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Iowa State College of Agriculture and
Mechanic Arts

# TEMPERATURE AND THE GROWTH OF YEAST

A Dissertation

Submitted to the Graduate
Faculty in Candidacy for the
Degree of

Doctor of Philosophy

by

Frank F. Sherwood

Approved:

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1924

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Page

I. Introduction ..... 1

II. The Influence of Temperature on Rates of Reaction:

1. Theoretical discussion ..... 3

2. Resume of previous work on rates of reactions. 4

III. The Influence of Temperature Upon the Growth of Yeast.

1. Historical Discussion ..... 14

IV. Statement of Problem ..... 18

V. Determination of the Optimum Medium for Various Temperatures..... 19

1. The Effect of Ammonium Chloride Upon the Growth of Yeast in Wort ..... 30

2. A Second Maximum of Ammonium Chloride for the Growth of Yeast in Medium B ..... 32

3. The Effect of Dipotassium Phosphate Upon the Growth of Yeast in Medium B ..... 35

4. The Effect of Calcium Chloride Upon the Growth of Yeast in Medium B ..... 35

5. The Effect of Calcium Carbonate Upon the Growth of Yeast in Medium B ..... 35

T 1211

Page

VI. Growth Rates and Temperature Coefficients for Growth of Yeast ..... 39

1. Growth Rates of Yeast in Wort Containing Ammonium Chloride ..... 40

2. Growth Rates of Yeast in Medium E ..... 46

3. Growth Rates for Medium E Optimum for 30° C. . 52

VII. Summary ..... 59

# TEMPERATURE AND THE GROWTH OF YEAST

## I. Introduction.

In carrying on investigations dealing with vital phenomena we must keep in mind the fact that we are dealing with very complex systems. If we attempt to analyze living matter by separating its chemical constituents and identifying them we find that we are no longer dealing with living matter because the reagents which we employ immediately and abruptly suspend its characteristic functions. For example, we may make an analysis of yeast and obtain some interesting products but we no longer have yeast cells and the products we obtain, although very interesting in themselves, may bear only a remote relationship to those which were present in the living organism. This method of investigation has been employed by numerous investigators in an attempt to arrive at or determine the nutritional requirements of yeast. That is, they would analyze a sample of yeast and assume that all the constituents found were absolutely necessary for the growth of yeast and consequently an artificial medium should contain all the constituents found. This is, of course, false reasoning as the mere presence of any given element is not an a priori reason for believing that this particular element is absolutely essential for the normal growth and reproduction of yeast.

A second method of study consists in an analysis of the chemical phenomena which accompany or underlie the activities of living, undisturbed and more or less functioning protoplasm.

As Robertson[1] says, "In the so-called 'exact sciences', namely mechanics and physics, we have, as a rule the power to isolate more or less completely any phenomenon or group of phenomena which we wish to study, and to guard them from disturbance by the intrusion of accidental variables. For example, it is not a difficult matter to demonstrate that a falling body experiences a constant acceleration, the most serious intrusive variable being the friction of the air, a variable which can now be very readily excluded in a variety of ways. Similarly, in chemistry, it is not a difficult matter to observe the progress and equilibrium of such a reaction, as, for example, the reduction of iron oxide by hydrogen. However, when we come to organic chemistry we encounter much more complex phenomena and when we take up the study of life processes we find the difficulties which are encountered in studying organic reactions in laboratory glass ware are enormously magnified in studying reactions which occur in living matter."

In making a study of life processes two methods have been employed, first, the influence of temperature upon life processes and, second, the growth rate. Before going fur-

ther it will, perhaps, be well to give a brief summary of the work that has been done regarding the influence of temperature on chemical reactions:

II. The Influence of Temperature on Rates of Reaction.

Due to the fact that the influence of temperature on chemical reactions is so pronounced it has been quite generally recognized as one of the most important factors in the study of chemical changes. Although many interesting facts have been brought to light and many phenomena explained there is still much to be learned concerning the influence of temperature upon chemical reactions.

The velocities of most purely chemical reactions increase rapidly with rise of temperature. For example, the inversion of cane sugar proceeds five times as fast at 55 degrees as it does at 25 degrees; the conversion of solid Ammonium Cyanate into Urea is fifty times as rapid at 57 degrees as it is at 33 degrees; the transformation of dibromosuccinic acid into bromomaleic acid goes three thousand times as rapidly at 101 degrees as at 15 degrees; and although the reaction between hydrogen and oxygen is so slow at 155 degrees that no sign of combination can be detected after many months, yet at about 600 degrees the combination takes place with explosive violence. Dewar, too, has shown that at the temperature of liquid air (-183 degrees) photographic action

is 20 percent; and at the temperature of liquid hydrogen (-250 degrees) it is but 10 percent of its value at ordinary temperature.

The first important work carried out upon reactions in homogenous systems was that of Wilhelmy[2] who measured the rate of the inversion of cane sugar in aqueous solution into dextrose and laevulose under the catylitic action of a small quantity of acid (hydrochloric or nitric). Using a polarimeter, Wilhelmy determined the rate of reaction from the change in the angle of rotation at different time intervals. Wilhelmy made the assumption that the rate at which the inversion took place, is proportional to the quantity of cane sugar still uninverted in the solution. This important assumption of Wilhelmy is the basis of the law of mass action. If the time be expressed by t and the quantity of sugar inverted after any given time be x, the velocity of inversion is $\frac{dx}{dt}$. This quantity $\frac{dx}{dt}$ is, according to Wilhelmy, proportional to the quantity of cane sugar remaining, which may be represented by (a-x), where (a) is the original quantity of cane sugar when t equals 0. Hence, Wilhelmy's equation is $-\frac{dx}{dt} = k(a-x)$, where k is a proportionality factor called the "velocity constant" of the reaction. On intergration one obtains,

$$k = \frac{1}{t} \log \frac{a}{a-x} \text{ or } k_1 = \frac{1}{t} \log_{10} - \frac{a}{a-x} \qquad (1)$$

where $k = 2.303\ k_1$.

Wilhelmy was the first to construct the differential equation quoted above for the calculation of a chemical reaction velocity. Using nitric acid as the catalyst (at 15 degrees) he succeeded in verifying the above experimentally.

In the above equation k is the specific velocity of the reaction, constant, provided we keep the temperature constant. If the temperature changes during the reaction, k is no longer constant, but increases proportionally with the temperature. For example, Harcourt and Esson[3] found that in the reaction -- $H_2O_2$ plus $2HI$ = $I_2$ plus $2H_2O$ k varied with the temperature, as indicated by the following numbers:

When t equals 0, 10, 20, 30, 40, 50,

k equals 1.00, 2.08, 4.32, 8.38, 16, 19, 30, 95.

Various formulae have been proposed, since Wilhelmy proposed his, by Berthelot, Harcourt and Essen, Warder, Urech, Hood, Vant Hoff, and Arrhenius.

In dealing with the influence of temperature on chemical equilibria if we let A and B represent two different reacting substances at the point of equilibrium, the velocities of the opposing reactions are the same, or--

$$k_1 C_A = k_2 C_B; \text{ therefore, } K = \frac{k_1}{k_2} - \frac{C_B}{C_A} \quad (2)$$

Since this relation holds good only when the temperature is constant, Nernst proposes to call K the "reaction isotherm". In his work, Etudes de dynamique chemie, vant Hoff

has deduced the expression--

$$\frac{d \log k_1}{dT} - \frac{d \log k_2}{dT} = \frac{q}{2T^2} \qquad (3)$$

from the mechanical theory of heat for the relation between $k_1$ and $k_2$ and the quantity (q) of heat set free when one gram molecule of A is transformed into B at the absolute temperature T.

"Although this equation, says vant Hoff, does not directly express the relation between the velocity constants of the two inverse reactions and the temperature, yet it does show that this relation must have the form --

$$\frac{d \log K}{dT} = \frac{P}{T^2} \text{ plus } Q \qquad (4)$$

where P and Q are constants." The differential coefficient on the left side of (4) refers to the variation of the value of K with temperature. This law of chemical equilibrium, it will be observed, deals only with the end state of a reaction, and it has nothing to say about the time in which that and state will be attained. Although thermodynamics gives us a relation between the state of equilibrium and the thermal value of a reaction, the time factor finds no place in that expression. P is not necessarily constant because the quantity of heat (q) absorbed or evolved in any reaction changes with the temperature. This change, however, is usually so small that we may often assume that P is really constant throughout a small interval of temperature, but the thermal value of some reactions varies considerably

with temperature. For example, the thermal value of the reaction,

$$H_2 + I_2 = 2HI,$$

at 10 degrees is-- 6100 cal.; at 180 degrees 1883 cal., and at 520 degrees 4444 cal.

P is really a function of temperature and for this reason van Hoff's solution of the problem is still indefinite, owing to the lack of any information as to the form of the function--

$$q = f(T) \text{ ---------- } (5)$$

Whenever we meet with a case like this it is the usual thing to write--

$$f(T) = A + BT + CT^2 + DT^3 + \text{ -- } (6)$$

where A, B, C, -- are constants, because we know that whenever a physical change is represented by such an expression we can generally approximate very close to the numerical value of (q) by increasing the number of terms included in the calculation.

Practically all the empirical formulae which have been proposed by different investigators to represent the unknown relation between the temperature and the velocity of a chemical reaction can be referred to the formula--

$$\frac{d \log K}{dT} = \frac{A + BT + CT^2}{T^2} \text{ ---- } (7)$$

which on intergration assumes the form--

$$\log.K = \frac{A}{T} + B \log. T + CT + \text{---} + \text{constant} \quad (8)$$

where A, B, and C are constants.

The first three terms of the series (6) were employed by C. Hahn[5] to represent the influence of temperature upon the reaction between hydrogen and carbon dioxide at high temperatures; and by M. Bodenstein[6] to represent the influence of temperature on K in the reaction--

$$H_2 + I_2 = 2HI$$

D. M. Kooij omitted all terms succeding the second, and thus obtained the expression--

$$\frac{d \log.K}{d T} = \frac{A + BT}{T}; \text{ or, } \log K = \frac{A}{T} + B \log T + \text{a constant} \quad (9)$$

for the influence of temperature on the decomposition of phosphine and arsine.

Arrhenius[8] only retained the first term of the series (7) and employed the equation--

$$\frac{d \log K}{d T} = \frac{A}{T^2}; \text{ or, } \log k_1 - \log k_0 = A\left(\frac{1}{T_1} - \frac{1}{T_0}\right) \quad (10)$$

i.e., $k = k_0 e^{\frac{a}{2}\left(\frac{T_0 - T_1}{T_1 T_0}\right)}$

The above equation has given fairly satisfactory results with a large number of measurements to which it has been applied. Arrhenius himself compared it with measurements by Hood[9], Warder[10], Schwab and Hecht[11] and Conrad, as well as with the experiments of Urach[12] and of Spohr[13] on the inversion of cane sugar. Price[14] employed it in his work on the hydrolysis of the esters; I. Remsen[15] and E. E. Reid for the hydrolysis of

nitrobenzamide by the bases; J. H. Kastle[16] and A. S. Loevenhart for the oxidation of formaldehyde by hydrogen peroxide; Goldschmidt[17] and Reinders for the conversion of diazo amido into amido diazo compounds; Ley[18] also obtained very fair results with the transformation of anisynaldoxime acetate into the corresponding nitrile, and for the intramolecular transformation of the acetates of anisynaldoxime, p-chlorbenzsynaldoxime, and thiophensynaldoxime.

Harcourt and Essen[18], in their study of the reaction--

$$H_2O_2 + 2HI = I_2 + 2H_2O,$$

sought the form of the function (T). They found that if temperature be designated on the absolute scale, and $k_0$ be the value of k at $T_0$, and k the value of the velocity at some other temperature, T, then--

$$\frac{k}{k_0} = \frac{(T)B}{(T_0)}, \qquad (11)$$

where B is a constant to be evaluated from the experimental data.

Harcourt and Esson used the equation--

$$\frac{d \log K}{d T} = \frac{B}{T}, \qquad (12)$$

which is a special case of the fundamental equation (7) which on intergration, assumes the form indicated in equation (11).

van t Hoff's equation--

$$\frac{d \log K}{d T} = \frac{A}{T} + C \qquad (13)$$

or, integrated--

$$\log K = \frac{A}{T} + CT + \text{a constant} \qquad (14)$$

is a special case of the fundamental equation obtained by putting B = 0. Schwab[19] applied this equation to the transformation of dibromosuccinic acid into bromo maleic acid, and to the reaction between sodium mon chloracetate and sodium hydroxide; J. Spohr[20] to the inversion of cane sugar; and G. Buchbock used it to represent his results on the influence of temperature on the decomposition of carbonyl sulphide by water.

If we neglect the first two terms of the series in the original equation we get--

$$\frac{d \log K}{d T} = C, \qquad (15)$$

which on integration becomes--

$$\log K = CT + \text{a constant.}$$

If natural logerithms are employed this may be written--

$$k = k_0 e^{CT} \qquad (16).$$

The above formula was employed by Pendlebury[22] and Seward in their study of the interaction of hydrogen iodide; by Tammann[23] to represent the velocity of crystallization at different temperatures; by Reid[24] to represent the hydrolysis of nitrobenzamide; and by Veley[25] for the reaction between nitric acid and copper.

If common logarithms be employed, expression (16) becomes—

$$k = k_0 \times 10^{CT}, \qquad (17)$$

which was used by Bugarszky to represent the influence of temperature on the reaction between bromine and ethyl alcohol; and by Hecht[27] and Conrad in their work on the action of alkyl iodides on sodium alkylates.

If we take the logarithms to some other base, say a, we get--

$$k = k_0 \; a^{CT} \qquad (18).$$

The above equation in a modified form was used by Berthelot in 1862, for the action of acetic acid upon ethyl alcohol; by Spring[29] for the dissolution of marble in mineral acids and by Hood[30] for the rate of oxidation of ferrous sulphate by potassium chromate.

Arrhenius maintains the view that the increase of velocity of a chemical reaction with temperature cannot be explained by any change in the physical properties of the solution with temperature. He proposes the hypothesis that cane sugar contains two kinds of molecules--active and passive. The active can alone be hydrolyzed by the acid, while the passive molecules are not acted upon. The amount of "active" cane sugar in solution is supposed to be very small in comparison with the "inactive" sugar. In order to explain the influence of temperature on the rate of inversion, Arrhenius still further assumes that the quantity of active cane sugar must increase very rapidly,--about 12 percent for each degree rise of temperature--and this at the cost of the inactive sugar. The transformation of inactive into active sugar is said to be due either to a rearrangement of the atoms or to the introduction of water into the molecule of inactive sugar. A state of equilibrium between the active and inactive molecules of cane sugar will be attained when the respective concentrations $C_a$ =

$C_1$; and from van't Hoff's equation (3).

$$\frac{d \log K}{d T} = \frac{q}{2T^2}; \quad K_1 = K_0 e^{\frac{q}{2}\frac{(T_1 - T_0)}{(T_1 \quad T_0)}}, \qquad (19)$$

where q denotes the thermal value of the transformation of inactive into active cane sugar, and $K_1$ and $K_0$ are the equilibrium constants at the two different temperatures.

If the velocities of the reactions at the two temperatures be $V_1$ and $V_0$, then it is supposed that--

$V_1 = V_0 e^{\frac{q(T_1 - T_0)}{2(T_1 T_0)}}$ ; or q is about 25,600 calories per gram molecule of inactive sugar.

Robertson says that, "the behavior of physical phenomena which are affected by the temperature, is quite different. That is, the effect of temperature is in these phenomena qualitatively much less than it is in phenomena which arise from chemical transformations, and for this reason we may, with a fair degree of confidence, employ the temperature coefficient of a complex phenomenon which involves physical as well as chemical changes as a means of gauging the extent to which the velocity of the process is governed by the chemical transformations which it involves. If the pace is set by the rate at which some chemical change transpires, then the rapidity of the process will be at least doubled and not improbably, more than doubled by a rise of 10 degrees in temperature. But if the chemical transformations are subordinate to some physical process and must await its development before they can proceed, or if they are simply consequent upon physical changes

the pace of the whole process will be set by this physical event and the temperature coefficient of the process may be expected to be less than two or even very considerably less than two."

Bayliss[32] does not agree with the above statements and believes that caution must be exercised in our conclusions as to whether a process is of a physical or chemical nature on the basis of observed temperature coefficients. His views are supported by the fact that a goodly number of chemical reactions exhibit a low value for temperature coefficients while some are even retarded by a rise in temperature.

For example, Benson[33] found that the rate of reaction in the case of the liberation of iodine from a mixture of KI, $FeSO_4$ and $CrO_3$ is less at 30 degrees than at 0 degrees while the rate of reduction of ferric sulphate by iron in acid solution appears to decrease with increase of temperature.

Trautz and Volkmann[34] found that the saponification of ethyl butyrate by barium hydroxide between 50 and 60 degrees has the low value for a chemical reaction of 1.33 for ten degrees whereas diffusion, a physical process, has a value nearly as high, viz., 1.28. Chick[35] and Martin find that the heat coagulation of haemoglobin has the extraordinarily high temperature coefficient of 13.8 for ten degrees, while that of albumin is even higher.

P. von Schroeder[36] finds that gelatine solutions, in a particular condition, have a viscosity at 21 degrees repre-

sented by 13.76, whereas at 31 degrees it is only 1.42. This is interesting due to the fact that colloids of the type of gelatine play a large part in vital processes.

We find that even in a simple uncomplicated chemical transformation the temperature coefficient for ten degrees temperature intervall is not constant, for, reverting to the equation--

$$\frac{K_1}{K_0} = e^{\frac{q}{2}\frac{(T_1 - T_0)}{(T_1 \quad T_0)}} \qquad (20)$$

we see that the temperature coefficient for 10 degrees is given by-- $\frac{K_1}{K_0} = e^{\frac{q}{2}\left(\frac{10}{T_1 T_0}\right)}$ it is therefore not independent of the temperature employed; in fact the temperature coefficient must invariably decrease as the temperature rises. Assuming a value of q = (13,200) which would yield a coefficient of 2 between the temperatures of 30 and 40 degrees, the following table shows the coefficients which might be anticipated at other temperatures:

| Temperature Interval. | Temperature Coefficient. |
|---|---|
| 0---------10 degrees | 2.34 |
| 10--------20 " | 2.22 |
| 20--------30 " | 2.11 |
| 30--------40 " | 2.00 |
| 40--------50 " | 1.92 |

III. The Influence of Temperature Upon the Growth of Yeast.

Temperature coefficients for yeast were determined by

Aberson[37] working between 12 and 33 degrees giving a mean temperature quotient for 10 degrees ($K_t \div 10/K_t$) = 2.72. Herzog[38] gives values of velocities from 14.5 to 28.5 degrees, with $K_{24.5}/K_{14.5}$ = 2.88. Slator[39] confirmed these results, but showed that the temperature quotient varied with the temperature. He obtained his temperature quotients by growing yeast in a closed vessel connected with a manometer and took the time required to raise the column of mercury 4 mm. at temperatures ranging from 5 to 40 degrees. The results of his experiments are shown in the following table:

| Temp. | Observed ratio | Percentage correction | $V_{t+5}/V_t$ | $V_{t+10}/V_t$ |
|---|---|---|---|---|
| 5 | | | | |
| 10 | 2.94 | 10 | 2.65 | 5.6 |
| 15 | 2.29 | 8 | 2.11 | 3.8 |
| 20 | 1.89 | 5 | 1.80 | 2.80 |
| 25 | 1.65 | 5 | 1.57 | 2.25 |
| 30 | 1.50 | 5 | 1.43 | 1.95 |
| 35 | 1.42 | 5 | 1.35 | 1.60 |
| 40 | 1.27 | 5 | 1.20 | |

Slator states that below five degrees the reaction proceeded too slowly to be measured while above forty degrees the enzyme was destroyed. However his experiments were carried out in a synthetic medium apparently made up on the basis of a chemical analysis of yeast and therefore probably not the best medium obtainable for his purpose. Furthermore, he did not consider the relation of temperature to the composition

of the medium. Since it has been shown, by Fulmer, Nelson and Sherwood[40], that there is an optimum concentration of the salts used that varies with the temperature, it is quite probable that he would have obtained entirely different results had he varied the composition of his medium with the temperature.

In a study of the growth rate of yeast A. Slator[41], in 1913, used four methods of estimating K. The validity of these equations was tested by measuring the rate of growth of a pure culture of a Burton yeast in highly hopped wort of specific gravity 1.040. It was found that K, the constant of unrestricted growth of this culture, could be determined by methods of yeast counting and also by measuring the rate of fermentation of the growing yeast cells. Slator also showed that until the yeast crop reached about ten million cells per cubic centimeter (a count of 40) the rate of increase at any moment is proportional to the number of cells then present; whence $k = \frac{\log C}{t}$.

About the same time T. Carlson[42] proposed a more complicated relation between C and t from the assumption that, besides being proportional to the number of cells present, the rate is also proportional to the square of the concentration of the foodstuff remaining available in the wort.

Clark[43] showed that under the conditions specified in his work, that the rate of reproduction is independent of the concentration of alcohol until this reaches 1.8 grams per 100 c.c.

In 1914 Horace T. Brown[44] determined the rate of reproduction of yeast when grown in mixtures of air-saturated wort, with

wort from which all air had been removed by boiling in an atmosphere of hydrogen.

Fulmer[45] measured the rate of reproduction of yeast in wort with and without ammonium fluoride and showed that the cells which are not killed outright undergo a period of paralysis, after which they reproduce, giving rise to fluoride-resistent cells.

As stated above in no case where thermal coefficients of growth rates have been determined have the investigators taken into consideration the composition of their medium. Reaction rates in solution are usually appreciably influenced by the presence of neutral salts which apparently have nothing to do with the reaction itself. In many cases this "neutral salt effect", as it is called, may be interpreted as due, at least partially, to the influence of the ions of the salt upon the thermodynamic environment prevailing within the solution, for the rate of any given reaction is very powerfully influenced by the nature of the medium in which it takes place.

In determining growth rates or thermal coefficients it is therefore essential that the experiments be conducted as far as possible under comparable conditions as regards thermodynamic environment, since the logarithmic rate of reproduction may be influenced by changes in the reaction of the medium, in surface tension, in osmotic pressure, in pressure, in temperature, in permeability and in the state of hydration of cell proteins.

In a previous communication, Fulmer[46], Nelson and Sherwood made a study, among other things, of the effect of ammonium salts upon the growth of yeast in synthetic media. The findings of the above authors may be summarized as follows:

1. There is an optimum concentration of ammonium salts for the growth of yeast at a given temperature.
2. For five ammonium salts tested the optimum concentration for the growth of yeast was found to be at the same normality, that is, the effect is due to the ammonium ion.
3. The higher the temperature the greater the concentration of ammonium salt required for optimum reproduction.
4. At all temperatures studied (21, 30, 35, and 40 degrees) the concentration of ammonium salt for the optimum growth of yeast was found to coincide with the concentration of the salt in which a protein, wheat gluten, was the least swollen.

## IV. Statement of Problem.

The object of the work presented by the author is to determine growth rates and temperature coefficients by adhering to the following:

1. Determine the best possible medium for each desired temperature.
2. After determining the best medium for each temperature, determine the growth rates and from these values calculate the temperature coefficients for the various time intervals.

V. Determination of the Optimum Medium for Various Temperatures.

Method: The method employed was identical with the method outlined in the preceding paper[49] except in the case of the determination of growth rates the flasks were gently rocked in a small rocker. James O'Sullivan[50] shows that agitation is absolutely necessary in making a quantitative study of the growth rates of yeast. Clark[43] also used this method in his work.

It was found that there is an optimum concentration of ammonium chloride for the growth of yeast at the following temperatures: 0, 10, 20, 25, 30, 35, 40, 42 and 43 degrees.

The results of the above investigations are shown for the different temperatures in Tables 1 to 9 inclusive and summarized in Table 10.

It was found that the concentration of ammonium salt for the optimum growth of yeast coincides with the concentration of the salt in which gluten is the least swollen[40]. It will be noticed that the best growths were obtained at 40 degrees. At 42 degrees the yeast were growing quite well while at 43 degrees there is a rather sharp break, the total crop for medium E being a count of 3.4 and for wort 3.8 at the end of 68 hours, as compared with 186 for medium E and 630 for wort at 42° C.

TABLE I.

Growth of Yeast at 0° C. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | I = 1 Count 24 hrs. | I = 1 Count 48 hrs. | I = 1 Count 98 hrs. |
|---|---|---|---|
| 0.000 | 2.3 | 2.2 | 2.1 |
| 0.0118 | 2.6* | 3.6* | 3.8* |
| 0.01475 | 1.8 | 2.4 | 2.3 |
| 0.02360 | 1.3 | 1.1 | 1.1 |
| 0.0295 | 1.2 | .6 | .9 |
| 0.0354 | 1.0 | .5 | .8 |
| 0.03835 | 1.0 | | |
| 0.0413 | .5 | | |
| 0.0472 | .4 | | |
| 0.0590 | .4 | | |

TABLE II.

Growth of Yeast at 10° C. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | I = 1 Count 37 hrs. | I = 1 Count 45 hrs. |
|---|---|---|
| 0.000 | 11.3 | 15 |
| 0.0118 | 19.6 | 21 |
| 0.02065 | 24.0 | 28 |
| 0.02360 | 31.50 | 39 |
| 0.02655 | 27.0 | 29.5 |
| 0.0295 | 19.0 | 22 |
| 0.0354 | 13.5 | 17 |
| 0.0413 | 10 | 12 |

TABLE III.

Growth of Yeast at 20° C. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | I = 1 Count 24 hr. | I = 1 Count 30 hr. | I = 1 Count 30 hr. | I = 1 Count 36 hr. | I = 1 Count 24 hr. | I = 1 Count 46 hr. | I = 1 Count 46 hr. |
|---|---|---|---|---|---|---|---|
| 0.000 | 43 | 26 | 45 | 45 | 54 | 67 | 89 |
| 0.0118 | 47 | 58 | 87 | 103 | 96 | 113 | 120 |
| 0.0236 | 53 | 92 | 103 | 109 | 108 | 166 | 143 |
| 0.0295 | 55 | 101 | 158 | 158 | 150 | 224 | 168 |
| 0.03009 | | 39 | 77 | 83 | 47 | 101 | 104 |
| 0.03068 | | 31 | 65 | 53 | 45 | 84 | 73 |
| 0.03127 | | 30 | 66 | 42 | 47 | 66 | 73 |
| 0.03186 | | 27 | | 43 | | 69 | 76 |
| 0.0324 | 50 | | 61 | 40 | 46 | 67 | 73 |
| 0.0354 | 32 | | | 38 | | 66 | |
| 0.03835 | 30 | 28 | 42 | 39 | 49 | | 76 |
| 0.0472 | 27 | 28 | 42 | 43 | 49 | 68 | 73 |
| 0.0590 | 22 | | | | | | |

TABLE IV.

Growth of Yeast at 25° C. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | I = 1 Count 24 hr. | I = 1 Count 36 hr. |
|---|---|---|
| 0.000 | 30 | 45 |
| 0.0118 | 40 | 56 |
| 0.02360 | 48 | 98 |
| 0.0295 | 78 | 103 |
| 0.03068 | 96 | 109 |
| 0.03186 | 122 | 156 |
| 0.0324 | 80 | 89 |
| 0.0354 | 60 | 69 |
| 0.0413 | 49 | 56 |
| 0.0472 | 45 | 49 |

TABLE V.

Growth of Yeast at 30° C. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | I = 0.5 Count 48 hr. | I = 0.5 Count 83 hr. | I = 1 Count 24 hr. | I = 1 Count 48 hr. |
|---|---|---|---|---|
| 0.000 | 53 | 46 | 56 | 80 |
| 0.0118 | 194 | 242 | 102 | 212 |
| 0.02360 | 256 | 280 | 115 | 260 |
| 0.02655 | 274 | 294 | 117 | 273 |
| 0.0295 | 295 | 312 | 138 | 280 |
| 0.0324 | 306 | 313 | 156 | 296 |
| 0.3422 | 356 | 335 | 174 | 311 |
| 0.0354 | 389 | 369 | 184 | 354 |
| 0.03658 | 270 | 283 | 120 | 276 |
| 0.03835 | 220 | 229 | 96 | 201 |
| 0.0413 | 213 | 226 | 85 | 161 |
| 0.0472 | 190 | 182 | 74 | 144 |
| 0.0590 | 173 | 177 | 67 | 140 |

TABLE VI.

Growth of Yeast at 35° C. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | 1 = 1 Count 72 hr. | I = 1 Count 24 hr. |
|---|---|---|
| 0.000 | 11.7 | 57 |
| 0.0118 | 99 | 108 |
| 0.02360 | 188 | 144 |
| 0.0295 | 201 | 196 |
| 0.0324 | 243 | 225 |
| 0.0354 | 277 | 257 |
| 0.03835 | 314 | 273 |
| 0.0413 | 186 | 202 |
| 0.0472 | 177 | 197 |
| 0.059 | 168 | 143 |

TABLE VII.

Growth of Yeast at 40° C. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | I = 1 Count 72 hr. | I = 1 Count 44 hr. | I = 1 Count 72 hr. | I = 1 Count 77 hr. |
|---|---|---|---|---|
| 0.000 | 11.5 | 57 | 59 | 62 |
| 0.0118 | 98 | 204 | 122 | 146 |
| 0.02360 | 190 | | 146 | 213 |
| 0.02655 | | | | |
| 0.0295 | 206 | 222 | 199 | 236 |
| 0.0324 | | | 242 | 260 |
| 0.03422 | | | | |
| 0.0354 | 244 | 237 | | |
| 0.03658 | | | 303 | 291 |
| 0.03835 | 280 | 276 | 363 | 310 |
| 0.0413 | 318 | 288 | 378 | 388 |
| 0.0472 | 174 | 216 | 251 | 164 |
| 0.059 | 160 | 198 | 156 | 142 |

TABLE VIII.

Growth of Yeast at 42°. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | 1 = 1 Count 24 hr. | 1 = 1 Count 48 hr. | 1 = 1 Count 30 hr. |
|---|---|---|---|
| 0.000 | 30 | 75 | 18 |
| 0.02360 | 59 | 123 | 35 |
| 0.0295 | 76 | 149 | 47 |
| 0.0354 | 135 | 173 | 63 |
| 0.03835 | 196 | 229 | 77 |
| 0.0413 | 239 | 282 | 99 |
| 0.04248 | 279 | 342 | 139 |
| 0.04366 | 206 | 289 | 156 |
| 0.0472 | 159 | 240 | 145 |
| 0.0590 | 143 | 186 | 139 |

TABLE IX.

Growth of Yeast at 43°. Varying $NH_4Cl$.

| Normality of $NH_4Cl$ | I = 1 Count 24 hr. | I = 1 Count 34 hr. | I = 1 Count 45 hr. |
|---|---|---|---|
| 0.0000 | 1.00 | 1.1 | 1.2 |
| 0.03835 | 2.5 | 2.7 | 2.9 |
| 0.0413 | 2.6 | 2.8 | 3.00 |
| 0.0424 | 2.7 | 3.00 | 3.1 |
| 0.04307 | 3.1 | 3.2 | 3.3 |
| 0.04366 | 2.8 | 2.9 | 3.1 |
| 0.04425 | 2.7 | 2.8 | 3.0 |
| 0.0472 | 2.6 | 2.6 | 3.1 |

TABLE X.

Summary of the Effect of Ammonium Chloride Upon the Growth of Yeast in Medium E.

| Temp. | Optimum $NH_4Cl$ | Time | Maximum Growth | Time | Maximum Growth | Time | Max. Growth | Time | Max. Growth |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.0118 | 24hr. | 2.6 | 48hr. | 3.6 | 96hr. | 3.8 | | |
| 10 | 0.02360 | 37 | 31.5 | 45 | 39 | | | | |
| 20 | 0.0295 | 24 | 55 | 30 | 101 | 36 | 158 | 46 | 224 |
| 25 | 0.03186 | 24 | 122 | 36 | 156 | | | | |
| 30 | 0.0354 | 48 | 389 | 24 | 184 | 48 | 354 | | |
| 35 | 0.03835 | 72 | 314 | 24 | 273 | | | | |
| 40 | 0.0413 | 72 | 318 | 44 | 288 | 72 | 378 | 77 | 388 |
| 42 | 0.04248 | 24 | 279 | 48 | 342 | 30 | 139 | | |
| 43 | 0.04307 | 45 | 3.3 | | | | | | |

It will also be noticed that yeast grow at 0 degrees although rather slowly. The results of the above investigations show that yeast will grow over a rather wide range of temperature if the proper environment is provided for them.

### 1. The Effect of Ammonium Chloride upon the Growth of Yeast in Wort.

During the course of the above investigations it occured that it might be well to make a similar study of the effect of ammonium chloride upon the growth of yeast in wort, which is commonly considered to be the best known medium for the growth of yeast.

A few preliminary experiments seemed to indicate that there were two maximal concentrations of ammonium chloride for the growth of yeast in wort. The influence of ammonium chloride upon the growth of yeast in wort was studied for 10, 20, 30, 40 and 42 degrees and in each case it was found that there are two maximal concentrations for the growth of yeast in wort. The results are shown in Table 11.

TABLE XI.

The Effect of Ammonium Chloride upon the Growth of Yeast in Wort.

| Temperature | 10 | | 20 | | 30 | | 40 | | 42 |
|---|---|---|---|---|---|---|---|---|---|
| Normality of $NH_4Cl$ | Count 36 hr. | Normality of $NH_4Cl$ | Count 30 hr. | Normality of $NH_4Cl$ | Count 22 hr. | Normality of $NH_4Cl$ | Count 42.5 hr. | Normality of $NH_4Cl$ | Count 25 hr. |
| 0.000 | 185 | 0.000 | 210 | 0.000 | 230 | 0.000 | 680 | 0.000 | 29 |
| 0.0059 | 210 | 0.0118 | 240 | 0.0118 | 270 | 0.0236 | 795 | 0.0118 | 63 |
| 0.0088 | 222 | 0.01475 | 253 | 0.0236 | 352 | 0.0259 | 825 | 0.0236 | 94 |
| 0.0118 | 235 | 0.0177 | 265 | 0.0294 | 232 | 0.02714 | 843 | 0.0294 | 157 |
| 0.0129 | 248 | 0.0182 | 273 | 0.0341 | | 0.0283 | 900 | 0.0353 | 83 |
| 0.0135 | 260 | 0.0188 | 340 | 0.0353 | 242 | 0.0295 | 720 | 0.0482 | 86 |
| 0.0141 | 321 | 0.0194 | 258 | 0.0470 | 245 | 0.0354 | 692 | 0.0470 | 83 |
| 0.0147 | 245 | 0.0200 | 245 | 0.0588 | 244 | 0.0590 | 700 | 0.0588 | 84 |
| 0.0177 | 239 | 0.0236 | 233 | 0.0820 | 246 | 0.0826 | 713 | 0.0700 | 77 |
| 0.0354 | 226 | 0.0354 | 225 | 0.106 | 281 | 0.1060 | 733 | 0.0820 | 72 |
| 0.0531 | 240 | 0.0590 | 220 | 0.118 | 235 | 0.1180 | 772 | 0.0940 | 81 |
| 0.0590 | 256 | 0.0708 | 239 | 0.129 | 233 | 0.1416 | 685 | 0.1230 | 142 |
| 0.0649 | 269 | 0.0767 | 242 | | | | | 0.140 | 111 |
| 0.0708 | 245 | 0.0826 | 253 | | | | | 0.153 | 84 |
| 0.0826 | 232 | 0.0826 | 261 | | | | | | |
| | | 0.0885 | 241 | | | | | | |
| | | 0.0896 | 236 | | | | | | |

A study of Table 11 brings out the following points:

1. There are two maximal concentrations, as in the synthetic medium, of ammonium chloride which vary with temperature, for the growth of yeast.
2. Less ammonium chloride is required for the growth of yeast in wort than in a synthetic medium, showing that there is present in the wort something which plays a similar role to that of ammonium ion.

### 2. A Second Maximum of Ammonium Chloride for the Growth of Yeast in Medium E.

In view of the above findings it was thought advisable to try higher concentrations of ammonium chloride in medium E, to ascertain whether or not there was a second maxima of ammonium chloride for the growth of yeast in this medium. Higher concentrations of ammonium chloride were tried at 10, 20, 30, and 40 degrees. In every case there was found a second maximum. The results are shown in Table 12. It was also found that the concentration of ammonium chloride for the second maxima corresponds to a second minimum point of hydration of gluten.

F. Boas[47], working with a bottom yeast in a 15% dextrose solution and various concentrations of ammonium sulphate, found a zone of maximum carbon dioxide production in salt concentrations between 0.5 and 0.05N. He also found two minima regions from 1.5 to 1.0N and from 0.016N down.

Pringsheim[48] carried out a series of experiments to determine the influence of the concentration of nitrogenous food on fermentation, with three races of yeast, "Lagor" yeast, "Frohberg" yeast, and a wine yeast. Peptone, synthetic leucine, leucine from molasses, asparagine and ammonium sulfate were used as sources of nitrogen. With synthetic leucine, asparagine and ammonium sulfate a well marked optimum concentration was found with two minima regions. Pringsheim explains these phenomena on the basis of the action of the different nitrogen concentrations on the plasma membrane at times causing a thickening and slow intake of sugar and consequently a slower fermentation, at times a loosening, resulting in the reverse action.

TABLE XII.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Medium E -- Second Maximum.

| Temperature | 10 | | 20 | | 30 | | 40 |
|---|---|---|---|---|---|---|---|
| Normality of $NH_4Cl$ | Count 30 hr. | Normality of $NH_4Cl$ | Count 35 hr. | Normality of $NH_4Cl$ | Count 28 hr. | Normality of $NH_4Cl$ | Count 30 hr. |
| 0.0000 | 48 | 0.000 | 58 | 0.000 | 61 | 0.000 | 67 |
| 0.059 | 53 | 0.059 | 65 | 0.118 | 75 | 0.118 | 76 |
| 0.118 | 61 | 0.118 | 71 | 0.177 | 84 | 0.177 | 84 |
| 0.1298 | 72 | 0.1475 | 80 | 0.2265 | 89 | 0.2265 | 91 |
| 0.1475 | 80 | 0.177 | 86 | 0.236 | 105 | 0.236 | 94 |
| 0.1534 | 92 | 0.182 | 89 | 0.295 | 84 | 0.265 | 97 |
| 0.1593 | 79 | 0.194 | 100 | 0.354 | 76 | 0.278 | 111 |
| 0.1652 | 60 | 0.206 | 88 | 0.472 | 63 | 0.277 | 79 |
| 0.177 | 55 | 0.236 | 65 | 1.118 | 58 | 0.295 | 72 |
| 0.236 | 40 | 0.354 | 56 | 1.483 | 50 | 0.354 | 65 |
| 0.2950 | 38 | | | | | 0.4720 | 59 |

### 3. The Effect of $K_2HPO_4$ Upon the Growth of Yeast in Medium E.

The effect of various concentrations of dipotassium phosphate upon the growth of yeast was tried at 25°, 30°, 35°, and 40° C. It was found that the addition of dipotassium to the medium increased the growth of yeast with increase of the salt up to a concentration of 0.00464 normal. Further addition showed no improvement. The results of these determinations are shown in Table 13.

### 4. The Effect of Calcium Chloride Upon the Growth of Yeast in Medium E.

The effect of various concentrations of calcium chloride upon the growth of yeast was tried at 25°, 30°, 35°, and 40° C. It was found that the addition of calcium chloride showed an improvement in the medium up to a concentration of 0.1800 normal. The addition of larger quantities showed no improvement over 0.18 or normal. The results are tabulated in Table 14.

### 5. The Effect of Calcium Carbonate Upon the Growth of Yeast in Medium E.

Various concentrations of calcium carbonate were tried in medium E at 25°, 30°, 35°, and 40° C. It was found that the addition of calcium carbonate showed an improvement of the medium with increase of calcium carbonate up to a concentration of 0.0160 normal. Further additions showed no improvement over 0.016 normal. The results are tabulated in Table 15.

TABLE XIII.

Growth of Yeast, Varying $K_2HPO_4$.

| Normality of $K_2HPO_4$ | 25° C. I=1 Count 30 hr. | 25° C. I=1 | 30° C. I=1 Count 66 hr. | 30° C. I=1 Count 42 hr. | 35° C. I=1 Count 24 hr. | 35° C. I=1 | 40° C. I=1 Count 24 hr. | 40° C. I=1 Count 72 hr. |
|---|---|---|---|---|---|---|---|---|
| 0.0000 | 180 | | 93 | 60 | 27 | | 26 | 91 |
| 0.00232 | 222 | | 227 | 162 | 172 | | 98 | 185 |
| 0.003480 | 246 | | 280 | 165 | 181 | | 126 | 241 |
| 0.00464 | 261 | | 305 | 205 | 190 | | 159 | 269 |
| 0.00696 | 260 | | 302 | 204 | 189 | | 158 | 267 |
| 0.00928 | 259 | | 304 | 203 | 187 | | 157 | 268 |
| 0.01160 | 261 | | 305 | 202 | 188 | | 158 | 269 |
| 0.01392 | 262 | | 302 | 203 | 187 | | 159 | 267 |

TABLE XIV.

Growth of Yeast, Varying $CaCl_2$.

| Normality of $CaCl_2$ | 25° C. I = 1 Count 24 hr | 25° C. I = 1 Count 48 hr | 30° C. I = 1 Count 72 hr | 30° C. I = 1 Count 48 hr | 35° C. I = 1 Count 42 hr | 35° C. I = 1 Count 98 hr | 40° C. I = 1 Count 24 hr | 40° C. I = 1 Count 48 hr |
|---|---|---|---|---|---|---|---|---|
| 0.000 | 61 | 92 | 217 | 186 | 157 | 202 | 160 | 210 |
| 0.0180 | 73 | 106 | 235 | 198 | | | | |
| 0.0360 | 91 | 117 | 247 | 202 | 161 | 257 | 209 | 269 |
| 0.0540 | 105 | 129 | 289 | 246 | | | | |
| 0.0720 | 130 | 136 | 295 | 266 | 177 | 260 | 212 | 272 |
| 0.0900 | 140 | 156 | 304 | 290 | | | | |
| 0.108 | 158 | 173 | 312 | 298 | 191 | 274 | 226 | 285 |
| 0.126 | 219 | 196 | 310 | 310 | | | | |
| 0.1440 | 256 | 218 | 318 | 318 | 211 | 278 | 245 | 299 |
| 0.1620 | 286 | 278 | 335 | 342 | 232 | 286 | 251 | |
| 0.1800 | 379 | 334 | 386 | 388 | 255 | 319 | 275 | 325 |
| 0.216 | 375 | 333 | 387 | 388 | 253 | 318 | 276 | 328 |
| 0.270 | 378 | 335 | 388 | 389 | 254 | 315 | 274 | 326 |
| 0.324 | 376 | 335 | 387 | 389 | 255 | 318 | 275 | 324 |
| 0.3600 | | | | | 254 | 317 | 276 | |

TABLE XV.

Growth of Yeast, Varying $CaCO_3$.

| Normality of $CaCO_3$ | 25° C. I = 1 Count 32 hr. | 30° C. I = 1 Count 48 hr. | 35° C. I = 1 Count 36 hr. | 40° C. I = 1 Count 46 hr. |
|---|---|---|---|---|
| 0.000 | 42 | 130 | 65 | 70 |
| 0.0080 | 115 | 179 | 170 | 196 |
| 0.0120 | 125 | 298 | 192 | 210 |
| 0.0160 | 170 | 371 | 220 | 251 |
| 0.0200 | 100 | 238 | 180 | 215 |
| 0.0240 | 80 | 226 | 164 | 190 |
| 0.032 | 65 | 206 | 150 | 140 |
| 0.040 | 52 | 186 | 148 | 139 |
| 0.048 | 51 | 175 | 139 | |

## VI. Growth Rates of Yeast.

Using a medium of uniform composition with plenty of food present, and plenty of room for the cells to grow, it is natural to expect that each cell would grow independently of the others, and that therefore the rate of increase of the number of cells in the flask at any moment would be proportional to the number of cells then present in the flask, i. e., (writing "C" for "Count"),

$$\frac{dC}{C} = kdt \qquad \text{Integrating, we have}$$

$$\int_{I}^{C} \frac{dC}{C} = \int_{t_1}^{t_2} kdt$$

Therefore,

$$\log C - \log I = k(t_2 - t_1)$$

Letting I = 1,

$$\log C = kt$$

then $k = \frac{\log C}{t}$

During the logarithmic period of growth the plot of log C against t is a straight line.

Having determined the optimum medium for the growth of yeast at various temperatures the author proceeded to determine the growth rates for the various temperatures using the optimum medium for each particular temperature and from these results calculated the thermal coefficients. The same determinations were also made upon wort, and wort with the

first and second maxima concentrations of ammonium chloride.

1. The Growth Rates of Yeast in Wort and Wort plus Ammonium Chloride.

The results obtained with wort and wort plus ammonium chloride are shown in Tables 16, 17, 18, 19, and 20. Tables 16 and 17 give the count for the various time intervals for wort and also for the first and second maxima. Table 18 shows the critical count and maximum crop of yeast for each temperature studied. It is interesting to note that both the critical count and the maximum crop are increased by the addition of ammonium chloride. Table 19 gives the time in hours and the corresponding log, C, for wort and the first and second maxima at each temperature. From these values if log C is plotted as ordinate and time as abscissae we get a straight line during the logarithmic growth rate. Table 20 shows the optimum concentration of ammonium chloride for the first and second maxima, k and $k_{10}/k$ or the thermal coefficient for each rise of 10 degrees in temperature.

TABLE XVI.

The Effect of Ammonium Chloride Upon the Growth Rates of Yeast in Wort.

| | 10 degrees | | | 20 degrees | | |
|---|---|---|---|---|---|---|
| Time | Wort Count | First Max. Count | Second Max. Count | Time | Wort Count | First Max. Count | Second Max. Count |
| 12 | 3.3 | 3.5 | 3.4 | 17 | 58 | 72 | 65 |
| 15 | 4.4 | 4.8 | 4.6 | 18 | 73 | 92 | 83 |
| 17 | 5.4 | 6.0 | 5.7 | 19 | 92 | 119 | 107 |
| 19 | 6.6 | 7.2 | 6.8 | 21 | 148 | 196 | 175 |
| 26 | 13.3 | 14.9 | 14.0 | 25 | 290 | 384 | 342 |
| 32 | 23.8 | 28.4 | 26 | 34 | 966 | 1159 | 1040 |
| 45 | 86 | 109 | 98 | 43 | 1085 | 1301 | 1180 |
| 50 | 102 | 153 | 133 | 55 | 1170 | 1404 | 1270 |
| 62 | 158 | 237 | 206 | 67 | 1218 | 1449 | 1296 |
| 74 | 160 | 244 | 210 | 75 | 1218 | 1444 | 300 |
| 81 | 161 | 246 | 212 | | | | |

TABLE XVII.

The Effect of Ammonium Chloride Upon the Growth Rates of Yeast in Wort.

| Time | Wort Count | First Max. Count | Second Max. Count | Time | Wort Count | First Max. Count | Second Max. Count | Time | Wort Count | First Max. Count | Second Max. Count |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | 22 | 29 | 25 | 10.3 | 99 | 131 | 113 | 9 | 54 | 64 | 59 |
| 10 | 48 | 56 | 54 | 11.3 | 159 | 209 | 178 | 11 | 131 | 161 | 146 |
| 11 | 74 | 87 | 82 | 12.3 | 242 | 332 | 277 | 12 | 206 | 255 | 228 |
| 12 | 104 | 130 | 122 | 15 | 810 | 1187 | 980 | 13 | 312 | 406 | 356 |
| 21.5 | 1245 | 1437 | 1300 | 25.5 | 950 | 1330 | 1200 | 23 | 450 | 600 | 525 |
| 34 | 1340 | 1470 | 1390 | 47 | 1365 | 1465 | 1380 | 35 | 504 | 652 | 617 |
| 58 | 1345 | 1510 | 1395 | 69 | 1550 | 1705 | 1565 | 48 | 612 | 667 | 655 |
| 70 | 1350 | 1515 | 1400 | 81 | 1555 | 1710 | 1560 | 59 | 620 | 670 | 662 |
| | | | | | | | | 80 | 622 | 665 | 660 |

TABLE XVIII.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Wort.

| | Wort | | First Maximum | | Second Maximum | |
|---|---|---|---|---|---|---|
| Temperature | Critical Count | Maximum Crop | Critical Count | Maximum Crop | Critical Count | Maximum Crop |
| 10 | 102 | 161 | 153 | 246 | 133 | 212 |
| 20 | 290 | 1218 | 384 | 1442 | 342 | 1300 |
| 30 | 1245 | 1350 | 1437 | 1515 | 1300 | 1400 |
| 40 | 950 | 1555 | 1330 | 1710 | 1200 | 1560 |
| 42 | 450 | 622 | 600 | 665 | 525 | 660 |

TABLE XIX

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Wort.

| 10 | | | | 20 | | | | 30 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time Hrs. | Wort Log C | First Max. Log C | Second Max. Log C | Time Hrs. | Wort Log C | First Max. Log C | Second Max. Log C | Time Hrs. | Wort Log C | First Max. Log C | Second Max. Log C |
| 12 | 0.5185 | 0.5490 | 0.5315 | 17 | 1.7634 | 1.8573 | 1.8129 | 8 | 1.3464 | 1.4683 | 1.3962 |
| 15 | 0.0435 | 0.6812 | 0.6628 | 18 | 1.8633 | 1.9638 | 1.9191 | 10 | 1.6839 | 1.7649 | 1.7396 |
| 17 | 0.7324 | 0.7782 | 0.7559 | 19 | 1.9638 | 2.0755 | 2.0294 | 11 | 1.8692 | 1.9395 | 1.9138 |
| 19 | 0.8195 | 0.8573 | 0.8325 | 21 | 2.1703 | 2.2923 | 2.2430 | 12 | 2.0171 | 2.1139 | 2.0864 |
| 26 | 1.1239 | 1.1734 | 1.1461 | 25 | 2.4624 | 2.5843 | 2.5340 | 21.5 | 2.0951 | 2.1574 | 2.1139 |
| 32 | 1.3766 | 1.4533 | 1.4150 | | | | | | | | |
| 45 | 1.9345 | 2.0374 | 1.9934 | | | | | | | | |
| 50 | 2.0086 | 2.1847 | 2.1239 | | | | | | | | |

| 40 degrees | | | | 42 degrees | | | |
|---|---|---|---|---|---|---|---|
| 10.3 | 1.9956 | 2.1139 | 2.0531 | 9 | 1.7324 | 1.8062 | 1.7709 |
| 11.3 | 2.2014 | 2.3201 | 2.2520 | 11 | 2.1173 | 2.2041 | 2.1644 |
| 12.3 | 2.3838 | 2.5239 | 2.4425 | 12 | 2.3139 | 2.4065 | 2.3579 |
| 15 | 2.9085 | 3.0735 | 2.9880 | 13 | 2.4942 | 2.6085 | 2.5574 |
| 25.5 | 2.9777 | 3.1239 | 3.0792 | 23 | 2.6532 | 2.7782 | 2.7202 |

TABLE XX.

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Wort.

| | Wort | | | First Maximum | | | Second Maximum | | |
|---|---|---|---|---|---|---|---|---|---|
| Temperature | Normality $NH_4Cl$ | k | $k_{10}/k$ | Normality $NH_4Cl$ | k | $k_{10}/k$ | Normality $NH_4Cl$ | k | $k_{10}/k$ |
| 10 | 0.00 | 0.0430 | | 0.0416 | 0.0453 | | 0.0649 | 0.0443 | |
| 20 | 0.00 | 0.1035 | 2.40 | 0.01888 | 0.1092 | 2.41 | 0.0826 | 0.1068 | 2.41 |
| 30 | 0.00 | 0.1686 | 1.62 | 0.0236 | 0.1780 | 1.63 | 0.1060 | 0.1740 | 1.63 |
| 40 | 0.00 | 0.1936 | 1.14 | 0.02832 | 0.2051 | 1.15 | 0.1180 | 0.1992 | 1.145 |
| 42 | 0.00 | 0.1923 | 0.99 | 0.0294 | 0.2005 | 0.977 | 0.1230 | 0.1964 | 0.972 |

It is a significant fact that although k has a higher value in the case of the two optima concentrations of ammonium chloride than in wort alone, the thermal coefficients in each case are the same. In other words, the percentage increase in the growth rate is apparently a constant.

2. Growth Rates of Yeast in Medium E.

The growth rates of yeast in medium E at various temperatures, using the optimum concentration of ammonium chloride for each temperature were determined and the thermal coefficients were calculated from these. Growth rates for normal wort were also obtained at the same time and the thermal coefficients calculated. The results are shown in Tables 21 to 25 inclusive. Tables 21, 22 and 23 give the time and the corresponding count for each temperature for wort and for Medium E, optimum for each temperature studied. Table 24 gives the critical count, maximum crop of yeast, k and $k_{10}/k$ for each temperature.

It will be noticed that the best results were obtained at 40 degrees. At 40 degrees we obtain not only the highest critical count but also the highest maximum crop of yeast. This is of interest in view of the fact that most investigators have held to the view that yeast will not grow well at 40 degrees.

TABLE XXI.

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Medium E.

| 0 degrees | | | | 10 degrees | | | |
|---|---|---|---|---|---|---|---|
| Time | Wort Count | Medium E (A) Count | Medium E (B) Count | Time | Wort Count | Medium E (A) Count | Medium E (B) Count |
| 28 | 2.2 | 2.1 | 2.00 | 26 | 13.2 | 11.3 | 11.6 |
| 35 | 2.7 | 2.6 | 2.50 | 29.5 | 18.8 | 15.7 | 15.8 |
| 48 | 3.9 | 3.6 | 3.50 | 36 | 35.5 | 28.5 | 29 |
| 32 | 4.3 | 3.8 | 3.9 | 40 | 53 | 42 | 41.5 |
| 64 | 4.4 | 4.2 | 4.3 | 52 | 128 | 105 | 106 |
| 76 | 4.6 | 4.1 | 4.3 | 62 | 162 | 134 | 132.5 |
| | | | | 77 | 164 | 137 | 134 |

TABLE XXII.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Medium E.

| 20 degrees | | | | 30 degrees | | | | 40 degrees | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | Wort Count | Med. E(A) Count | Med. E (B) Count | Time | Wort Count | Med. E (A) Count | Med. E (B) Count | Time | Wort Count | Med. E (A) Count | Med. E (B) Count |
| 17 | 57.3 | 34.2 | 32.7 | 7 | 15.5 | | | 6 | 4.5 | | |
| 119 | 92.4 | 49.2 | 48.6 | 11 | 70.5 | 30 | 33 | 8 | 24.6 | | |
| 21 | 149 | 74 | 73 | 12 | | 45.5 | 45 | 9.5 | 67 | | |
| 223 | 243 | 111 | 112 | 13 | 153 | | | 11 | | 43.5 | 46 |
| 25 | 281 | 128 | 129 | 14 | 225 | 77 | 77 | 12 | 223 | | |
| 29.5 | 792.5 | 149 | 150 | 15.25 | | 110.5 | 107.5 | 12.5 | | 63.5 | 61 |
| 39.5 | 1165 | 224 | 226 | 16 | | 137 | 140 | 13 | 320 | | |
| 48 | 1190 | 280 | 285 | 23 | 677 | | | 13.5 | | 91.5 | 92.5 |
| 63.5 | 1205 | 283 | 281 | 29 | 1050 | | | 14 | 505 | | |
| | | | | 35.25 | 1092 | 156 | 164 | 14.5 | | 112.5 | 113.5 |
| | | | | 39 | | 191 | 192 | 22 | | 169 | 166 |
| | | | | 40.5 | 1197 | | | 23.5 | 837.5 | | |
| | | | | 43.25 | | 254 | 258 | 29 | 872.5 | | |
| | | | | 46.5 | 1205 | 261 | 265 | 34 | | 223 | 228 |
| | | | | 48 | 1220 | | | 37 | 1342 | | |
| | | | | 54 | | 274 | 279 | 45 | | 395 | 390 |
| | | | | 59.25 | 1215 | | | 48 | 1345 | | |
| | | | | 65 | | 270 | 272 | 58 | | 393 | 392 |
| | | | | 73 | 1223 | | | 60 | 1332 | | |
| | | | | | | | | 68 | | 399 | 399 |
| | | | | | | | | 72 | 1342 | 398 | 391 |

TABLE XXIII.

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Medium E.

| 42 degrees | | | | 43 degrees | | | |
|---|---|---|---|---|---|---|---|
| Time | Wort Count | Medium E (A) Count | Medium E (B) Count | Time | Wort Count | Medium E (A) Count | Medium E (B) Count |
| 9 | 53 | 13.3 | 13 | 12 | 1.6 | 1.5 | 1.5 |
| 10 | 83 | 17.2 | 18 | 18 | 2.2 | 1.8 | 1.8 |
| 11 | 131 | 22.7 | 22.5 | 24 | 2.8 | 2.2 | 2.1 |
| 13 | 308 | 41.5 | 41 | 30 | 3.6 | 2.6 | 2.7 |
| 15 | 316 | 70.5 | 72.5 | 38 | 3.7 | 3.3 | 3.4 |
| 17 | 340 | 131 | 129 | 45 | 3.8 | 3.4 | 3.5 |
| 21 | 355 | 162 | 159 | 68 | 3.8 | 3.5 | 3.4 |
| 33 | 408 | 258 | 250 | | | | |
| 41.5 | 521 | 332 | 336 | | | | |
| 56.5 | 625 | 349 | 343 | | | | |
| 68.5 | 630 | 345 | 342 | | | | |

TABLE XXIV.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Medium E at Different Temperatures.

| Temperature | Critical Count | Max. Crop | k | $k_{10}/k$ | Critical Count | Max. Crop | Critical Count | Max. Crop | k | $k_{10}/k$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 4.4 | 4.6 | 0.0122 | 3.5250 | 4.1 | 4.2 | 4.2 | 4.3 | 0.0113 | |
| 10 | 128 | 164 | 0.0430 | 3.5250 | 105 | 137 | 106 | 134 | 0.0405 | 3.521 |
| 20 | 281 | 1205 | 0.1035 | 2.407 | 149 | 283 | 150 | 281 | 0.0883 | 2.180 |
| 30 | 677 | 1223 | 0.1686 | 1.630 | 156 | 270 | 107 | 272 | 0.1361 | 1.541 |
| 40 | 837 | 1342 | 0.1936 | 1.149 | 169 | 398 | 166 | 391 | 0.1452 | 1.066 |
| 42 | 308 | 630 | 0.1923 | 0.99 | 162 | 345 | 159 | 342 | 0.1239 | 0.853 |
| 43 | 3.7 | 3.8 | 0.0180 | 0.093 | 3.3 | 3.4 | 3.4 | 3.50 | 0.0143 | 0.115 |

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Medium E.

| Temperature:- | 0 degrees | | | | 10 degrees | | | |
|---|---|---|---|---|---|---|---|---|
| Wort: | | Med.: (A) | | (B) | Wort: | | Med.: (A) | (B) |
| Time | Log.C. | Time | Log.C. | Log.C. | Time | Log.C. | Time | Log.C. | Log.C. |
| 28 | 0.3424 | 28 | 0.3222 | 0.3010 | 26 | 1.1205 | 26 | 1.0529 | 1.9645 |
| 35 | 0.4314 | 35 | 0.4150 | 0.3979 | 29.5 | 1.2742 | 29.5 | 1.1959 | 1.1987 |
| 48 | 0.5911 | 48 | 0.5563 | 0.5441 | 36 | 1.5502 | 36 | 1.4548 | 1.4624 |
| 52 | 0.6335 | 52 | 0.5798 | 0.5911 | 40 | 1.7243 | 40 | 1.6232 | 1.6180 |
| 64 | 0.6435 | 64 | 0.6232 | 0.6335 | 52 | 2.1072 | 52 | 2.0212 | 2.0253 |
| | 20 degrees | | | | | 30 degrees | | | |
| 17 | 1.7597 | 17 | 1.5105 | 1.5132 | 7 | 1.1903 | 11 | 1.4771 | 1.5185 |
| 19 | 1.9657 | 19 | 1.6920 | 1.6866 | 11 | 1.8482 | 12 | 1.6580 | 1.6532 |
| 21 | 2.1732 | 21 | 1.8692 | 1.8633 | 13 | 2.1847 | 14 | 1.8865 | 1.8865 |
| 23 | 2.3856 | 23 | 2.0453 | 2.0492 | 14 | 2.3522 | 15.25 | 2.0433 | 2.0305 |
| 25 | 2.4487 | 25 | 2.1072 | 2.1106 | 23 | 2.8309 | 16 | 2.1367 | 2.1461 |
| | 40 degrees | | | | | 42 degrees | | | |
| 6 | 0.6532 | 11 | 1.6385 | 1.6628 | 9 | 1.7243 | 9 | 1.1271 | 1.1139 |
| 8 | 1.3909 | 12.5 | 1.8028 | 1.7853 | 10 | 1.9191 | 10 | 1.2340 | 1.2553 |
| 9.5 | 1.8325 | 13.5 | 1.9614 | 1.9661 | 11 | 2.1239 | 11 | 1.3560 | 1.3522 |
| 12 | 2.3483 | 14.5 | 2.0511 | 2.0550 | 13 | 2.4886 | 13 | 1.6180 | 1.6128 |
| 13 | 2.5051 | 22 | 2.2279 | 2.2201 | 15 | 2.5079 | 15 | 1.8482 | 1.8693 |
| 14 | 2.7033 | 34 | 2.3483 | 2.3579 | 17 | 2.5315 | 15 | 2.1173 | 2.1106 |
| | 43 degrees | | | | | | | | |
| 12 | 0.2041 | 12 | 0.1761 | 0.1761 | | | | | |
| 18 | 0.3424 | 18 | 0.2553 | 0.2780 | | | | | |
| 24 | 0.4472 | 24 | 0.3424 | 0.3222 | | | | | |
| 30 | 0.5563 | 30 | 0.4150 | 0.4314 | | | | | |
| 38 | 0.5682 | 38 | 0.5185 | 0.5315 | | | | | |

At 42 degrees the yeast are growing fairly well but at 43 degrees there is a very sharp break in the growth rate and also in the total crop of yeast. In fact the break is so sudden that it is quite difficult to obtain a very accurate growth rate.

Table 25 gives the time in hours and Log. C. for the corresponding time.

In Figure 2 the values of k for the various temperatures are plotted as ordinates and temperature as abscissa. Plotting k against temperature we obtain a straight line, except from 0 to 10 degrees where there is apparently a lag phase, and from 40 degrees on, where there is a gradual falling off until 42 degrees is reached while from 42 degrees to 43 degrees there is a sudden drop, k dropping to 0.0143 for Medium E and to 0.0180 for wort.

3. Growth Rates for Medium E Optimum for 30 degrees.

Growth rates were determined for Medium E using the optimum concentration of Ammonium Chloride for 30 degrees, at 10, 20, 30, 40 and 42 degrees. The results are shown in Tables 26 to 28 inclusive and Figure 2.

TABLE XXVI.

Growth Rates of Yeast in Medium E. (Optimum for 30 degrees).

| 10 deg. | | | 20 deg. | | | 30 deg. | | | 40 deg. | | | 42 deg. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | E (A) Count | E (B) Count | Time | E (A) Count | E (B) Count | Time | E (A) Count | E (B) Count | Time | E (A) Count | E (B) Count | Time | E (A) Count | E (B) Count |
| 15 | 3 | 3.1 | 18 | 24 | 25 | 11 | 33 | 30 | 8 | 11.2 | 11.4 | 8 | 7.5 | 7.3 |
| 19 | 4.2 | 4.1 | 21 | 42 | 43 | 12 | 45 | 46 | 10 | 21 | 20 | 10 | 13.5 | 13.2 |
| 23 | 5.5 | 5.9 | 24 | 72 | 73 | 14 | 77 | 79 | 11 | 28 | 28.5 | 13 | 26.5 | 26 |
| 30 | 92 | 9.7 | 28 | 80 | 82 | 15.25 | 110 | 108 | 12 | 38 | 38.5 | 16 | 55 | 56 |
| 39 | 18.2 | 18 | 41 | 174 | 177 | 25.5 | 137 | 140 | 16 | 97 | 99 | 25 | 89 | 90 |
| 55 | 37 | 39 | 50 | 184 | 187 | 35.25 | 156 | 164 | 32 | 144 | 141 | 39 | 132 | 133 |
| 64 | 67 | 70 | 65 | 205 | 207 | 39 | 192 | 191 | 42 | 188 | 183 | 51.5 | 139 | 137 |
| 72 | 69 | 73 | 75 | 203 | 210 | 43.25 | 258 | 254 | 56 | 195 | 198 | 63 | 138 | 138 |
| | | | | | | 48 | 261 | 265 | 69 | 196 | 201 | | | |
| | | | | | | 59.25 | 274 | 279 | 80 | 202 | 206 | | | |
| | | | | | | 71 | 270 | 272 | | | | | | |

TABLE XXVII.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Medium E.
(Optimum for 30 deg.).

| 10 deg. | | | 20 deg. | | | 30 deg. | | | 40 deg. | | | 42 deg. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | Log. C | Log. C | Time | Log. C | Log. C | Time | Log. C | Log. C | Time | Log. C | Log. C | Time | Log. C. | Log. C. |
| 15 | 0.4771 | 0.4914 | 18 | 1.3909 | 1.3979 | 11 | 1.4771 | 1.5185 | 8 | 1.0492 | 1.0569 | 8 | 0.8751 | 0.8633 |
| 19 | 0.6232 | 0.6128 | 19 | 1.4744 | 1.4771 | 12 | 1.6580 | 1.6532 | 10 | 1.3222 | 1.3118 | 10 | 1.1303 | 1.1206 |
| 23 | 0.7404 | 0.7709 | 21 | 1.6274 | 1.6335 | 14 | 1.8865 | 1.8865 | 11 | 1.4472 | 1.4548 | 13 | 1.4232 | 1.4150 |
| 30 | 0.9638 | 0.9868 | 22 | 1.7050 | 1.7076 | 15.25 | 2.0433 | 2.0305 | 12 | 1.5798 | 1.5855 | 16 | 1.7404 | 1.7482 |
| 39 | 1.2691 | 1.2553 | 24 | 1.8573 | 1.8637 | 16 | 2.1367 | 2.2461 | 16 | 1.9868 | 1.9956 | 25 | 1.9494 | 1.9542 |
| 55 | 1.5740 | 1.5911 | 28 | 1.9031 | 1.9138 | | | | | | | | | |

TABLE XXVIII.

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Medium E. at Various Temperatures Using a Medium Optimum for 30 degrees C.

| | E - I | | E - II | | | |
|---|---|---|---|---|---|---|
| Temperature | Critical Count | Maximum Crop | Critical Count | Maximum Crop | k | $k_{10}/k$ |
| 10 | 37 | 69 | 39 | 73 | 0.0324 | |
| 20 | 80 | 203 | 82 | 210 | 0.0777 | 2.398 |
| 30 | 156 | 270 | 107 | 272 | 0.1361 | 1.751 |
| 40 | 97 | 202 | 99 | 206 | 0.1316 | 0.966 |
| 42 | 89 | 138 | 90 | 139 | 0.1098 | 0.834 |

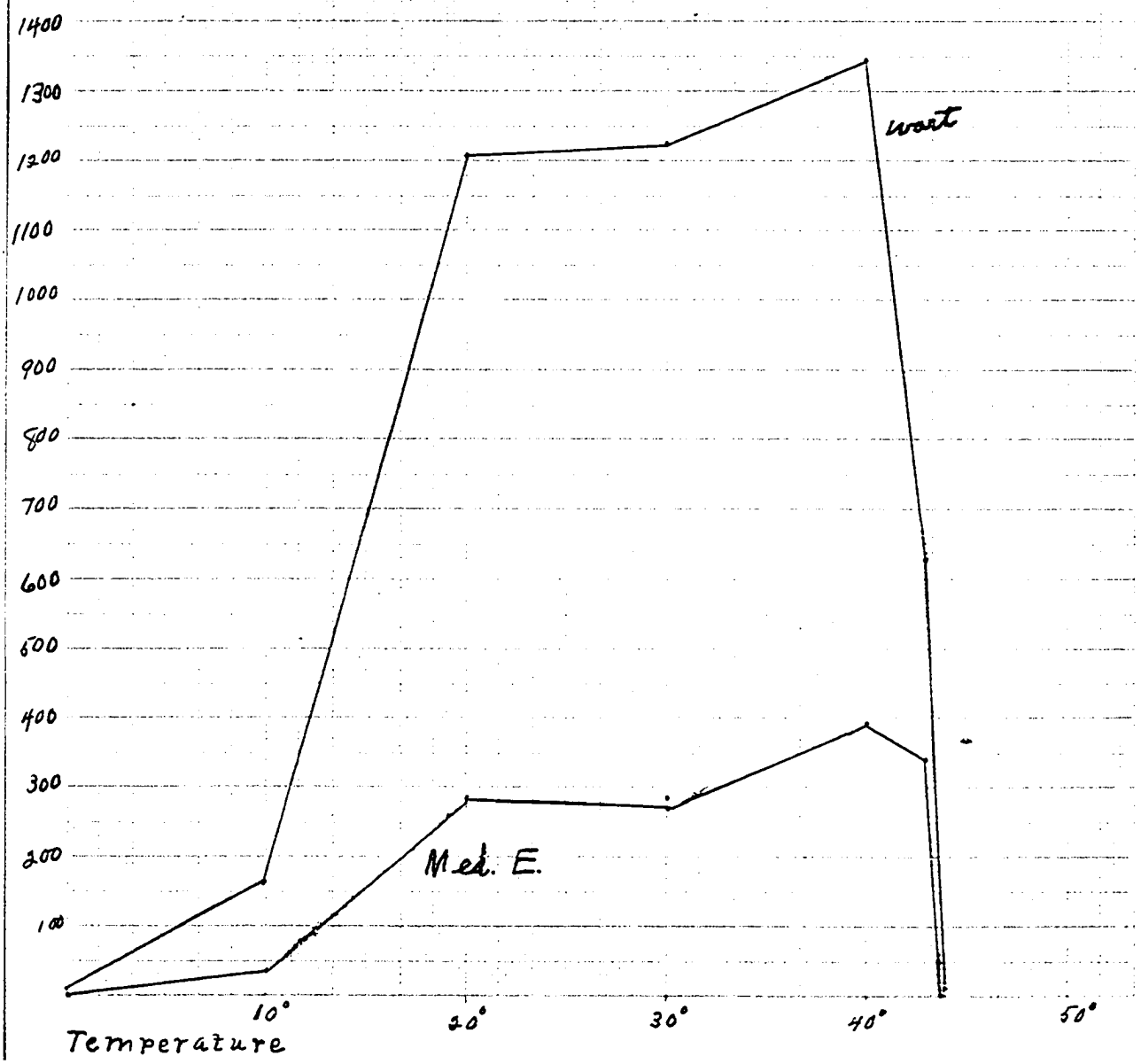

1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
wart
Med. E.
10°
20°
30°
40°
50°
Temperature

Table 26 shows the time and the count for the temperatures studied while table 27 gives the time and log. C. for the corresponding temperatures. Table 28 gives the critical count, the maximum crop, and k and $k_{10}/k$ for the various temperatures studied.

It will be noticed in Figure 2 that k for Medium E (Optimum for 30 degrees) is below the corresponding k for the optimum medium for that particular temperature; for example at 10 degrees the value of k for the optimum medium E. is 0.0405 while the value for k at 10 degrees using a medium E. optimum for 30 degrees gives a lower value, namely 0.0324. The same is true for 20 degrees while at 30 degrees, where the composition of the medium is optimum, k is on the curve plotted for media optimum for each temperature. However at 40 degrees the value of k shows a very decided drop again, k dropping from 0.1452 to 0.1316 while at 42 degrees it drops from 0.1239 to 0.1098. It is evident from a study of Figure 2 that the values of k (using a medium optimum for 30 degrees) at 10 degrees and 30 degrees are too low and again above 30 degrees they are too low, while the value for k or $k_{10}/k$ are too high for temperatures below 30 degrees and too low for values above 40 degrees, as shown by a comparison of Tables 24 and 28. The explanation for this is that as we increase the temperature we are also approaching the optimum temperature for the medium used, so that we have two factors tending to increase the growth rate,

Fig. a.

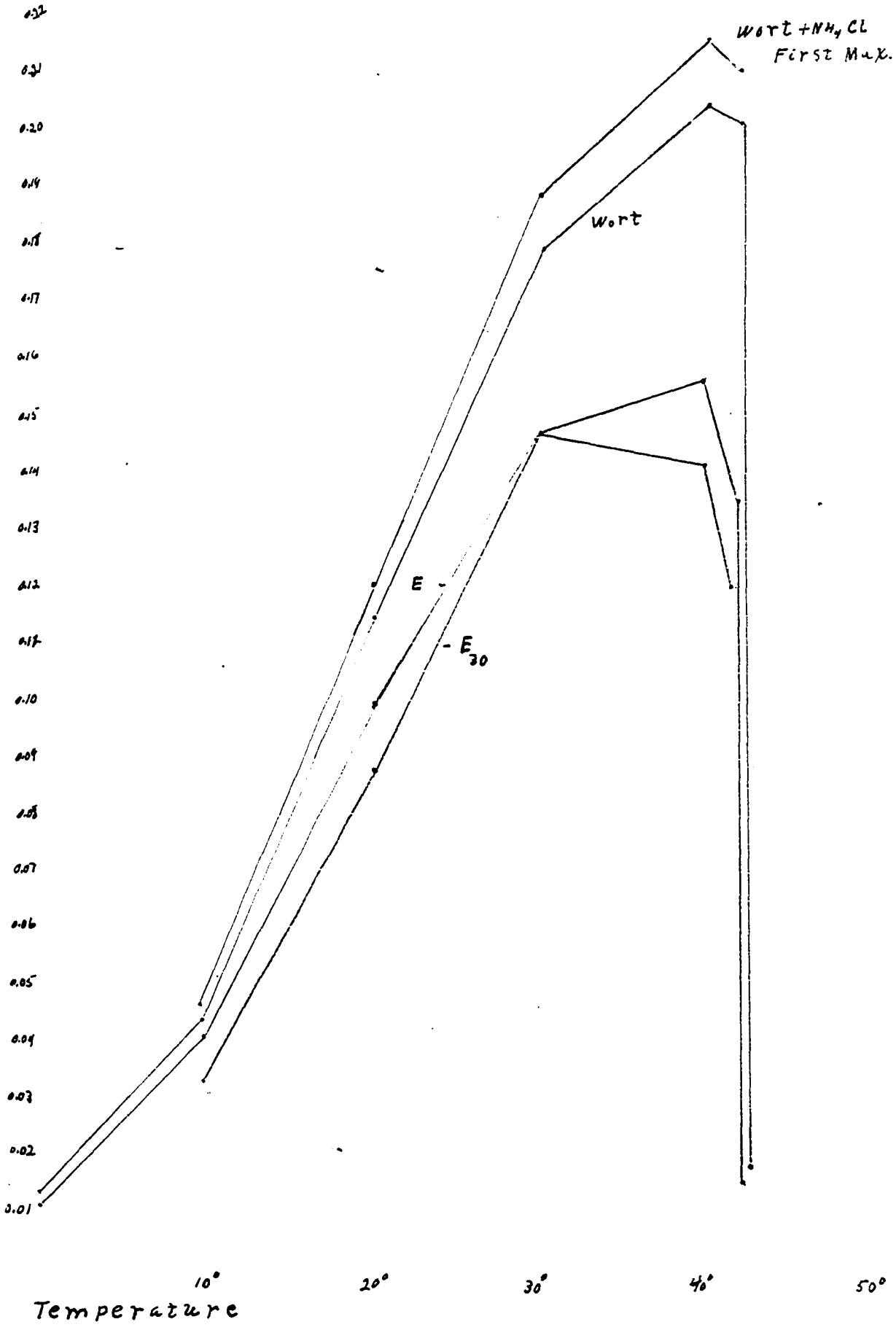
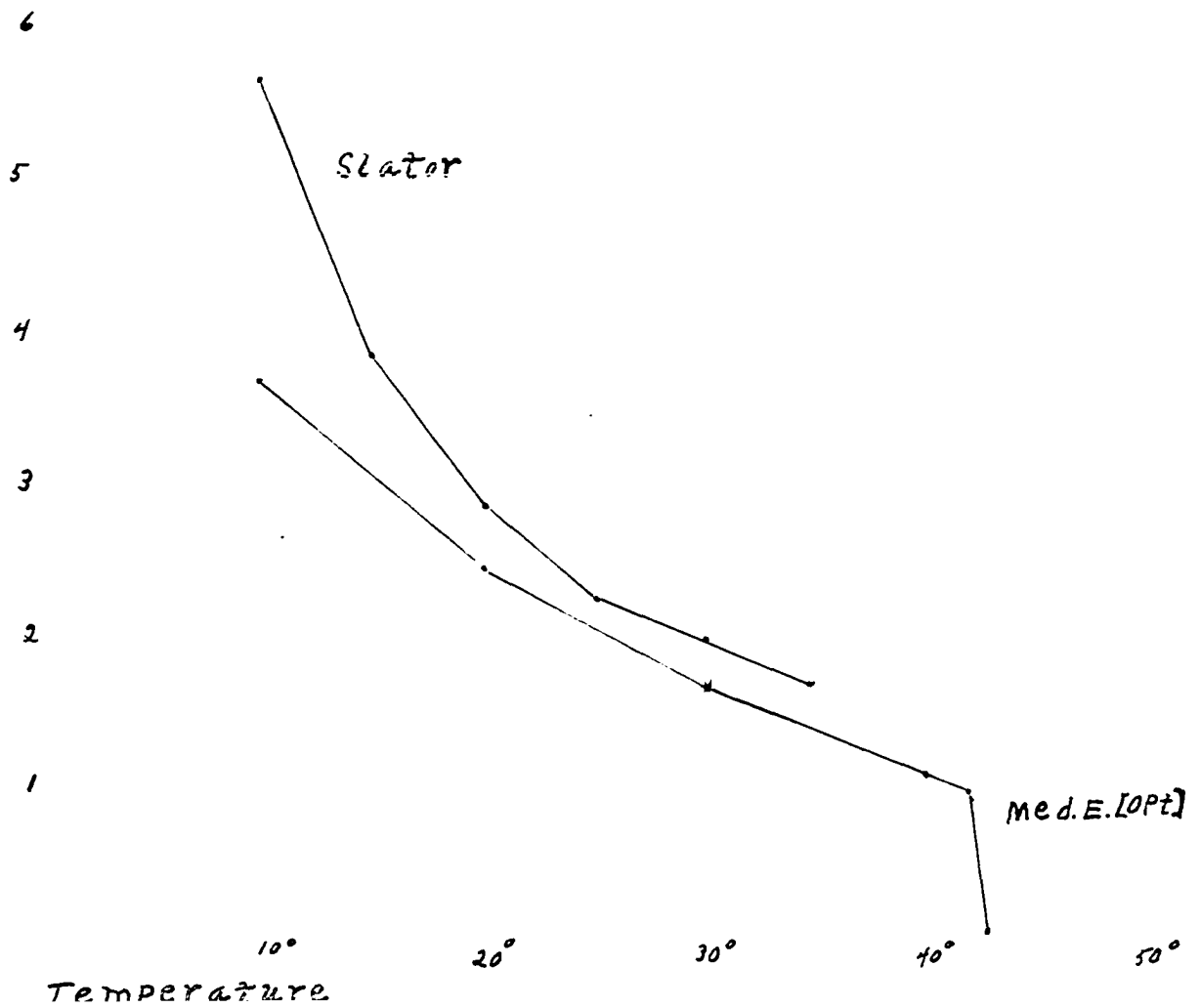

Wort + $NH_4Cl$
First Max.
Wort
E
$E_{30}$
0.22
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
10°
20°
30°
40°
50°
Temperature

TABLE XXIX.

| Slator | | Medium E. | |
|---|---|---|---|
| $k_{10}/k$ Experiment | $k_{10}/k$ Calculated | $k_{10}/k$ Experiment | $k_{10}/k$ Calculated |
| 5.6 | 2.162 | 3.521 | 1.770 |
| 2.25 | 2.017 | 2.180 | 1.65 |
| 1.60 | 1.886 | 1.541 | 1.541 |
| | | 1.066 | 1.443 |


6
5
4
3
2
1
Slater
Med.E.[OPt]
10°
20°
30°
40°
50°
Temperature

rise in temperature and the change in the thermodynamic environment until the optimum temperature for the given medium is reached, while from there on up these two factors work against each other, that is, a rise in temperature will tend to increase the growth rate, while the change in the thermodynamic environment of the medium will tend to decrease the growth rate, hence the values for $k_{10}/k$ will fall below the value of $k_{10}/k$ where physico-chemical conditions are optimum.

Table 29 gives the thermal coefficients for 10 degree intervals for the growth of yeast from Slator's work and those obtained from Medium E., together with the calculated values (using formula (20), page 14) in each case. The experimental values do not agree very closely with the calculated values. However they do agree somewhat more closely in the case of Medium E. than they do with Slator's figures.

These experiments were carried out in the Bio-Physical Chemistry Laboratory of the Iowa State College under the direction of Dr. E. I. Fulmer.

## VII. Summary.

1. The method given in a former paper[51] by which the optimum concentration of each of the constituents of the medium for the growth of yeast has been extended to include other temperatures. The results confirm the former findings.

2. The effect of ammonium chloride upon the growth of yeast in wort was studied. Two maxima concentrations of ammonium chloride were found which varied with the temperature.

3. A second maximum concentration of ammonium chloride was also found for the growth of yeast in Medium E.

4. Thermal coefficients for the growth of yeast in various media were determined in similar physico-chemical environments for each temperature.

5. The average $K_{10}/K$ for four investigators was 2.72 to 2.88. Determined as in 4, the average was 2.05, showing a material difference in methods.

BIBLIOGRAPHY.

1. Principles of Biochemistry, Robertson.

2. Wilhemly, L., Pogg. Ann. 81; 422, 499, 1850.

3. Harcourt, A. V., and Esson, W., Phil. Trans. 167; 117, 1867.

4. Van't Hoff, J. H., Lectures on Theoretical and Physical Chemistry.

5. Hahn, O., Zeit. phys. chem., 42; 705, 1903.
Hahn, O., Zeit. phys. chem., 48; 735, 1904.

6. Bodenstein, M., Zeit. phys. chem. 29; 298, 1899.

7. Kooij, D. M., Zeit. phys. chem. 12; 155, 1893.

8. Arrhenius, S., Zeit. phys. chem. 4; 226, 1889.

9. Hood, J. J., Phil. Mag. (5), 20; 323, 1885.

10. Warder, R. H., Amer. Chem. Journ., 3; 203, 1881.

11. Hecht, W., and Conrad, M.; Zeit. phys. chem. 3; 450, 1889.

12. Urech, F., Ber. 16; 762, 1883.

13. Spohr, J., Zeit. phys. chem. 2, 194; 1818.

14. Price, T. S., Journ. Chem. Soc. 79, 303, 1901.

15. Remsen, I., and Reid, E. E., Am. Chem. Journ. 21; 281, 1899.

16. Kastle, J. H., and Loevenhart, A. S., Am. Chem. Journ. 26; 539, 1901.

17. Goldschmidt, H., and Reinders, R. V., Ber., 29; 1369, 1899.

18. Harcourt, A. V., and Esson, W., Phil. Trans., 157, 117, 1867.

19. Van't Hoff's, J. H., Études, 113, 1884.

20. Spohr, J., Zeit. phys. chem., 2; 194, 1888.

21. Buchbock, G., Zeit. phys. chem., 23; 123, 1897.

22. Pendlebury, W. H., and Seward, M., Proc. Royal Soc. 45; 396, 1889.

23. Tammann, G., Zeit phys. chem. 28; 317, 1899.

24. Remsen, I., and Reid, E. E., Amer. Chem. Journ. 21; 281, 1899.

25. Veley, V. H., Journ. Chem. Soc., 54, 200, 361, 1899.

26. Bugarsky, S., Zeit phys. chem. 42; 545, 1903.

27. Hecht, W., and Conrad, M., Zeit. phys. chem. 3; 450, 1889.

28. Berthelot, M., Ann. Chim. Phys. 3, 66, 110, 1862.

29. Spring, W., Zeit. phys. chem. 1; 209, 1887.

30. Hood, J. J., Phys. Mag.(5), 20; 323, 1885.

31. Principles of Biochemistry, Robertson.

32. Principles of General Physiology, Bayliss, W.M.

33. Benson, C. C., Journ. Phys. Chem. 8; 116, 1904.

34. Nernst, 1888, page 624.

35. Nernst, 1910, page 415.

36. Nernst, 1903, page 88.

37. Aberson, M. J. H., Rec. trav. chim., 22; 78, 1903.

38. Herzog, Zeit. phys. chem., 37; 149, 1902.

39. Slator, A., Journ. Chem. Soc. 89; 128, 1906.

40. Fulmer, E. I., Nelson, V. E., and Sherwood, F. F., Journ. Amer. Chem. Society, 43; 186, 1921.

41. Slator, A., Biochem. Journ. 7; 197, 1913.

42. Carlson, T., Biochem. Zeit. 57; 313, 1913.

43. Clark, N. A., Journ. Phys. Chem. 26; 42-60, 1922.

44. Brown, Horace T., Annals of Botany, 28; 197, 1914.

45. Fulmer, E. I., University of Toronto Studied, No. 124.

46. Fulmer, E. I., Nelson, V. E., and Sherwood, F. F., Journ. Amer. Chem. Soc., 43; 191, 1921.

47. Boas, F., Biochem. Z. 105; 193-8, 1920.

48. Pringsheim, Hans, Biochem. Z. 3; 121, 286, 1875.

49. Loc. Cit., page 187.

50. O'Sullivan, James, Journ. of the Soc. of Chem. Ind. 17, 1898, p. 555; Jour. Chem. Soc., 1892.

51. Loc. Cit., page 191.