Kinetics of Microbial Growth on Pentachlorophenol

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Batch and fed-batch experiments were conducted to examine the kinetics of pentachlorophenol utilization by an enrichment culture of pentachlorophenol-degrading bacteria. The Haldane modification of the Monod equation was found to describe the relationship between the specific growth rate and substrate concentration. Analysis of the kinetic parameters indicated that the maximum specific growth rate and yield coefficients are low, with values of 0.074 h⁻¹ and 0.136 g/g, respectively. The Monod constant (Kᵣ) was estimated to be 60 μg/liter, indicating a high affinity of the microorganisms for the substrate. However, high concentrations (Kᵣ = 1,375 μg/liter) were shown to be inhibitory for metabolism and growth. These kinetic parameters can be used to define the optimal conditions for the removal of pentachlorophenol in biological treatment systems.

Pentachlorophenol has been extensively used as a wood preservative, insecticide, and herbicide. In view of its widespread application, the feasibility of biological treatment of pentachlorophenol-containing wastewaters has been the subject of numerous investigations (5, 7, 10, 13, 25). Although the compound has been shown to be extensively degraded in both laboratory and full-scale systems, relatively few studies have been conducted to evaluate the basic parameters which describe the kinetics of pentachlorophenol utilization. Since the efficient operation of biological treatment systems is largely dependent on the kinetic properties of the microbial population, determination of these parameters is essential for the development of operational strategies for the optimum removal of pentachlorophenol during wastewater treatment.

Stanlake and Finn (21) have recently described the isolation and kinetic properties of an Arthrobacter sp. capable of degrading pentachlorophenol. Based on cell yield (0.15 g of cells per g of substrate), pentachlorophenol was a poor substrate for growth of the organism, presumably due to the high chlorine content of the molecule. The effect of pentachlorophenol on the growth of the organism was examined in batch cultures, using a defined medium at substrate concentrations ranging from 50 to 300 mg/liter. The specific growth rate of the cultures increased with increasing pentachlorophenol concentrations to a maximum of 0.1 h⁻¹ at 130 mg/liter. However, substrate concentrations greater than 130 mg/liter significantly decreased the specific growth rate to a level of approximately 0.05 h⁻¹ at 300 mg/liter.

Moos et al. (16) have also examined the kinetics of pentachlorophenol degradation by a mixed population of sewage microorganisms. Continuous-flow reactors were operated at fixed hydraulic residence times ranging from 3.2 to 18.3 days and were supplied a complex medium containing 20 mg of pentachlorophenol per liter and an additional 600 mg of soluble organic substrates per liter. During operation of the reactors, a high degree of variability was noted in effluent pentachlorophenol concentrations. These results were presumably due to substrate inhibition, since greater stability was noted in reactors operated at longer residence times. Analysis of the reactor performance data by using a mathematical model based on the Monod equation indicated that the biodegradation of pentachlorophenol was first order with respect to substrate, with a rate constant (μᵣmax/Kᵣ) of 0.0074 liters/μg per day.

Recent concern has been expressed over the use of mathematical models based on Monod kinetics for describing the biodegradation of inhibitory substrates in wastewater treatment systems (20). Although such models may be valid for describing the kinetics of substrate removal at low specific growth rates (and hence low effluent concentrations), they do not permit evaluation of system performance over a wider possible range of operating conditions. Since the kinetics of pentachlorophenol degradation appear to involve substrate inhibition, the present study was conducted to define the relationship between substrate concentration and the specific growth rate of an enrichment culture of pentachlorophenol-utilizing bacteria.

MATERIALS AND METHODS

Isolation and maintenance of enrichment cultures. Two mixed bacterial cultures, capable of utilizing pentachlorophenol as a sole carbon source, were isolated from samples of industrial sewage, using a continuous-culture enrichment technique. Culture vessels were constructed from 2-liter Pyrex resin kettles, with an overflow tube placed at the 1-liter level (Fig. 1). Culture medium was supplied to the reactors from 8-liter reservoirs via glass and silicone rubber tubing. The medium flow rate was controlled by a Manostat model 72-500-000 peristaltic pump. Cultures were aerated with filtered and humidified air at a rate of 250 ml/min and agitated with a star-head magnetic stirrer. The reactors were maintained in a constant-temperature incubator at 20°C.

The two continuous cultures were maintained on a mineral salts medium containing either 10 or 100 mg of pentachlorophenol per liter as the sole carbon source. The medium was composed of, per liter: K₂HPO₄, 8.7 g; MgSO₄, 0.1 g; NH₄NO₃, 0.05 g; tap water, 50 ml; and deionized water, 950 ml. Pentachlorophenol was added to the medium from a stock solution (10 g/liter) prepared in 0.01 N NaOH. The medium was adjusted to pH 7.5 with H₃PO₄. The reactors were initially operated on a discontinuous basis until consistent substrate removal was noted. At that point a continuous flow of medium was supplied to the reactors. Samples were removed daily from the reactors for pentachlorophenol analysis and periodically for cell mass determinations. Once stable operation of the reactors was noted, the continuous

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cultures provided the source of inoculum for all subsequent kinetic experiments.

**Kinetic experiments.** Batch culture experiments were conducted in 2-liter flasks containing 450 ml of the mineral salts medium previously described. Flasks were amended with various concentrations of pentachlorophenol (200 to 2,000 μg/liter) and inoculated with 50 ml of the mixed culture obtained from the appropriate continuous-culture reactor. Flasks were incubated at 20°C on a rotary shaker at 200 rpm. Samples of the cultures were removed periodically and analyzed for pentachlorophenol concentration as described below.

A fed-batch experiment was also conducted to examine the kinetics of pentachlorophenol degradation. To perform the test, the volume of the continuous-culture reactor was initially decreased by removing approximately 500 ml of the culture. The reactor was then allowed to fill to the original volume by the slow continuous addition of mineral salts

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**FIG. 1.** Continuous-flow enrichment culture reactor.
medium containing pentachlorophenol. Samples were removed periodically from the fed-batch culture and analyzed for pentachlorophenol concentration as described below.

**Analytical methods.** Pentachlorophenol was analyzed by high-pressure liquid chromatography. Culture samples were initially filtered through 0.2-μm polycarbonate membrane filters (Nuclepore Corp., Pleasanton, Calif.). During sample preparation, the first 3 ml of culture filtrate was discarded. The second portion of the filtrate was collected in a sample vial and analyzed as described below. Preliminary experiments indicated that >90% of the pentachlorophenol present in standard solutions was recovered in the second portion of the filtrate.

Liquid chromatography was performed with a system composed of a Waters model 710B automatic sample processor and model M6000A pump and an LDC Spectromonitor III variable-wavelength detector adjusted to 214 nm. Separations were achieved on a ZORBAX tetramethylsilane reverse-phase analytical column (DuPont Co., Wilmington, Del.), using a solvent system composed of acetonitrile-water (45:55) and 0.02 M H3PO4. The flow rate was 0.9 ml/min at approximately 1,000 lb/in2. Output of the detector was connected to a Waters model 730 recording integrator.

Pentachlorophenol concentrations were calculated on the basis of peak height measurements by comparison with an external standard. The detector response was linear over the concentration range of interest (10 to 2,000 μg/liter), with a detection limit of approximately 10 μg/liter.

Cell mass concentrations were determined by a total organic carbon analysis. Two samples (10 ml) of the culture were removed for each determination; one sample was sonicated for 1 min at the full output of a Labline Ultrasonic system, whereas the cell mass was removed from the other sample by centrifugation at 20,000 × g for 20 min. The total organic carbon contents of both the culture supernatant solution and the sonicated sample were determined with a Beckman model 915B total carbon analyzer. The instrument was calibrated with potassium biphthalate as the standard. Total carbon concentration of the cell mass was calculated from the difference of the organic carbon present in the sonicated sample and the supernatant solution. By assuming that the molecular formula for a microbial cell was C8H7NO2 (8), the organic carbon concentration was multiplied by a factor of 1.88 (C8H7NO2/C8) to obtain the dry weight of the cell mass.

**Data analysis.** Results of the kinetic experiments were analyzed with an IBM model 370 computer, using the Dow advanced computer simulation language (DACSL [1]). Batch test data were analyzed by using modified Monod equations for growth and substrate utilization (2, 6).

\[
\frac{dX}{dt} = \mu_{max} \frac{X}{\left( K_s + S + S^2/K_i \right)} - k_d X
\]

\[
\frac{dS}{dt} = \frac{\mu_{max} X}{Y \left( K_s + S + S^2/K_i \right)} - \frac{Q_i}{V} \left( \frac{S}{Y} - \frac{Q_i}{S} \right) - \frac{Q_e}{V} \frac{S}{Y} \left( \frac{dV}{dt} \right)
\]

where \( \mu_{max} \) is the maximum specific growth rate coefficient, \( X \) is the cell mass concentration, \( Y \) is the cell yield coefficient, \( S \) is the substrate concentration, \( K_s \) is the Monod constant, \( K_i \) is an inhibition constant, and \( k_d \) is the endogenous decay coefficient. To simplify the model calculations, the value for the endogenous decay coefficient was assumed to be 0.002 h⁻¹, which is within the range of values frequently observed for heterogeneous microbial popula-

**RESULTS**

**Operation of enrichment cultures.** Pentachlorophenol-degrading bacterial populations were obtained by continuous enrichment from a sample of industrial sewage. Two continuous cultures were maintained during the investigation on mineral salts media containing pentachlorophenol as the sole carbon source. Reactor 1 was operated with a medium containing 10 mg (nominal) of pentachlorophenol per liter; the medium for reactor 2 contained 100 mg (nominal) of the compound per liter. During the initial enrichment, the reactors were operated for a few days on a discontinuous basis until consistent substrate removal was noted. At that point,
a continuous flow of medium was supplied to the cultures. Initial operation of both reactors at a flow rate of 10 ml/h proved unstable, and thus the flow rates were decreased to approximately 3 ml/h. After 55 days of continuous operation, stable performance was noted in both reactors (Table 1). Pentachlorophenol removal efficiencies of >99.8% were maintained in the cultures during the following 3 months of incubation.

During the period of stable operation, the mixed cultures in the two reactors consisted primarily of small suspended floculent particles. Microscopic examination of the particles indicated the presence of bacteria and protozoa in the cultures; both suspended and stalked protozoa were noted. Qualitative examination of samples of the two cultures indicated that a greater number of protozoa were present in the culture maintained on the higher concentration of pentachlorophenol. As a result, the low yields of cell mass (0.01 to 0.04 g/g) in the continuous cultures may be attributed to predation.

**Kinetics of pentachlorophenol degradation.** Batch experiments were conducted to examine the effects of both cell mass and substrate concentration on the rate of pentachlorophenol degradation. Studies were conducted at two different initial cell mass concentrations by using a 10% (vol/vol) inoculum of mixed culture obtained from either reactor 1 or reactor 2. Biodegradation rates were examined at nominal pentachlorophenol concentrations ranging from 200 to 2,000 μg/liter.

Results of batch kinetic experiments inoculated with cells from reactor 1 are shown in Fig. 2. The initial cell mass concentration (X0) in the tests was 16 μg/liter. Note that the rate of pentachlorophenol degradation increased with time, due to growth of the microorganisms. However, high concentrations (800 to 1,600 μg/liter) of pentachlorophenol were inhibitory to growth, as the rates of substrate utilization were greater at low initial concentrations (160 to 400 μg/liter).

The results were analyzed with a computer simulation model to obtain values for the kinetic parameters (μmax, Y, Ks, Kj). During computer analysis, the values for the kinetic parameters and the initial cell mass concentration were varied individually to obtain the best fit of the experimental data. For the purpose of the coefficients were obtained by averaging the results of numerous simulations of each data set. Average values estimated for the parameters were as follows: μmax = 0.074 h⁻¹, X0 = 17.3 μg/liter; Y = 0.136 g/g; Ks = 60 μg/liter; and Kj = 1,375 μg/liter. The validity of the average values was tested by simultaneously applying the coefficients to describe the kinetics of pentachlorophenol degradation over the entire range of initial substrate concentrations. Excellent agreement was noted between the measured and predicted pentachlorophenol concentrations for three of the four data sets (Fig. 3). Although the agreement between the measured and predicted values was not as good for the remaining data set, this may be due to differences in the quantity of cell mass actually present in the batch tests. However, the reasonable agreement observed over the entire range of initial substrate concentrations suggests that the average values adequately describe the kinetics of pentachlorophenol degradation.

Batch experiments were conducted in an identical manner to investigate the biodegradation of pentachlorophenol by cells from reactor 2. The initial cell mass concentration in the tests was 170 μg/liter. Results of the study are shown in Fig. 4. Note that the rate of pentachlorophenol utilization was greater than observed in the previous batch tests due to the higher level of cell mass used. Analysis of the results with the computer simulation model indicated that the kinetic coefficients previously obtained from the analysis of reactor 1 batch cultures also described the rate of pentachlorophenol degradation by cells from reactor 2.

**Kinetics of pentachlorophenol degradation in fed-batch culture.** Conventional continuous-culture kinetic experiments were not feasible since washout of the population would be expected to occur as a result of substrate inhibition as steady-state concentrations of pentachlorophenol increased in the reactor with increases in the dilution rate. Consequently, a fed-batch technique was used to examine the kinetics of pentachlorophenol utilization under conditions resembling continuous-culture operation. Theoretical calculations for the operation of a fed-batch culture with pentachlorophenol indicated that decreasing the volume of the culture in the reactor (and hence the level of active cell mass) should result in a transient increase in substrate concentration in the reactor, followed by a decline to the original level. These calculations were tested in a fed-batch experiment conducted with reactor 2. The culture was initially adjusted to a volume of 500 ml and then allowed to fill to the original level by the constant addition of mineral salts medium containing 86.2 mg of pentachlorophenol per liter at a flow rate of 2.9 ml/h. During the experiment, the concentration of cell mass in the reactor was nondetectable. This was presumably due to technical difficulties in measuring low concentrations of cell mass or the inability to obtain a representative sample because of the flocculant nature of the culture. However, the transient increase in pentachlorophenol concentration observed during operation of the fed-batch culture was shown to be predicted by the computer simulation model, using values of μmax = 0.074 h⁻¹, Ks = 60 μg/liter, Kj = 1,375 μg/liter, and Y = 0.136 g/g for the kinetic coefficients (Fig. 5). The best fit of the data was obtained when the initial cell mass concentration was assumed to be 920 μg/liter, which is consistent with the low levels of cell mass previously measured during continuous operation of the reactor.

**DISCUSSION**

Pentachlorophenol has often been considered to be relatively resistant to biodegradation due to the high chlorine content of the molecule. However, numerous reports have appeared in the literature describing the microbial degrada-

<table>
<thead>
<tr>
<th>Reactor vol (ml)</th>
<th>Flow rate (ml/h)</th>
<th>Hydraulic/ solids residence time (days)</th>
<th>Influent (pentachlorophenol) (μg/liter)</th>
<th>Effluent (pentachlorophenol) (μg/liter)</th>
<th>Cell mass in reactor (mg/liter)</th>
<th>Cell yield (g of cells/g of substrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,050</td>
<td>3.4</td>
<td>12.9</td>
<td>8.0</td>
<td>&lt;10</td>
<td>0.13</td>
</tr>
<tr>
<td>2</td>
<td>1,110</td>
<td>3.2</td>
<td>14.5</td>
<td>80.7</td>
<td>37.8</td>
<td>0.139-0.17</td>
</tr>
</tbody>
</table>

* Detection limit was 10 μg/liter; a value of 5 μg/liter was used for calculations.
The flocculant from reactor 1, of various reactors.

FIG. 2. Kinetics of pentachlorophenol degradation in batch cultures of cells from reactor 1. Batch tests were conducted in 2-liter flasks containing 500 ml of mineral salts medium, 8 μg of cells from reactor 1, and various concentrations of pentachlorophenol. Cultures were incubated on a rotary shaker at 200 rpm and 20°C. Kinetic parameters were estimated by using the computer simulation model as described in the text.

FIG. 3. Comparison of measured (O) and predicted (—) pentachlorophenol concentrations for batch cultures of cells from reactor 1. Values used for the computer simulation were as follows: \( \mu_{\text{max}} = 0.074 \text{ h}^{-1} \); \( X_0 = 17.3 \mu\text{g/liter} \); \( Y = 0.136 \text{ g/liter} \); \( K_s = 60 \mu\text{g/L} \); \( K_l = 1.375 \mu\text{g/liter} \).

The flocculant nature may have been due to the growth conditions, since Gaudy and Gaudy (8) have noted that flocculation is frequently observed in heterogeneous cultures at low growth rates. Low cell yields were noted during operation of the reactors, with yield coefficients estimated in the range of 0.01 to 0.04 g (dry weight) of cell mass per g of substrate consumed. These low yields were presumably due to predation by protozoa present in the cultures. However, Yang and Humphrey (26) have noted that cell yield decreases as the specific growth rate decreases. Thus, low yields would be expected for continuous cultures operating at dilution rates of approximately 0.07 day\(^{-1}\). Computer analysis of the batch test data indicated that the actual cell yield is much higher, with a value estimated to be 0.136 g/g.
This value is consistent with the cell yield of 0.15 g/g reported by Stanlake and Finn (21) for bacterial growth on pentachlorophenol as the sole carbon source.

Batch culture experiments were conducted to evaluate the kinetics of pentachlorophenol utilization. The rate of degradation was found to be proportional to the concentration of cell mass used in the test. Degradation rates were also related to the pentachlorophenol concentration; however, high substrate concentrations (800 to 1,600 µg/liter) were inhibitory, as the removal rates were greater at low concentrations (160 to 400 µg/liter). These observations indicated that the relationship between the specific growth rate and pentachlorophenol concentration deviates from the classical hyperbolic function described by the Monod equation. Various kinetic relationships have been devised to depict the joint dependence of the specific growth rate on substrate concentration when the compound furnished for growth serves as both a substrate and an inhibitor. One that is often used to describe experimental data is based on the equation developed by Haldane for the kinetics of enzyme catalyzed reactions (2, 6). The Haldane modification of the Monod equation has been previously used to describe the kinetics of microbial growth on phenol (9, 12, 20, 26) and was found in the present study to be suitable for describing the kinetics of pentachlorophenol utilization. The relationship between specific growth rate and pentachlorophenol concentration is shown in Fig. 6. The specific growth rate increases with increases in pentachlorophenol concentration and reaches a maximum at approximately 300 µg/liter. Concentrations above this level decrease the specific growth rate as the effects of substrate inhibition become more pronounced. These effects would not be predicted by the hyperbolic Monod function and indicate that the Haldane modification should be used to describe the growth rate-substrate relationship for inhibitory compounds. Rozich et al. (20) have also stressed the importance of including substrate inhibition effects into predictive models for describing the removal of inhibitory compounds in biological systems.

Due to the inhibitory nature of pentachlorophenol, conventional continuous-culture kinetic studies were not feasible since washout of the microbial population was likely at high dilution rates. Moos et al. (16) have previously observed considerable instability during operation of continuous cultures on pentachlorophenol at high flow rates, complicating a kinetic analysis of the results. Thus, a feed-batch approach was used in the present study to examine the kinetics of pentachlorophenol utilization under conditions resembling continuous-culture operation. Computer analysis

![Graph](image-url)
The latter of the techniques evaluated from those nated at properties of Jannasch may correlate affinities. The good substrate growth is preferentially directed during experiments that is, the average time a cell remains in the reactor before it is wasted or lost, has been widely used as a control parameter for describing the steady-state performance of biological treatment systems. Mean cell residence time, indicated that the kinetic coefficients determined in batch experiments also described the behavior of fed-batch cultures. The transient increase in pentachlorophenol concentration during fed-batch operation can be attributed to the effects of substrate inhibition and to decreasing the amount of active cell mass in the system. The good agreement between the experimental data and model predictions indicate that the kinetics of pentachlorophenol utilization are adequately described by the Haldane modification of the Monod equation. Furthermore, the studies illustrate the utility of fed-batch experiments for examining the kinetics of microbial growth on inhibitory substrates.

The enrichment conditions used to isolate the mixed culture preferentially selected for organisms capable of growth at low pentachlorophenol concentrations. Analysis of the kinetic properties of the culture indicated that both the maximum specific growth rate \( \mu_{\text{max}} \) and the Monod constant \( K_s \) are low, with values of 0.074 h\(^{-1}\) and 60 \( \mu \)g/liter, respectively. Jannasch (11) has previously noted that a correlation may exist between microbial growth rates and substrate affinities. Organisms capable of high growth rates at high substrate concentrations typically grow less efficiently at lower concentrations due to low substrate affinities (low \( K_s \)). Alternatively, organisms that grow efficiently at low substrate concentrations generally exhibit low growth rates at high substrate affinities (high \( K_s \)). Note that the kinetic properties of the mixed culture differ considerably from those reported by Stanlake and Finn (21) for an Arthrobacter sp. capable of growth on pentachlorophenol. The latter organism was isolated from soils highly contaminated with pentachlorophenol, using batch culture enrichment techniques at high substrate concentrations. Analysis of the kinetic properties of the Arthrobacter sp. suggest that the Monod constant is high, with a value estimated to be approximately 60 mg/liter. These observations illustrate that the conditions used for enrichment play an important role in selecting for a particular type of microbial population.

An understanding of the kinetics of pentachlorophenol degradation provides a foundation for process analysis and design for the optimum removal of the compound in wastewater treatment systems. The low growth rate, cell yield, and effects of substrate inhibition indicate that relatively long cell residence times will be required for the efficient removal of pentachlorophenol. Mean cell residence time, as a result, is inversely proportional to the net specific growth rate, and thus the effluent substrate concentration for a given residence time can be calculated from knowledge of the kinetic parameters. The relationship between effluent pentachlorophenol concentration and mean cell residence time for a continuous-flow reactor is shown in Fig. 7. Operation of the system at short residence times leads to high effluent concentrations; washout of the population is likely to occur at a residence time of \(<2\) days. Alternatively, high removal efficiencies are predicted at cell residence times of \(>10\) days, which is consistent with the results obtained during operation of the laboratory enrichment culture reactors. Thus, long mean cell residence times are an important control parameter for the efficient removal of pentachlorophenol in biological systems. Further applications of the kinetic parameters in conjunction with predictive models, such as the one recently described by Rozich et al. (20), may reveal additional process control strategies for the optimum performance of wastewater treatment facilities.

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LITERATURE CITED


