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# Growth Kinetics of *Colpoda steinii* on *Escherichia coli*

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*Colpoda steinii* was grown in two-stage continuous cultures with *Escherichia coli* as prey species. The concentration of prey and the ciliate mean cell volume, dry weight, and number per milliliter were determined at known growth rates. Steady states were reached in the second-stage continuous cultures at all growth rates. Although changes occurred in mean cell size of the ciliates and in the number per milliliter at various growth rates, the yield of protozoan biomass per unit of prey consumed was constant at all growth rates. The data were compared with several equations proposed to describe the kinetics of protozoan growth as a function of prey density.

In natural aquatic systems, the bacterial flora may provide the primary source of nutrients for many ciliated protozoa. The extent to which protozoan predation can affect either the composition or the total numbers of bacteria in such microbial communities could be considerable. Predation is only one process in the dynamics of a natural microbial community, and other processes may contribute to gains or losses in composition or total numbers of bacteria. Quantitative, or qualitative, estimates of the losses due to predation cannot be made from measurements on the populations of predator and prey unless the other processes are known or negligible. Measurements of the predator and prey populations can yield estimates of the losses due to predation if the relationships between prey density and predator growth rate are known.

A few descriptions of the growth rate of ciliated protozoa as functions of the prey concentration have been reported. Harding (6) used batch cultures of *Tetrahymena glaucoma* (syn. *T. pyriformis*) to show a dependence of the growth rate of ciliates on the concentration of a pseudomonad. No quantitative interpretation of the data was given, but the ciliate growth rate increased as the prey concentration increased until saturating levels of prey were reached where the growth rate became constant. Batch cultures of *Colpoda steinii* studied by Proper and Garver (11) behaved in a similar fashion. The data were assumed to fit an equation of the Michaelis-Menten, or Monod, type.

Studies in continuous cultures of a marine ciliate fed washed, antibiotic-treated *Serratia marinorubra* yielded data which fit a Monod equation except at low levels of prey (5). In addition, the volume of the ciliate cells varied considerably. The volume changes were not related to the growth rate of ciliate but seemed related to the protozoan density. Staged continuous cultures were used by Curds and Cockburn (3) to study the feeding kinetics of *T. pyriformis*. Both the ciliate maximum growth rate and mean cell volume were reported as functions of the specific growth rate. The data over a range of growth rates did not fit a Michaelis-Menten expression. An equation giving the ciliate growth rate as a function of prey concentration and ciliate mean size did adequately represent the data. Further studies on *T. pyriformis* in staged continuous cultures (7) gave similar results. An equation describing the protozoan growth rate as a function of the bacterial concentration only provided an adequate fit to the observed data.

Observations on the growth of *C. steinii* grown in staged continuous cultures are presented here. The data are analyzed in terms of several of the equations proposed to describe the kinetics of protozoan growth.

## MATERIALS AND METHODS

**Medium.** Cultures were grown in a glucose-limited C-medium described earlier (12). For continuous cultures, glucose at 0.5 mg/ml served as the limiting substrate. The organisms were diluted for Coulter counting in 0.6% NaCl.

**Organisms.** *Escherichia coli* B/r was obtained from K. B. Raper, University of Wisconsin. *C. steinii* was isolated by a continuous culture enrichment process as described earlier (4).

**Counting methods.** The numbers and size distribution in *C. steinii* cultures were determined by using a model A Coulter Counter with a 100-$\mu$m aperture. The total count was determined at a threshold setting that included all of the ciliates. The threshold was progressively increased, and du-

plicate counts were recorded until the count had fallen to 5 or 10% of the total count. The difference in counts at successive threshold settings was used to determine the fraction of the total count in each of the threshold intervals. The threshold scale was calibrated in terms of volume by using 6- to 14-$\mu$m polystyrene latex spheres (Dow Chemical Co.) with a mean size of 10 $\mu$m. The bacterial numbers were determined with a model $Z_b$ Coulter Counter equipped with a 30-$\mu$m aperture tube. Cells were diluted to yield counts of 10,000 to 20,000/0.05 ml. At these levels, coincidence corrections were negligible. Repeat determinations had coefficients of variation of about 5.0%.

**Dry weights.** For dry-weight determinations of *Colpoda* or *E. coli*, the organisms were collected on washed, tared membrane filters (type HA, Millipore Corp.). The collected cells were washed with an equal volume of filtered distilled water, dried in a vacuum desiccator over phosphorus pentoxide, and weighed. Amounts of 10 or 20 ml of culture were filtered, and duplicated determinations were made for each sample. A blank filter placed under the collection filter served to correct for filter weight losses during washing and drying. Filters were weighed to the nearest microgram on a Mettler microbalance. For *Colpoda* dry weights no correction was made for the small numbers of bacteria present.

**Continuous cultures.** Staged continuous cultures were run in 100-ml culture vessels similar to the design of Novick and Szilard (9). Water jackets were added for temperature regulation. The continuous cultures were fed from a common medium reservoir by a capillary feed mechanism (10). Flow rates were determined by weighing the collected medium overflow. The culture vessels were constructed with matching ground-glass tapered fittings on the overflow siphon and medium port, and thus could be connected in a cascade. The overflow from the first chemostat was the feed to the second-stage chemostat. Up to six stages can be staged in this fashion.

The medium reservoir and attached feed assemblies were filled with medium and autoclaved. After cooling, sterile glucose was added. The culture vessels were assembled and, after sterilization, were connected to the medium reservoir. The vessels were filled with medium, and 0.2-ml samples were removed for glucose assay. *E. coli* were inoculated into the first stage and allowed to grow to steady state. During this time, the culture flow rates were adjusted to the required values, and samples were removed for contamination checks. When the bacterial population had reached steady state, an inoculum of *C. steinii* was added to the second-stage culture vessel.

The chemostats were checked microscopically and by plates for contaminants. They were invariably negative.

## RESULTS

Staged continuous cultures were run with several holding times in the second stage. Figure 1 shows a typical set of results. After inoculation, *Colpoda* reached nearly steady levels in both numbers and mean volume within 4 to 5 days. Similarly, the bacterial levels fell to a constant low level as the protozoa increased. The ciliates remained active, and no evidence of encystment was seen upon microsopic examination of samples removed from the cultures. Encystment did occur in the overflow flasks as evidenced by settling and accumulation of cysts on the walls of the flasks. The steady-state values in the second stage for *Colpoda* numbers and bacteria numbers were computed by averaging the last five daily values. The cultures were then terminated, and samples were taken from both stages for dry-weight determinations.

The size distributions of *Colpoda* in steady-state second-stage continuous cultures at three different holding times are shown in Fig. 2. Each size distribution is a composite formed by averaging the last five measured cell size distributions. The measured size distributions were

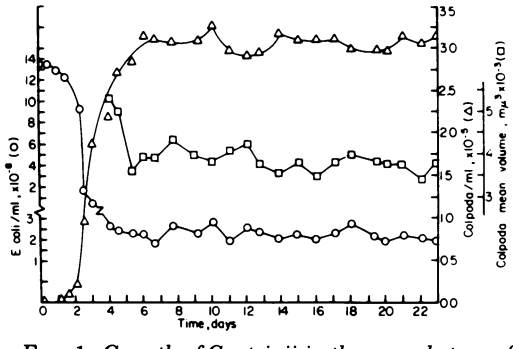


FIG. 1. *Growth of C. steinii in the second stage of a two-stage continuous culture. E. coli grown as pure culture in the first stage. Holding time of second stage, 8.0 h (D = 0.125 h$^{-1}$). Temperature, 25°C.*

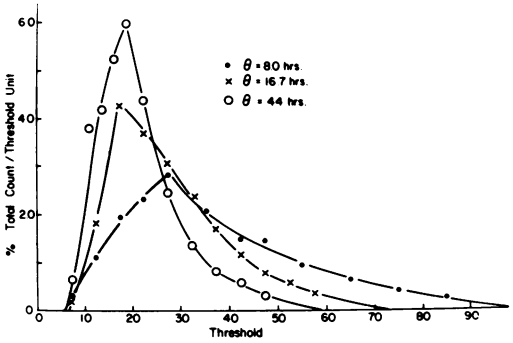


FIG. 2. *Size distributions of C. steinii at steady state in staged continuous cultures. Threshold scale is linear with volume. Plotted points are average values of the last five daily determinations of the size distributions from the cultures shown in Fig. 1 and other data.*

integrated for computation of the mean cell volume.

The average concentrations of the bacterial prey in the second stage, as measured by Coulter counts, are listed in Table 1 for a range of holding times from 8 to 54 h.

## DISCUSSION

The continuous-culture data provide values for the mean cell volume, dry weight, and number per milliliter of *Colpoda* growing at a fixed, known growth rate. In addition, the concentration of bacteria necessary to maintain the *Colpoda* growth rate and the amount of prey consumed were measured. From these data, some of the characteristics of the growth kinetics of *Colpoda* were determined. As shown in Fig. 3,

TABLE 1. *Ciliate growth rates and steady-state prey levels in staged continuous cultures*[a]

| *Colpoda* growth rate | | Steady-state bacteria (no./ml) |
|---|---|---|
| Exponential rate ($h^{-1}$) | Doubling time (h) | |
| 0.115 | 6 | $2.28 \times 10^7$ |
| 0.080 | 8 | $1.59 \times 10^7$ |
| 0.060 | 11.5 | $1.45 \times 10^7$ |
| 0.045 | 15 | $1.07 \times 10^7$ |
| 0.023 | 30 | $0.94 \times 10^7$ |
| 0.019 | 38 | $0.89 \times 10^7$ |

[a] Data from second stage of two-stage continuous cultures at 25°C. Ciliate growth rates were computed from measured flow rates into chemostat vessels of known volume. Bacterial levels at steady state are the average value of five or more daily determinations.

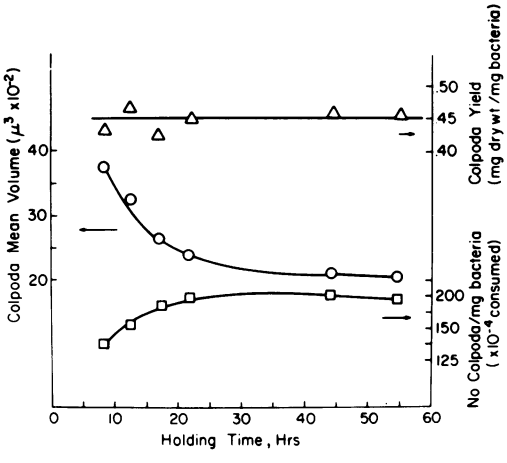


FIG. 3. *Mass yield, mean volume, and number yield for C. steinii growing on E. coli in two-stage continuous cultures. Plotted values computed from measurements of dry weight and number of C. steinii after steady states were reached.*

the yield of *Colpoda* in terms of biomass was constant over the range of holding times studied. A mass yield of 0.45 mg of *Colpoda* per mg of bacteria consumed was obtained at all holding times from 8 to 54 h.

The *Colpoda* mean cell volume did change as the growth rate of the culture varied. As the holding time increased (growth rate decreased), the mean cell volume decreased as shown in Fig. 3. Since the mass yield of *Colpoda* is constant, the decreasing mean cell volume must be matched by an increase in the number of *Colpoda* produced per unit of prey mass consumed. Thus, the number yield of *Colpoda* was not constant but increased as the growth rate decreased. Similar results were reported with *T. pyriformis* in staged continuous cultures (3). The yield of *T. pyriformis* biomass was constant over a range of growth rates. However, the ciliate mean cell volume, and hence the number yield, changed as the growth rate was varied.

The concentrations of bacteria required to maintain the ciliate growth rate in continuous culture are given by the steady-state bacterial concentrations, as shown in Table 1. A description of the rate of *Colpoda* growth as a function of prey concentration can be obtained by fitting the observed data with a suitable equation. Figure 4 is a Lineweaver-Burke plot of the data from Table 1. Two equations, which have been used to predict microbial growth rates as functions of the food concentration, were fitted to the data. The dotted straight line is the best-fitting least-squares linear fit corresponding to the reciprocal of the Monod equation (8)

$$\mu = \mu_m b/(k + b) \quad (1)$$

where $\mu$ is the observed ciliate growth rate at a bacterial concentration of $b$. The maximum growth rate, $\mu_m$, and the saturation coefficient, $k$, have the same significance as in the Michaelis-Menten equation. Equation 1 has been widely used to describe the dependence of growth rate on substrate concentration for bacteria and yeast in continuous cultures. Several alternative equations have been proposed and are discussed by Boyle and Berthouex (1). A general feature of these equations is that the growth rate is linear at low substrate values. As can be seen in Fig. 4, the growth rate of *Colpoda* is not linear at low bacterial levels. Rather, the growth rate falls sharply as the concentration of bacteria decreases. This curvature in the data results in meaningless negative values for both the maximum growth rate and the saturation coefficient if a linear fit of the data is made. Since the observed data for ciliate growth rate versus bacterial concentration are clearly not

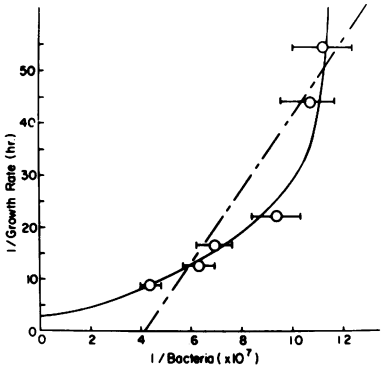


FIG. 4. *Bacterial levels in second-stage continuous cultures of C. steinii. Reciprocal plot of bacterial number at steady state versus dilution rate. Solid line is the best fitting quadratic. Dotted line is best-fitting straight line.*

linear for low growth rates, equations similar to Monod's, which are linear at low substrate levels, would not be expected to represent the data adequately.

The equation derived by Jost et al. (7) and Tsuchiya et al. (13) to describe the growth rate of *T. pyriformis* as a function of prey concentration is not linear at low growth rates. Jost's equation is

$$\mu = \mu_m b^2/(k_1 + b)(k_2 + b) \quad (2)$$

Equation 2 is similar in form to equation 1 with the observed growth rate, $\mu$, defined in terms of the maximum growth rate, $\mu_m$, and the saturation coefficients $k_1$ and $k_2$. The observed growth rate at low bacterial levels, $b$, however, varies as the square of $b$. Thus, as the concentration of prey decreases, the predicted ciliate growth rate decreases rapidly. The reciprocal form of Jost's equation is

$$1/\mu = (k_1k_2/\mu_m)\,1/b^2 + 1/\mu_m\,(k_1 + k_2)/b + 1/\mu_m \quad (3)$$

This represents a quadratic in $1/b$ and gives a sigmoid-shaped curve instead of the rectangular hyperbola. The values of the parameters $k_1$, $k_2$, and $\mu_m$ were evaluated from the coefficients of the least-squares quadratic fitted to the data. The solid, curved line in Fig. 4 represents a quadratic corresponding to the values of $\mu_m$ = 0.37 $h^{-1}$, $k_1 = 1.3 \times 10^7$ *E. coli* cells/ml, and $k_2$ = $1.4 \times 10^7$ *E. coli* cells/ml. The predicted maximum growth rate of 0.37 $h^{-1}$ for *Colpoda* in steady-state continuous cultures is higher than the maximum growth rate of 0.22 $h^{-1}$ observed in batch cultures with excess prey present. A similar phenomenon was seen for *T. pyriformis* (3), where a predicted maximum growth rate in continuous culture of 0.43 $h^{-1}$ was contrasted with the observed batch-culture maximum growth rate of 0.22 $h^{-1}$. These observations are unique in that the maximum growth rates of bacteria and yeasts in continuous and batch cultures are generally similar. The wide discrepancy between the maximum growth rate of both *Colpoda* and *T. pyriformis* in batch or continuous culture implies that different mechanisms are operating in these ciliates to control the maximum rate of growth.

The very complete study of the feeding kinetics of *T. pyriformis* by Curds and Cockburn (3) provides results similar to those reported here. Based on their data, they proposed an equation describing the ciliate growth rate as a function of the concentration of bacterial prey and the mean cell volume of the ciliates. Their proposed equation is

$$\mu = (k_3Y/M)b/\{(k_3/k_1M) + b\} \quad (4)$$

where $\mu$ is the observed growth rate for a culture with mean cell mass $M$ feeding on bacteria at a concentration $b$ (milligrams per milliliter). The parameters $k_1$ and $k_3$ are rate constants for the capture and processing of food. The yield coefficient, $Y$, is the ratio of ciliate mass produced to the amount of bacteria consumed. As with *Colpoda*, the mass yield coefficient of *T. pyriformis* was constant over a wide range of growth rates. Equation 4 was derived by modifying the equation proposed by Caperon (2) for uptake of food by microorganisms. The terms $(k_3Y/M)$ and $(k_3/k_1M)$ correspond to the maximum growth rate, $\mu_m$, and the saturation coefficient, $k$, of equation 1.

These terms are not, however, constant but are functions of the mean ciliate mass, $M$. The reciprocal form of equation 4 is

$$b/\mu = 1/k_1Y + (1/k_3Y)Mb \quad (5)$$

and a plot of $b/\mu$ versus $Mb$ should be linear. Such a plot for the results from the staged continuous cultures of *Colpoda* is shown in Fig. 5. The results are clearly not linear for *Colpoda*. The data of Curds and Cockburn for *T. pyriformis* did yield an acceptable linear fit to equation 5, and a possible explanation for the discrepancy may be the difference in the range of growth rates studied. At the lower growth rates studied here, neither the ciliate mean size nor the bacterial concentration at steady state decrease fast enough to yield a linear plot. The mean size and the bacterial concentration both become relatively constant at low growth rates. Thus, the term $Mb$ approaches a constant value, whereas $b/\mu$ increases as the growth rate decreases. Thus, a plot of $b/\mu$ versus $Mb$ tends to curve upward at low growth rates, as shown in Fig. 4.

Curds and Cockburn found that the use of

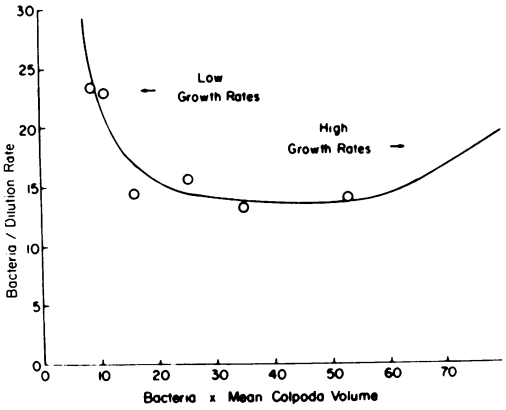


FIG. 5. *Plot of data from second-stage continuous cultures of C. steinii and E. coli according to linear transformation of equation 3 (Curds and Cockburn [3]). Nonlinear trend of the data is evident.*

both the bacterial concentration and the ciliate mean size in equation 4 gave a better prediction of the ciliate growth rate than equation 1, which uses the bacterial concentration only. A difficulty with equation 4 arises if the calculation is reversed. That is, given the growth rate of the protozoan, neither the bacterial concentration nor the ciliate mean size can be computed. Equation 4 represents a three-dimensional surface. The specification of a ciliate growth rate establishes a plane which cuts this surface. The line of intersection of the plane and the surface represents all combinations of bacterial concentration and ciliate mean size which combine to yield the specified growth rate. There are an uncountable number of such combinations. The experimental observations for both *Colpoda* and *T. pyriformis* indicate that, at a fixed growth rate, a stable steady state is reached. Both the bacterial concentration and the ciliate mean size reach steady, reproducible values. Therefore, the other combinations of prey concentration and ciliate mean size predicted by equation 4 are extraneous.

Equation 2 uses a single independent variable, the prey concentration. The calculation of the prey concentration, given the growth rate, does yield a single solution if the positive value of the quadratic is chosen as the only meaningful result.

Both equations are, of course, abstractions which only partially represent the complex kinetics of protozoan feeding.

The fit of the models does not depend upon the units used for $b$ or $k$. These terms appear as dimensionless ratios in which the units cancel. To convert $b$ or $k$ from units of numbers per milliliter to biomass (or volume) per milliliter requires multiplication by the mean cell mass or volume. This multiplication factor will appear in both the numerator and denominator and thus not change the value of the ratio.

At steady state in a continuous culture, growth is balanced. Thus, the growth rate in terms of number of biomass is identical to the dilution rate of the vessel. Therefore, the data would not be changed by reformulation in terms of number or biomass is identical to the advantage of continuous cultures in kinetic studies such as this, since all rates of growth must equalize at steady state.

If the characteristics of microorganisms are determined in response to their environment, then in a fixed environment with varying prey density both the mean size and the growth rate of ciliates should be functions of only the concentration of the prey species. Staged continuous cultures provide an experimental situation in which these conditions may be realized. Experimental characterization of ciliate growth kinetics is a required first step toward a fuller understanding of the ecological role played by these ubiquitous microbial predators.

## ACKNOWLEDGMENT

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