INABILITY OF BACTERIA TO DEGRADE LOW CONCENTRATIONS OF TOLUENE IN WATER

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Abstract—Microbial utilization of low concentrations of toluene in water was investigated. When bacteria that were not actively degrading toluene were incubated in solutions containing 2.1 to 11 μg of toluene/L, the concentrations remaining in solution after 8 d ranged from 0.69 to 0.99 μg/L. However, when the initial concentration was ≥31 μg/L, the concentration remaining was <0.2 μg/L. When bacteria actively degrading toluene were inoculated into solutions containing 0.9 μg of toluene/L, the concentration remaining after 8 d was 79 ng/L. We propose that the inability of some bacteria to maintain or attain high metabolic activity on a compound present at low concentrations may prevent its rapid degradation in natural environments.

Keywords—Concentration Persistence Threshold Toluene

INTRODUCTION

Toxic organic compounds may persist in natural waters at low concentrations even though they are readily degradable by microorganisms at high concentrations. When the concentration falls below a threshold value, the metabolism of the compound can be too slow to provide the cells with energy at a rate needed for the maintenance of their metabolism [1–3]. As a consequence, the cells will not multiply, and the rate of degradation will remain slow if the population of bacteria able to metabolize the compound is initially small. However, if the population is large, low concentrations of a chemical can be degraded [4].

Enzyme induction is required prior to the metabolism of many compounds [5,6]. If the concentration of a chemical is too low to induce the synthesis of enzymes required for metabolism of the compound in the cells, then the degradation of that compound may be slow or fail to occur even if the population of bacteria is large. Robertson and Button [7] reported that the minimum concentration of toluene above which an increase in activity would occur could be as high as 132 μg/L, and Reber [8] reported that 3-chlorobenzoate could induce its degradation by Acinetobacter calcoaceticus only at concentrations above 157 μg/L.

The purpose of this research was to investigate whether or not the degradation of a test compound by large populations of a bacterium is affected when the concentration of the chemical in water falls below a certain level. The test compound, toluene, was selected because it is readily degradable by bacteria and is a common pollutant of soil and ground water, where it is derived from accidental spills or leaking storage tanks.

MATERIALS AND METHODS

Chemicals

Methanol, toluene, 1-butanol, and ethanol (all certified ACS) were from Fisher Scientific Rochester, NY, USA. Triton X-100, styrene (>99%), benzene (>99% ACS), and dimethyl sulfoxide (>99%) were from Aldrich Chemical, Milwaukee, WI, USA. d-Glucose was from Mallinckrodt Chemical, St. Louis, MO, USA. Air (grade 0.1), N₂ (grade 5.0), and H₂ (grade 5.0) were from Airco, Murray Hill, NJ, USA. Radioactive [methyl ¹⁴C] toluene was from Amersham, Arlington Heights, IL, USA.

The inorganic salts solution contained (in g/L): MgSO₄·7H₂O, 0.2; CaCl₂·2H₂O, 0.2; NH₄NO₃, 0.15; K₂HPO₄, 0.4; NaH₂PO₄, 0.6; and FeCl₃·6H₂O, 0.01. The solution was adjusted to pH 6.1 with HCl and sterilized by autoclaving.

Microorganisms

The bacterium (strain T) is a short, gram positive rod that was isolated from forest soil by streaking an enrichment culture on a solid medium containing the inorganic salts and 1.6% agar. After being streaked, the plates were placed inside a 1.0-L glass jar and 30 µL of toluene was introduced into the jar as a carbon source and allowed to evaporate. In liquid media, the isolate grew rapidly on benzene, toluene, styrene, glucose, ethanol, and 1-butanol at 100 mg/L, but it did not grow on methanol or dimethyl sulfoxide. Results similar to those reported here were obtained for a second bacterium, which was isolated together with strain T [9]. The population density is given either as colony-forming units (CFU) determined by plating or as the number of cells/L determined by a calculation based on the observation that 1 mg of toluene gave rise to approximately 9 × 10⁸ CFU.

The cultures were incubated on a rotary shaker (operating at 90 rpm) at 23°C in 64-ml glass bottles (Qorpack clear Boston Rounds, Fisher Scientific, Rochester, NY, USA) closed with screw caps with Teflon-coated silicon septa or Mininert valves (Baxter Scientific, Edison, NJ, USA). To avoid artifacts because small amounts of toluene sorbed onto the Teflon septa and then slowly desorbed, the caps were replaced with uncontaminated caps during some experiments.

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Tolouene concentration was determined by injecting 500 μl of the headspace into a gas chromatograph (GC) (Model HP-5890A, Hewlett Packard, Kennett Square, PA, USA) equipped with a 25 HP-1 capillary column (Hewlett Packard) and a flame ionization detector. The temperature of the oven, injector, and detector were 70, 160, and 150°C, respectively, and the retention time of toluene was 2.2 min. The carrier gas was N₂. The concentration of toluene in water was calculated from its concentration in the headspace using Henry’s law. The lowest concentration detected in water was approximately 0.2 μg/L [9].

Mineralization of radioactive toluene was determined by trapping ¹⁴CO₂ in 0.6 ml of an aqueous solution of 2.0 N NaOH present in a 76-mm test tube placed inside each bottle. The alkali was mixed with 2.4 ml of water and 2.0 ml of Liquiscent scintillation cocktail (National Diagnostics, Manville, NJ, USA), and then added to a 7-ml plastic minivial. The mixture was analyzed with a liquid scintillation counter (Model LS 7500, Beckman Instruments, Irvine, CA, USA).

An additional method was sometimes used to measure the amount of radioactive tolouene remaining in the bottle. To trap the remaining toluene after removal of the alkali from the test tube, 2.5 ml of 1-butanol was placed into the test tube and the butanol was analyzed for radioactivity after 1 or 2 d by mixing it with 2.4 ml of ethanol and 2.0 ml of scintillation cocktail. This procedure was repeated until radioactivity was no longer present in the butanol.

Degradation of different concentrations of toluene

Concentrations of radioactive toluene (28,000 disintegrations per min per bottle) ranging from 0.56 to 10,000 μg/L were introduced into unreplicated bottles containing 25 ml of an aqueous solution of inorganic salts and an empty test tube. In addition, sterile bottles containing 0.9, 3.9, 6.9, 30.9, 300, or 3000 μg of toluene were used to assess abiotic losses of toluene. The culture was grown for 10 d in 10 ml of the inorganic salts solution containing 1.0 mg of ethanol and 5.5 × 10⁵ CFU/ml of the inoculum. Six days later, the headspace was analyzed, and the NaOH was replaced with 2.5 ml of 1-butanol. At the same time, 0.9 ml of 6.0 N NaOH was introduced into the aqueous solution to stop biodegradation and to increase the water solubility of metabolically produced volatile alcohols and acids so that they would not be trapped in the butanol. The butanol was replaced at 48 and 96 h, and 2.0 ml of an aqueous solution of 1% Triton X-100 then was added to the water phase. At 120 h, the test tube was removed, the inside of the bottles was scraped with a brush to remove adhering biomass, and the solution was removed for analysis of radioactivity. The total recovery represents the sum of the radioactivity trapped in NaOH, trapped in butanol, and remaining in aqueous solution.

Degradation in the presence of other carbon sources

The same procedures as above were used to prepare the bottles and sample the cultures. The medium contained 0.9 μg of toluene and 50 mg of benzene, styrene, ethanol, 1-butanol, or d-glucose/L. The inoculum was prepared as in the previous study. In an additional treatment, toluene was the only carbon source, and the medium was inoculated with bacteria (1.6 × 10⁵ CFU/ml) that were preconditioned so that they exhibited high metabolic activity on toluene. This inoculum was prepared by growing the bacteria in a medium to which toluene (1 mg/L) was repetitively added prior to introduction of the cells into the bottles. All treatments were duplicated.

Mathematical analysis

The activity of the bacteria living on toluene varied with time. A model was derived to simulate the effect of variation in bacterial activity on the degradation of low concentrations of toluene. This model was conceptually based on the assumption that the activity was related to the relative concentrations of the enzymes involved in the rate-determining step in toluene degradation in the cells. Button [10] used the term “affinity” to describe a similar property, but this term is inappropriate for the mathematical model.

The model is a simplified version of the model of Ely et al. [11], and therefore, only a short description is presented. The rate of disappearance of toluene is given by Equation 1,

\[
\frac{dT}{dt} = \frac{-V_i}{V_i + PV \frac{r_A X T}{K_T + T}}
\]

where \( T \) is the toluene concentration (μg/L), \( t \) is the time (h), \( K_T \) is the half-saturation constant (μg/L), \( r_A \) is the rate constant (μg of toluene per cell and per h), \( X \) is the cell concentration (cells/L), \( P \) is the partition coefficient between air and water for toluene (\( P = 0.232 \) [9]), and \( V_i \) and \( V_l \) are the volumes in the bottle of air and liquid, respectively. The last three parameters take into account the toluene in the headspace. A unitless, which represents the relative activity of the cells, varies from zero (no activity) to one (maximum activity).

The increase in activity is assumed to be proportional (with \( \Omega \), in cells/μg of toluene, as the proportionality constant) to the amount of toluene the cells degrade (Eqn. 2).

\[
\frac{dA}{dt} = \frac{V_i + PV \frac{r_A X T}{K_T + T}}{X} f \frac{dT}{dt}
\]

This assumption is based on the hypothesis that the cells do not store sufficient carbon and energy to synthesize significant amounts of the enzymes involved in the metabolism of toluene and that they require the degradation of toluene to obtain the energy to synthesize these enzymes. In Equation 2, the loss of activity is assumed to be first order, with \( f \) (per h) as a first-order parameter.

The variation in cell density (Eqn. 3) is assumed to be proportional to the quantity of toluene degraded. Knowing that 1 g of toluene yields 0.9 × 10¹² CFU (see above), the yield parameter \( Y \) is equal to 0.9 × 10⁸ cells/μg of toluene.

\[
\frac{dX}{dt} = -Y \frac{V_i + PV \frac{r_A X T}{K_T + T}}{V_i} \frac{dT}{dt}
\]

However, little growth occurred in the simulated experiments.

The model was derived for three purposes. The first was to obtain a mathematical expression of the variation in activity that was consistent with experimental observations. The second purpose was to use this expression to test whether or not variation in metabolic activity might be responsible for the incomplete degradation that was observed. The third purpose was to test whether or not an increase in activity was proportional to the amount of toluene degraded. Equations 1 and 2 were not sufficient for these purposes, and additional conditions were imposed on parameters \( \Omega \) and \( f \). These conditions are described below.

Parameter \( \Omega \) controls the increase in activity. Because Equation 2 would allow the activity to increase beyond the
maximum ($A > 1$), $\Omega$ was set to zero in the simulations as soon as $A$ reached a value of one. Parameter $f$ controls the loss of activity and, for simplicity, it was assumed that a loss of activity did not occur as the activity of the bacteria increased. Therefore, the simulation of the degradation of toluene by bacteria with a low initial activity was made with $f$ set to zero.

The values of the parameters were found using experimental data given in the Results. The data obtained during the degradation of toluene by inocula having the highest metabolic activity were used to estimate the values of $r_T$ and $K_T$. The data obtained with inocula initially inactive on toluene were used to estimate $\Omega$, and the data obtained when measuring the loss of activity on toluene were used to estimate $f$. Parameters $r_T$, $K_T$, and $\Omega$ were estimated by fitting the model to the data by weighted least squares fitting [12], and the rules describing the variation of $f$ per h (and indicated in Results) were obtained by trial and error.

### RESULTS

#### Degradation of different concentrations of toluene

The concentrations of toluene below which the metabolism of an initially inoccule degrading the toluene will be markedly reduced were determined. The extent of degradation after 8 d was determined in three ways: trapping $^{14}$CO$_2$, measuring the remaining concentration of toluene by gas chromatography, and collecting toluene in an organic trap. Finally, the radioactivity remaining in aqueous solution was measured to obtain the total recovery.

At initial concentrations of toluene from 0.56 to 11 $\mu$g/L, from 0.34 to 0.99 $\mu$g/L was not metabolized (Table 1). This represents from 61.6 to 6.3%, respectively, of the initial concentration. In contrast, when the initial concentration was $\geq$31 $\mu$g/L, less than 0.2 $\mu$g/L (which was the detection limit of the gas chromatographic method) remained, demonstrating that the bacteria were able to degrade toluene below this concentration.

Calculations from the data in Table 1 show that 2.5 to 33.9% of the toluene utilized was mineralized. At lower initial concentrations, the percentages were low; they were approximately 3 to 5% for concentrations $<7.1$ $\mu$g/L. The percentages increased at levels $>7.1$ $\mu$g/L.

The average total recovery of radioactivity for all concentrations was 91.2 $\pm$ 6.1% for the cultures, and the value was 84.0 $\pm$ 5.8% for the uninoculated media. Small losses of radioactive toluene and CO$_2$ may have occurred, but the recoveries were still high.

#### Degradation in the presence of other carbon sources

In the experiment described above, it is possible that the bacteria degrading low concentrations of the substrate did not have a sufficient supply of carbon or energy to increase their activity on toluene. Using the same inoculum and concurrent with the above experiment, we tested whether or not the activity of cells metabolizing 0.9 $\mu$g of toluene/L would increase if the bacteria were provided with an additional carbon source at 50 mg/L. In addition, to determine whether or not the period of degradation of toluene might be shortened by an inoculum with a high initial activity on toluene, an additional carbon source was not included in one treatment, but the bacteria were preconditioned prior to inoculation.

Toluene was degraded in 8 d to below the detection limit of 2 ng/L only in the presence of benzene and styrene (Table 2). In media containing glucose, ethanol, or 1-butanol, 53, 203, and 414 ng/L of toluene remained, respectively. The final concentration of toluene (79 ng/L) remaining in the solution that received a more active inoculum was appreciably lower than the concentrations that were left at comparable initial concentrations in the previous experiments, with less active inocula.

From these data, we calculate that 19.1 and 37.0% of the

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**Table 1. Biodegradation in 8 d of toluene at different initial concentrations by bacteria not initially active on toluene**

<table>
<thead>
<tr>
<th>Initial toluene concn. ($\mu$g/L)</th>
<th>Toluene mineralized (%)$^a$</th>
<th>Toluene remaining ($\mu$g/L)$^b$</th>
<th>$^{14}$C remaining in aqueous solution (%)$^c$</th>
<th>Total $^{14}$C recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56</td>
<td>1.2</td>
<td>0.34</td>
<td>37.2</td>
<td>89.7</td>
</tr>
<tr>
<td>2.1</td>
<td>2.1</td>
<td>0.84</td>
<td>57.8</td>
<td>83.4</td>
</tr>
<tr>
<td>3.1</td>
<td>1.7</td>
<td>0.99</td>
<td>66.4</td>
<td>90.0</td>
</tr>
<tr>
<td>4.1</td>
<td>3.7</td>
<td>0.89</td>
<td>74.6</td>
<td>96.2</td>
</tr>
<tr>
<td>5.1</td>
<td>4.1</td>
<td>0.93</td>
<td>77.7</td>
<td>90.0</td>
</tr>
<tr>
<td>6.1</td>
<td>4.5</td>
<td>0.91</td>
<td>80.6</td>
<td>92.3</td>
</tr>
<tr>
<td>7.1</td>
<td>6.3</td>
<td>0.82</td>
<td>82.1</td>
<td>101.5</td>
</tr>
<tr>
<td>9.1</td>
<td>6.9</td>
<td>0.79</td>
<td>84.4</td>
<td>93.0</td>
</tr>
<tr>
<td>11</td>
<td>10.7</td>
<td>0.69</td>
<td>84.9</td>
<td>92.9</td>
</tr>
<tr>
<td>31</td>
<td>16.6</td>
<td>$&lt;0.2$</td>
<td>83.1</td>
<td>97.1</td>
</tr>
<tr>
<td>100</td>
<td>24.0</td>
<td>$&lt;0.2$</td>
<td>76.0</td>
<td>77.2</td>
</tr>
<tr>
<td>300</td>
<td>24.5</td>
<td>$&lt;0.2$</td>
<td>75.3</td>
<td>88.9</td>
</tr>
<tr>
<td>1,000</td>
<td>29.3</td>
<td>$&lt;0.2$</td>
<td>70.5</td>
<td>93.8</td>
</tr>
<tr>
<td>3,000</td>
<td>28.8</td>
<td>$&lt;0.2$</td>
<td>71.0</td>
<td>94.7</td>
</tr>
<tr>
<td>10,000</td>
<td>33.9</td>
<td>$&lt;0.2$</td>
<td>66.0</td>
<td>91.8</td>
</tr>
<tr>
<td>Controls$^d$</td>
<td>0.6 $\pm$ 0.2$^d$</td>
<td>96.3 $\pm$ 0.4$^d$</td>
<td>3.1 $\pm$ 0.4$^d$</td>
<td>84.0 $\pm$ 5.8$^d$</td>
</tr>
</tbody>
</table>

$^a$ As a percentage of total $^{14}$C recovered.

$^b$ Calculated from the amount of radioactive toluene trapped in an organic solvent for initial concentrations $\leq$11 $\mu$g/L and from gas chromatographic analysis for higher initial concentrations.

$^c$ Percentage of total recovered $^{14}$C remaining in solution after removal of $^{14}$CO$_2$ and [1$^{14}$C]toluene.

$^d$ Values from 6 uninoculated bottles containing from 1.1 to 3000 $\mu$g of toluene/L.
Degradation of individual additions

This experiment determined how quickly bacterial cells lose and recover their metabolic activity when grown on toluene. The method consisted of successively adding small amounts of toluene (approximately 1.0 mg/L of water) over increasing time periods. The inoculum was fully active. It was expected that the longer the time between two additions of toluene, the larger the decrease in the activity of the cells and the slower the degradation. The bottle contained 12 ml of inorganic salts solution and an initial population of 3.4 \( \times 10^7 \) CFU/ml.

To assess whether the increase in activity observed in Fig. 1 was due to the metabolism of toluene, the increase in metabolic rate during the degradation was not the consequence of marked variation in cell density. Moreover, the population density of this strain grown on toluene (100 \( \mu \)g/L) did not decrease appreciably after a 49-d period of starvation (data not shown).

Degradation by bacteria grown on ethanol and 1-butanol

Another study determined how quickly the activity of strain T would increase. Inocula with low activities on toluene were grown on ethanol or 1-butanol (70 mg/L), and a low concentration of toluene (approximately 1.3 mg/L) was added 8 d later. Duplicate bottles contained 12 ml of inorganic salts solution and an initial population of 3.4 \( \times 10^3 \) cells/ml.

The degradation of toluene by ethanol- and butanol-grown cells was initially slow, but the rate increased after 1 h (Fig. 2). Toluene was no longer detectable after approximately 4 h. The cell densities before and immediately after the degradation of the toluene were 3.2 \( \times 10^7 \) and 3.3 \( \times 10^7 \) CFU/ml for ethanol-grown cells and 4.3 \( \times 10^7 \) and 4.2 \( \times 10^7 \) CFU/ml for butanol-grown cells.

Relationship between increase in activity and degradation

To assess whether the increase in activity observed in Figures 1 and 2 was related to the amount of toluene metabolized, the goodness of fit of a mathematical model consistent with this hypothesis to the experimental data was tested. The decrease in activity was not a first order function, as described
in the model. Therefore, the curve fit was made assuming that there was no loss of activity with time (i.e., \( f = 0 \)) during the degradation of toluene, and the curves were fit as if they were independent of each other. That is, the initial activity corresponding to each curve was independent of the activity of the other curves. The sum of weighted squares obtained for the curve fit was 193 with 158 degrees of freedom. Assuming that this sum follows a chi-square distribution [13], the probability that a sum would be obtained that is greater or equal to the one calculated is 0.030; the fit would be accepted at the 97% level. The lines in Figures 1 and 2 are the values predicted by the model. Because visual examination shows a good fit, the hypothesis that the increase in activity is proportional to the amount of toluene degraded is acceptable.

The values and standard deviations of the parameters obtained from the curve fit are: \( r_t = 308 \times 10^{-9} \pm 7 \times 10^{-9} \) (\( \mu g \) of toluene/cell/h), \( \Omega = 4.7 \times 10^6 \pm 0.2 \times 10^6 \) (cells/\( \mu g \)), and \( K_f = 202 \pm 14 \) (\( \mu g/L \)). The results from the curve fit for the activities before and after each addition of toluene are presented in Table 3.

The values for the activities and standard deviations of the parameters obtained from the curve fit are: \( r_t = 308 \times 10^{-9} \pm 7 \times 10^{-9} \) (\( \mu g \) of toluene/cell/h), \( \Omega = 4.7 \times 10^6 \pm 0.2 \times 10^6 \) (cells/\( \mu g \)), and \( K_f = 202 \pm 14 \) (\( \mu g/L \)). The results from the curve fit for the activities before and after each addition of toluene are presented in Table 3.

The times given in column 1 of Table 3 correspond to those in Figure 1. The activities for cells grown on ethanol or 1-butanol, which were provided with toluene only at the time of inoculation, are shown in the last 2 rows of the table, and the curves depicting degradation are shown in Figure 2. The highest activity was evident at 1.9 h. The data show that the initial activity declined when the cells were without toluene for increasingly long periods; for example, the relative activities of cells incubated without substrate for 6.4, 14.7, and 167 h were 0.208, 0.0547, and 0.0243, respectively. The initial activities of cultures grown on ethanol or 1-butanol were much less than the lowest activities of the bacteria receiving individual additions of toluene, but the activity of these cells increased 48 and 67 times, respectively, by the time they degraded the added toluene.

**Simulations**

Model simulations assessed whether or not the results obtained during the degradation of varying amounts of toluene by inactive and active inocula (shown in Tables 1 and 2) could be a consequence of differences in the activity of the inocula. For this purpose, the values calculated for the initial and final degradative activities of the top 9 rows of Table 3 were used to provide a mathematical description of the loss of activity with time. This description was made by assuming that the loss of activity is a first order function (i.e., described with the parameter \( f \)) but only during a certain period of time. Accordingly, the description is the following: the loss is zero (\( f = 0 \)) when the concentration of toluene in water is above a threshold value of 4 \( \mu g/L \). When the concentration of toluene falls below this threshold and during the first 2.2 h, \( f \) equals 0.06 per h. The value of \( f \) equals 0.34 per h until the relative activity is 0.055, and the value of \( f \) equals 0.005 per h when the relative activity is \( \leq 0.055 \). Strain T did not metabolize toluene initially sorbed onto granular activated carbon when the toluene concentration in solution fell below approximately 4 \( \mu g/L \) [9]; hence, this value was assumed to be the concentration below which bacterial activity decreases.

A simulation was made of the degradation of 0.56 and 100 \( \mu g \) of toluene/L under conditions similar to those in the experiment presented in Table 1. The initial activity of this inoculum was assumed to be the lowest observed; that is, \( A = 0.0032 \). The metabolism of toluene (0.56 \( \mu g/L \)) was simulated assuming no loss of activity over time. The values of \( f \) cited above were obtained from data corresponding to activities ranging from A = 1 to A = 0.0243 (Table 3). To avoid extrapolating those values of \( f \) to conditions of lower activities (that is, \( A = 0.0032 \)), it was assumed that no loss of activity would occur (i.e., \( f = 0 \)) in simulating the degradation of 0.56 \( \mu g/L \). In this experiment, the degradation was slow because too little toluene was degraded to increase the activity substantially (Fig. 3A). Calculation of the final concentration of toluene gave a value of 0.33 \( \mu g/L \), which was almost the same as the experimental value of 0.34 \( \mu g/L \) (Table 1). In media with 100 \( \mu g \) of toluene/L, the activity of the bacteria increased to the maximum (\( A = 1 \)) and then fell sharply until it reached a value for \( A = 0.055 \) (Fig. 3B). The amount of toluene remaining after 196 h (1.1 ng/L) was below the detection limit of 0.2 \( \mu g/L \), which is consistent with the experimental results shown in Table 1. The cell density increased by approximately 24%.

Degradation of 0.9 \( \mu g \) of toluene/L by an active inoculum was simulated using conditions similar to those for the experiment presented in Table 2. Because of the appreciable decline in activity in the first few hours (Fig. 3C), the calculated concentration of toluene after 196 h was 99 ng/L, a

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**Table 3. Metabolism of individual additions of toluene**

<table>
<thead>
<tr>
<th>Time of toluene addition (h)</th>
<th>Degradation period (h)</th>
<th>Time without toluene (h)</th>
<th>Initial conc (mg/L)</th>
<th>Relative degradation activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Final</td>
</tr>
<tr>
<td>0.0</td>
<td>0.84</td>
<td>0.0</td>
<td>1.09</td>
<td>0.996 ± 0.031</td>
</tr>
<tr>
<td>1.9</td>
<td>0.67</td>
<td>0.2</td>
<td>0.93</td>
<td>1.000 ± 0.032</td>
</tr>
<tr>
<td>3.2</td>
<td>0.76</td>
<td>0.1</td>
<td>1.18</td>
<td>0.968 ± 0.032</td>
</tr>
<tr>
<td>6.0</td>
<td>0.74</td>
<td>2.1</td>
<td>1.20</td>
<td>0.853 ± 0.028</td>
</tr>
<tr>
<td>13.1</td>
<td>1.3</td>
<td>6.4</td>
<td>1.14</td>
<td>0.208 ± 0.007</td>
</tr>
<tr>
<td>29.1</td>
<td>2.3</td>
<td>14.7</td>
<td>1.25</td>
<td>0.0547 ± 0.0032</td>
</tr>
<tr>
<td>59.1</td>
<td>2.4</td>
<td>27.7</td>
<td>1.33</td>
<td>0.0343 ± 0.0031</td>
</tr>
<tr>
<td>130.4</td>
<td>2.4</td>
<td>68.9</td>
<td>1.05</td>
<td>0.0343 ± 0.0021</td>
</tr>
<tr>
<td>299.8</td>
<td>2.5</td>
<td>167.0</td>
<td>1.09</td>
<td>0.0243 ± 0.0017</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4.0</td>
<td>—</td>
<td>1.30</td>
<td>0.00334 ± 0.00050</td>
</tr>
<tr>
<td>1-Butanol</td>
<td>3.5</td>
<td>—</td>
<td>1.33</td>
<td>0.00315 ± 0.00043</td>
</tr>
</tbody>
</table>

\(^a\) Time for the complete disappearance of the added toluene.

\(^b\) Time interval from disappearance of previous addition of toluene to the time of the new addition.

\(^c\) The higher activity was considered to be 1.0.

\(^d\) NC, not calculated because the values would be greater than 1.0.
value somewhat similar to the experimental result of 79 ng/L shown in Table 2. It was calculated that if the bacteria had not lost their activity, the concentration of toluene after 38 and 196 h would have been \(1.0 \times 10^{-3}\) and \(3.7 \times 10^{-16}\) ng/L, respectively. It is therefore appropriate to assume that the cells lost their activity.

**DISCUSSION**

The experiments and the mathematical simulation provide evidence that the inability of bacteria to maintain or reach high metabolic activity on a substrate present at low concentrations may prevent its rapid degradation.

The simulation indicated that the incomplete degradation of toluene at 0.56 \(\mu\)g/L by bacteria with a low initial activity on that compound was consistent with an insufficient increase of activity because of a lack of adequate carbon and energy. By contrast, sufficient toluene was available to increase the activity of the cells when the initial concentration of toluene was 100 \(\mu\)g/L. The simulations in Figure 3C suggested that the concentrations of toluene (72 ng/L) remaining after 8 d in solutions initially containing 0.9 \(\mu\)g/L and inoculated with active bacteria were consistent with a decrease in the activity of the cells.

When benzene and styrene were present at 50 mg/L, toluene initially at 0.9 \(\mu\)g/L was degraded to concentrations below 2 ng/L, but low concentrations of toluene remained in the presence of ethanol, butanol, or glucose. It is not unexpected that an additional substrate enhances the degradation of a test substrate present at low concentrations. For example, Schmidt and Alexander [14] reported that *Salmonella typhimurium* mineralized glucose at low initial concentrations when arabinose was present but not in the absence of a second carbon source. Similarly, Law and Button [15] observed an increase in toluene metabolism by bacteria in the presence of benzene. In contrast, it is possible that the synthesis of enzymes required for toluene metabolism was repressed during the degradation of ethanol, butanol, or glucose or that the concentration of toluene might have been too low to induce the synthesis of these enzymes [7,8].

The toluene-degrading activity of strain T varied about 300- to 400-fold (Table 3). Similarly Robertson and Button [7] reported that the metabolic activity of a *Pseudomonas* sp. degrading toluene varied by a factor of 10,000, and Gaal and Neujahr [16] reported a 50- to 400-fold variation in the phenol-metabolizing activity of *Trichosporon cutaneum*. The increase in activity of strain T was proportional to the amount of toluene metabolized, probably because the cells require the energy and carbon provided by the degradation of toluene for the synthesis of additional enzymes involved in its metabolism. Law and Button [15] reported that the activity of a *Pseudomonas* sp. following exposure to toluene increased with increasing concentrations of toluene and was characterized by a hyperbolic relationship. At low concentrations of toluene, this type of relationship predicts an increase of activity proportional to the amount of toluene degraded.

Guerin and Boyd [17] reported that the activity of starved cells of a *Pseudomonas* sp. and a *Alcaligenes* sp. decreased exponentially with half-lives of 115.5 h and 69.3 h, respectively. In the studies reported here, the most rapid decrease in activity \(f = 0.34\) per h corresponds to a half-life of 2.0 h.

It is possible that the fraction of toluene utilized that is mineralized increased with the activity of the cells. This is suggested by the study of Robertson and Button [7], who reported that the amount of CO\(_2\) produced during the degradation of toluene was greater when a *Pseudomonas* sp. culture was fully active than when it was only partly active. The fraction of toluene utilized that was mineralized by strain T ranged from 2.5 to 33.9%. Therefore, although only one bacterium was tested, it is plausible that the estimation of degradation of low concentrations of an organic compound solely from data on its mineralization may give incorrect results.

The results show that bacteria may be unable to maintain a high metabolic activity during the metabolism of low concentrations of an organic substrate. As a consequence, the rate of degradation of low concentrations of a chemical may be several orders of magnitude slower than the rate expected based on kinetic studies involving high concentrations. The inability of some bacteria to maintain or increase their activity in the presence of low concentrations of a chemical may account for the persistence of degradable molecules in polluted environments. The results also suggest that adding large numbers of bacterial cells to remediate soils contaminated with low concentrations of a pollutant may have no benefit if the pollutant concentration is too low for these bacteria to maintain their metabolic activity.

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**REFERENCES**


