Kinetic Constants for Aerobic Growth of Microbial Populations Selected with Various Single Compounds and with Municipal Wastes as Substrates

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The general applicability of the Monod relationship between the logarithmic growth rate constant and substrate concentration was studied for heterogeneous populations metabolizing a variety of substrates including concentrated municipal sewage. It was found that growth could be described by the Monod equation,

\[ \mu = \frac{\mu_m(s)}{k_s + s} \]

where \( \mu \) is the logarithmic growth rate constant, \( s \) is the substrate concentration, \( \mu_m \) is the maximum logarithmic growth rate constant for the culture under the conditions used, and \( k_s \) is the substrate concentration at which \( \mu = \mu_m/2 \). Several modifications of this equation have been suggested [for review, see (5)]. Applications of the original and modified equations to heterogeneous populations or waste treatment processes have been proposed [for review, see (2)], but many design formulations in present use assume a linear relation between \( \mu \) and \( s \) rather than the hyperbolic one described by the Monod equation.

We have previously reported that the Monod relationship best describes the growth of heterogeneous populations in a synthetic waste containing glucose as the sole carbon source and limiting nutrient (3, 7). Glucose was employed in these studies because it is readily used by many organisms and probably permits the maximum heterogeneity of species which can be obtained in a minimal medium. The studies reported herein were undertaken to extend the usefulness of the previous work by testing the applicability of the Monod equation to heterogeneous populations growing on a variety of substrates. As the most severe possible test of the utility of the equation in biological waste treatment, municipal sewage was concentrated and the soluble portion was used as substrate.

MATERIALS AND METHODS

Microbial populations were developed on each carbon source from initial inocula of sewage obtained from the municipal treatment plant at Stillwater, Okla. Five milliliters of primary clarifier effluent were added to 45 ml of synthetic medium containing a specific carbon source (1,000 mg/liter of final concentration, see Table 1) and aerated on a shaker apparatus (250-ml Erlenmeyer flasks, 90 oscillations per min, temperature 23 ± 2°C). Daily, 5 ml of the cell suspension was transferred into 45 ml of fresh medium. After seven such transfers, a portion of the mixed culture was employed in growth rate studies. Using the remainder of the culture, the daily transfer procedure was continued for several weeks, and the growth rate experiment was repeated.

In the growth rate experiments, 2.5 ml of the cell suspension was placed in each of the growth flasks (250 ml, Erlenmeyer), and the volume was made up to 50 ml with growth medium containing various concentrations of the carbon source under study. The inorganic salt concentrations were those given in Table 1, except that for substrate concentrations above 1,000
mg/liter the mineral components were proportionately increased. The concentration of carbon source was varied from 10 to 1,500 mg/liter. In a few experiments, growth on nine concentrations of substrate was assessed, but in most cases eleven concentrations were employed. The course of growth in each flask was assessed optically as percent transmittance at 540 nm (spectrophotometer, Bausch & Lomb Inc., Rochester, N.Y.) converted to optical density.

### RESULTS AND DISCUSSION

The logarithmic growth rate constant, \( \mu \), for each substrate concentration was obtained from a plot of optical density versus time on semilogarithmic paper. These values were then plotted versus initial substrate concentration, \( s \), for examination of the shape of the curve developed and estimation of \( \mu_m \) and \( k_s \). Values of \( \mu_m \) and \( k_s \) were also calculated from plots of values of \( s/\mu \) versus \( s \) (slope intercept form). In general, values of the "growth constants" calculated from either type of plot were comparable. However, use of the slope intercept plot allows a somewhat more mathematically precise estimate of the constants from the experimental data, and only these values are reported.

In all, 27 sets of experiments were undertaken, 25 with various pure compounds as sole source of carbon and 2 with sewage. An example of the relationship which was generally observed for the pure carbon sources is shown in Fig. 1. The graph of \( \mu \) versus \( s \) shows that a continuous (single-phase) hyperbolic curve (as described by the Monod equation) provides a much better fit to the data than does a straight line intersecting a horizontal line through \( \mu_m \) (see dotted lines). Only at very low substrate concentrations could a straight line be fitted through the data. Significant differences in the values of the growth constants were observed in the two experiments. Because of the heterogeneity of the population and the attendant opportunities for changes in predominance in such systems, a usable range rather than a precise value must be expected for the constants in any kinetic model which is applied to depict growth of heterogeneous populations.

The values of growth constants obtained for all experiments are given in Table 2. In general, hyperbolic curves were obtained in plots of \( \mu \) versus \( s \) for all experiments. In 7 of the 27 experiments the hyperbolic fit was questionable, but in each of these cases a continuous hyperbolic curve provided a better fit of the data than a straight line (see Fig. 1). With most of the substrates used, different values for \( \mu_m \) and \( k_s \) were obtained in experiments carried out at different times. In most
TABLE 2. Values of \( \mu_m \) and \( k_s \) for heterogeneous populations growing on various substrates

| Substrate     | Expt. no. | \( \mu_m \) (hr\(^{-1}\)) | \( k_s \) (mg/liter) |
|---------------|-----------|-----------------------------|----------------------|
| Glucose       | 1         | 0.49                        | 29                   |
| Glucose       | 2         | 0.38                        | 11                   |
| Lactose       | 1         | 0.53                        | 55                   |
| Lactose       | 2         | 0.44                        | 37                   |
| Lactose       | 3         | 0.20                        | —                    |
| Lactose       | 4         | 0.43                        | 33                   |
| Sucrose       | 1         | 0.55                        | 17                   |
| Sucrose       | 2         | 0.28                        | 6                    |
| Sorbitol      | 1         | 0.60                        | 18                   |
| Sorbitol      | 2         | 0.44                        | 13                   |
| Alanine       | 1         | 0.33                        | 27                   |
| Alanine       | 2         | 0.18                        | 15                   |
| Glutamic acid | 1         | 0.78                        | 47                   |
| Glutamic acid | 2         | 0.59                        | 95                   |
| Serine        | 1         | 0.43                        | 50                   |
| Serine        | 2         | 0.54                        | 30                   |
| Histidine     | 1         | 0.50                        | 17                   |
| Histidine     | 2         | 0.67                        | 50                   |
| Phenylalanine | 1         | 0.33                        | 41                   |
| Phenylalanine | 2         | 0.33                        | 54                   |
| Cysteine      | 1         | 0.16                        | 23                   |
| Acetic acid   | 1         | 0.36                        | 41                   |
| Acetic acid   | 2         | 0.29                        | 47                   |
| Propionic acid| 1         | 0.38                        | 6                    |
| Propionic acid| 2         | 0.37                        | 17                   |
| Sewage        | 1         | 0.49                        | 41                   |
| Sewage        | 2         | 0.43                        | 62                   |

*Value of \( \mu_m \) and \( k_s \) were obtained from plots of \( s/\mu \) versus \( s \).

cases there was macroscopic evidence of a change in predominance in the mixed culture during the interval of several weeks between replicate experiments, i.e., the color and appearance of the culture usually changed noticeably.

The experiments in which sewage was employed as substrate are particularly interesting since it is one of the most complex and heterogeneous carbon sources known, and it was important to determine the existence and nature of a relationship between \( \mu \) and \( s \). For growth rate studies, sewage is a difficult medium to use, since it represents a rather dilute carbon source system and contains a considerable amount of suspended organic material and its composition can vary widely. The total organic content measured as chemical oxygen demand (COD, reference 1) of the effluent from the primary clarifier (i.e., settled sewage) of the municipal treatment plant at Stillwater varied, during the period of sampling, from 150 to 400 mg/liter. Approximately 50% of this COD was retained on a membrane filter, pore size of 0.45 \( \mu \)m. Thus, in order to obtain the required range of substrate concentrations for growth rate studies, it was necessary to concentrate the settled sewage. The substrates used in the two growth rate experiments on sewage consisted of a series of dilutions of the soluble (filterable) fraction of settled sewage which had been concentrated to \( \frac{1}{320} \) of its original volume. Portions of 500 ml each were evaporated under vacuum to a final volume of 25 ml in an evaporator (Buchler Instrument Co.) Evaporation temperatures of 65 and 55 C were employed (substrates for experiments 1 and 2, respectively). There was a significant loss of COD during concentration. Determinations of the COD of the settled sewage before concentration and at concentrations of 5-, 10-, 15-, and 20-fold indicated that, at all concentration ratios, approximately 35% of the sewage COD was volatilized and swept out of the system when

![Fig. 2. Relationship between \( \mu \) and \( s \) for heterogeneous microbial populations grown on a heterogeneous carbon source, the soluble fraction of concentrated sewage. (a) Sewage concentrated under vacuum at 65 C. (b) At 55 C.](image-url)
Concentration was conducted at 65°C. At 55°C, 20 to 25% of the sewage COD was volatilized during concentration. The COD removed from the evaporating flask was not recovered in the condensation flask, indicating that the material was quite volatile (strippable). The condensate was analyzed for COD and never contained more than 30 mg/liter COD. Check runs were made in which a known concentration of glucose solution was placed in the apparatus at 65°C and 100% of the glucose was retained in the evaporation flask. Also, a solution of acetic acid was concentrated. Complete recovery of this compound was attained; 30% remained in the evaporation flask and 70% was found in the condensation flask. Thus, it appears that the apparatus functioned properly and that the material stripped from the settled sewage under the conditions of evaporation was of such volatility that it could not be condensed in the cold flask and was forced from the system. Concentration studies at lower temperatures were conducted, but the time required to concentrate a 500-ml portion to 25 ml was sufficient to allow microbial growth and it was undesirable to add microbial inhibitors. Each 25 ml of concentrated sewage was filtered immediately through membrane filters, 0.45-μm pore size, and the filtrates were pooled and stored at 0°C for use in a growth rate experiment. Thus, the sewage substrate consisted, not of all the organic components of sewage, but of the soluble organic portion minus the highly volatile material in the sewage. In any event, there should be no doubt about the complexity and heterogeneity of this substrate. The concentrate exhibited the characteristic odor of fresh sewage and the appearance of urine. It was slightly alkaline, pH 8.5. This material diluted to various concentrations (expressed as COD) in distilled water comprised the sole growth medium (i.e., no mineral salts or buffer was added). The pH remained at 8.5 during the growth experiments. The heterogeneous populations used as initial inocula in the growth experiments consisted of cells grown from random sewage samples through 3 to 5 serial, 24-hr transfers in the sewage medium.

Plots of μ versus s for experiments run on sewage concentrated at 65 and 55°C are shown in Fig. 2. The decrease in growth rate constant with high substrate concentrations (1,000 and 1,300 mg/liter COD, Fig. 2b) suggests that an inhibitory compound may have been removed from the sewage at 65 but not at 55°C. The results leave little doubt concerning the validity of the Monod relationship for this complex “substrate.” Values of μm are approximately the same as those observed in the many experiments reported previously using glucose as carbon source (3, 7), and they are in general agreement with the other values obtained in the present study (see Table 2).

The agreement between values obtained with sewage and with some pure compounds, e.g., glucose, as substrates is perhaps not unpredictable since a simple medium may often be converted to a “complex” one by the accumulation of partially metabolized products (2).

In general, based upon the massive amounts of data previously reported (3, 7) for heterogeneous populations using glucose as substrate, the experimental results presented here in which various single carbon sources were employed and, of even greater importance, those in which sewage medium was employed, it is concluded that the hyperbolic function can be employed to describe the relation between growth kinetics and substrate concentration for systems which may be heterogeneous with respect to both species of microbe and carbon sources. Clearly, the kinetic constants μm and Ks cannot be considered as precise values for systems in which species predomination is subject to fluctuations and a usable range of values must be employed in depicting the behavior of the system.

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