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Effect of Starvation on Induction of Quinoline Degradation for a Subsurface Bacterium in a Continuous-Flow Column

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Differences in the induction response and the initial two reactions of quinoline degradation between short-term (2 days)- and long-term (60 to 80 days)-starved cells of a subsurface *Pseudomonas cepacia* strain were examined by using continuous-flow columns. The ability of bacteria that are indigenous to oligotrophic environments to respond to a contaminant was assessed by using long-term starvation to induce a cell physiology that simulates the in situ physiology of the bacteria. With quinoline concentrations of 39 and 155 μM, long-term-starved cells converted quinoline to degradation products more efficiently than did short-term-starved cells. Quinoline concentrations of 155 μM and, to a greater extent, 775 μM had an inhibitory effect on induction in long-term-starved cells. However, only the length of the induction process was affected with these quinoline concentrations; degradation of quinoline at the steady state for long-term-starved cells was equal to or better than that for short-term-starved cells. The induction time for short-term-starved cells did not increase progressively with increasing quinoline concentration. Experiments with starved cells are important for the development of accurate predictive models of contaminant transport in the subsurface because starvation, which induces a cell physiology that simulates the in situ physiology of many bacteria, may affect the induction process.

Microorganisms, including aerobic and facultative aerobic heterotrophic bacteria, are present in many subsurface sediments (5–8, 15, 33, 34, 37). Populations of these heterotrophic bacteria are typically 10^4 to 10^6 CFU g^{-1}, and their population and metabolic potential do not decrease with depth (5, 15, 17, 22, 33). Many deep subsurface aquifers that have been studied are highly oligotrophic or nutrient limited, often containing less than 10 mg of organic carbon $liter^{-1}$ (12, 15, 21, 37). The morphology (5, 6, 8) and biochemical structure (36) of cells recovered from the subsurface and estimates of in situ subsurface microbial activity (12) indicate that the microorganisms are under severe nutrient stress with very low metabolic activity. Long-term time-course studies of changes in bacterial morphology and physiology after a transition from high-nutrient conditions to a lack of nutrients have been conducted with bacteria isolated from oligotrophic marine (2–4, 13, 20, 25, 27, 30, 31) and terrestrial (10, 14, 29) environments. Because cells that have undergone long-term starvation (LTS) in the laboratory closely approximate the morphology and physiology of cells existing in the environment, the starvation process is believed to induce a suite of physiological stresses that approximate those experienced by indigenous bacteria (24).

Organic contaminants introduced to the subsurface represent a potential energy and carbon source for heterotrophic microorganisms. The ability of subsurface microbial communities to degrade a variety of recalcitrant compounds in batch (17, 32, 37, 38) and continuous-flow column (9, 18, 19, 24, 35, 39) experiments has demonstrated the potential for using indigenous microbial communities to remove contaminants from ground water (i.e., in situ bioremediation). However, an increased understanding of the factors that control microbial processes in the subsurface is necessary to increase rates of contaminant degradation and construct predictive models of contaminant transport.

Investigations of subsurface systems typically attempt to approximate subsurface conditions in the laboratory. Continuous-flow column experiments simulate in situ aquifer conditions because they incorporate factors such as hydrodynamic flux, bacterial adhesion, a constant influx of contaminant, and removal of metabolites. Two general types of continuous-flow column experiments have been conducted. In the first type, columns contain aquifer material and its indigenous microbial community (or the community supplemented with a specific substrate-degrading bacterium) (18, 19, 39). This type of experiment simulates field conditions well. However, because the microbial and physical properties of the aquifer material are already established and spatially heterogeneous, a mechanistic understanding of the effect of specific variables is often not possible. The second general type of column experiment uses an artificial, homogeneous medium such as glass beads that is colonized by a specific bacterium or bacterial consortia (9, 24, 35). Although these experiments are less representative of field conditions, they offer several significant advantages. First, low spatial heterogeneity allows for greater replicability. A second advantage is the greater ability to manipulate and control a variety of microbial and physical properties such as microbial population, distribution, and physiological state and flow conditions, particle size, and porous media reactivity (sorption). Thus, it is easier to interpret results in terms of the effect of specific variables and to quantify these effects to model processes in the more complex field environment.

A variable that has been omitted in the latter type of column experiment is the physiological state of indigenous bacteria. For biodegradation experiments, bacteria are typically grown in the laboratory on labile nutrients or an inoculum is taken from a culture growing on labile nutrients. An improved technique that more closely simulates subsur-

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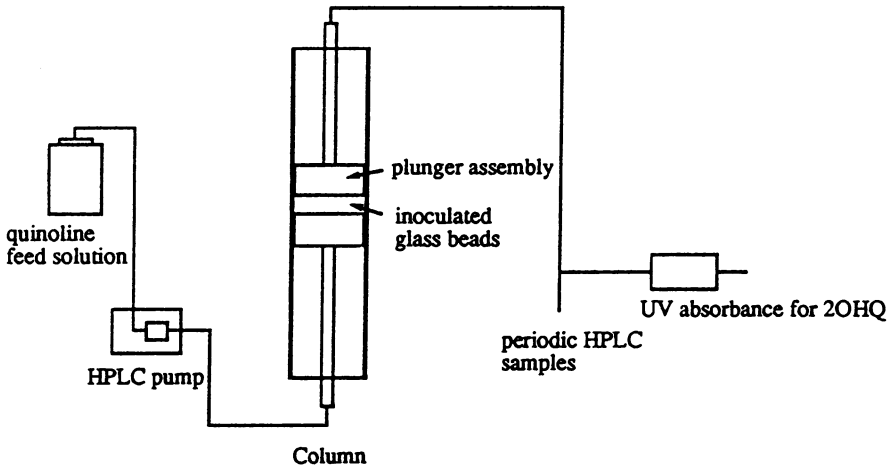

FIG. 1. Continuous-flow column apparatus used to study the induction response and initial two reactions of quinoline degradation for a *P. cepacia* strain.

face aquifer conditions in biodegradation experiments is the use of cells that have undergone LTS.

The objective of this study was to improve the understanding of how LTS affects the ability of a bacterial culture to respond to a recalcitrant substrate. Experiments with starved cells are important for the development of accurate predictive models of contaminant transport in subsurface environments, because the physiology induced by starvation simulates the in situ physiology of many bacteria. Degradation of quinoline by cells starved for 2 days (short-term-starved [STS] cells) or 60–80 days (LTS cells) was examined at three different quinoline concentrations in continuous-flow columns. The parameters measured were the time required to induce degradative enzymes, the time from induction to steady-state degradation, the maximum rate of change of column effluent constituent concentrations, and the concentration of each constituent in the effluent at the steady state.

MATERIALS AND METHODS

Cultural conditions and starvation protocol. A pure culture of a bacterium that was isolated from subsurface sediments at a depth of 203 m (strain 866A) (11) and that uses quinoline as a sole source of carbon, nitrogen, and energy was used in this study. The bacterium was identified as *Pseudomonas cepacia* by 16S rRNA sequencing (32a). Degradation of quinoline by this bacterium proceeds by an inducible enzyme system (11). Stock cultures (stationary-phase cells, 10^9 CFU ml^{-1}) were stored at −80°C. Bacteria were grown aerobically in electrolyte solution (ES) (24) amended with 100 μg of succinate (Sigma Chemical Co., St. Louis, Mo.) ml^{-1} and 5 μg of nitrate (supplied as KNO_3) ml^{-1}. Stationary-phase cells (10^9 CFU ml^{-1}) were obtained by inoculating 50 μl of the stock culture in the growth medium (150 ml) and incubating at 30°C (150 rpm, r = 1.5 cm) for 48 h. Cells were harvested by centrifugation (10,000 × g, 20 min, 4°C), resuspended in sterile ES, and then washed and resuspended (150 ml) a second time. To reduce endogenous nutrient reserves and sorbed nutrients, cells were rested in ES (25°C, 150 rpm, r = 1.5 cm) for 2 days after the washing procedure. Cells were then concentrated by centrifugation, resuspended in sterile ES to the appropriate density, and used for STS experiments. For LTS experiments, rested cells (400 ml) were washed as described above and transferred to 1-liter, screw-top Erlenmeyer flasks (starvation flasks) that were fitted with a filtered air inlet (activated carbon, cotton, and 0.22-μm-pore-size in-line filter (Millex-GV; Millipore Corp., Bedford, Mass.) and a septum for sampling. The bacteria in the flasks were starved at 14°C (100 rpm) in the dark for 60 to 80 days before use. Aliquots from starvation flasks were concentrated by centrifugation to the appropriate density and used as the inoculum in LTS experiments. One to three column experiments were performed with LTS cells from each starvation flask.

Degradation experiments. For both STS and LTS cells, four replicate experiments (room temperature, 22°C) were performed with 39, 155, and 775 μM quinoline input concentrations. The apparatus shown in Fig. 1 was used for all degradation experiments. To obtain a final cell density of 10^8 CFU g of glass $beads^{-1}$, 2.1 ml of cells concentrated to 10^9 CFU ml^{-1} was slurried with 8.0 g of autoclaved glass beads (100- to 150-μm diameter) in a sterile beaker. The inoculated beads were packed in glass chromatography columns (2.5-cm inside diameter; Beckman, Cedar Grove, N.J.) to a length of 1.0 cm. Beads were constrained in the column by plunger assemblies fitted with 30- to 60-μm-pore-size Teflon filters. All column components and tubing were autoclaved or soaked in disinfectant (5% Roccal II) overnight and thoroughly rinsed with sterile ES before use.

Quinoline (reagent grade; Sigma) feed solutions (quinoline at the specific concentration in ES) were contained in autoclaved feed bottles (Ultraware; Kontes, Vineland, N.J.). A constant flow rate (high-performance liquid chromatography [HPLC] pump model 302; Gilson, Middleton, Wis.), resulting in a 10-min packed-column residence time was used for all experiments. The first two catabolic reactions in quinoline degradation were monitored: the production of 2-hydroxyquinoline (2OHQ) from quinoline and the transformation of 2OHQ to the next metabolite. The concentration of 2OHQ was continuously measured with an in-line variable-wavelength detector (Waters Associates, Milford, Mass.) at a 330-nm wavelength. Periodic effluent samples were collected, and quinoline and 2OHQ concentrations were analyzed by HPLC (model 1090 C-18 column; Hewlett-Packard Co., Palo Alto, Calif.) with 50% acetonitrile–50% 0.01 M NaH_2PO_4 at 0.4 ml min^{-1} and 25°C. Influent samples were also collected during experiments and

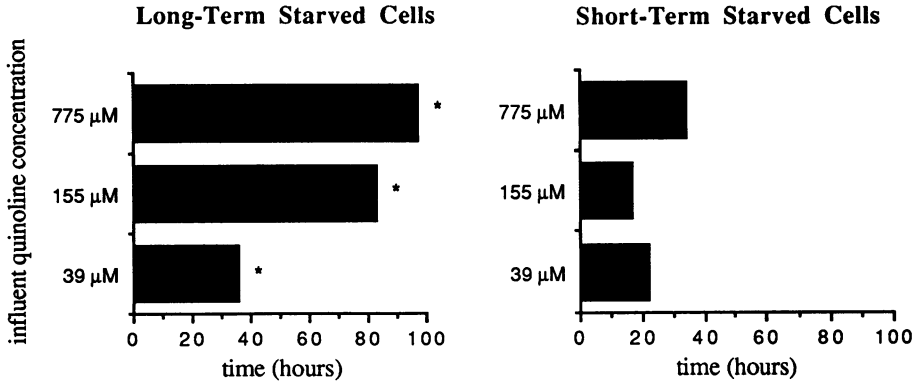

FIG. 2. Time required to induce quinoline degradation in a *P. cepacia* strain. An asterisk indicates that the difference between the induction times for LTS and STS cells was statistically significant ($P \leq 0.01$).

analyzed by HPLC to ensure that the quinoline influent concentration remained constant. Metabolites beyond 2OHQ (hereafter termed "other metabolites") were calculated as the difference between the input quinoline concentration and the sum of the effluent quinoline and 2OHQ concentrations. The concentrations of other metabolites were expressed as micromoles of 2OHQ degraded.

Monitoring the starvation-survival response. Periodic (6-day interval) samples were withdrawn from starvation flasks with a sterile syringe and a 27-gauge needle. Culturable cell numbers were determined on 10% tryptic soy agar spread plates (1). Triplicate samples (1.5 ml) were frozen for later protein and gross cell morphology analyses. The protein concentration was measured by using the Lowry et al. method (21a) (Micro BCA Assay; Pierce Chemical Co., Rockford, Ill.). Samples (1.0 ml) were mixed with 1.0 ml of 1 M NaOH and heated at 90°C for 10 min to extract protein (16). After the samples were cooled to room temperature, the pH was adjusted to <3 by adding 180 μl of 6 M HCl. Samples were then analyzed according to the protocol of the manufacturer of the assay kit. Gross cell morphology was determined by negative staining. Cells were placed on a carbon-coated 200-mesh copper specimen grid, stained with 1% phosphotungstinic acid for 3 min, and photographed on a Philips model 400 transmission electron microscope at 100 kV. Volumes for rod-shaped cells were calculated by assuming that the cell shape was a cylinder with a hemisphere on each end.

Bacterial adhesion and growth in the columns. Adhesion of STS and LTS cells to the glass beads was assessed by using columns like those used for degradation experiments, except that quinoline was omitted from the feed solution and the experiment duration was 45 min. The protein concentrations of the inoculum and the column contents at the completion of the experiment were compared. The protein concentrations rather than enumeration of culturable bacteria were used because of difficulties in recovering culturable bacteria from the glass beads because of cell adhesion. At the completion of the experiment, the glass beads, column plunger inlet filter, and column plunger outlet filter were separately suspended in 10 ml of 0.5 M NaOH and heated at 90°C for 10 min. After the suspensions were cooled to room temperature, samples (2.0 ml) were adjusted to pH <3 by adding 180 μl of 6 M HCl and analyzed for protein content. In addition, the column effluent (9 ml) was collected and enumerated by using the spread plate technique to determine the number of culturable bacteria that were eluted from the column.

Bacterial growth during degradation experiments was assessed by comparing the protein concentrations of the inoculum and the column contents at the completion of the experiment. The glass beads, column plunger inlet filter, and column plunger outlet filter were separately suspended in 10 ml of 0.1% sodium pyrophosphate (pH 7.0) and vortexed for 15 min to dislodge bacteria, and aliquots were spread plated to enumerate bacterial contaminants via colony morphology. The remaining solution was assayed for protein concentration as described above for monitoring the starvation-survival response.

Column data analysis. Real-time UV absorbance spectra specific for 2OHQ and HPLC concentrations from periodic samples were used to construct profiles of time versus concentration of effluent constituents for each experiment. These profiles were used to determine the time required for induction, time from induction to steady-state degradation, and steady-state effluent constituent concentrations. The maximum rate of change in the concentration of each effluent constituent was obtained from a best fit of the HPLC concentration data (r^2, typically >0.92) from each experiment. The Student t statistic was used to make pairwise comparisons among the six treatments (two starvation regimens, each at three quinoline concentrations). Quinoline degradation was defined in two steps. The formation of 2OHQ was the first reaction (10a), and initiation of this step defined induction for the purpose of this study. This reaction occurs approximately 4 to 7 times faster than subsequent reactions, and 2OHQ is released to solution before being utilized as a substrate (24). The second reaction was the degradation of 2OHQ to further products, which were grouped as other metabolites.

RESULTS

Degradation experiments. The average times required for induction for each treatment are shown in Fig. 2. LTS cells required significantly ($P \leq 0.01$) longer times to induce than STS cells did at all quinoline input concentrations. The induction times for LTS cells were 3 to 5 times longer than those of STS cells with 155 and 775 μM quinoline but only 1.6 times longer with 39 μM quinoline. For LTS cells, the induction time was significantly ($P \leq 0.01$) greater with 155 and 775 μM quinoline than with 39 μM quinoline. In con-

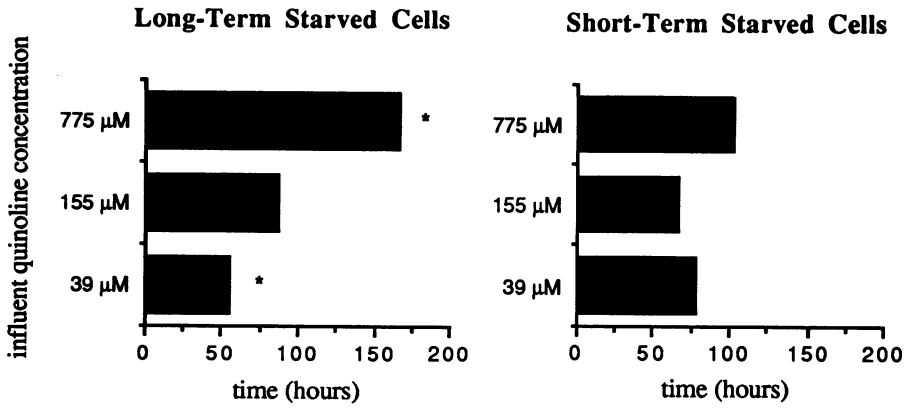

FIG. 3. Time required to progress from induction to steady-state degradation of quinoline for a *P. cepacia* strain. An asterisk indicates that the difference between the times for LTS and STS cells was statistically significant ($P \leq 0.02$).

trast, for STS cells, the induction times with 39 and 775 μM quinoline were the same and the induction time with 155 μM quinoline was significantly less than those with 39 μM ($P \leq 0.02$) and 775 μM ($P \leq 0.01$) quinoline.

The times from induction to steady-state degradation for each treatment are shown in Fig. 3. LTS cells required a significantly ($P \leq 0.01$) longer time to reach the steady state than STS cells did with 775 μM quinoline. The time to reach the steady state was the same for STS and LTS cells with 155 μM quinoline and significantly ($P \leq 0.02$) shorter for LTS cells than for STS cells with 39 μM quinoline. For LTS cells, the differences between the times to reach the steady state with 775 μM quinoline and both 39 μM ($P \leq 0.01$) and 155 μM ($P \leq 0.02$) quinoline were significant. The same trend was evident for STS cells, for which the time to the steady state with 775 μM quinoline was significantly different from those for both 39 μM ($P \leq 0.05$) and 155 μM ($P \leq 0.01$) quinoline.

The effluent constituents at the steady state for each treatment are shown in Fig. 4. With 39 μM quinoline, only other metabolites were present at the steady state for both STS and LTS cells. A transient peak of 2OHQ was observed in the effluent before this steady-state profile was reached for both STS and LTS cells. With 155 and 775 μM quinoline, all three constituents were present at the steady state. Transient peaks were not observed at these concentrations. The percentages (and concentrations) of other metabolites at the steady state were significantly ($P \leq 0.01$) greater for LTS cells than for STS cells with 155 μM quinoline.

The maximum rates of change in concentrations of effluent

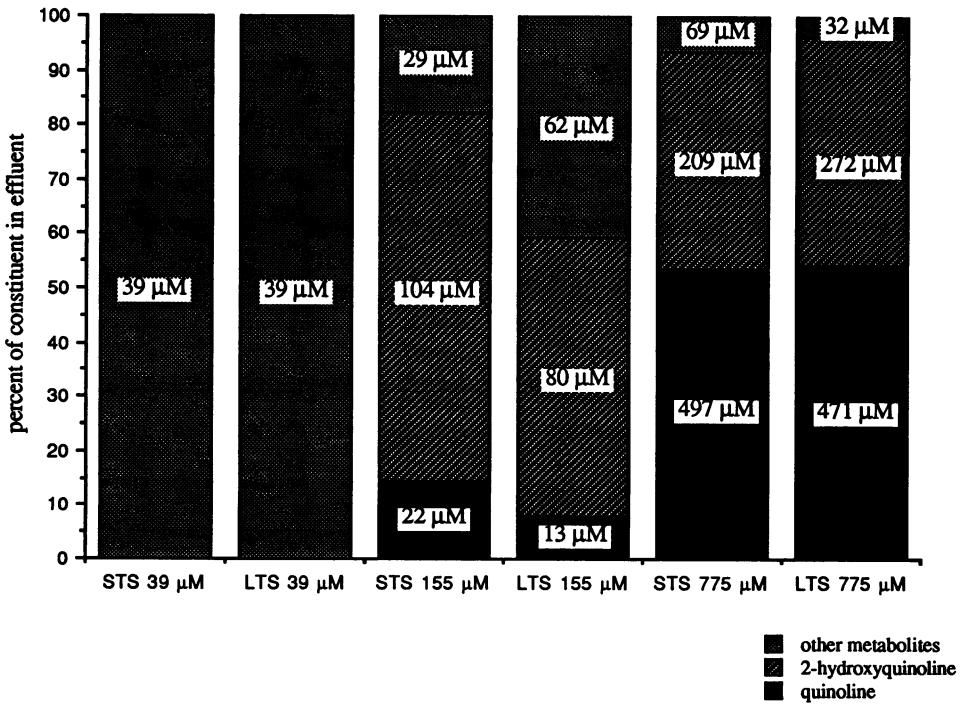

FIG. 4. Column effluent constituents at steady-state quinoline degradation for a *P. cepacia* strain. Constituents are shown as the average percents and the corresponding concentrations.

TABLE 1. Maximum rate of change of effluent concentrations during the progression from induction to steady-state degradation

Quinoline input concn (μM)	Cell type	Rate of change of effluent concn (nmol ml^{-1} h^{-1})		
		Quinoline	2OHQ	Other
39	STS	−1.25	0.39	1.30
39	LTS	−1.68	1.10	1.55
155	STS	−3.56	3.34	0.83
155	LTS	−3.02	1.47	1.33
775	STS	−3.50	2.83	2.5
775	LTS	−2.99	2.31	0.4

constituents are shown in Table 1. The maximum rate of change of the concentration of 2OHQ in the effluent was significantly ($P \leq 0.02$) greater for LTS cells than for STS cells with 39 μM quinoline. However, the maximum rate of change of the concentration of 2OHQ was significantly ($P \leq 0.02$) greater for STS cells than for LTS cells with 155 μM quinoline. With 775 μM quinoline, the maximum rate of change in the concentration of other metabolites (i.e., rate of 2OHQ degradation) was 6 to 7 times slower for LTS cells than for STS cells.

Less than 0.001% of the bacterial inoculum was eluted from columns, as determined by plate counts in experiments designed to determine the extent of bacterial adhesion. In addition, the combined protein contents of the beads and filters were equal to that of the inoculum in these adhesion experiments. The average amounts of total protein in the column were 1 to 1.5 (39 μM quinoline) and 2 to 2.5 (155 and 775 μM quinoline) times greater at the completion of the degradation experiments than at the beginning. Therefore, changes in the bacterial population were assumed to be not significant relative to the measured parameters during degradation experiments. The percentage of bacteria with a colony morphology different than that of the inoculated bacterium in columns at the completion of degradation experiments was typically less than 1% of the total.

Starvation-survival response. The average number of culturable bacteria and average protein concentration remained essentially constant for the duration of starvation (2 to 90 days). The average culturable count was 6.4×10^7 (1.6×10^7) CFU ml^{-1}. The average protein concentration was 7.49×10^{-5} (0.7×10^{-5}) ng $cell^{-1}$. Cells retained their rod shape for the duration of starvation monitoring. The average cell volume decreased by 29% during the first 40 days of starvation and by a total of 57% by day 62, after which the volume remained constant through 96 days of starvation.

DISCUSSION

To our knowledge, this is the first comparison of the ability of STS and LTS cultures to biodegrade an aromatic contaminant in continuous-flow column experiments. Induction of a biodegradative pathway by a cell that has undergone LTS is of importance because it involves a large commitment of energy and other cellular reserves. During starvation, the endogenous nutrient reserves of cells are decreased (13, 25, 26). Yet the activation and polymerization reactions of mRNA and protein syntheses require 4,300 high-energy phosphate bonds for the production of a single 1,000-amino-acid residue protein (28). These ribonucleotides and amino acids are initially made available at the sacrifice of other cellular macromolecules (23). In addition, the initial reactions in the degradation of recalcitrant compounds rarely yield energy for metabolism and, in some cases (e.g., monooxygenase reactions), may even require energy in the form of reduced NAD as a cofactor.

We examined the induction response and the initial two reactions of quinoline degradation to better understand how LTS effects the ability of a bacterial culture to respond to the appearance of a recalcitrant biodegradable substrate and the efficiency by which the culture processes quinoline through the initial reactions. Quinoline degradation by the *P. cepacia* used in this study proceeds via a membrane-associated dehydrogenase that forms 2OHQ (34a). This metabolite is then prepared for ring cleavage by a dioxygenation and dehydrogenation of the benzene ring (10a). The parameters selected for these experiments were chosen to measure the physiological response of the bacteria to the imposed experimental conditions. The time required for induction of a biodegradative pathway can be conceptualized as a measure of the ability of the bacteria to realign their physiology (i.e., regulation and production of the appropriate macromolecules) in response to new environmental conditions. The time from induction to steady-state degradation can be conceptualized as the time required for the bacteria to come into a temporary equilibrium with their new environment. The maximum rate of change in the concentration of the initial substrate and metabolites can be conceptualized (as a first approximation) as the cellular concentration and/or activity of the appropriate enzyme(s).

In this study, the in situ physiology of the bacterium was simulated by subjecting the bacterium to LTS. Many bacteria exposed to LTS reach a stable physiological state (starvation survival) after a specific period of time (typically 30 to 40 days) (2, 3, 20, 25, 30, 31). It is this stable starvation-survival state that simulates the in situ physiological state of the bacteria. Measurements of the number of culturable cells, protein concentration, and cell size were used in this study to determine when the bacterium reached the stable starvation-survival state. The *P. cepacia* strain was apparently well adapted to survival in an oligotrophic environment, because the number of culturable cells and the protein concentration remained constant for the duration of starvation. Cells starved for more than 60 days were determined to be in the stable starvation-survival state because their size remained constant.

To more closely approximate the physiological state that bacteria in the environment experience, both STS and LTS cells were subjected to two forms of biosynthetic stress before starvation. First, the use of succinate as a sole carbon and energy source resulted in suboptimal growth, as indicated by a cell size 59% smaller and a generation time 102% slower than those of cells grown on 10% tryptic soy broth (34a). Second, an oxidized form of nitrogen (NO_3^-) and a high carbon-to-nitrogen ratio (200:1) were used to simulate nitrogen stress in the environment. Although both STS and LTS cells were grown under this nutrient stress, the starvation process induced further physiological changes in LTS cells that were important to the response of the bacterium to an introduced substrate (i.e., quinoline).

With 39 μM quinoline, LTS cells were more efficient than STS cells at processing quinoline. LTS increased the time required to induce quinoline degradation but decreased the time from induction to steady-state degradation and increased the maximum rate of change in quinoline, 2OHQ, and other metabolite concentrations in the column effluent. With 155 μM quinoline, LTS cells also required more time to induce than STS cells but, once induced, were more efficient

at processing quinoline. In the column effluent for the LTS treatment, the quinoline concentrations were lower and the concentrations of 2OHQ converted to other metabolites at steady state were higher. This latter result was associated with a lower maximum rate of change in the 2OHQ concentration and a higher maximum rate of change in the concentration of 2OHQ converted to other metabolites for the LTS cells. LTS cells (155 μM quinoline) required longer to induce than did STS cells (all concentrations) or LTS cells with 39 μM quinoline, indicating some inhibitory effect on induction of biodegradation in LTS cells with 155 μM quinoline.

In contrast to the results at the lower concentrations, with 775 μM quinoline, LTS cells responded much more slowly and were less efficient at processing quinoline than were STS cells. LTS cells (775 μM quinoline) took longer to induce and longer to progress from induction to steady-state degradation than STS cells did with 775 μM quinoline and longer to progress from induction to the steady state than LTS cells did with 155 μM quinoline. In addition, the maximum rate of change in the concentration of 2OHQ converted to other metabolites in LTS experiments was only 16% of the rate in STS experiments with this quinoline concentration. Thus, 775 μM quinoline had a general inhibitory or toxic effect on quinoline degradation in LTS cells or induced the LTS cells to regulate the expression and/or activity of catabolic enzymes for slower quinoline utilization.

The greater efficiency with which LTS cells with 39 and 155 μM quinoline processed the substrate through the initial reactions is indicative of the development of an efficient physiological state poised for nutrient capture and rapid utilization. Upon nutrient deprivation, bacteria induce the synthesis of a variety of novel proteins to escape starvation, prepare for LTS, and allow development of a highly stress-resistant cell state (23). Because these responses utilize a large fraction of endogenous energy reserves and other macromolecules that are not critical to the cell's survival, bacteria exposed to LTS reach a stable starvation-survival state characterized by very low energy reserves, a very low maintenance energy requirement or rate of respiration, and a small size (13, 25, 26, 30, 31). Because of more depleted endogenous energy reserves, LTS cells utilized the substrate at low concentrations more efficiently than did STS cells.

An inhibitory effect was manifested in the longer induction time and time from induction to steady-state degradation for LTS cells than for STS cells with 155 and 775 μM quinoline. The oxygen concentration in the column influent and effluent for an experiment with a 775 μM influent quinoline concentration was measured; inhibition was not an artifact of oxygen limitation (34a). Although it has been reported that starved cells may survive exposure to a variety of physical and chemical stresses better than nonstarved cells (23), sensitivity to aromatic substrates may be related to the localization of the compound and intermediates in the cell interior during catabolism. Kong (17a), who used another deep subsurface bacterium, demonstrated that cells starved for 2 days required a concentration of toluene or *o*-xylene that was 1 order of magnitude greater than that required by cells starved for 60 days to reduce viability by 10%. To verify that the differences in induction for LTS versus STS cells were not due to substantial cell mortality, cells starved for 118 days were subjected to 775 μM quinoline in a batch experiment (34a). The transient reduction in viability (20%) before the onset of quinoline utilization was not enough to account for all of the differences in the induction response observed in the column experiments. Inhibitory effects were not noted during steady-state degradation. Therefore, by utilizing quinoline (i.e., lowering the extracellular concentration) and recovering from starvation (i.e., increased cellular energy), LTS cells were able to overcome the initial inhibitory effects of quinoline.

Using the same bacterium and similar column design, McBride et al. (24) performed experiments to examine maximum quinoline degradation rates. They used a cell density that was 1 order of magnitude greater and induced bacteria that had undergone an acclimation process to obtain maximum degradation rates. In our experiments, both STS and LTS cells reached steady-state degradation rates similar to the rates observed by McBride et al. (24). The higher biomass in the previous study may have affected nutrient diffusion (i.e., formation of a biofilm), resulting in a lower biodegradation rate per unit of biomass.

Several conclusions can be inferred from this study relative to in situ biodegradation of organic contaminants. Bacteria indigenous to oligotrophic subsurface environments (as simulated by LTS in the laboratory) may initiate more complete degradation of a substrate than may be indicated by laboratory experiments with recently cultured, actively metabolizing cells. Thus, more complete degradation of contaminants may result in lower concentrations of metabolites that may also be contaminants. However, contaminant concentration appears to be very important in that higher concentrations may inhibit recovery from starvation and/or the induction process in indigenous bacteria. In summary, characteristics of in situ contaminant degradation must be carefully studied under conditions that closely simulate the physiological state of bacteria in the environment to construct successful predictive models of biodegradation in subsurface environments.

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REFERENCES

1. **American Public Health Association.** 1989. Standard methods for the examination of water and wastewater, 17th ed. American Public Health Association, Washington, D.C.
2. **Amy, P. S., and R. Y. Morita.** 1983. Starvation-survival patterns of sixteen freshly isolated open-ocean bacteria. Appl. Environ. Microbiol. **45:**1109–1115.
3. **Amy, P. S., C. Pauling, and R. Y. Morita.** 1983. Starvation-survival processes of a marine vibrio. Appl. Environ. Microbiol. **45:**1041–1048.
4. **Amy, P. S., C. Pauling, and R. Y. Morita.** 1983. Recovery from nutrient starvation by a marine *Vibrio* sp. Appl. Environ. Microbiol. **45:**1685–1690.
5. **Balkwill, D. L.** 1989. Numbers, diversity, and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina. Geomicrobiol. J. **7:**33–52.
6. **Balkwill, D. L., and W. C. Ghiorse.** 1985. Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma. Appl. Environ. Microbiol. **50:**580–588.
7. **Beloin, R. M., J. L. Sinclair, and W. C. Ghiorse.** 1988. Distribution and activity of microorganisms in subsurface sediments of a pristine study site in Oklahoma. Microb. Ecol. **16:**85–97.
8. **Bone, T. L., and D. L. Balkwill.** 1988. Morphological and cultural comparison of microorganisms in subsurface soil and subsurface sediments at a pristine study site in Oklahoma.

Microb. Ecol. **16:**49–64.

9. **Bouwer, E. J., and P. L. McCarthy.** 1983. Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. **45:**1286–1294.
10. **Boylen, C. W., and J. C. Ensign.** 1970. Intracellular substrates for endogenous metabolism during long-term starvation of rod and spherical cells of *Arthrobacter crystallopoietes*. J. Bacteriol. **103:**578–587.

10a. **Brockman, F. J.** Unpublished data.

11. **Brockman, F. J., B. A. Denovan, R. J. Hicks, and J. K. Fredrickson.** 1989. Isolation and characterization of quinoline-degrading bacteria from subsurface sediments. Appl. Environ. Microbiol. **55:**1029–1032.
12. **Chapelle, F. H., and D. R. Lovely.** 1990. Rates of microbial metabolism in deep coastal plain aquifers. Appl. Environ. Microbiol. **56:**1865–1874.
13. **Dawes, E. A.** 1985. Starvation, survival and energy reserves, p. 43–79. *In* M. Fletcher and G. Floodgate (ed.), Bacteria in their natural environments. Academic Press, Inc., New York.
14. **Ensign, J. C.** 1970. Long-term starvation survival of rod and spherical cells of *Arthrobacter crystallopoietes*. J. Bacteriol. **103:**569–577.
15. **Fredrickson, J. K., T. R. Garland, R. J. Hicks, J. M. Thomas, S. W. Li, and K. M. McFadden.** 1989. Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties. Geomicrobiol. J. **7:**53–66.
16. **Hanson, R. S., and J. A. Phillips.** 1981. Chemical composition, p. 358. *In* P. Gerhardt (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
17. **Hicks, R. J., and J. K. Fredrickson.** 1989. Aerobic metabolic potential of microbial populations indigenous to deep subsurface environments. Geomicrobiol. J. **7:**67–78.

17a. **Kong, S.** 1988. M.S. thesis, Washington State University, Pullman.

18. **Kuhn, E. P., P. J. Colberg, J. L. Schnoor, O. Wanner, A. J. Zehnder, and R. P. Schwarzenbach.** 1985. Microbial transformations of substituted benzenes during infiltration of river water to groundwater: laboratory column studies. Environ. Sci. Technol. **19:**961–968.
19. **Kuhn, E. P., J. Zeyer, P. Eicher, and R. P. Schwarzenbach.** 1988. Anaerobic degradation of alkylated benzenes in denitrifying laboratory aquifer columns. Appl. Environ. Microbiol. **54:**490–496.
20. **Kurath, G., and R. Y. Morita.** 1983. Starvation-survival physiological studies of a marine *Pseudomonas* sp. Appl. Environ. Microbiol. **45:**1206–1211.
21. **Leenheer, J. A., R. L. Malcolm, P. W. McKinley, and L. A. Eccles.** 1974. Occurrence of dissolved organic carbon in selected ground-water samples in the United States. J. Res. U.S. Geol. Surv. **2:**361–369.

21a. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.

22. **Madsen, E. L., and J. M. Bollag.** 1989. Aerobic and anaerobic microbial activity in deep subsurface sediments from the Savannah River Plant. Geomicrobiol. J. **7:**93–102.
23. **Matin, A., E. A. Auger, P. H. Blum, and J. E. Schultz.** 1989. Genetic basis of starvation survival in nondifferentiating bacteria. Annu. Rev. Microbiol. **43:**293–316.
24. **McBride, J. F., F. J. Brockman, J. E. Szecsody, and G. P. Streile.** 1992. Kinetics of quinoline degradation, sorption, and desorption in a clay-coated model soil containing a quinoline-degrading bacterium. J. Contam. Hydrol. **9:**133–154.
25. **Morita, R. Y.** 1985. Starvation and miniaturisation of heterotrophs, with special emphasis on maintenance of the starved viable state, p. 111–130. *In* M. Fletcher and G. Floodgate (ed.), Bacteria in their natural environments. Academic Press, Inc., New York.
26. **Morita, R. Y.** 1988. Bioavailability of energy and its relationship to growth and starvation survival in nature. Can. J. Microbiol. **34:**436–441.
27. **Moyer, C. L., and R. Y. Morita.** 1989. Effect of growth rate and starvation-survival on the viability and stability of a psychrophilic marine bacterium. Appl. Environ. Microbiol. **55:**1122–1127.
28. **Neidhardt, F. C., J. L. Ingraham, and M. Schaechter.** 1990. Physiology of the bacterial cell: a molecular approach. Sinauer Associates, Inc., Sunderland, Mass.
29. **Nelson, L. M., and D. Parkinson.** 1978. Effect of starvation on survival of three bacterial isolates from an arctic soil. Can. J. Microbiol. **24:**1460–1467.
30. **Novitsky, J. A., and R. Y. Morita.** 1977. Survival of a psychrophilic marine vibrio under long-term nutrient starvation. Appl. Environ. Microbiol. **33:**635–641.
31. **Novitsky, J. A., and R. Y. Morita.** 1978. Possible strategy for the survival of marine bacteria under starvation conditions. Marine Biol. **48:**289–295.
32. **Phelps, T. J., E. G. Raione, D. C. White, and C. B. Fliermans.** 1989. Microbial activities in deep subsurface environments. Geomicrobiol. J. **7:**79–92.

32a. **Reeves, R.** Unpublished data.

33. **Sinclair, J. L., and W. C. Ghiorse.** 1989. Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. Geomicrobiol. J. **7:**15–31.
34. **Sinclair, J. L., S. J. Randtke, J. E. Denne, L. R. Hathaway, and W. C. Ghiorse.** 1990. Survey of microbial populations in buried-valley aquifer sediments from northeastern Kansas. Ground Water **28:**369–377.

34a. **Truex, M. J.** 1991. M.S. thesis, Washington State University, Pullman.

35. **Vogel, R. M., and P. L. McCarthy.** 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. **49:**1080–1083.
36. **White, D. C., G. A. Smith, M. J. Gehron, J. H. Parker, R. H. Findlay, R. F. Martz, and H. L. Fredrickson.** 1983. The groundwater aquifer microbiota: biomass, community structure, and nutritional status. Dev. Ind. Microbiol. **24:**201–211.
37. **Wilson, J. T., J. F. McNabb, D. L. Balkwill, and W. C. Ghiorse.** 1983. Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. Ground Water **21:**134–142.
38. **Wilson, J. T., J. F. McNabb, B. H. Wilson, and M. J. Noonan.** 1983. Biotransformation of selected organic pollutants in ground water. Dev. Ind. Microbiol. **24:**225–233.
39. **Zeyer, J., E. P. Kuhn, and R. P. Schwarzenbach.** 1986. Rapid microbial mineralization of toluene and 1,3-dimethylbenzene in the absence of molecular oxygen. Appl. Environ. Microbiol. **52:**944–947.