

EXPERIMENTAL STUDIES ON THE STRUGGLE FOR EXISTENCE

I. MIXED POPULATION OF TWO SPECIES OF YEAST

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(With Four Text-figures.)

ALTHOUGH the phenomena of variation and heredity have in recent years become amenable to quantitative treatment, our knowledge of the struggle for existence has been increased to an almost negligible extent during the past fifty years. In the present paper an account is given of a series of experiments which constitute an attempt to apply experimental methods to the study of the struggle for existence. The work has been carried out with two species of yeast, and observations have been made on the effect of the presence of one species on the rate of reproduction of another. This situation has recently been considered from a mathematical point of view by Lotka (1925) and by Volterra (1931).

THE THEORY OF GROWTH OF A MIXED POPULATION.

The growth of a homogeneous population within a limited environment has been studied by Raymond Pearl (1925), who showed that the growth of such a population may be expressed by a logistic curve.

The logistic curve is of the following form:

$$\frac{dy}{dx} = -by \frac{K-y}{K} \quad \dots\dots(i).$$

This indicates that the rate of growth of a population (dy/dx) is proportional to the value of the population already accumulated (y), the inherent rate of growth for the given species (b), and the unutilised potentialities of growth $\left(\frac{K-y}{K}\right)$.

Equation (i) expresses the idea that the smaller be the difference $K-y$, the slower is the rate of growth of the population. This difference is a measure of the degree to which the accumulated value of the population (y) has approached to the limiting value, which is possible in a given microcosm and to which the population tends to attain (K). A given microcosm can only support a population of a limited size. As the population increases, the number of "yet unoccupied places" decreases, and the growth of the population becomes correspondingly reduced. The

unutilised possibility of growth is expressed in the units of a given population. This factor in the logistic equation shows how many free places are available for the population of a given species at a given moment.

Turning to the growth of a mixed population we must take into account that the independent growth of the population of each separate species may be expressed by a logistic curve. What happens in the course of the growth of a mixed population of two species if one species affects the other by an alteration of the environment? If, during the process of the independent growth of each species, the accumulation of its own waste products reduces the number of available places and is of decisive importance, then in a mixed population each species may be influenced by the excretory products of the other species as well as by its own. The investigations of Woodruff (1914) and of Allee (1931) have shown the important rôle of the waste products, as well as their complex influence upon the growth and multiplication of organisms. It will be noted, however, that the logistic equation is not restricted to populations where the unutilised opportunity for growth is responding solely to the effects of accumulation of waste products.

The rate of growth of each species in a mixed population will depend on (i) the inherent rate of growth of the given species, (ii) the value of the population of this species accumulated already, and (iii) the unutilised opportunity for growth, just as is the case of populations of the first and second species growing separately. But the unutilised opportunity for growth of a single species in a mixed population is a complex variable. It measures the number of places which are still vacant for the species in spite of the effect produced by the second species which is consuming the common food and excreting waste products. If we measure the population in units of the mass of living matter (= the weight of the organisms present), then the unutilised possibility for growth of the first species may be expressed in the following form: $\frac{K_1 - (y_1 + m)}{K_1}$, where K_1 is the asymptotic mass of the species for separate growth in the given conditions, y_1 is the already accumulated mass of this species at a given moment in the mixed population, m is the number of the places of the first species which are taken up by the second species at a given moment, expressed in units of the mass of the first species. The "unutilised opportunity for growth" of the first species in the mixed population is more understandable if we compare it with the value of the unutilised opportunity for the separate growth of the same species. In the latter case the unutilised opportunity for growth is expressed by the difference between the asymptotic number of places and the number of places already occupied by the species. Instead of this for the mixed population we write the difference between the asymptotic number of places and that of the places already occupied by our species together with the second species growing simultaneously.

An attempt may be made to express the value m directly by the mass of the second species at a given moment, but it is unlikely that two species will utilise their environment in an absolutely identical way for equal masses of two different species will not consume (on an average) equal quantities of food, and excrete equal quantities of

metabolic products of the same chemical composition. The degree to which at any moment a given mass of a second species has utilised the environment will not be the same as that of an exactly equal mass of another species. If y_2 be the mass of the second species at a given moment, then the places of the first species which it occupies, expressed in units of mass of the first species, will be $m = \alpha y_2$. The coefficient α shows the intensity of influence of the second species upon the growth of the first. The coefficient α is a measure of the degree to which the mass of one species influences the unutilised opportunity for growth of another species.

Taking the coefficient α we can now express in the following form the unutilised opportunity for growth of the first species in a mixed population: $\frac{K_1 - (y_1 + \alpha y_2)}{K_1}$. The unutilised opportunity for growth of the second species will be analogous: $\frac{K_2 - (y_2 + \beta y_1)}{K_2}$. Here β indicates the intensity of the influence of the first species on the unutilised opportunity for growth of the second.

An important feature of a mixed population is the simultaneous influence upon each other of the species constituting it. The rate of growth of the first species depends upon the number of places already occupied by it as well as by the second species at a given moment. As growth proceeds the first species increases the number of places already occupied and thus influences the growth of the second species as well as its own.

To express the growth of a mixed population we may write a system of two logistic equations reflecting the continuous influence of one species upon another. Let dy_1/dx , dy_2/dx be the rates of growth of the first and of the second species in the mixed population at a given moment; y_1 , y_2 being the quantities of the first and of the second species in the mixed population at a given moment; b_1 , b_2 are inherent rates of the separate growth of the first and of the second species; K_1 , K_2 are the asymptotic masses of the first and of the second species when grown separately; α , β are the intensities of influence of the mass of one species on the unutilised opportunity of growth of another species in a mixed population.

The rate of growth of the mass of the first species in the mixed population (dy_1/dx) is proportional to its inherent rate of growth (b_1), the already accumulated mass of the species at a given moment (y_1), and the unutilised opportunity for growth at a given moment $\left(\frac{K_1 - (y_1 + \alpha y_2)}{K_1}\right)$. An analogous relationship holds true for the second species. The growth of the first and of the second species are simultaneous. It can be expressed by the following system of simultaneous differential equations:

$$\left. \begin{aligned} \frac{dy_1}{dx} &= -b_1 y_1 \frac{K_1 - (y_1 + \alpha y_2)}{K_1} \\ \frac{dy_2}{dx} &= -b_2 y_2 \frac{K_2 - (y_2 + \beta y_1)}{K_2} \end{aligned} \right\} \dots\dots(ii).$$

Whether the first species will supplant the second, or whether it will be supplanted by the latter, depends firstly on their inherent rates of growth (b_1 , b_2) and

their maximal masses (K_1 , K_2), which we know from a study of the separate growth of the species. But when we mix two species and a mixed population begins to develop, then new coefficients α and β appear. These coefficients characterise the relations of one species to another as regards the influence of one species on the unutilised opportunity of growth of another. The system of equations (ii) enables us to express quantitatively the growth of a mixed population.

For an adequate study of mixed populations the following plan may be adopted. We begin by a series of experiments on the growth of populations of two species taken separately. The experimental data on the growth of each species may be fitted by means of logistic curves. These curves will give us the parameters b_1 , b_2 , K_1 , K_2 . We next determine the growth of the mixed population of the same two species. The experimental data obtained may be fitted by our system of logistic differential equations. This operation will give us the parameters α and β showing how one species influences the growth of another species in a mixed population.

MATERIAL AND METHODS.

For the experimental study of a mixed population the growth of two species of yeast, *Saccharomyces cerevisiae* and *Schizosaccharomyces kefir*, has been investigated. Their rate of growth is very different, and each species can be easily distinguished by the size and form of its cells. Both species influence the environment by alcoholic fermentation.

The experiment consisted of the inoculation into the culture medium of a certain quantity of yeast belonging to the first, to the second, or to both species together. The increase of the volume of yeast was measured by centrifugation. In the mixed population the number of cells of the first and of the second species were counted in a counting chamber under the microscope. The number of cells constituting a unit of yeast volume in each species, was established by counting the cells in the separate cultures. From these data it is possible to calculate the masses of each of the species in the mixed culture.

Saccharomyces cerevisiae (stock 12) and *Schizosaccharomyces kefir* had been obtained from the Alcohol Research Institute in Moscow. As a nutritive medium the so-called "yeast fluid" was employed; it was prepared in the following manner: 20 gm. of dry pressed beer yeast were mixed with 1 litre of distilled water, boiled for half an hour in Koch's boiler, and then filtered through infusorial earth. To this mixture 5 per cent. of sugar was added, and then the medium was sterilised in an autoclave. The sterile medium was aseptically poured into the test-tubes which had been stopped by cotton-wool and first sterilised by dry heat. All the experiments were made with tubes of about 13 mm. diameter, 10 c.c. of nutritive medium being poured into every tube. This technique was that already employed in another investigation (Gause, 1932). The experiments were made in a thermostat at a temperature of 28° C.

Young cultures, having recently finished their growth, were used for inoculation: *Saccharomyces* 48 hours old, and the slow-growing *Schizosaccharomyces* aged 5 days.

The inoculation was made with a sterilised pipette. It was found that the density of population, or the amount of yeast in 10 c.c. of nutritive medium, for *Schizosaccharomyces* in tubes intended for inoculation was 2.5 times smaller than that in the tubes with *Saccharomyces*. Therefore in order to inoculate an equal initial quantity of each species into the mixed population, two drops of a uniform suspension of yeast cells of *Saccharomyces* and five drops of *Schizosaccharomyces* were added to each tube prepared for the growing of the mixed population. Into the tubes for pure cultures of *Saccharomyces* and *Schizosaccharomyces* two drops and five drops of the yeast suspension were added respectively. After inoculation, at fixed intervals of time, the determination of the yeast volume and the counts of the number of cells were made. For this purpose three tubes were taken. An even suspension of cells was obtained in them by shaking. One cubic centimetre of this suspension was taken from the tube and poured into another clean tube, where 3 c.c. taken from three tubes were fixed by 3 c.c. of 20 per cent. sulphuric acid. After this the fixed material was counted in the Thoma counting chamber, and the number of cells in a unit of volume was determined. The buds in these experiments were not taken into account. In the tables the number of cells in a square of the Thoma counting chamber has been reduced to the dilution obtained after fixation, which was taken as a standard of comparison. There was no difficulty in distinguishing the cells of *Saccharomyces* and *Schizosaccharomyces*. Those of the former species are larger and possess a characteristic structure.¹

When the sample for counting the number of cells had been taken from the tube, the yeast volume was determined in the remaining 9 c.c. of the suspension. For this the suspension was centrifuged for 1 min. in a special tube, placed in an electric centrifuge making 4000 revolutions per minute. The liquid was then poured off and the yeast cells sedimented on the bottom were shaken up with a small quantity of the remaining liquid. The mixture thus obtained was transferred by means of a pipette into a short graduated glass tube of 3.5 mm. diameter. This mixture in the graduated tube was again centrifuged for 1.5 min. and then the volume of the sediment was rapidly measured with the aid of a magnifying glass. A quantity of the mixture was always introduced into the short graduated tube such that the sediment should not exceed 10 divisions of the tube, and, if necessary, the secondary centrifugation was repeated several times. Everywhere in this paper the volume of yeast occupying one division of the graduated tube has been taken as a unit.

In some of the cultures a determination of the quantity of alcohol was made as well. For this 27 c.c. of the liquid, taken from three tubes after the removal of the cells by centrifugation, were distilled and the alcohol determined pycnometrically according to the tables in Abderhalden's *Handbuch der biochemischen Arbeitsmethoden*.

Four series of experiments were carried out, and the results of two of them are given in Table I. In these two series 27,300 individual yeast cells have been counted.

¹ For technical references see Guilliermond, 1912, and Buchanan and Fulmer, 1928.

EXPERIMENTAL RESULTS.

Table I presents the data on the growth of the yeast volume and of the number of cells in *Saccharomyces*, *Schizosaccharomyces* and in the mixed population according to the first and second series of experiments. Fig. 1 represents graphically the growth of the yeast volume. It is obvious that *Schizosaccharomyces* grows slower than *Saccharomyces*, and that the final volume of the former species is smaller. The volume of the mixed population attains a lower level than that of *Saccharomyces* growing by itself.

The volumes occupied by each species in the mixed culture have been evaluated in the following way. First of all a calculation was made of the average number of cells of the given species which occupied a unit of yeast volume when separately grown (Table I). It appears that the number of cells occupying a unit of yeast

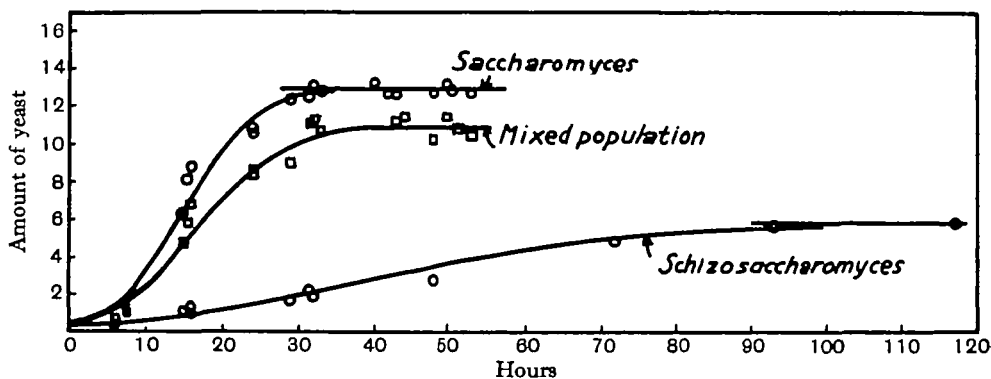


Fig. 1. The growth of the volume of *Saccharomyces cerevisiae*, *Schizosaccharomyces kefir* and the mixed population according to the first and second series of experiments.

volume varies in the course of the growth of the culture. However, this variation is not great, and for further calculations average values can be taken. These give the average number of cells in a unit of yeast volume for the entire cycle of growth of a given species. In *Saccharomyces* according to the first series of experiments 16.59 cells in a square of the Thoma counting chamber after fixation corresponds to a unit of yeast volume, and in *Schizosaccharomyces* 57.70 cells. Starting from these averages we have calculated the volumes occupied by each species in the mixed population at a given moment according to the observed number of cells of each species in the mixed population. The sum of the calculated volumes of both species in the mixed culture at a given moment should agree with the observed volume of the mixed population. In the first series the sum of the calculated volumes is smaller than the observed volume of the mixed population, but the causes of this disagreement is known. In the second series these causes have been eliminated, and it is clear that the sum of the calculated volumes nearly coincides with the observed volume.

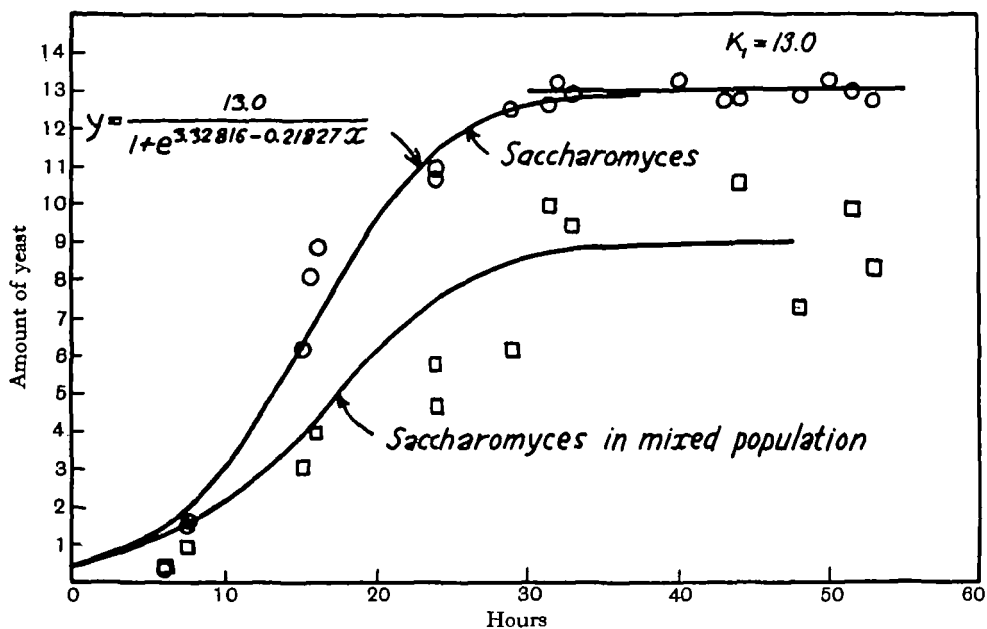


Fig. 2. The growth of the volume of *Saccharomyces cerevisiae* cultivated separately and in the mixed population according to the first and second series of experiments.

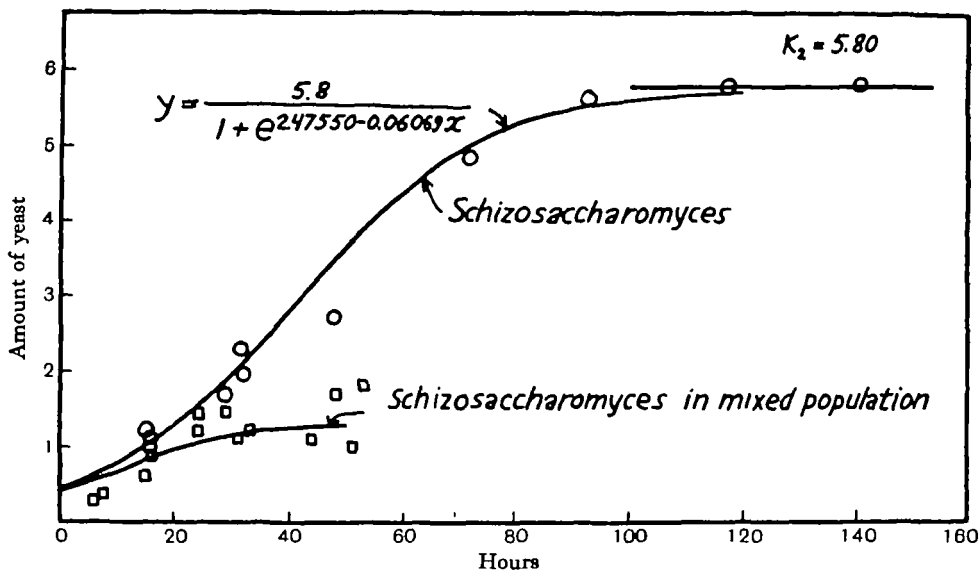


Fig. 3. The growth of the volume of *Schizosaccharomyces kefir* cultivated separately and in the mixed population according to the first and second series of experiments.

Figs. 2 and 3 give the curves of the growth of volume of *Saccharomyces* and *Schizosaccharomyces* in separate cultures and in the mixed population. Other experiments (Richards, 1928; Gause, 1932) have shown that the curve of the separate growth of *Saccharomyces* is somewhat asymmetrical, but this asymmetry is not marked and it is possible to fit the independent growth of each species to a symmetrical logistic function of the following form:

$$y = \frac{K}{1 + e^{a+bx}}$$

Here the constant a defines the original value of the ordinate. The curves of the separate growth of *Saccharomyces* and *Schizosaccharomyces* have been fitted so that in both species with $x = 0$ we have $y = 0.45$. The fitting of logistic equations has been described elsewhere (Pearl, 1930; Gause and Alpatov, 1931). The equations used are given in Figs. 2 and 3. The values of parameters in these equations are as follows (species No. 1 is *Saccharomyces*, species No. 2 is *Schizosaccharomyces*):

$$\begin{array}{ll} K_1 = 13.0 & K_2 = 5.8 \\ b_1 = -0.21827 & b_2 = -0.06069 \end{array}$$

QUANTITATIVE EXPRESSION OF THE EXPERIMENTAL RESULTS.

The growth of the mixed population of the two species of yeast can now be fitted by means of the logistic equations mentioned on p. 391. This operation consists of two parts: (1) the determination of the coefficients α and β , and (2) the construction of the growth curves according to the system of the differential equations.

(1) Determination of the coefficients α and β .

Although the accuracy of the method of calculating these coefficients is sufficient for our present purposes, it may well be improved by further work. The system defined in (ii) may be considered as two equations with two unknown values: α and β . The coefficient α from the first equation was directly expressed by all the other parameters of this equation, and the same was done as regards β in the second equation¹:

$$\alpha = \frac{K_1 + \frac{\frac{dy_1}{dx} \cdot K_1}{b_1 y_1} - y_1}{y_2}; \quad \beta = \frac{K_2 + \frac{\frac{dy_2}{dx} \cdot K_2}{b_2 y_2} - y_2}{y_1}.$$

The experimental data expressing the growth of volume of each species in the mixed population were marked as points on the graph. These points were connected by a smooth curve by hand, and the coefficient α was calculated for several points of this curve as follows: (1) b_1 and K_1 are known from the logistic curve of the independent growth of the first species; (2) y_1 and y_2 , or the volumes of the first and second species in the mixed population at a given moment, were taken from the graph by measuring the ordinates of the corresponding curves; (3) dy_1/dx represents

¹ It must be noted that b is a negative value and therefore the second term in the numerator is subtracted.

the increase of the volume of the first species per unit of time in the mixed population, or graphically the slope of the tangent at a given point. The tangent at a given point was drawn on the graph designed on a large scale, and dy_1/dx was measured graphically. In this manner for three points on the curve of growth of volume of *Saccharomyces* in the mixed population the values α were estimated, and for three points corresponding to the same moments of time, the values β were estimated from the growth curve of *Schizosaccharomyces* in the mixed population. Table II gives the data obtained. It is seen that the coefficient α diminishes with time, but to a first approximation we may calculate the average value of α and thus make this coefficient constant for the whole cycle of growth. The average values of the parameters α and β are: $\alpha = 3.15$; $\beta = 0.439$. The parameter α clearly shows that the influence of the volume of *Schizosaccharomyces* upon the unutilised opportunity of growth of *Saccharomyces* is decidedly marked. A unit of volume of *Schizosaccharomyces* diminishes the unutilised opportunity for growth of *Saccharomyces* in the same measure as 3.15 units of the *Saccharomyces*' own volume. With its comparatively small volume *Schizosaccharomyces* takes up "a great number of places" in the microcosm. It is interesting to note that inversely *Saccharomyces* influences the growth of *Schizosaccharomyces* but slightly. The coefficient β shows that a unit of volume of *Saccharomyces* diminishes the unutilised opportunity for growth of *Schizosaccharomyces* to the same extent as 0.439 unit of volume of the latter species. These relationships will be discussed later.

Table II. *The coefficients α and β ; α shows the intensity of the influence of Schizosaccharomyces on Saccharomyces; β shows the intensity of the influence of Saccharomyces on Schizosaccharomyces.*

Age in hours	α	β
20	4.79	0.501
30	2.81	0.349
40	1.85	0.467
	Mean 3.15	Mean 0.439

(2) *Construction of growth curves from differential equations.*

We can now write our system of simultaneous logistic differential equations (ii) for *Saccharomyces* (species No. 1) and *Schizosaccharomyces* (species No. 2) in the following form:

$$\left. \begin{aligned} \frac{dy_1}{dx} &= 0.21827 y_1 \frac{13 - (y_1 + 3.15 y_2)}{13} \\ \frac{dy_2}{dx} &= 0.06069 y_2 \frac{5.8 - (y_2 + 0.439 y_1)}{5.8} \end{aligned} \right\}.$$

From this system of equations, the growth of volume of each species in the mixed population can be calculated. For these calculations we have chosen the so-called method of approximate numerical integration of Runge-Kutta (described by Runge and König, 1924).

The data thus obtained are represented in the form of curves on Figs. 2 and 3. It is seen that the calculated curves of the growth of *Saccharomyces* and *Schizosaccharomyces* describe accurately the observed growth of these species in the mixed population. Consequently our system of logistic differential equations presents a convenient quantitative expression for the growth of the mixed population consisting of two species.

We may conclude that:

- (1) The system of simultaneous logistic differential equations describes satisfactorily the observed growth of each species in the mixed population.
- (2) According to these equations the coefficient of the influence of *Schizosaccharomyces* upon *Saccharomyces* is 3.15 and that of *Saccharomyces* upon *Schizosaccharomyces* 0.439.

FACTORS LIMITING THE GROWTH OF THE YEAST POPULATION.

What are the factors that determine the cessation of growth in a population of yeast cells under the conditions of the present experiments?

The curves of growth of the yeast volume and of the accumulation of alcohol in *Saccharomyces* are shown in Fig. 4. The second experiment on Fig. 4 corresponds to the second series of experiments recorded in Table I. The first experiment, however, on Fig. 4, does not correspond to the first series of experiments on Table I, but represents another experiment where the composition of the nutritive medium was somewhat different from that in the first series. Fig. 4 shows that a certain time after the experiment has begun the accumulation of alcohol takes place almost in proportion to the accumulation of the volume of yeast. In other terms there exists a proportionality between the metabolism of the yeast cells and the growth of their volume. Later, conditions appear in which the growth of the yeast volume decreases and finally stops. However, the yeast cells remain active, fermentation goes on and alcohol continues accumulating as long as there is sugar in the nutritive medium. This demonstrates that the critical concentration of waste products for the growth of the yeast volume appears earlier than the exhaustion of the food resources in the medium. Recently Richards (1928 *a, b*, 1931) arrived at the same conclusion in his investigations on the growth of population of the yeast cells.

Among the waste products alcohol is undoubtedly the most important, but it is uncertain how far the alcohol itself causes the cessation of growth, or how far it only constitutes an indicator for the accumulation of other waste products, present in small quantities though influencing in a decisive manner the cessation of the growth of the yeast. To elucidate this point experiments were made in which a small quantity of ethyl alcohol was added to the nutritive medium before the inoculation of the yeast. A decrease in the maximal yeast volume was the result. Apparently the critical concentration of the waste products was reached here with a smaller quantity of accumulated yeast volume. Therefore the alcohol itself seems to be one of the most important factors in the complex of conditions determining the cessation of growth of the population of yeast cells.

A determination of the quantity of waste products excreted by a unit of volume in *Saccharomyces* and *Schizosaccharomyces* is of primary importance in all studies involving the rates of growth. The production of alcohol per unit of yeast volume in *Saccharomyces* and *Schizosaccharomyces* is given in Table III. If the production of alcohol per unit of volume in *Saccharomyces* be taken as 1, then in *Schizosaccharomyces* the same production will be 2.186. The comparison of the alcohol production shows why the far from abundant *Schizosaccharomyces* produces such a

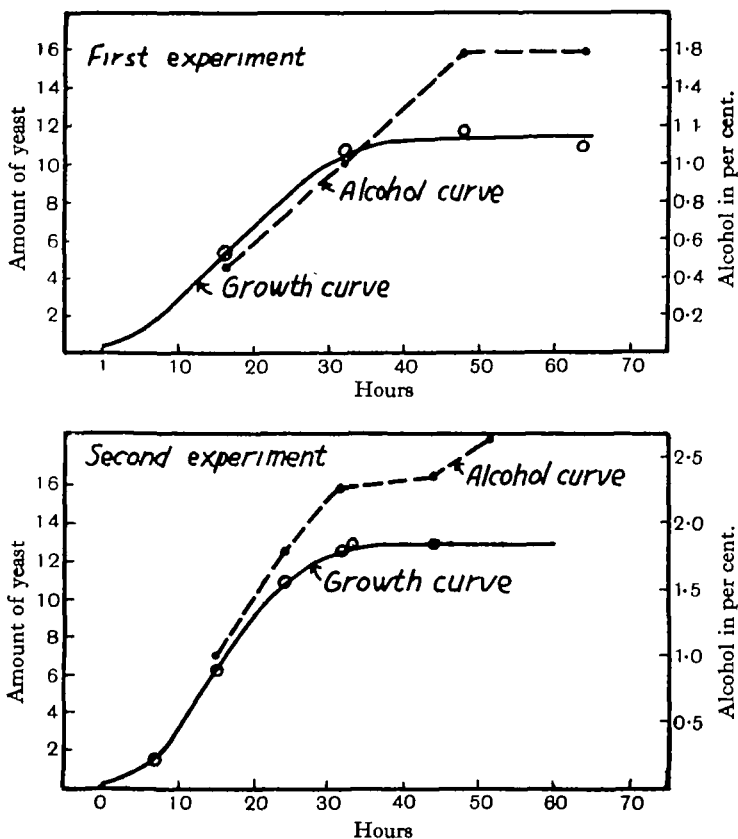


Fig. 4. The growth of the yeast volume and the accumulation of alcohol in *Saccharomyces cerevisiae*.

strong diminution of the volume of *Saccharomyces* in the mixed population. The last species ceases to grow when a certain quantity of waste products becomes accumulated in the nutritive medium. A unit of volume of *Schizosaccharomyces* excretes a quantity of waste products 2.186 times larger than the same volume of *Saccharomyces*. Consequently *Schizosaccharomyces* with a comparatively small volume occupies a great many places in the microcosm. The smaller volume of the mixed population in comparison with the volume of the separately growing *Saccharomyces* can be explained by the same property of *Schizosaccharomyces*.

Table III. *Alcohol production in Saccharomyces cerevisiae and Schizosaccharomyces kefir.*

<i>Saccharomyces</i>				<i>Schizosaccharomyces</i>			
Age in hours	Alcohol %	Yeast volume in 10 c.c. of the medium	Alcohol per unit of yeast volume	Age in hours	Alcohol %	Yeast volume in 10 c.c. of the medium	Alcohol per unit of yeast volume
16	1.100	10.20	0.108	48	0.728	3.08	0.236
16	0.480	5.33	0.090	72	1.425	5.51	0.259
24	1.690	12.22	0.138				
			Mean = 0.113				Mean = 0.247

$$\alpha_1 = \frac{0.247}{0.113} = 2.186.$$

It is interesting to note that the coefficients of the influence of one species upon the other, obtained from the analysis of the growth of the species in the mixed population, coincide in general with the relationships found in the study of the quantity of waste products excreted by a unit of volume in these species. The strong influence of *Schizosaccharomyces* upon *Saccharomyces* is associated with the great quantity of waste products excreted by the unit of volume of the former species. But is the influence of one species of yeast upon the other to be solely determined by their alcohol production? The evidence seems to indicate that it is not so. *Schizosaccharomyces* excretes a quantity of alcohol per unit of volume 2.186 times greater than *Saccharomyces*, but its influence on the latter species as expressed by the coefficient α is 3.15. We mention this as a preliminary indication of the probable rôle of metabolic products other than alcohol. This phenomenon requires further investigation.

The material of this section can be summed up as follows: (1) In the course of the growth of a population of yeast cells in anaerobic conditions, before the food resources of the medium become exhausted, the accumulation of the waste products leads to the cessation of growth. Among the waste products alcohol is of primary importance.

(2) The determination of the quantity of alcohol excreted by a unit of the yeast volume shows that in *Schizosaccharomyces* the quantity of alcohol per unit of volume is 2.186 times greater than in *Saccharomyces*. As a result the former species with a comparatively small volume occupies in the microcosm a great number of places.

(3) The coefficients of the influence of one species upon another, estimated by an analysis of the curves of growth of these species in the mixed population, coincide in general with the results of the determination of their alcohol production. The strong influence of one species upon another is due to the great quantity of waste products excreted by a unit of volume in this species.

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