RELATIONSHIPS BETWEEN INITIAL NUTRIENT CONCENTRATION AND TOTAL GROWTH[1]

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ABSTRACT

Ecker, R. E. (Iowa State University, Ames), and W. R. Lockhart. Relationships between initial nutrient concentration and total growth. J. Bacteriol. **82**:80–84. 1961.—The relationship observed between maximal attainable growth in a microbial population and initial concentration of limiting nutrient depends upon the parameter selected for measurement of growth. A linear relationship is found only when cell mass is the parameter. When population is used as the measure of growth, the relationship is:

$$N_{\max} = K'C_0^s,$$

where $N_{\max}$ is the maximal attainable population, C_0 is the initial concentration of limiting nutrient and K' and s are constants. The use of this expression in demonstrating subtle differences between strains is shown.

A linear relationship between total growth attainable and the initial concentration of limiting nutrient in batch cultures of microorganisms has been demonstrated by Monod (1942). A somewhat different, nonlinear relationship was reported by Hinshelwood (1946), who attempted to reconcile the two observations with the suggestion that exhaustion of the supply of limiting nutrient is not the only cause for termination of growth in such populations. He advanced the hypothesis that, at higher maximal populations, the adverse effect of metabolic products in the medium becomes a growth-limiting factor and causes a deviation from the linear relationship observed by Monod.

It is suggested here that such an assumption is unnecessary, for it can be shown that the observed differences arise from the use of different growth parameters by the respective authors, one of whom employed cell mass as the measure of maximal growth, whereas the other used cell numbers.

MATERIALS AND METHODS

Escherichia coli strain K12 was cultivated in a defined medium containing: $K_2HPO_4 \cdot 3H_2O$, 0.7%; KH_2PO_4, 0.3%; $MgSO_4 \cdot 7H_2O$, 0.01%; $(NH_4)_2SO_4$ and glucose, variable quantities to provide the desired limiting factor; deionized water. pH of the medium was 7.0. Glucose was sterilized separately by autoclaving and added aseptically before use.

The apparatus used for aeration control, and its calibration for rates of oxygen availability, have been previously described (Ecker and Lockhart, 1959*b*, 1961; Lockhart and Ecker, 1958). Aeration rates were in excess of those which had been found to be limiting at the population levels attained. Inoculum cultures were grown in a glucose-salts medium containing 1.0% glucose, 0.1% $(NH_4)_2SO_4$, and 0.5% sodium citrate in addition to the constituents previously mentioned. Inocula were centrifuged at 1,500 × *g* for 20 min and resuspended in pH 7.0 phosphate buffer before use. All incubation was at 37 C. Total counts were made by a membrane filter method (Ecker and Lockhart, 1959*a*). Estimations of total cell mass (as protein) were made as follows: to 0.5 ml of sample, containing from 10^7 to 10^8 washed cells, were added 0.3 ml of 4.0 N NaOH. Samples were then heated in a boiling water bath for 3 to 4 min. After cooling rapidly, 0.2 ml of 4.0 N HCl was added. Then the method of Lowry et al. (1951) was followed, with volumes appropriately adjusted for the larger sample. Optical densities of samples were read in a Coleman Universal spectrophotometer at 700 mμ and compared to a standard curve made

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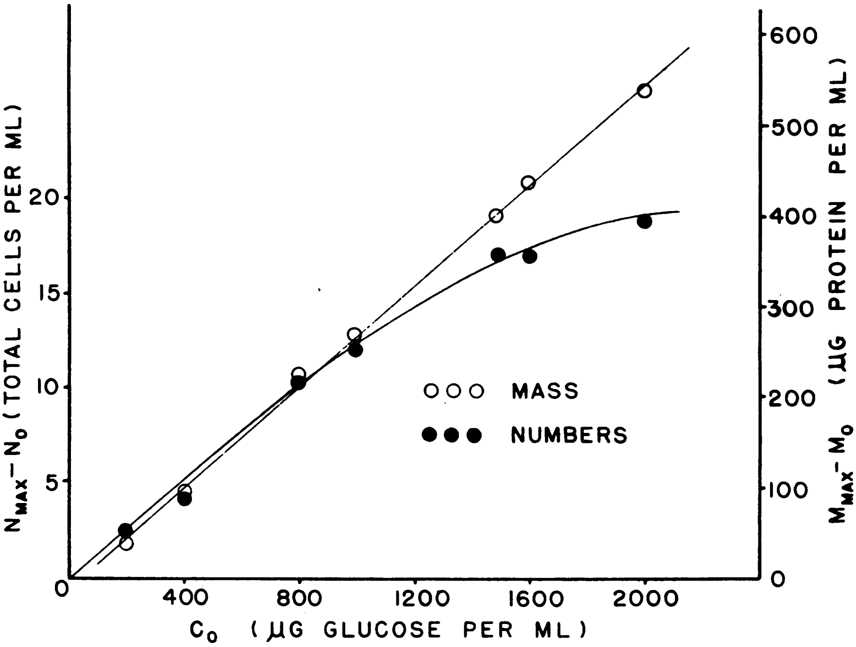

FIG. 1. *Maximal numbers and mass produced as a function of initial glucose concentration for a series of cultures of Escherichia coli strain K12 in 500 ml of aerated defined media at 37 C.*

using weighed amounts of crystalline bovine serum albumin (Armour Laboratories, Chicago).

RESULTS AND DISCUSSION

The curves in Fig. 1 show the basic relationships between total attainable growth and initial nutrient concentration, and demonstrate their dependence on the choice of growth parameter. These are cultures in which glucose was limiting; similar results are obtained when the nitrogen source is the factor limiting growth.

The relationship shown in Fig. 1 between nutrient concentration and mass is the same as that reported by Monod (1942) and can be expressed numerically as

$$M_{\max} = KC_0, \tag{1}$$

where $M_{\max}$ is the maximal attainable mass,[3] C_0 is the initial concentration of the limiting nutrient and K is a constant of proportionality, the "yield constant" of Monod (1942).

Over a significant range of concentrations the curve in Fig. 1, showing maximal attainable population[3] ($N_{\max}$) as a function of initial nutrient concentration, can be given by the expression

$$N_{\max} = K'C_0{}^s, \tag{2}$$

where s has a positive value less than 1.0. K' and s are constants, the significance of which can be observed more easily using the logarithmic form of equation 2,

$$\log N_{\max} = s \log C_0 + \log K'. \tag{3}$$

A plot of $\log N_{\max}$ as a function of $\log C_0$ provides a straight line with slope s and with intercept of $\log K'$ on the $\log N_{\max}$ axis. Figure 2 shows such a log-log plot for two series of cultures, one limited by the supply of glucose and the other by the availability of $(NH_4)_2SO_4$.

The data given by Hinshelwood (1946) resemble closely this $N_{\max}$ vs. C_0 relationship and can be represented by equation 2. This phenomenon was also reported by Butterfield (1929), who presented an expression identical to equation 2 for population growth in a medium containing

[3] More accurately, the values in equations 1 and 2 should be ($M_{\max} - M_0$) and ($N_{\max} - N_0$), respectively. That is, the actual yield is the maximal mass (or numbers) attained, minus the inoculum. Since in most cultures the inoculum values M_0 and N_0 are negligible in comparison with $M_{\max}$ and $N_{\max}$, this consideration has been omitted from the mathematical expressions for the sake of simplicity.

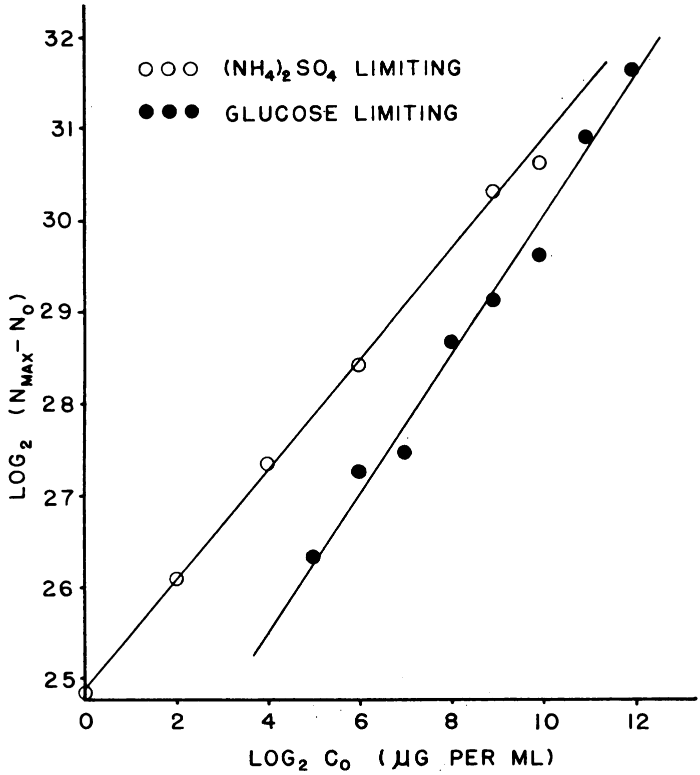

FIG. 2. *Log of the maximal attainable population as a function of log of initial concentration of limiting nutrient for cultures of Escherichia coli strain K12 in 10 ml of aerated defined media at 37 C.*

variable amounts of glucose and peptone. The generally accepted explanation for the differences between such results and those of Monod, that the large populations obtained at greater nutrient concentrations create adverse changes in the medium which exert limiting effects, has not been supported by experimental evidence.

It seems likely that the different results obtained are due to the choice of growth parameter rather than to any differences in culture conditions. This is not to say that accumulation of toxic substances in the medium may not eventually limit the growth of a population, but such effects are not observed at the relatively low population levels reported here.

It is not surprising that growth and cell division operate independently. Many chemical agents selectively inhibit either growth or division without appreciably affecting the other (*see* Loveless, Spoerl and Weisman, 1954). Henrici (1928) showed that average cell sizes may change appreciably between different phases in the normal growth of a population. Monod (1949) emphasized the danger of indiscriminate exchange of growth variables, pointing out that there is no justification for considering bacterial density (mass) and cell concentration (numbers) to be equivalent.

Figure 1 indicates that, as the maximal attainable population increases, the average cell size increases. Not only is it invalid, then, to assume that cell size is constant between phases of growth, but it is impossible to make this assumption concerning cells at any point in the stationary phase if the maximal populations are not also constant. The relationship between initial substrate concentration and nephelos or optical density readings is the same as that shown in equation 1, indicating that these parameters are related to cell mass rather than to numbers.

Monod (1949) suggests three basic growth constants: the length of the lag, the rate of exponential growth and the total attainable growth. However, in many cases the differences in these constants for closely related strains are not great enough to be detected. Monod (1942) also

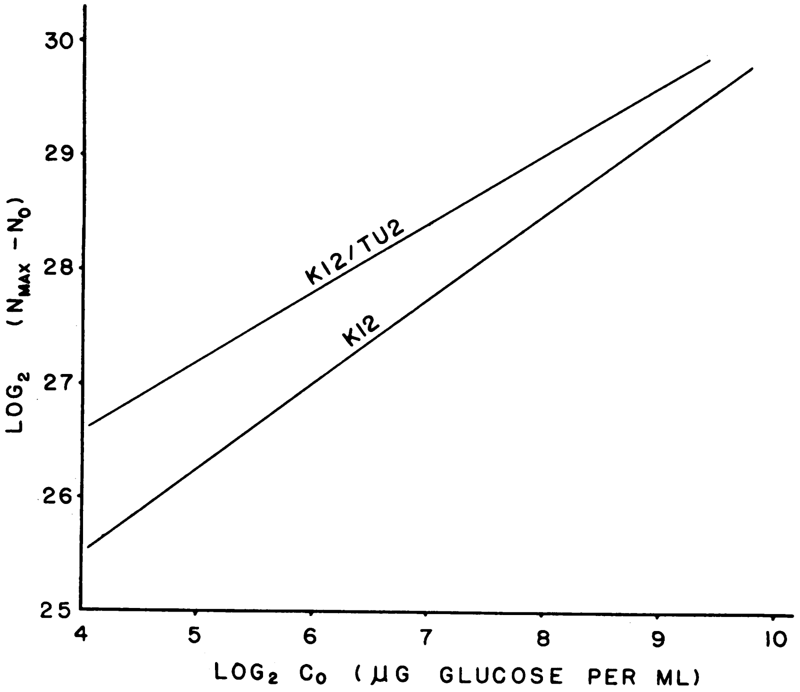

FIG. 3. *Log of the maximal attainable population as a function of log of initial glucose concentration for cultures of two strains of Escherichia coli in 10-ml volumes of aerated defined media at 37 C.*

demonstrated that the mass yield constant (K in equation 1) is characteristic for a given strain in a given medium, but, as can be seen from his data, these values do not vary a great deal from one species to another so the yield constant would not be a particularly useful diagnostic tool in demonstrating subtle differences between closely related strains. On the other hand, the relationship in equation 3 can be very helpful in pointing out some of these differences.

Although equation 3 provides two constants of use in comparing the growth characteristics of different strains, care must be taken not to attribute overdue significance to them. The relationship shown in equation 2 is an approximation for what is probably a small portion of the curve relating total attainable population and initial nutrient concentration. It is not suggested that this is necessarily the correct equation for the total relationship between these two parameters, nor that the constants of this expression have any precise physiological significance. In general, however, the curve whose nature is reflected in these constants is a true representation of physiological events and the use of s and K' for qualitative description of these events is thus justified.

The total efficiency of growth, as measured by increase in numbers, decreases with increasing maximal population. K' is the theoretical maximal population produced from a single unit of nutrient, so this constant in general reflects the efficiency at low maximal populations. s indicates the nature of the change, or decrease, from this efficiency as the maximal population increases. For strain K12 of *Escherichia coli*, grown under glucose-limiting conditions, the values of these constants are: $s = 0.75$ and $K' = 6.0 \times 10^6$.

In comparing this parent strain with several mutants, little significant difference is found in the basic growth characteristics. Exponential growth rate remains extremely stable and the lag, within the limits of its usefulness as a growth constant, does not appear to vary. Maximal attainable growth, without consideration of other factors, does not provide a meaningful comparison. On the other hand, the N_{max} vs. C_0 relationships are often quite different between strains which are otherwise similar in their growth characteristics. For example, when strain K12 is compared to strain K12/TU2, a mutant strain selected for its resistance to thiourea, their basic growth characteristics are the same. Yet Fig. 3 shows that they are not at all the same in efficiency of glucose utilization for increase in cell numbers. The values of the constants for K12/TU2 are: $s = 0.62$ and $K' = 1.8 \times 10^7$. These values, when compared with those given for strain K12, indicate that the mutant is more efficient at lower maximal populations but decreases in efficiency more rapidly with increased maximal population than does the parent strain.

Although work on the subject is continuing, no explanation is presently available for this phenomenon. During the course of the growth of such populations it is assumed that nutrients will be utilized for at least three general processes, two generative and one "sustentative." The former are mass synthesis and cell division and the latter is a still obscure process in which the cells are observed to continue in their utilization of nutrient after growth has ceased (*see* Lockhart and Powelson, 1954). We have as yet no clear idea as to the cellular and environmental factors controlling the relative predominance of these (and possibly other) processes at any given time in the growth of a population. However, this characteristic behavior of a particular strain can provide a tool to assist in the search for an understanding of growth control mechanisms.

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