AN APPLICATION OF THE AUTOCATALYTIC GROWTH CURVE TO MICROBIAL METABOLISM[1]

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Received for publication, December 22, 1931

Growth does not take place at a constant rate in living organisms. In bacterial cultures it is initially exceptionally slow, then increasingly rapid, and finally exceptionally slow. This is most conspicuous if the initial inoculum into fresh media is very small. We have found that the rate of accumulation of microbial metabolic products likewise is not constant but begins slowly, increases rapidly, and again slows down.

The work reported concerns the accumulation of nitrates by soil bacteria and of carbon dioxide by yeasts. During the earlier part of the incubation period the accumulation of nitrates was meagre. Then followed a period of rapid increase and following this a very slow or negative accumulation. In some cases as much as 70 mgm. of nitric nitrogen had accumulated between the twelfth and the sixteenth days, whereas only 20 mgm. had accumulated up to the twelfth day. The same relationship holds for carbon dioxide production by yeasts, but the changes in rate are less extreme.

If the concentration of nitrates or carbon dioxide be plotted against time it may be observed that the increase is regular (not constant) and that the plotted points conform closely to a smoothly drawn S-shaped curve. This is typical of growth curves to which

[1] Contribution from Department of Bacteriology, Utah Agricultural Experiment Station.

Publication authorized by Director, December 19, 1931.

This study was planned by Dr. J. E. Greaves and carried out under his supervision. Valuable assistance was also given by Dr. Willard Gardner.

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considerable study has been devoted. An inclusive list of references to the literature on the subject of time growth relations up to 1926 has been published by the Missouri Agricultural Experiment Station (Brody, Hogan et al. (1926)). Both weight and linear increase have been used to indicate growth.

Robertson (1923) has applied the differential equation which expresses the speed of a monomolecular autocatalyzed chemical reaction to the growth of plants and animals. In this paper that equation is applied to our data on nitrate and carbon dioxide accumulation.

The equation implies that the catalyst is produced by the reaction in exactly the same proportion as the end-product y accumulates. It then becomes one of the reacting substances. By letting a represent the original concentration of the substrate, y the amount used by the reaction (the product) and also, by our assumption, the catalyst, and k_1 the proportionality constant, the rate of reaction may be expressed as

$$\frac{dy}{dt} = k_1 y(a - y) \tag{1}$$

However, the amount of nutrient substrate a is not generally the limiting factor of growth, but the reaction must be considered reversible, and an equilibrium is reached which is expressed by subtracting the reverse from the forward reaction.

$$\frac{dy}{dt} = k_1 y(a - y) - k_2 y^2 \tag{2}$$

It is understood that the catalyst is active in both directions. A second constant k_2 must be introduced for the reverse reaction. By a little manipulation and substituting A for $\frac{k_1 a}{k_1 - k_2}$,

$$\frac{dy}{dt} = y \frac{k_1 a}{A} (A - y) \tag{3}$$

which is, since $\frac{k_1 a}{A}$ is a constant, identical in form with (1). But A is a fractional part of a and defines the limit of growth or

maximum amount of end-product due to equilibrium between the forward and reverse reactions rather than the total amount of nutrient substrate originally present.

Integrating (3) and simultaneously substituting K for $\frac{k_1 a}{2.3 A}$ (changing to Napierian logarithms), we obtain

$$\log \frac{y}{A - y} = Kt + C \tag{4}$$

We wish to evaluate C as $-Kt_1$ where t_1 is the time at which the reaction is half completed, that is, $y = \frac{A}{2}$.

$$\log \frac{y}{A - y} = K(t - t_1) \tag{5}$$

At the point of maximum velocity the acceleration, expressed by the second derivative, is 0, from which we calculate $y = \frac{A}{2}$.

The curve is symmetrical with its center at $y = \frac{A}{2}$, $t = t_1$.

When solved explicitly for y

$$y = \frac{A \cdot 10^{K(t - t_1)}}{1 + 10^{k(t - t_1)}} \tag{6}$$

EVALUATION OF CONSTANTS

In applying this equation to experimental data the three constants, A, K, and t_1, must be properly evaluated. They were calculated from a modified form of equation (3) in which $b = Kt_1$.

$$\log \frac{y}{A - y} = Kt - b \tag{7}$$

The constant A, representing the amount of end-product, in most cases was evaluated by inspection after plotting the data. It is approximately an arithmetic average of the several determinations made after the maximum had been reached. In some cases

A was evaluated by a method Robertson uses (1923, pp. 63–64), substituting in four equations four values of t and y such that when the first is subtracted from the second and the third from the fourth, the two differences are equal. This means the four points must be chosen in two pairs separated by equal time intervals. A can then be calculated. It is desirable to choose points well distributed along the curve. However, the choice is quite arbitrary and the method so overweights those points chosen that a more satisfactory A can be chosen by inspection. The constants K and b were evaluated by the method of least squares.

NITRIFICATION

The data plotted in the nitrification curves which follow (figs. 1 to 16, inclusive) were obtained by inoculating soil (1 gram) into Fred's nitrite medium (200 cc.) and incubating for five weeks. The nitrates present in 5 cc. of solution were determined daily for twenty-one days by the phenoldisulphonic-acid method and after that on the twenty-ninth and thirty-sixth days.

Both productive and alkali soils were used to inoculate. The productive soil was also artificially made alkaline by the addition of salts. Each soil was used both before and after being leached with water (Greaves and Pulley (1931)). In table 1 are listed the various soils used, together with a summary of the equation constants for nitrification.

The curves plotted conform so uniformly to the data obtained experimentally that there can be no doubt that the progress of metabolism indicated by nitrate formation is autocatalytic, conforming to the general mathematical expressions given above.[3]

DISCUSSION OF EQUATION CONSTANTS

The velocity constant K is influenced by the type of soil. This may be due in part to the modifying effect the soil has on the type of microflora present and in part to the stimulating or the toxic effects of the salts added with the soil. A salt which stimulates nitrification increases K. One which depresses lowers its value.

[3] A similar study is being conducted at the Utah Station on the time relationship of NH_3 accumulation by soil organisms.

Sodium carbonate is highly stimulating if present in small quantities but becomes toxic in slightly larger amounts. Media inoculated with soil (1 gram in 200 cc.) carrying 1 per cent Na_2CO_3

TABLE 1

Soils used to inoculate nitrite media for nitrification, also calculated equation constants

SOIL NUMBERS	DESCRIPTION OF SOIL	EQUATION CONSTANTS		
		K	t_1 (days)	A (milligrams nitric nitrogen)
53–54	Native soil + 1 per cent each NaCl, Na_2CO_3	1.05	21.0	88
45–46	Native soil + 1 per cent each NaCl, Na_2SO_4	0.62	19.9	93
35–36	Native soil + 2 per cent Na_2CO_3, leached	0.51	13.1	95
21–22	Native soil + 2 per cent Na_2SO_4	0.41	17.2	94
69–70	Native soil + ⅔ per cent each, NaCl, Na_2SO_4, Na_2CO_3	0.38	14.1	92
79–80	Corinne soil, alkali	0.37	21.5	90
81–82	Corinne soil, alkali leached	0.37	16.8	92
55–56	Native soil + 1 per cent each, NaCl, Na_2CO_3, leached	0.37	13.4	94
71–72	Native soil + ⅔ per cent each, NaCl, Na_2SO_4, Na_2CO_3, leached	0.37	15.5	95
89–90	Richland acres soil, alkali	0.35	15.7	98
61–62	Native soil + 1 per cent each, Na_2SO_4, Na_2CO_3	0.35	15.9	94
93–94	Richland acres soil, alkali, leached	0.32	15.3	94
11–12	Native soil + 2 per cent NaCl, leached	0.31	16.4	96
23–24	Native soil + 2 per cent Na_2SO_4, leached	0.30	16.8	98
47–48	Native soil + 1 per cent each, NaCl, Na_2SO_4, leached	0.26	16.9	90
1–2	Native soil	0.26	16.7	105
3–4	Native soil, leached	0.25	16.7	104
63–64	Native soil + 1 per cent each Na_2SO_4, Na_2CO_3, leached	0.24	15.0	94
101–102	Benson soil, alkali	0.22	28.2	88
105–106	Benson soil, leached	0.16	26.0	92
33–34	Native soil + 2 per cent Na_2CO_3	0.12	39.9	95

and 1 per cent NaCl have an exceptionally high K, 1.05, which by leaching is lowered to 0.37. That inoculated with soil carrying 2 per cent Na_2CO_3 is toxic; here K is only 0.12. But the same soil leached has a K of 0.51. The native alkali soil of Benson

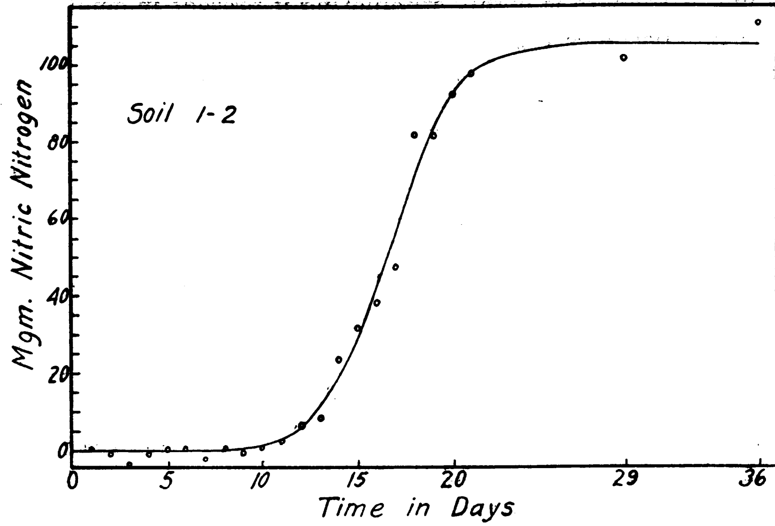

FIG. 1

FIGS. 1–16, inclusive. Time rate of nitrate accumulation in nitrite media inoculated with 1 gram of various soils. Soil numbers refer to table 1. The disconnected points represent experimental observations. The smooth curve is calculated from formula 3.

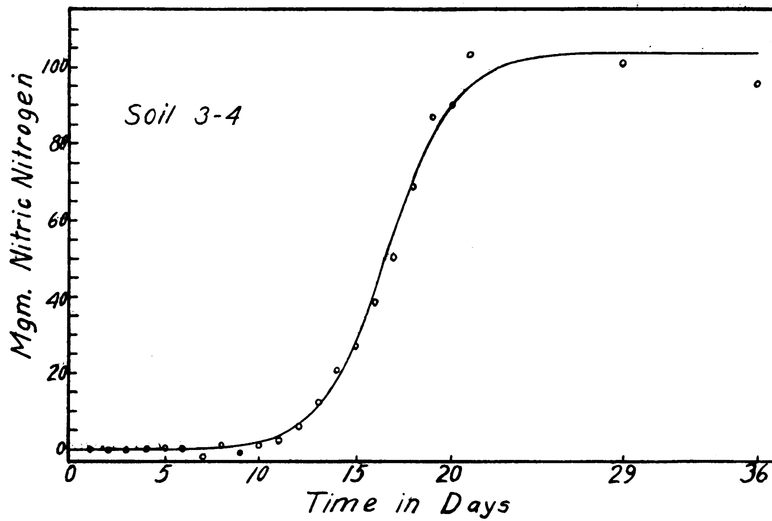

FIG. 2

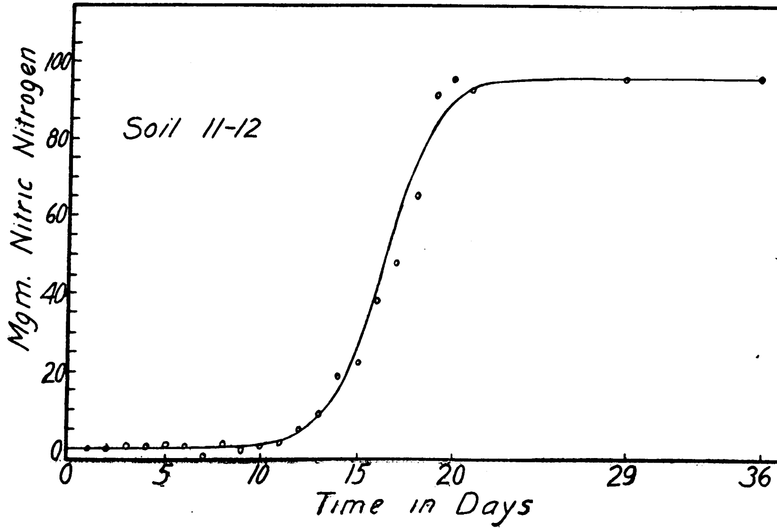

FIG. 3

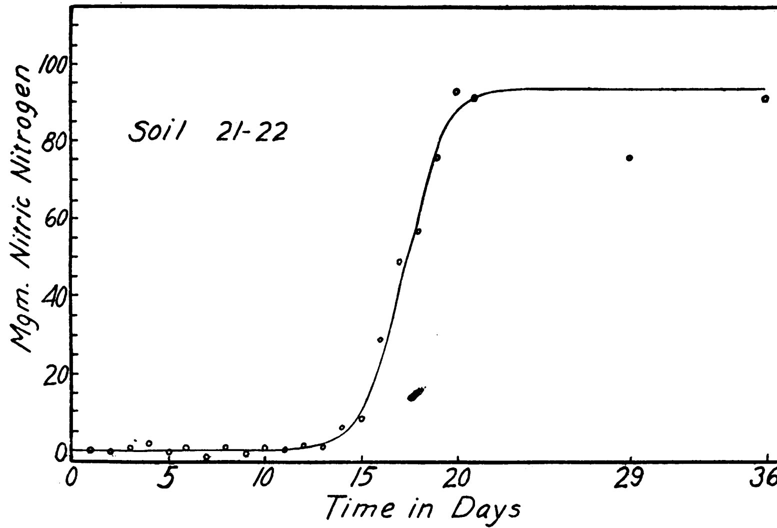

FIG. 4

gives a low K most comparable to the toxic carbonate soil, but in this case it is not increased by leaching. Sodium carbonate in

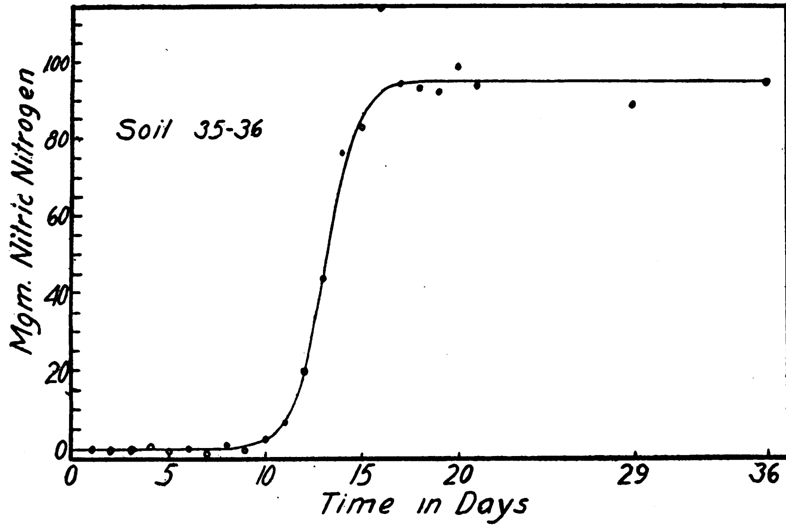

FIG. 5

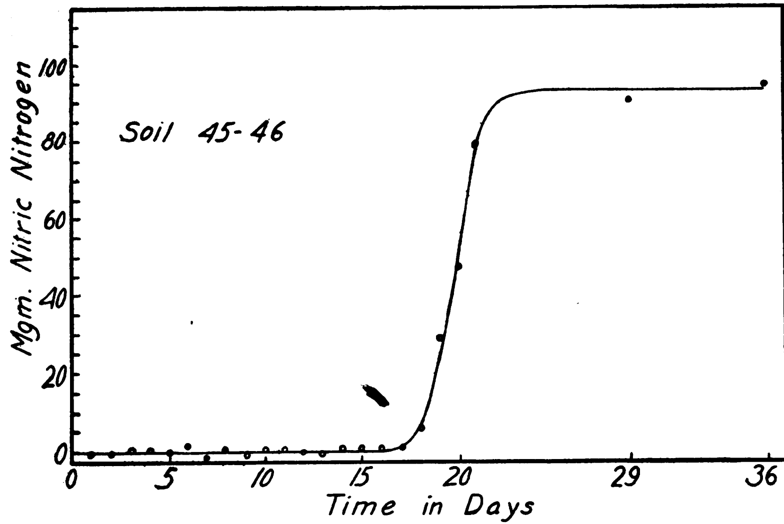

FIG. 6

combination with the sulfate and with the sulfate and chloride is less stimulating.

Nitrifiers are less sensitive to sulfate than to carbonate. The

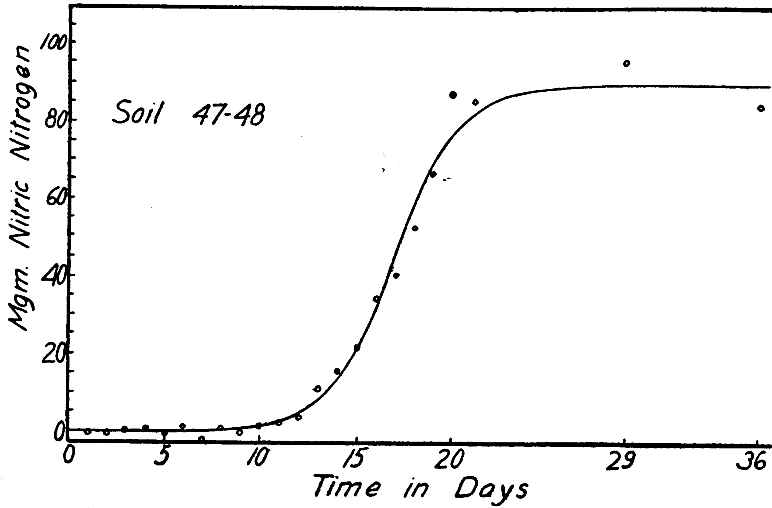

FIG. 7

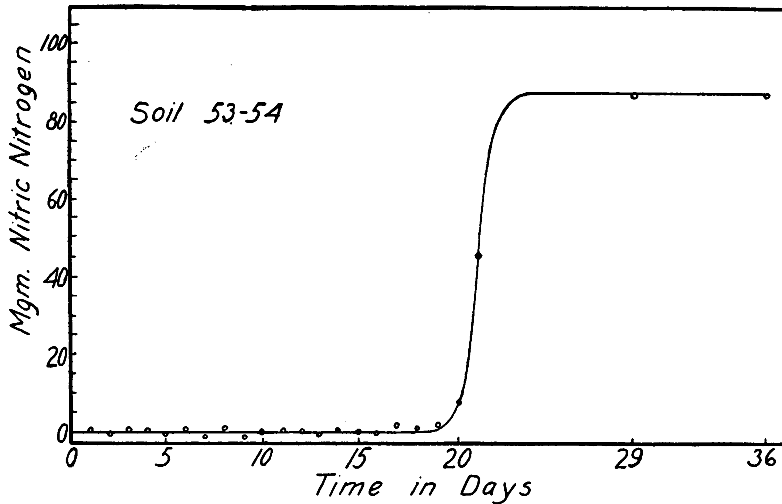

FIG. 8

soil carrying 2 per cent Na_2SO_4 gives $K = 0.41$. The same leached is only 0.30. In smaller amounts stimulation is less pronounced. Sodium chloride also stimulates in the concentrations used but more so in non-leached soils. The 2 per cent NaCl

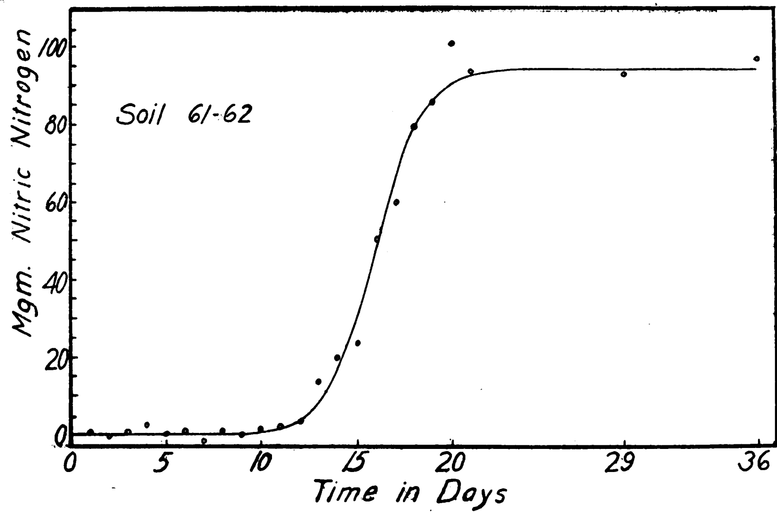

FIG. 9

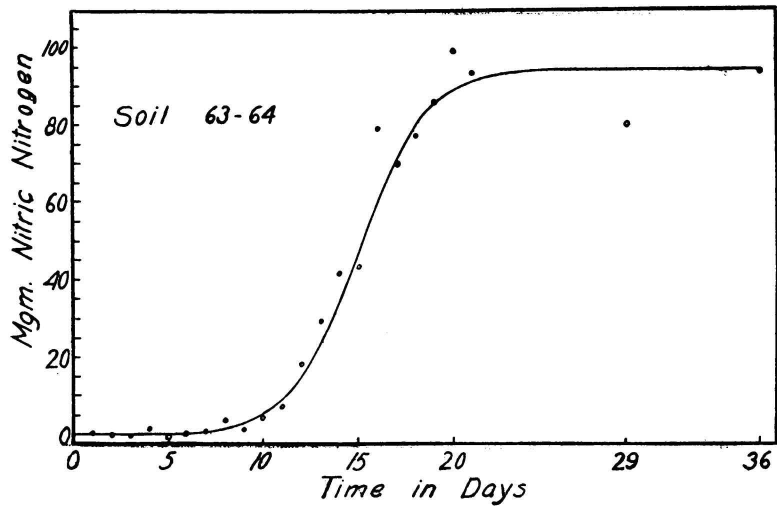

FIG. 10

non-leached soil was lost from the series. The $NaCl$-Na_2SO_4 combination is stimulating, K being 0.62.

The time t_1, in which the "nitrate cycle" is half completed, is not without significance. With the 2 per cent Na_2CO_3 soil it is

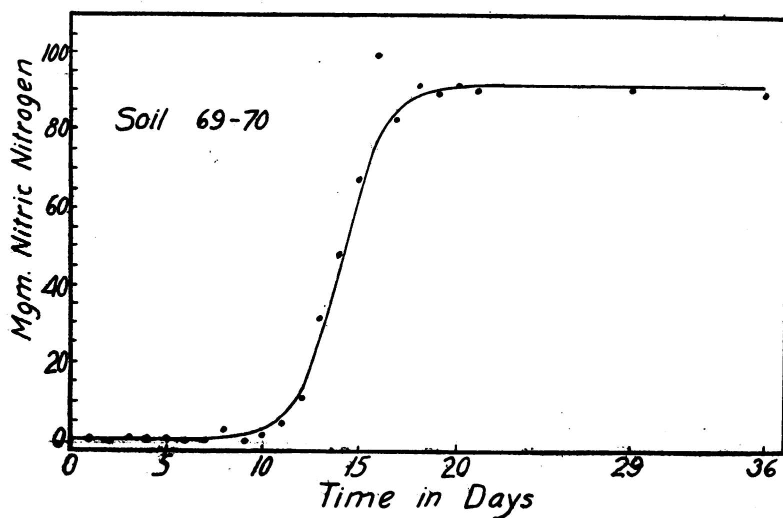

FIG. 11

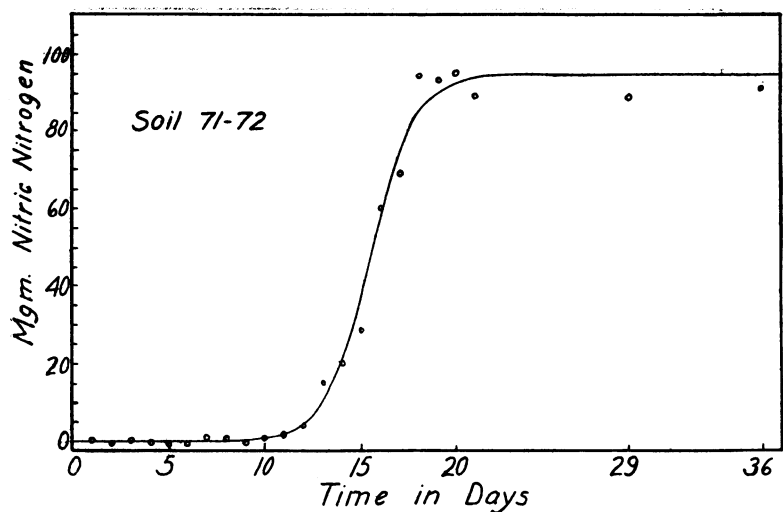

FIG. 12

39.9 days. After leaching this soil, t_1 becomes 13.1 days. The alkali Benson soil is also "slow." The most usual effect of leaching is to shorten t_1. A long lag period (high t_1) is accompanied by a low velocity constant K.

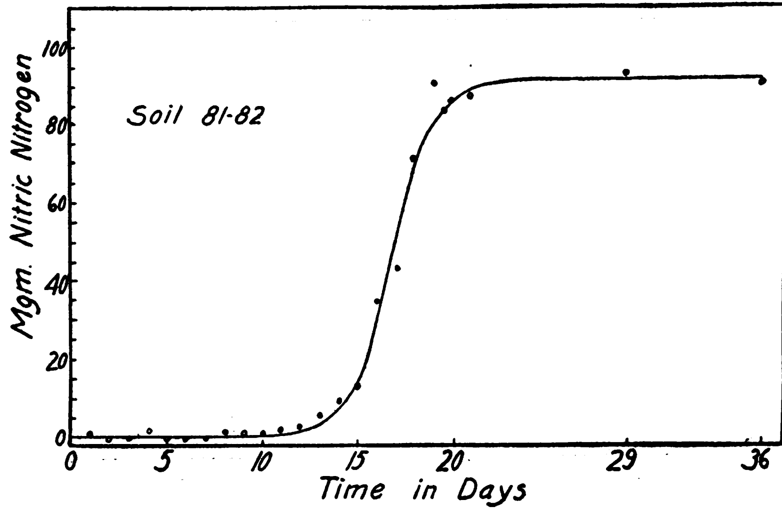

FIG. 13

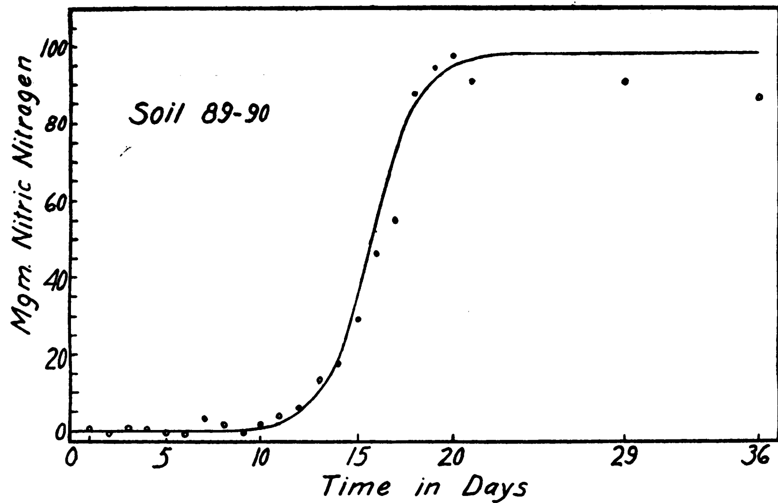

FIG. 14

Leaching is without significant influence on A, the ultimate amount of nitrates formed. This is maximum in native productive soil, both leached and non-leached, but is minimum in 1 case in a toxic alkali soil and in another case in a highly stimulated

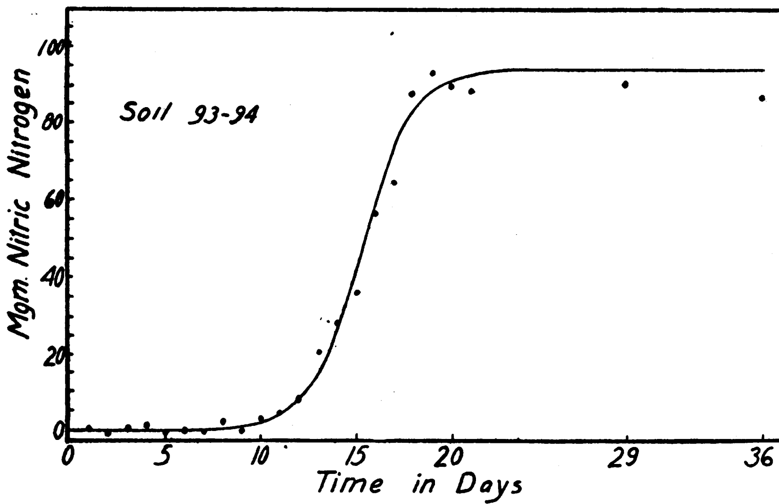

FIG. 15

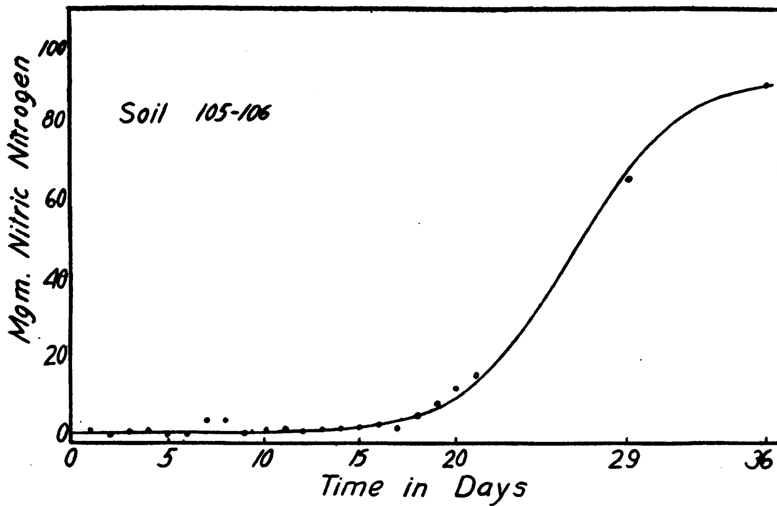

FIG. 16

soil. The constant A is not highly variable but t_1 is. Hence, a better insight into nitrifying powers can be obtained, at least in liquid media, by using an incubation period of fifteen to eighteen days rather than longer. At this time, t_1, the respective curves

are most widely divergent, tending in the progress of time to converge to an approximately uniform maximum, A.

YEAST METABOLISM

In a study on the influence of iodine on the growth and metabolism of yeasts (Greaves, Zobell and Greaves (1928)) considerable data were collected on the rate of carbon dioxide evolution. Both commercial yeasts and pure cultures of *Saccharomyces cerevisiae* were cultured. The initial inoculation varied in different cases from 1.5 to 480,000 cells per cubic centimeter of solution. During short incubation periods the carbon dioxide was measured every two hours after an initial lapse of twenty-four hours. Other tests were run for seven, nine, fourteen, and thirty-one days, the carbon dioxide being measured either daily or every two days. Mayer's culture fluid was used as the medium, modified in various cases by the addition of different sugars and of NaI, KI or I_2 in amounts from 0 to 8000 p.p.m.

Counts of the yeast were made by diluting to the required extent and then placing a drop on the disk of a hemocytometer. Ten groups of 25 squares were counted. In the absence of agreement among duplicates this was repeated. The reported results are the average of a number of determinations and represent the number of yeasts found in one cubic millimeter of the cultural solution at the specific time. One of the greatest obstacles encountered in the work was the obtaining of representative samples as the yeasts tend to adhere.

The data obtained furnish an excellent opportunity to apply the autocatalytic growth formula to yeast metabolism. The determinations were made with reasonable accuracy, and check samples show that they are consistent. The data used in our curve study were obtained from seven series of flasks. The various series differed in the iodine treatment. Each consisted of from 6 to 12 different concentrations of the iodine, in duplicate or triplicate. The data, therefore, include more than 150 culture flasks. The curves which follow are typical of those studied. The equation constants for the theoretical curves are given in table 2.

TABLE 2

Data to accompany figures 17 to 24

The amount of initial inoculation, the incubation period and the media are listed, also the equation parameters for the curves.

Figure number		Incubation period	Initial inoculation	Media	Iodine treatment	Equation constants: K	K'	Days: t_i	t'_i	Milligram CO_2: A	A'
		days									
17	I	1.54	Commercial yeast, 480,000 cells per cubic centimeter	Mayer's with commercial beet sugar	No NaI	1.66		1.65		623	
	II	1.54			1 p.p.m. NaI	1.63		1.76		900	
	III	1.54			10 p.p.m. NaI	1.87		1.66		825	
	IV	1.54			100 p.p.m. NaI	1.85		1.55		627	
18	I	1.5	Commercial yeast, 480,000 cells per cubic centimeter	Mayer's with commercial beet sugar	1 No I_2	2.45		1.09		170	
	II	1.5			1 p.p.m. I_2	1.73		1.14		200	
	III	1.5			10 p.p.m. I_2	2.02		1.17		170	
	IV	1.5			100 p.p.m. I_2	2.26		1.31		130	
19		9	Commercial yeast, 480,000 cells per cubic centimeter	Mayer's with commercial beet sugar	No KI	1.78	0.31	1.89	538	530	280
20		9			100 p.p.m. KI	1.73	0.31	1.86	555	500	330
21	I	7	*Saccharomyces cerevisiae*, 100 cells per cubic centimeter	Mayer's with sucrose	100 p.p.m. KI	0.52		5.17		550	
	II	7			1,000 p.p.m. KI	0.63		4.73		530	
22	III	7	*Saccharomyces cerevisiae*, 50 cells per cubic centimeter	Mayer's + dextrose	10 p.p.m. KI	0.25		10.3		260	
	IV	14		Mayer's + purified sucrose	1,000 p.p.m. KI	0.22		6.83		280	
22	I	31	*Saccharomyces cerevisiae*, 150 cells per flask of 100 cc.	Mayer's from purified chemicals with especially purified sucrose	No KI	0.10		21.0		245	
	II	31			10 p.p.m. KI	0.09		24.7		500	
23		31			100 p.p.m. KI	0.11		23.4		650	
24		31			1,000 p.p.m. KI	0.15		17.9		536	

In the first experiments conducted, each culture flask received an initial inoculation of 480,000 cells per cubic centimeter of culture solution. With this inoculation rapid growth took place and the carbon dioxide evolved was measured every two hours from the twenty-fourth and thirty-sixth hours. From the de-

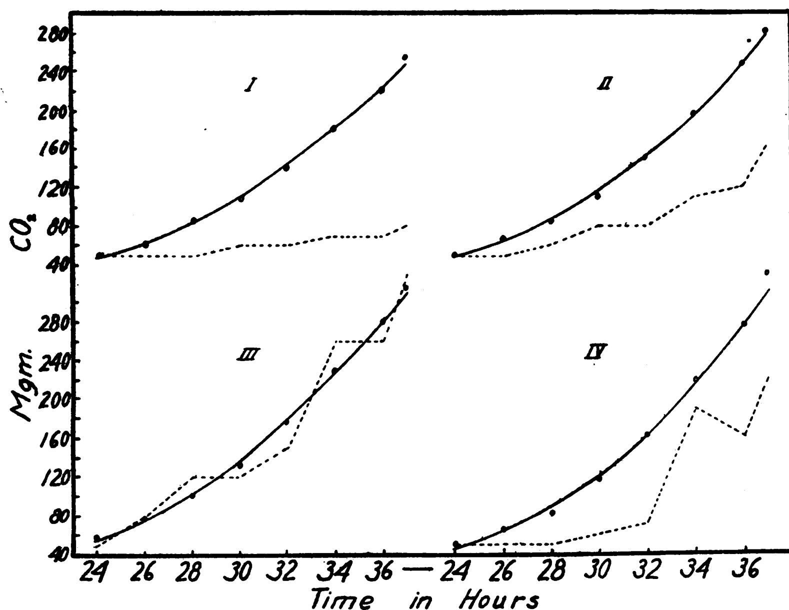

FIG. 17

FIGS. 17–24, inclusive. Time rate of CO_2 evolution by commercial and pure yeast cultures in Mayer's culture fluid varying the carbohydrate and the iodine content of the media and the amount of the initial inoculation. The dots represent experimental observations and the smooth curve the theoretical values. The ordinate scale represents also in hundreds of thousands the counts made of the number of yeast cells per cubic centimeter of solution, which is indicated by the dotted line.

The variations in treatment and inoculation, together with the equation constants for each of the smooth curves, are given in table 2.

terminations curves were calculated by the method already described to fit the experimental observations. Some of these curves are shown in figures 17 and 18 and their equation constants in table 2. K is large for these curves and t_1 is low.

Another set of flasks were similarly inoculated and observed, but the incubation and carbon dioxide measurements were con-

tinued for nine days. Curves from this set are shown in figures 19 and 20. Up to the thirty-seventh hour they closely correspond to those in figure 17. The equation constants are also similar. However, the later observations during the longer period of incubation digress considerably from the theoretical curve. But this digression again fits an autocatalytic curve calculated from

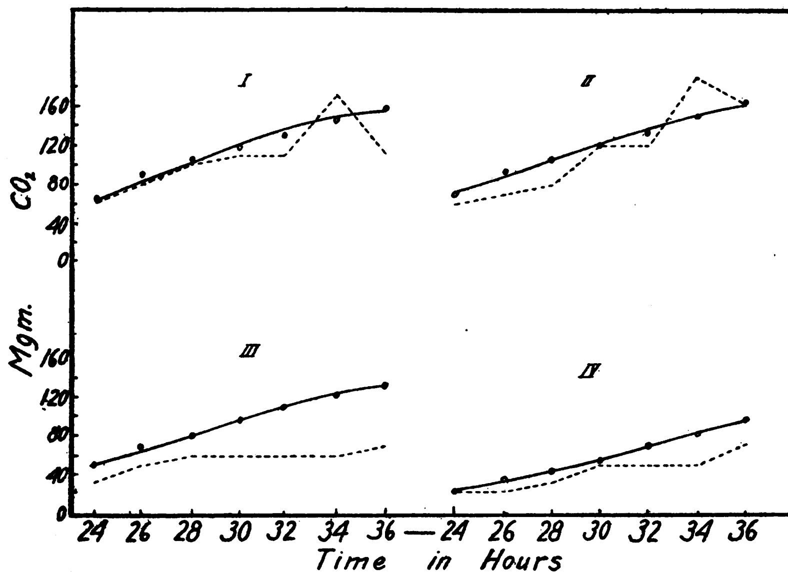

FIG. 18

the same general equation (Equation 3) with a different set of constants. The value of y is the sum of two others obtained for the two curves separately.

$$y = y_1 + y_2 \qquad \begin{aligned} y_1 &= \frac{A \cdot 10^{K(t - t_1)}}{1 + 10^{K(t - t_1)}} \\ y_2 &= \frac{A' \cdot 10^{K'(t' - t_1')}}{1 + 10^{K(t' - t_1')}} \end{aligned} \tag{8}$$

When the two equations are thus added together, the resulting curve fits the observed data with the accuracy indicated in figures 19 and 20. The second curve reaches a lower maximum than the first and the carbon dioxide is evolved at a slower rate.

This compound curve occurs only in the nine-day data with a heavy initial inoculation of a mixed culture. Would such a second cycle curve follow all of the first cycles if incubated long enough? Would the second cycle be followed by still a third and a fourth as time progresses? Would a pure culture act similarly, or is the mixed culture responsible for the double curve? Un-

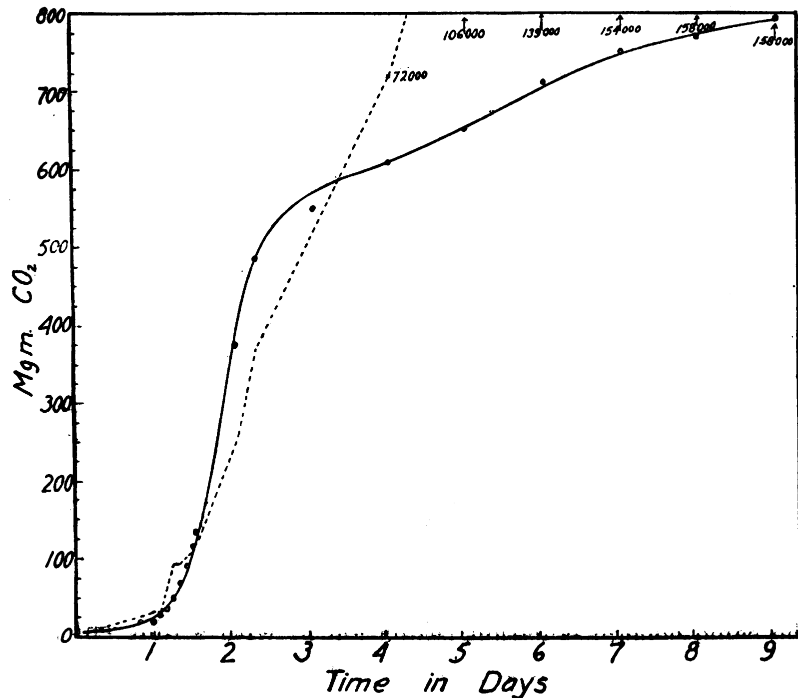

FIG. 19

fortunately, our data do not answer these queries, although Figure 21 (curve IV) and figures 23 and 24 give some indication of a second cycle. But even with these the possibility remains that contamination entered into the cultures toward the close of the experiment after being opened so many times to obtain material for the cell count. Many were terminated for this reason. Furthermore, it is evident particularly in figure 23 that a higher value for A could be chosen which would bring the curve up

through the last data points without increasing its departure from the other data points beyond experimental error, although the fit will not be as good as the one shown. Figure 21 (curve IV) is from a single flask without duplication.

The figures following figure 20 are from data using pure yeast cultures and a more limited inoculation. The incubation period

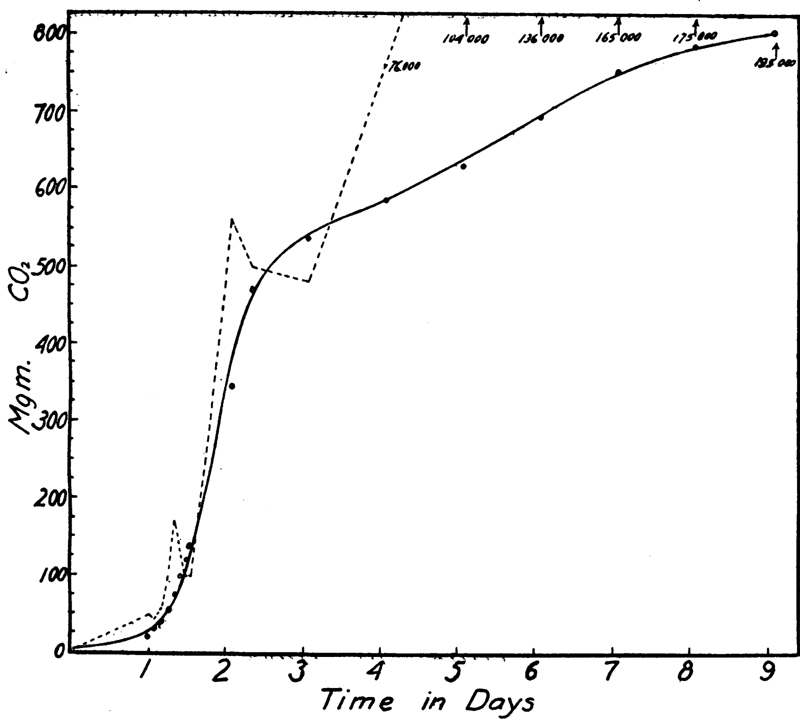

Fig. 20

was in no case shorter than seven days and in some it was thirty-one days.

The constant k decreases as the growth curve becomes longer and slower. The magnitude of A is from 250 to 900 mgm. of carbon dioxide. This varies more with the cultural media and iodine treatment than with the amount of the initial inoculation. The value of t_1 is controlled mostly by the amount of the initial inoculation, being highest where least inoculum is used.

Simultaneously with the carbon dioxide determination, a count was made of the number of cells present in the media. The broken curves on the graphic figures indicate the count obtained. It was more erratic than the carbon dioxide determination, and for this reason does not justify curve fitting. At times the count would suddenly drop, due to clumping of the cells; the numbers were also counted only above 320 thousand cells per cubic centi-

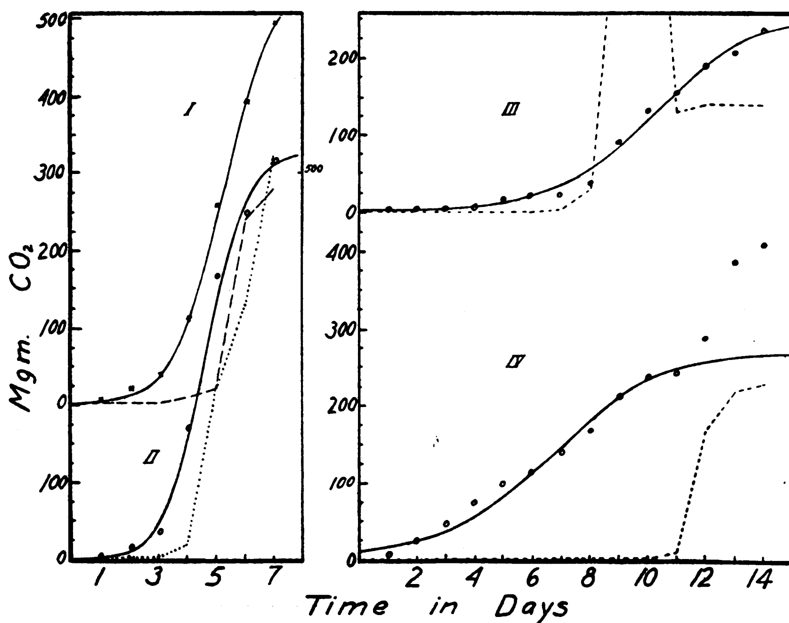

FIG. 21

meter; the data record, no growth, where the numbers are fewer than this. In spite of these inaccuracies, it is evident that the multiplication of cells is most rapid during the period of most rapid carbon dioxide evolution, or immediately following it. The iodine acts as a stimulant up to a concentration of about 1000 p.p.m. and in higher concentration as a depressant. Cell multiplication is more responsive to stimulation by iodine than is carbon dioxide evolution and is also more sensitive to the toxic effects of the higher concentrations.

We may conclude from this study that carbon dioxide is evolved according to the autocatalytic formula. Our data suggest that one curve succeeds another, the succeeding one being more prolonged and of smaller magnitude than the first. Our data on cell multiplication do not contradict other data which show that increase in numbers of cells follows the formula we have used. The increase in the number of cells is not proportional to the carbon

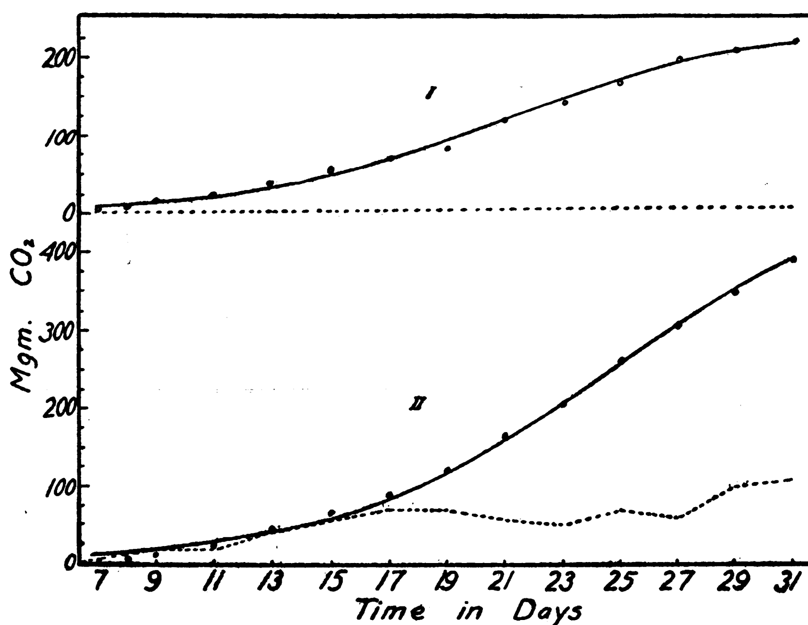

FIG. 22

dioxide production. The cell count will eventually reach a maximum, but the total carbon dioxide evolved will not, as long as any living cells remain. This consideration supports the idea that the evolution of carbon dioxide takes place in cycles, one following another and each decreasing in magnitude but more prolonged than its predecessor, rather than discontinuing at the definite maximum of a single-growth curve.

Our attempt has been to show that the theory advanced by Robertson (1923) to explain the rate of growth is also applicable

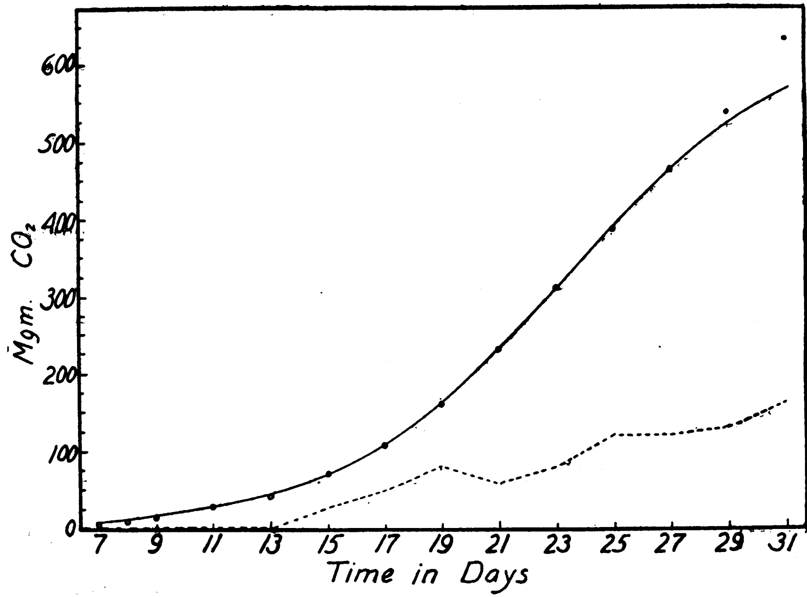

FIG. 23

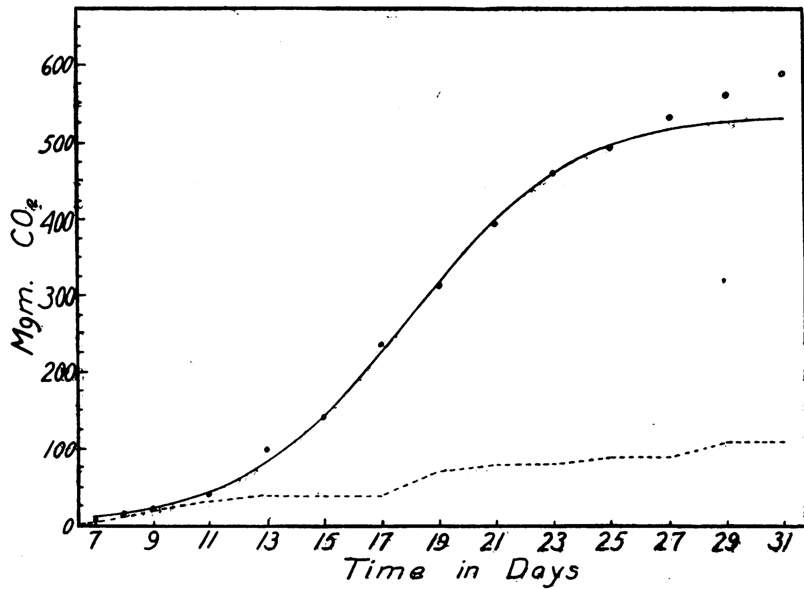

FIG. 24

to the various products of microbial metabolism. Both growth and metabolism are governed by the principle of the "master reaction." Protoplasmic synthesis is the result of a large number of interdependent chemical reactions. If any one reaction is retarded, all are retarded by a proportional amount. A measure of any one then gives a measure of the progress of all the others. It is for this reason that the laws governing the speed of a single monomolecular reaction immediately apply to the sum total of the metabolic activity. Our experimental indices of metabolism are the end products formed, e.g., total growth, nitrates, and carbon dioxide.

SUMMARY

The progress of nitrate accumulation by mixed cultures of soil bacteria and of carbon dioxide by pure cultures of *Saccharomyces cerevisiae* and by commercial yeast cultures can be defined by the equation

$$y = \frac{A \cdot 10^{K(t - t_1)}}{1 + 10^{K(t - t_1)}}$$

which is derived from the equation expressing the speed of a monomolecular autocatalyzed chemical reaction:

$$\frac{dy}{dt} = ky(a - y)$$

In the case of carbon dioxide production it is suggested from limited data that one cycle of evolution follows another, the curve being calculated by adding for the value of y the several separate values at any given time.

In the equation are two variables y, the metabolic product, and t the time, and three constants. The constant K is significant of the conditions of culture—whether favorable, stimulating or depressing. The constant t_1 is influenced most by the amount of the initial inoculation. The constant A is rather independent of environmental conditions until they become deleterious to the organisms. Then, A is notably decreased.

The carbon dioxide produced by yeasts under varying conditions is not proportional to the increase in the number of cells.

Illustrative graphs are given to show the application of the above formula to experimental data.

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