	20·2	0·3	19·9	67·33	0·150	0·149	68·1	0·0714	68·1	1·162	0·2204
20	29	37·4	22·8	0·3	22·5	76·0	0·148	0·172	61·0	0·0704	61·0	1·160	0·2424
43	15·5	48·6	13·7	3·2	10·5	4·28	0·0938	0·39	21·6	0·0446	21·6	1·098	0·4346

From the figures obtained the appropriate calculations can be made as in Table I in order to obtain the values of the fermentation coefficient (s) and the growth coefficient (r). The results obtained are illustrated in Fig. 2.

DISCUSSION

Growth coefficient (r).—This has a value of 0·149 at 20° C. (68° F.) and, when the temperature is increased to 30° C. (86° F.), the value rises to 0·311, at which point the curve flattens somewhat to reach a maximum of 0·342 at 36° C. (96·8° F.). At higher temperatures the value of r diminishes rapidly and is only 0·20 at 40° C. (104° F.).

Fermentation coefficient (s).—The value of s rises steeply and steadily up to 40° C. (104° F.) when $s = 0·656$; at higher temperatures the value of s is very rapidly diminished.

Rate of total sugar usage ($s + 0·476\ r$). This increases steeply and in almost linear fashion to a maximum of 0·735 at 40° C. (104° F.); the value falls rapidly at higher temperatures.

Yeast yield on total sugar used.—Yeast yield is, as explained above, a function of the ratio of $\frac{s}{r}$. The yield gradually falls with increasing temperature, the decrease up to 30° C (86° F.) being comparatively small. Yield becomes significantly affected above 30° C. (86° F.), and at temperatures above 36° C., when the assimilation rate itself is falling and fermentation rate increasing, yield falls very rapidly. Yield is only 26·6% at 40° C. (104° F.).

It may be noted that, in the growth experiments at 43° C. (109·4° F.), no growth occurred with the use of a small seeding rate and the values quoted were obtained with a high seed rate (3·2 grm. instead of 0·3 grm.).

SUMMARY

1. The total rate of usage of sugar by the yeast strain employed increases almost linearly between 20° C. (68° F.) and 40° C. (104° F.).

2. Rate of sugar usage by yeast depends on sugar used in the production of new yeast and sugar fermented to alcohol. The assimilation rate, r, is maximal at 36° C. (96·8° F.), but the fermentation rate, s, is maximal at 40° C. (104° F.). It follows that the yeast yield is rapidly decreased between 36° C. and 40° C.

3. Apart from evaporation difficulties, which could be overcome by suitable condensing arrangements, a greater *alcohol yield* efficiency may be expected from fermentations conducted at 36°–40° C. than from fermentations at lower temperatures.