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Comparison of relative rates of BTEX biodegradation using respirometry

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Biodegradation of BTEX by a microbial consortium isolated from a closed municipal landfill was studied using respirometric techniques. The kinetics of biodegradation were estimated from experimental oxygen uptake data using a nonlinear parameter estimation technique. All of the six compounds were rapidly degraded by the microbial culture and no substrate inhibition was observed at the concentration levels examined (200 mg L^{-1} as COD). Microbial growth and contaminant degradation were adequately described by the Monod equation. Considerable differences were observed in the rates of BTEX biodegradation as seen from the estimates of the kinetic parameters. A three-fold variation was seen in the values of the maximum specific growth rate, μ_{max}. The highest value of μ_{max} was 0.389 h^{-1} for *p*-xylene while *o*-xylene was characterized by a μ_{max} value of 0.14 h^{-1}, the lowest observed in this study. The half saturation coefficient, K_s, and the yield coefficient, *Y*, varied between 1.288–4.681 mg L^{-1} and 0.272–0.645 mg mg^{-1}, respectively. Benzene and *o*-xylene exhibited higher resistance to biodegradation while toluene and *p*-xylene were rapidly degraded. Ethylbenzene and *m*-xylene were degraded at intermediate rates. In biodegradation experiments with a multiple substrate matrix, substrate depletion was slower than in single substrate experiments, suggesting an inhibitory nature of substrate interaction.

Keywords: biodegradation; BTEX; microbial kinetics, respirometry

Introduction

The widespread release of petroleum hydrocarbons from leaking underground storage tanks, pipelines and accidental spills is a major cause of subsurface contamination by hydrocarbons. The primary concern with gasolene release in the subsurface is groundwater contamination by the relatively mobile and toxic components of gasoline such as benzene, toluene, ethylbenzene and xylene isomers, collectively referred to as BTEX. The BTEX hydrocarbons are a serious environmental concern because of the carcinogenic potential of the benzene component [12]. Of the several methods available for remediating subsurface contamination by BTEX, bioremediation is especially attractive because it is less expensive and has the potential for complete destruction of the toxic compound to innocuous products. It is now widely recognized that BTEX hydrocarbons can be biodegraded in a wide variety of redox conditions with varying terminal electron acceptors. Several studies have shown that BTEX can be degraded under aerobic [6,16], nitrate reducing [4], sulfate reducing [13], iron reducing [21] and methanogenic [19] conditions. However, not many studies have focused on a detailed quantitative interpretation of biodegradation data. Information on biodegradation kinetics is essential in understanding the transport and fate of organic contaminants in the subsurface and in the design of biological systems to treat organic compound contamination [27].

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It is common practice to describe contaminant biodegradation and associated microbial growth by the Monod equation. Kinetic studies typically involve estimation of the maximum specific growth rate, μ_{max}, the Monod half saturation coefficient, K_s and the true growth yield, *Y*. While classic chemostat experiments can be used to estimate biodegradation kinetics, they can be laborious and time consuming [17]. Batch experiments on the other hand, offer a simple and convenient means of kinetic parameter estimation [30,31]. The advantages of batch experiments can be further extended through the use of respirometry which employs oxygen uptake by microorganisms as an indicator of contaminant biodegradation. As collection of oxygen uptake data in respirometric experiments can be automated, relatively less experimental and analytical effort is required. Respirometry has been used to estimate biodegradation kinetics of a wide variety of contaminants including nonvolatile organics [10,14,17,29], volatile organics [25] and sparingly soluble compounds [1].

While several researchers have investigated the kinetics of BTEX biodegradation [2,9,16,20,26,33], no study was encountered that encompassed all six compounds. Information on the relative rates of BTEX biodegradation is important as it is likely that all of these compounds will be encountered at a contaminated site. As the kinetics of biodegradation can be influenced by a variety of factors [18], comparison of kinetic parameters from different studies becomes difficult. In the present investigation, BTEX biodegradation was studied using respirometric techniques under identical experimental conditions. Experimental oxygen uptake data were used to estimate the kinetics of biodegradation by a nonlinear parameter estimation technique. Monod kinetic parameters of the different compounds

varied considerably and the biodegradability trend observed in this study was consistent with that observed in several other studies. Biodegradation experiments with a mixture of BTEX as the carbon source suggested an inhibitory nature of substrate interactions.

Materials and methods

Growth medium and chemicals

The mineral salts medium used in this study had the following composition: KH_2PO_4, 160 mg L^{-1}; K_2HPO_4, 420 mg L^{-1}; Na_2HPO_4, 50 mg L^{-1}; NH_4Cl, 40 mg L^{-1}; $MgSO_4 \cdot 7H_2O$, 50 mg L^{-1}; $CaCl_2$, 50 mg L^{-1}; $FeCl_3 \cdot 6H_2O$, 0.5 mg L^{-1}; $MnSO_4 \cdot 4H_2O$, 0.05 mg L^{-1}; H_3BO_3, 0.1 mg L^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.05 mg L^{-1}; $(NH_4)_6Mo_7O_{24}$, 0.03 mg L^{-1}. Reagent grade benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene were obtained from Sigma Chemical Co, St Louis, MO, USA.

Microorganisms

BTEX degrading microorganisms were isolated from leachate samples obtained from the closed Norman Landfill (Norman, OK, USA). Enrichment experiments were carried out in a BOD Apparatus (Model 2173B, HACH Co, Loveland, CO, USA). Ten milliliters of leachate were added to 150 ml of the mineral salts medium and 10 mg L^{-1} of each of the BTEX compounds was added as the carbon source. Biodegradation was monitored by measuring oxygen uptake over time. Several serial transfers were made until successive enrichments were characterized by similar oxygen uptake curves. The microbial culture at the end of these enrichment experiments was used as inoculum in respirometric studies to estimate the kinetics of contaminant biodegradation. Microbial cultures from respirometric experiments were used in subsequent biochemical assays for species identification.

Respirometry

Batch biodegradation experiments were carried out in a respirometer (Challenge Environmental Systems, Fayetteville, AR, USA) interfaced with a microcomputer that allowed automatic collection of oxygen uptake data at defined time intervals. The bioreactors had a volume of 500 ml and consisted of 250 ml of mineral medium to which 200 mg L^{-1} as COD of the test compound and 5–10 mg L^{-1} as COD of the acclimated biomass were added. Glass tubes containing 5 ml of 40% KOH were placed in the bioreactors using a Teflon™ adapter. Potassium hydroxide was used to scavenge the carbon dioxide produced during biodegradation. The temperature of the reactors was maintained at 25°C by means of a water bath.

With the onset of biodegradation, oxygen concentration in the aqueous phase decreased, resulting in the transfer of oxygen from the reactor head space to the aqueous phase. This resulted in a partial vacuum in the reactor head space causing oxygen to be drawn into the reactor until the pressure in the reactor head space was restored to its initial value. The amount of oxygen drawn into the bioreactors was measured by means of a flow measuring cell. The flow measuring cell consisted of a silicone-based oil that allowed oxygen bubbles of a fixed volume to pass through. The number of oxygen bubbles passing through the flow measuring cell was recorded by the computer. This information was converted to oxygen concentration as the volume of the oxygen bubbles passing through the flow measuring cell was previously calibrated.

Mixed-substrate biodegradation

Mixed-substrate biodegradation experiments were carried out in closed 500-ml sidearm reactors. The reactors consisted of 250 ml of mineral salts medium and 25 mg L^{-1} each of benzene, toluene and *p*-xylene (BTpX). Benzene, toluene and *p*-xylene were chosen to constitute the mixed-substrate matrix, as this combination has been used in most studies on mixed substrate BTEX degradation [3,9,16,26]. This facilitated comparison of the results obtained in this study with those previously published. The same inoculum was used for both the mixed-substrate experiments and single-substrate respirometric experiments. Before adding BTpX, the reactors were air sparged for 30 min to prevent oxygen from being limiting. The contents of the flask were continuously stirred at 150 rpm throughout the course of the experiment. Substrate depletion and microbial growth were monitored by periodically withdrawing 3-ml samples from the reactors through a specially modified sidearm using a gas tight syringe. BTpX concentrations were measured by gas chromatography while biomass concentration was determined as absorbance at 660 nm.

Analytical methods

BTEX: Residual BTEX concentrations in the respirometric experiments were determined in a HP 5840A gas chromatograph equipped with a flame ionization detector by a purge and trap procedure. Five milliliters of the sample were placed in the purge and trap vessel which was sparged for 25 min with nitrogen at a flow rate of 20 ml min^{-1}. The volatile hydrocarbons were trapped in a desorb column (10 cm × 0.7 cm, stainless steel) packed with Tenax (Supelco, Bellefonte, PA, USA). The oven temperature was initially at 80°C and was ramped at 4°C min^{-1} to 180°C and was then held constant for 8 min. The injector and detector temperatures were 275°C. The carrier was helium at a flow rate of 30 ml min^{-1}. The column consisted of 3% SP-1500 supported on 80/120 Carbopack (Supelco, Bellefonte, PA, USA; Lot No. 102196). The above procedure was also used to determine the concentrations of benzene, toluene and *p*-xylene in the mixed-substrate experiments.

Biochemical assay: Biochemical tests in conjunction with selective media were used to examine the most prevalent microorganisms. Characterization was based on conventional tests such as API 20E, API 20A, API 20NE and API 20C and IAPI System (bioMerieux Vitek, Hazelwood, MO, USA). Enrichment cultures described previously were plated on nutrient agar (Fisher Scientific, St Louis, MO, USA) and mineral salts medium with 15 g L^{-1} bacto agar. Nutrient agar plates were incubated at room temperature and were not exposed to BTEX. The mineral salts media plates were placed in a closed plate incubation reactor with the corresponding BTEX compounds in the vapor phase. Colonies obtained on the mineral salts media were used for

strain identification after they were differentiated based on morphology, gram-stain reaction and oxidase reaction.

Biomass: Biomass concentration was determined on a dry weight basis by syringe filtering a known volume of the microbial suspension through a 25-mm diameter, 0.45 μm cellulosic filter (MSI, Westboro, MA, USA) and drying the filter paper at 105°C for about 24 h. The dry cell weight was converted to COD units by a theoretical stoichiometric relationship [15].

Residual soluble COD: The soluble COD in the bioreactor at the end of biodegradation was measured by filtering a known volume of the reactor contents to remove the biomass. The COD of the filtrate was then determined spectrophotometrically in a HACH DR/2000 spectrophotometer (HACH, Loveland, CO, USA). Final product concentration was obtained by adjusting the filtrate COD for the presence, if any, of residual substrate.

Kinetic parameter estimation

The use of respirometry or oxygen uptake data for determining biodegradation kinetics parameters is based on the concept of oxygen consumption as an energy balance [17]. According to this concept, all the electrons available from the electron donor are either incorporated into new biomass and microbial products or are transferred to the terminal electron acceptor. Thus, for aerobic biodegradation in a batch reactor, the cumulative oxygen uptake is stoichiometrically related by the energy balance equation to the substrate, biomass and product concentrations as [31]:

$$O = \frac{S - YX - Y_p P}{1 - Y - Y_p} \tag{1}$$

where O, S and X denote oxygen, substrate and biomass concentrations, respectively, and Y and Y_p are the true growth yield and the product yield coefficients, respectively. If microbial growth is coupled to degradation of a volatile substrate and Monod type kinetics are assumed to describe microbial growth, then the substrate, biomass and product concentrations over time can be described by the following equations [25]:

$$\frac{dX}{dt} = \frac{\mu_m \left[\dfrac{S^{tot}}{1 + H\dfrac{V_g}{V_{aq}}} \right] X}{K_s + \left[\dfrac{S^{tot}}{1 + H\dfrac{V_g}{V_{aq}}} \right]} - \frac{bK_s X}{K_s + \left[\dfrac{S^{tot}}{1 + H\dfrac{V_g}{V_{aq}}} \right]} \tag{2}$$

$$\frac{dS}{dt} = -\frac{1}{Y} \left[\frac{\mu_m \left[\dfrac{S^{tot}}{1 + H\dfrac{V_g}{V_{aq}}} \right]}{K_s + \left[\dfrac{S^{tot}}{1 + H\dfrac{V_g}{V_{aq}}} \right]} \right] X \tag{3}$$

$$\frac{dP}{dt} = \frac{Y_p}{Y} \left[\frac{\mu_m \left[\dfrac{S^{tot}}{1 + H\dfrac{V_g}{V_{aq}}} \right]}{K_s + \left[\dfrac{S^{tot}}{1 + H\dfrac{V_g}{V_{aq}}} \right]} \right] X \tag{4}$$

where S^{tot} refers to the total substrate concentration in the system, H is the Henry's coefficient and V_g and V_{aq} are the volumes of the gas phase and liquid phase, respectively, in the bioreactor. The cumulative oxygen uptake at any instance of time can then be written as

$$O = (S_0 - S) - (X - X_0) - (P - P_0) + O_s + O_0 \tag{5}$$

The parameter estimation technique used in this study was similar to the one developed by Grady *et al* [17], incorporating modifications proposed by Naziruddin *et al* [25] to account for the volatile nature of BTEX hydrocarbons. The parameters to be estimated include Y_p, b, μ_{max}, K_s, and Y. The product yield coefficient, Y_p, was determined from the residual substrate and product concentrations, while the microbial decay coefficient, b, was determined from oxygen uptake data following the plateau region in the oxygen uptake curves. Estimation of μ_{max}, K_s and Y involved simultaneously solving Eqns (2)–(4) using the values of Y_p and b determined above and appropriate initial estimates of μ_{max}, K_s and Y. The initial estimates of Y, μ_{max}, and K_s were obtained as described by Brown *et al* [8]. A computer program, MONRESV, was written in FORTRAN 77 to solve Eqns (2)–(4) using the above obtained estimates of μ_{max}, K_s and Y as starting values. This system of nonlinear differential equations was numerically integrated using the fourth-order Runge–Kutta method. The resulting values of substrate, biomass and product concentrations were used in Eqn (5) to obtain the theoretical oxygen uptake curve. These values were then compared with experimental oxygen uptake values and the residual sum of squares error (RSSE) between the two data sets was determined. As the objective of the parameter estimation technique was to obtain estimates of μ_{max}, K_s and Y such that the RSSE between the experimental and theoretical data sets was minimized, a suitable strategy of varying the kinetic parameters had to be employed. Grady *et al* [17] observed that a grid search routine was robust and gave reliable estimates of the kinetic parameters. Consequently, a grid search routine was used for parameter estimation in this study. While performing biodegradation experiments, care was taken to maintain the S_0:X_0 ratio greater than 20 and the S_0:K_s ratio greater than 5. The high S_0:X_0 value ensured that oxygen uptake was a consequence of substrate depletion and not due to microbial decay. Moreover, these conditions are also necessary for optimal parameter estimation by the nonlinear parameter estimation technique [8]. The S_0:K_s ratio is important as unique estimation of the Monod kinetic parameters is possible only in the mixed order region [28]. In the zero and first order regions, the sensitivity equations of

the parameters are almost exact multiples of each other making it extremely difficult to obtain unique estimates of the kinetic parameters. The program was truncated when the RSSE between the experimental and theoretical oxygen uptake data sets was minimized.

Results

Biodegradation of individual BTEX compounds

Three distinctly different colonies were observed on nutrient agar while only two (designated Colony 1 and Colony 2) were seen on the mineral salts medium. Based on the index numbers in the API method, these two colonies were tentatively identified as *Pseudomonas putida* and *Pseudomonas aeruginosa*. While the characteristics of Colony 1 were in close agreement with those for *Pseudomonas putida* in the API method, this was not the case for *Pseudomonas aeruginosa*. Results of a *t*-test indicated significant differences ($P<0.05$) between the characteristics observed for Colony 2 and those suggested for *Pseudomonas aeruginosa* in the API method. It was observed that degradation of benzene, toluene and *m*-xylene was carried out by *Pseudomonas putida* alone while that of *o*-xylene was carried out by *Pseudomonas aeruginosa* alone. Both microbial species were present in the degradation of ethylbenzene and *p*-xylene.

Biodegradation experiments with the six BTEX hydrocarbons were carried out at initial substrate concentrations of 200 mg L^{-1} as COD. Representative oxygen uptake curves for *p*-xylene biodegradation are shown in Figure 1. The points represent experimental data while the lines are the theoretical oxygen uptake curves obtained from Eqns (2)–(5) with the best fit parameters. The kinetic parameters obtained from the nonlinear parameter estimation technique were able to accurately describe experimental oxygen uptake data. Interflask variability in oxygen uptake as evident from Figure 1 was also seen for all the other BTEX hydrocarbons tested. The oxygen uptake curve along with the oxygen uptake rate and specific growth rate curves for benzene biodegradation are shown in Figure 2. While a total of 127 oxygen uptake measurements were made, only 25 have been shown for the sake of clarity. The rate curve helps determine if some factor other than substrate concentration is rate limiting [32]. Rate curves that did not exhibit a constantly increasing rate followed by a sharp decline corresponding to substrate exhaustion were not used for parameter estimation. The specific growth rate curve had an initial linear portion that corresponded to the exponential

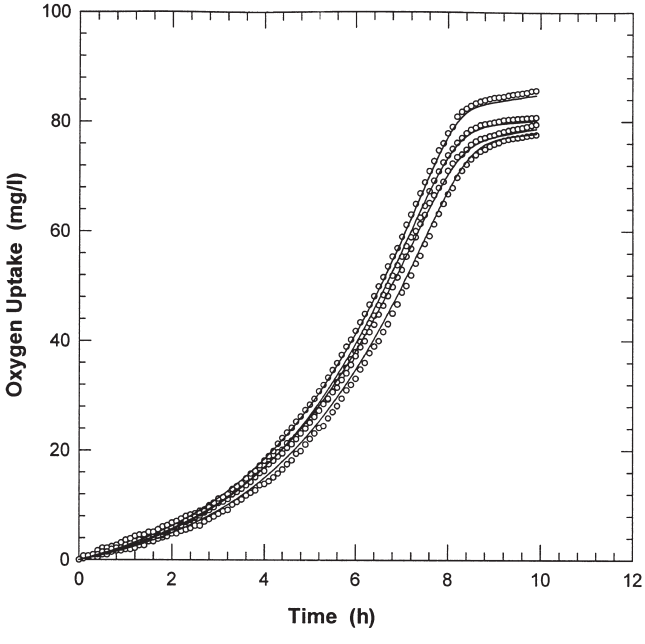

Figure 1 Oxygen uptake curves for *p*-xylene biodegradation. The points are experimental data while the lines are theoretical curves obtained from the best fit parameters.

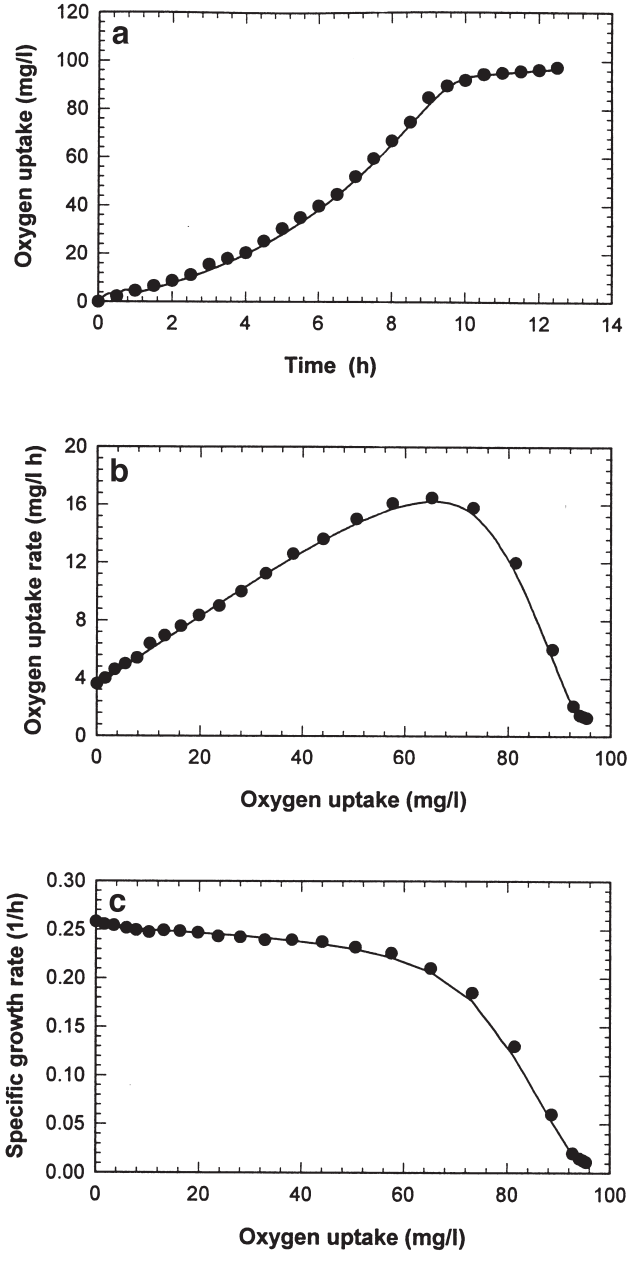

Figure 2 Oxygen uptake (a), oxygen uptake rate (b) and specific growth rate (c) curves for benzene biodegradation. The points are experimental data while the lines are theoretical curves obtained from the best fit parameters.

Table 1 Kinetic and stoichiometric parameters for BTEX biodegradation

Compound[a]	No. of experiments	μ_{max} (h^{-1})	K_s (mg L^{-1})	Y (mg mg^{-1})	Y_p (mg mg^{-1})	b (h^{-1})	RSSE ($\times 10^4$)
Benzene	4	0.205 ± 0.021	2.690 ± 0.550	0.477 ± 0.028	0.053 ± 0.019	0.010 ± 0.001	3.803 ± 0.587
Toluene	4	0.366 ± 0.003	3.253 ± 0.567	0.427 ± 0.060	0.053 ± 0.005	0.006 ± 0.003	4.899 ± 2.199
Ethylbenzene	5	0.211 ± 0.009	1.288 ± 0.406	0.272 ± 0.011	0.057 ± 0.012	0.008 ± 0.003	4.679 ± 0.953
o-Xylene	3	0.140 ± 0.021	3.540 ± 0.580	0.345 ± 0.018	0.053 ± 0.019	0.010 ± 0.003	2.705 ± 0.547
m-Xylene	4	0.241 ± 0.007	4.681 ± 0.920	0.331 ± 0.014	0.050 ± 0.008	0.007 ± 0.002	3.803 ± 1.346
p-Xylene	4	0.389 ± 0.024	3.451 ± 0.752	0.645 ± 0.016	0.005 ± 0.005	0.008 ± 0.003	9.024 ± 4.470

[a]All compounds were tested at initial concentrations of 200 mg L^{-1} as COD.

phase of microbial growth and served as a good initial estimate of μ_{max}. Once the microbes reached the stationary phase of growth, there was a sudden decline in the specific growth rate.

The kinetic parameter estimates for BTEX obtained in this study are shown in Table 1. All values are reported as the mean of several replicate experiments along with the associated standard deviations. Among all the kinetic and stoichiometric parameters, the variations were the highest for K_s. This is more due to the inability of the parameter estimation technique to accurately determine K_s than to the metabolic variability of the microbial culture [11]. All compounds were characterized by low values of Y_p indicating that almost complete degradation of the substrate had occurred. Further evidence to this effect was obtained by measuring residual contaminant concentration in the bioreactors. In most cases, contaminant levels were below the detection limit of the analytical procedure employed.

Stoichiometric calculations for BTEX biodegradation were performed using the approach proposed by McCarty [23] to verify if experimental oxygen uptake values were consistent with theoretical requirements from stoichiometry. As ammonia was the nitrogen source, the fraction of substrate associated with synthesis was assumed to be the experimentally observed true growth yield, *Y*. It is seen from Table 2 that experimental oxygen uptake values were in excess of 95% of the theoretical value for all BTEX implying almost complete conversion of BTEX to biomass, carbon dioxide and water.

Once the final estimates of the kinetic parameters were obtained, it was possible to predict substrate depletion, microbial growth and product formation over time from Eqns (2)–(5). The representative results of nonlinear parameter estimation for *o*-xylene are shown in Figure 3. Biodegradation of *o*-xylene at initial concentrations of 200 mg L^{-1} as COD required about 16 h. This was accompanied by oxygen uptake, microbial growth and product formation.

Table 2 Comparison of experimental and theoretical oxygen uptake values for BTEX biodegradation

Compound	Theoretical oxygen uptake[a] (mg L^{-1})	Mean experimental oxygen uptake (mg L^{-1})	% of theoretical oxygen uptake
Benzene	104.69	101.34	96.81
Toluene	114.60	111.20	97.03
Ethylbenzene	145.65	139.40	95.71
o-Xylene	131.05	125.24	95.57
m-Xylene	133.85	133.69	99.88
p-Xylene	71.03	78.79	110.93

[a]Computed by McCarty's approach [23] using experimentally determined growth yields.

Multiple substrate experiments

While a detailed investigation of substrate interactions during BTEX degradation was beyond the scope of this study, preliminary experiments were performed with BTpX as the carbon source. The substrate depletion profiles from these experiments are shown in Figure 4. All three compounds were simultaneously utilized and toluene was degraded the fastest while benzene and *p*-xylene were degraded at similar rates. However, as observed from the respirometric experiments, the degradation times were considerably longer than when the substrates were present as the sole carbon source.

Discussion

All six BTEX hydrocarbons were completely degraded by the mixed microbial culture used in this study. Examination

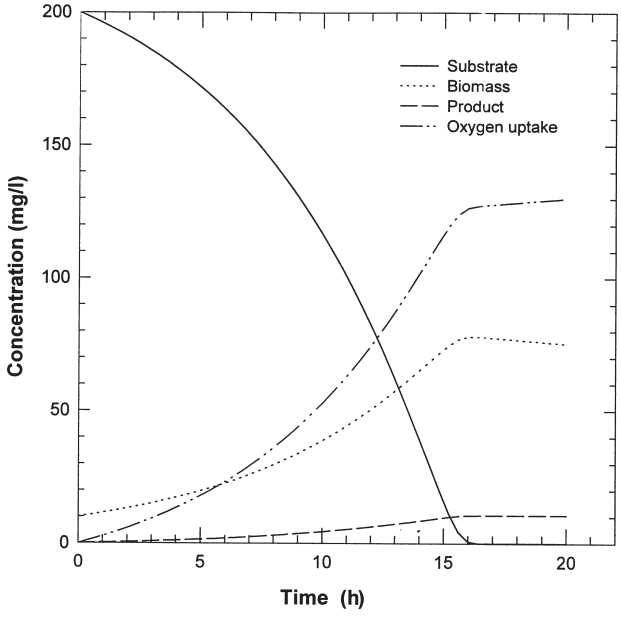

Figure 3 Substrate depletion and associated oxygen uptake, microbial growth and product formation for *o*-xylene.

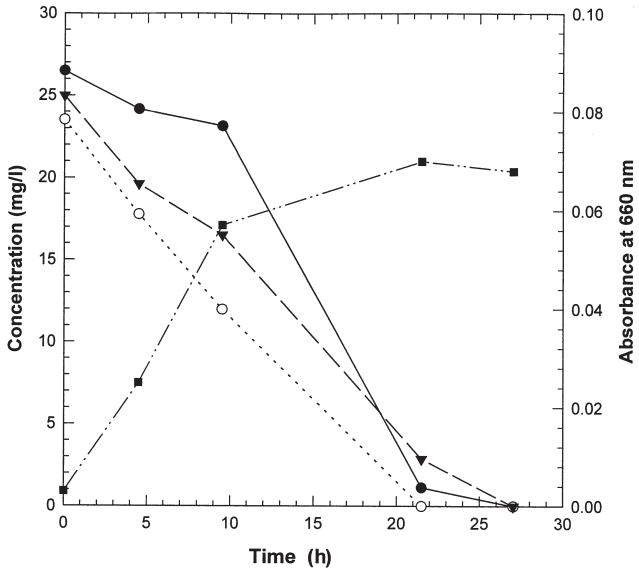

Figure 4 Substrate depletion curves for benzene (—●—), toluene (--○--) and *p*-xylene (—▼—) in a mixed substrate environment. Biomass (--■--).

of the oxygen uptake rate curves revealed no substrate inhibition for any of the compounds at the concentrations examined (200 mg L^{-1} as COD) and consequently microbial growth was described by the Monod equation. The acclimation periods for the various compounds however varied considerably and were as follows: benzene 20 days, toluene 3 days, ethylbenzene 21 days, *o*-xylene 74 days, *m*-xylene 15 days and *p*-xylene 7 days. Once the microbial community was acclimated to the substrate of interest, rapid contaminant biodegradation followed. Also, repeated transfer of the acclimated microorganisms to fresh medium considerably reduced the lag period and increased the rate of contaminant biodegradation.

Of the Monod kinetic parameters, the maximum specific growth rate, μ_{max}, significantly impacts the rate of contaminant degradation and is an important parameter in the design of biological treatment systems. It can be seen from Table 1 that there is almost a three-fold variation in the μ_{max} values among the BTEX hydrocarbons. High values of μ_{max} are desirable, as they result in rapid degradation of the contaminant. Further information on the relative ease/difficulty of contaminant biodegradation can be obtained from the half saturation coefficient, K_s as low K_s values imply rapid degradation. Relatively less variation was seen in the K_s values. Ethylbenzene was the only exception with relatively low values of K_s (Table 1). A comparison of yield coefficients also indicated a variability among BTEX, although not to the extent seen for μ_{max}. The yield coefficient was lowest for ethylbenzene while *p*-xylene was characterized by the highest value of *Y*. The other four compounds were characterized by fairly similar values of *Y* (Table 1).

As both μ_{max}, and K_s affect the biodegradation of a compound, they must be collectively considered in a comparison of relative biodegradation rates. Using the average kinetic parameters reported in Table 1 and assuming initial substrate and biomass concentrations of 200 and 10 mg L^{-1} as COD, respectively, substrate depletion profiles of BTEX were obtained and these are shown in Figure 5. It is seen from Figure 5 that toluene was most rapidly utilized, requiring less than 7 h for complete biodegradation. *p*-Xylene required more time for biodegradation than toluene in spite of having a larger value of μ_{max}. This is because *p*-xylene had a K_s value that was greater than that of toluene. Ethylbenzene and *m*-xylene were degraded at similar rates while benzene and *o*-xylene were utilized considerably slowly with biodegradation times of about 12 and 16 h, respectively. Based on the kinetic parameters obtained in this study, the relative biodegradability of BTEX is toluene > *p*-xylene > ethylbenzene, *m*-xylene > benzene > *o*-xylene.

This trend is similar to that seen in several other studies in the literature. Morgan *et al* [24] observed that in gas-condensate contaminated groundwater, *o*-xylene and benzene (in that order) exhibited the most resistance to biodegradation while toluene, ethylbenzene and the *m*- and *p*-isomers of xylene were rapidly degraded. In laboratory studies with a mixed microbial culture, Mallakin and Ward [22] reported that benzene resisted biodegradation by a mixed microbial culture while toluene, ethylbenzene and the *o*- and *p*-isomers of xylene were almost completely degraded after 20 days. The average biodegradation rates in the first 5 days were the highest for toluene (4–6 mg L^{-1} day^{-1}) and the lowest for benzene (0.2–0.5 mg L^{-1} day^{-1}) while the *o*- and *p*-isomers of xylene were degraded at intermediate rates. Barker *et al* [7] reported that the BTEX compounds were readily degraded in the laboratory as well as in an unconfined sandy aquifer. While the *m*- and *p*-isomers of xylene were most readily degraded, benzene exhibited the most resistance to biodegradation with *o*-xylene being degraded at intermediate rates. It is thus reasonable to expect that under appropriate environmental conditions, all the BTEX compounds will be aerobically degraded by an acclimated microbial culture. The rates of degradation however, may vary considerably.

No study was found in which the biodegradation kinetics

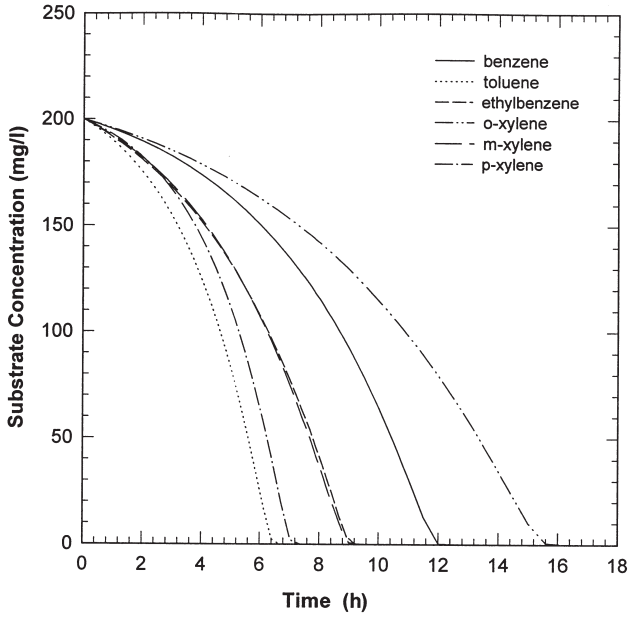

Figure 5 Comparison of relative rates of BTEX biodegradation.

Table 3 Comparison of kinetic parameters for BTEX biodegradation

Reference	Benzene			Toluene			*p*-Xylene		
	μ_{max}	K_s	Y	μ_{max}	K_s	Y	μ_{max}	K_s	Y
[16][a]	0.61	20	–	0.90	20	–	0.36	18.0	–
[33][a]	0.39	22.2	0.23	0.38	56.7	–	0.35	67.0	0.26
[2][a]	0.35[c]	12.2	–	0.41[c]	17.4	–	–	–	–
[9][b]	0.34	3.17	1.04	0.54	1.96	1.22	–	–	–
[9][b]	–	–	–	0.45	1.88	0.99	0.54	4.55	0.25
[26][a]	0.68	12.2	0.71	1.50[d]	11.03[d]	0.71[d]	–	–	–
[26][b]	0.44	3.36	0.65	1.56[d]	15.07[d]	0.64[d]	–	–	–
[20][a]	0.05	–	0.2	0.04	–	0.22	–	–	–
This study[a]	0.21	2.7	0.48	0.37	3.25	0.43	0.39	3.45	0.65

[a]Mixed microbial culture.
[b]Pure microbial culture.
[c]Maximum specific substrate utilization rate (mg substrate mg cells^{-1} h^{-1}).
[d]Biodegradation was described by Andrews kinetics.

of all the BTEX compounds were systematically investigated. A collection of studies where kinetic information on two or more of the BTEX hydrocarbons was available is shown in Table 3. Among the xylene isomers, *p*-xylene is more commonly studied probably because it is more readily biodegraded than the other two isomers. It follows from Table 3 that in most cases, toluene was characterized by a higher value of μ_{max} than benzene [2,9,16,26]. However, no such trend was observed for K_s. Tabak *et al* [33] present kinetic information for ethylbenzene and *m*-xylene in addition to that shown in Table 3. They observed that ethylbenzene was degraded at rates comparable to those of benzene, toluene and *p*-xylene, while *m*-xylene was the most rapidly utilized compound.

A comparison of Figures 4 and 5 indicates that the biodegradation times of benzene, toluene and *p*-xylene increased considerably when they were present as a mixture. Similar observations were made by Chang *et al* [9] for simultaneous degradation of benzene and toluene by *Pseudomonas* B1. Toluene acted as a competitive inhibitor and lowered the rate of benzene degradation. Also, for the simultaneous degradation of toluene and *p*-xylene by *Pseudomonas* X1, xylene acted as a competitive inhibitor lowering the rate of toluene degradation [9]. Competitive inhibition effects were also observed by Oh *et al* [26] for degradation of a mixture of benzene and toluene by *Pseudomonas* strain PP01. Hence, it is likely that increased biodegradation times observed in Figure 4 are a consequence of inhibitory effects due to the multiple substrate environment. However, competitive inhibition is not the only phenomenon observed in a mixed BTEX environment. Benzene degradation by a mixed microbial culture was enhanced by the presence of either toluene or *o*-xylene while a negative effect was seen when both were present [5]. Presence of either *p*-xylene or benzene reduced the rate of toluene degradation by a mixed bacterial culture [3]. Also, toluene enhanced benzene biodegradation by *Pseudomonas* sp CFS-215 while *p*-xylene lowered the rate of toluene degradation [3]. This rather complex nature of substrate interactions limits the extrapolation of kinetic results obtained from single substrate experiments to a multiple-substrate environment.

Each of the six BTEX hydrocarbons was rapidly degraded by a mixed bacterial culture under aerobic conditions. Respirometry coupled with a nonlinear parameter estimation technique was used to estimate the kinetics of BTEX biodegradation. Respirometry generates high quality experimental data with minimum experimental and analytical effort. As more than 100 data points are obtained in a single biodegradation experiment, confidence can be placed in the estimates of the kinetic parameters. None of the compounds exhibited substrate inhibition at the concentrations tested and the rates of biodegradation were adequately described by the Monod equation. Considerable variability was seen in the rates of BTEX biodegradation and the relative biodegradability was as follows: toluene $>$ *p*-xylene $>$ ethylbenzene, *m*-xylene $>$ benzene $>$ *o*-xylene. This trend or portions of it are consistent with several other studies in the literature.

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