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Research Article

Cometabolic Degradation Kinetics of Trichloroethylene Based on Toluene Enhancement by Encapsulated *Burkholderia cepacia* G4

The ability of encapsulated *Burkholderia cepacia* G4 (ATCC 53617) for trichloroethylene (TCE) degradation (1.5, 5, 10, and 20 mg/L) in the presence of toluene (10 and 60 mg/L) as enhancement substrate was evaluated experimentally. *Burkholderia cepacia* G4 cultures were encapsulated in cylindrical pellets (4 mm in diameter and 4 mm in height (preferred)) using polyethylene glycol (PEG). Higher transformation capacities were observed for the encapsulated cultures for both toluene concentrations. The highest transformation capacities measured for the encapsulated cultures and suspended cultures were 46.98 and 5.94 μg TCE/mg biomass, respectively. The Monod equation was used to simulate the degradation rates of toluene and Haldane's equation was employed to describe the degradation kinetics of TCE. The first-order reaction rate constant (k/K_s) for toluene degradation in the encapsulated cultures was 2.3-fold higher than the value of the suspended cultures, whereas the k_c/K_{sc} value for TCE was 4.3-fold higher compared to the suspended cultures. The higher kinetic values of the encapsulated cultures indicate that the degradation efficiency and capability of *B. cepacia* G4 was enhanced through PEG encapsulation. Moreover, the higher inhibition constant value for the encapsulated cultures compared with the suspended cultures demonstrated that PEG-encapsulated *B. cepacia* G4 can tolerate and degrade much higher TCE concentrations.

Keywords: Biodegradation; Gram-negative bacteria; PEG encapsulation; Transformation capacity

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1 Introduction

Trichloroethylene (TCE) is an industrial chemical and considered a priority pollutant that may cause several types of cancer along with numerous other effects on human health [1]. TCE is frequently found in groundwater in many countries such as United States of America, Korea, Thailand, and Japan due to extensive industrial use [2, 3]. Although biologically persistent in the environment, the biological degradation of TCE by anaerobic and aerobic bacteria is possible through cometabolism [4–6]. TCE bioremediation through anaerobic degradation produces less chlorinated byproducts and intermediates which are toxic to microorganisms and relatively persistent as compared to other pollutants [6]. For example, vinyl chloride is produced during anaerobic dechlorination of TCE, which is a known carcinogen. In situ- or ex situ-engineered bioremediation of TCE through aerobic cometabolism is the safest way to reduce TCE to simpler and harmless end products such as CO_2 [7]. Through bio-augmentation, bacterial strains are introduced that can perform biotransformation using one or more contaminants as the substrate or under substrate-fed supply conditions [7]. Bio-augmentation is particularly beneficial when one or more contaminants can be used as the primary substrate, for example, toluene. To overcome the physical (i.e., changes in pH, temperature, organic solvent poisons, and flushing), chemical or biological (such as competition, enzymes, bacteria, and viruses) challenges, additional efforts are needed in bio-augmentation.

TCE co-oxidation is toxic to microbial communities, especially at higher concentrations of TCE, upon direct contact [8]. Due to such adverse effects, the application of biodegradation to mineralize TCE has been restricted [9]. To achieve sustainable metabolic ability to degrade pollutants, maintain viability and regulate access to the target pollutant as well as substrates, the encapsulation of suitable microorganisms is most likely the best solution [10, 11]. Encapsulation may offer numerous benefits over bare cells, including the protection of microorganisms from tough ecological circumstances, easier recovery from solution, and the establishment of higher cell mass conditions to increase total substrate transformation rates and compact reactor sizes [10, 12].

A number of materials have been studied for the encapsulation of microbial cultures. Polyethylene glycol (PEG) polymer has been tested and determined to be the one of the best encapsulation materials because it is stable, durable, inert, environmentally safe, easy to assemble with good encapsulation properties and less resistant to diffusion [13]. The encapsulation of *Burkholderia cepacia* G4 by PEG to degrade TCE and toluene has not been yet evaluated. The objective of this study was to encapsulate *B. cepacia* G4 in PEG

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Abbreviations: CI, confidence interval; **PBS**, phosphate buffer saline; **PEG**, polyethylene glycol; **TCE**, trichloroethylene

pellets and to evaluate the efficiency and kinetics of this system for the removal of TCE using toluene as a growth substrate at higher TCE concentrations.

2 Materials and methods

2.1 Biomass and growth culture

Burkholderia cepacia G4 (ATCC 53617) was purchased from the American Type Culture Collection. The microorganisms were cultured in autoclaved nutrient broth at 28°C to obtain a high culture density. The nutrient broth solution containing the high-biomass culture was centrifuged and rinsed with sterile phosphate buffer saline (PBS) for 10 min. The composition of PBS was as follows: 8.1 g L^{-1} NaCl, 2.302 g L^{-1} Na_2HPO_4, and 0.194 g L^{-1} $Na_2H_2PO_4$. The rinsed *B. cepacia* G4 was then cultured in the phosphate-buffered medium. The medium contained: 1.234 g L^{-1} $(NH_4)_2SO_4$, 0.854 g L^{-1} K_2HPO_4, 0.694 g L^{-1} KH_2PO_4, 0.460 g L^{-1} $MgSO_4 \cdot H_2O$, 0.176 g L^{-1} $CaCl_2 \cdot 2H_2O$, 0.001 g L^{-1} $FeSO_4 \cdot 7H_2O$, and 5 mL of trace metal solution. The trace metal solution contained: 20 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 60 mg L^{-1} H_3BO_3, 2 mg L^{-1} $CuCl_2 \cdot 2H_2O$, 40 mg L^{-1} $CoCl_2 \cdot 6H_2O$, 6 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 4 mg L^{-1} $NiCl_2 \cdot 6H_2O$, and 6 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$ [14].

To acclimatize the *B. cepacia* G4 to toluene uptake, toluene was fed as the sole energy and carbon source. Shaking of the toluene-fed culture was performed at 150 rpm, at a temperature of 28°C and at pH 7.2. The detailed parameter and conditions are summarized in Table 1. Pure oxygen was purged after every 24 h to maintain the aerobic conditions, that is, >30 mg/L of oxygen to maintain bacterial activity. 99.8% pure toluene and 99.5% pure TCE (Sigma Aldrich) were used for all experiments. Nutrient broth powder (Difco) was used for the growth of the cultures. The nutrient broth powder was dissolved in deionized water (Millipore). All of the media, glassware, needles and solutions were autoclaved, and all of the procedures were performed using a clean bench space [15].

2.2 Analysis of TCE, toluene, and biomass concentrations

The optical density at 540 nm (OD_{540}) was measured in the solution using a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) to determine the concentration of biomass. A SMART™ BCA Protein Assay Kit (iNTRON BIOTECHNOLOGY) was used for protein assay. The readings for protein assay were obtained at 562 nm (UV-1601, Shimadzu, Kyoto, Japan).

Table 1. Experimental parameters and conditions

Parameter	Value
TCE concentration (mg/L)	1.5, 5, 10, 20
Toluene concentration (mg/L)	10, 60
Temperature (°C)	28
pH	7.2
Packing ratio of encapsulation experiments (v/v)	50%

For TCE and toluene analysis, a gas chromatograph (Varian GC 3400CX, USA) equipped with a flame ionization detector and a capillary column (DB-5, 0.53 mm, 30 m, J&W Scientific) was used. The injector and detector temperature were 200 and 230°C, respectively. The column temperature was maintained at 40°C for 3 min and increased by 8°C/min until reaching 120°C [15]. The detection limit of the equipment used for both TCE and toluene was 1 μg/L. The concentration in the liquid phase was calculated using Henry's law dimensionless constant (H_c) at 28°C. The H_c values for TCE and toluene were 0.445 and 0.298, respectively [15]. The TCE and toluene concentrations were calculated using the ratio of the chromatogram peak areas. For low ranges of TCE and toluene, an aliquot of 100 μL was taken from the head space of the serum bottle using of a gas-tight syringe (Hamilton) and injected into the GC. For the high ranges of TCE and toluene, that is, 5, 10, 20 mg/L TCE and 60 mg/L toluene, the aliquot volume was taken at the rate of 10 μL, and the final values were multiplied by a factor of ten.

2.3 Procedure for the synthesis of PEG encapsulated cell pellets

Figure 1 shows the layout of the encapsulation process. For encapsulation, the centrifuged cells were washed with sterile phosphate buffer twice and resuspended in 80.8 mL of phosphate mineral medium. PEG prepolymer (18% w/v), initiator ($K_2S_2O_8$) and 80 mL ($OD_{540} = 2$, i.e., approximately 3200 mg/L) of cells were mixed together for 3 min, and 0.9 g sorbitol (as thickener) was added to this mixture with subsequent stirring for 1 min. Then, 2.5 mL of borax

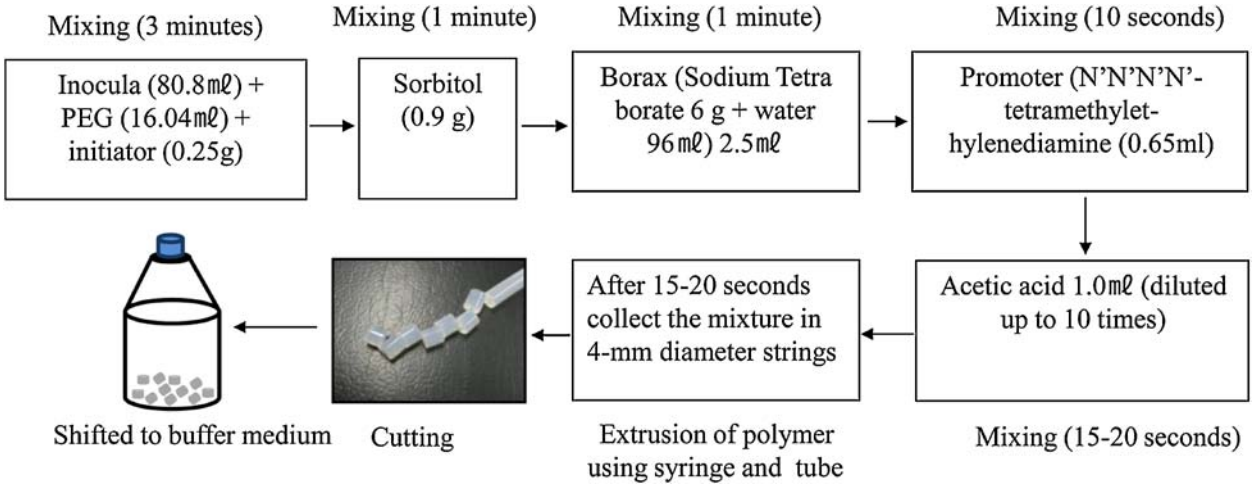

Figure 1. Procedure for synthesizing encapsulated cell pellets.

(*N*′,*N*′,*N*′,*N*′-tetramethylethylenediamine) were added to the mixture. After mixing for 10 s, 1 mL of acetic acid (diluted tenfold) was added. After mixing for a further 15–20 s, the final mixture was drawn into 4-mm-diameter cords using sterile PVC tubes and a syringe. These cords were then cut into 4-mm-long pellets using a specially designed cutting arrangement [15]. The specific surface area of each pellet was 1500 m^2/m^3. These pellets were kept in mineral solution under the conditions described above. The final weight of dry biomass was 174 mg/L in PEG pellets. The detailed procedures are described elsewhere [15].

2.4 Degradation of TCE and toluene by encapsulated and suspended cells

The degradation of different concentrations of TCE (1.5, 5, 10, and 20 mg/L) was investigated using two concentration of toluene (10 and 60 mg/L). A 50-mL volume of phosphate-buffered mineral solution (composition described above) was poured into a 120-mL brown serum bottle to avoid photocatalysis. The serum bottle was then sealed with silicon/PTFE septum and an aluminum cap. Once the requisite concentration of TCE and toluene was established, the cells were inserted into the serum bottle [15].

The same range of TCE and toluene concentrations was investigated using encapsulated cells. A packing ratio of 50% (v/v) was used to investigate the removal of TCE versus the degradation of toluene. Cells encapsulated in pellets were washed with PBS three times for 5 min to remove any growing cells on the surface before use. The pellets (equal to 25 mL of liquid volume) were put into the serum bottle containing phosphate-buffered mineral solution (25 mL) and the bottle was sealed using a Teflon-lined stopper and an aluminum cover. The serum bottle containing these pellets (in mineral solution) was then supplied with oxygen gas. The control samples were prepared using same method as described in above lines for degradation experiments. Control serum bottles for encapsulated cultures were prepared employing following steps; (1) PEG pellets with G4 culture were prepared and put in the serum bottle under 50% packing ratio (25 mL mineral solution and PEG pellets equal to 25 mL volume), (2) serum bottles containing empty PEG pellets were then sealed with silicon/PTFE septum and an aluminum cap to avoid volatilization of TCE and toluene, (3) oxygen was then supplied (at saturated concentration) using sterile needle to avoid any bacterial contamination, and (4) TCE and toluene concentrations equal to that of experimental values were then injected and measured periodically along with real sample values. The conditions of temperature and pH were the same as for the cell suspension. Once all the conditions were set, TCE and toluene was then supplied to carry out the encapsulated cell experiments [15].

2.5 Kinetics analysis

Monod kinetics is commonly used to calculate the kinetics of toluene, and the mathematical expressions are as follows:

$$q_p = \frac{dS}{dt}\frac{1}{X} = -\frac{kS}{K_s + S} \quad (1)$$

$$X = X_0 + (S_0 - S)Y \quad (2)$$

where q_p is the specific substrate (toluene) utilization rate ($mg\,L^{-1}\,h^{-1}\,mg\,cells^{-1}$), X is the biomass (*B. cepacia* G4) concentration ($mg\,L^{-1}$), k is the maximum specific rate of substrate utilization (mg toluene $L^{-1}\,h^{-1}\,mg\,biomass^{-1}$), S is the substrate (toluene) concentration ($mg\,L^{-1}$) and K_s is the concentration giving one-half the maximum rate ($mg\,L^{-1}$) of toluene degradation, also termed the half saturation concentration. X_0 and S_0 are the initial biomass and substrate concentrations, respectively, and Y is the biomass yield, which is defined as the biomass produced per unit mass of substrate consumed [7].

Combining Eqs. (1) and (2) generates Eq. (3):

$$\frac{dS}{dt} = -\frac{kSX}{K_s + S} \quad (3)$$

After integrating Eq. (3) over time (t) for batch reactors, the following relationship is established:

$$t = \frac{K_s Y \ln(S_0/S) + (K_s Y + X_0 + S_0 Y)\ln(X_0 + (S_0 - S)Y)}{X_0}\frac{1}{kY(X_0 + S_0 Y)} \quad (4)$$

In Eq. (4), X_0 and S_0 are already known from the experimental data, and the value of Y is obtained from previous studies. The remaining two unknown kinetic parameters (k and K_s) can be determined by comparing the model (Eq. (4)) and observed values for S at different times (t). A spreadsheet method, based on the weighted nonlinear least square method, which is commonly used for the integrated Monod equation, was employed in this study [16]. Microsoft® Excel 2010 was used to solve the Eq. (4) as described above.

The degradation of TCE at higher concentrations is described be Haldane's equation for accounting inhibition and toxicity [17].

$$q_c = \frac{dS_c}{dt}\frac{1}{X} = -\frac{k_c S_c}{K_{sc} + S_c + S_c^2/K_i} \quad (5)$$

where q_c is the specific substrate (TCE) utilization rate ($mg\,L^{-1}\,h^{-1}\,mg\,biomass^{-1}$), k_c is the maximum specific rate of substrate utilization ($mg\,TCE\,L^{-1}\,h^{-1}\,mg\,biomass^{-1}$) for TCE, S_c is the TCE concentration ($mg\,L^{-1}$) and K_{sc} is the concentration giving one half the maximum rate ($mg\,L^{-1}$). TCE and its cometabolites can affect the cometabolic degradation kinetics due to toxicity. To solve k_s and K_{sc} the initial rate method was employed in Eq. (5) [8]. As inhibition is only vital at higher TCE concentrations, k_c and K_{sc} were first calculated at lower TCE concentrations. Once the kinetics coefficients k_c and K_{sc} were established, K_i was then calculated at higher TCE concentrations. Microsoft® 2010 Excel was employed to calculate all the kinetic coefficients with a 90% confidence interval.

3 Results

3.1 Simultaneous degradation of TCE and toluene at a low toluene concentration (10 mg/L)

Figures 2 and 3 show the degradation of TCE (1.5, 5, 10, and 20 mg/L) and toluene (10 mg/L), respectively. No lag was observed in TCE degradation for either the suspended or encapsulated cultures, or TCE was degraded simultaneously along with toluene. There was a clear difference between the removal efficiencies of the two systems, with the encapsulated culture displaying higher transformation rates than the suspended culture (Figs. 2a and 3a).

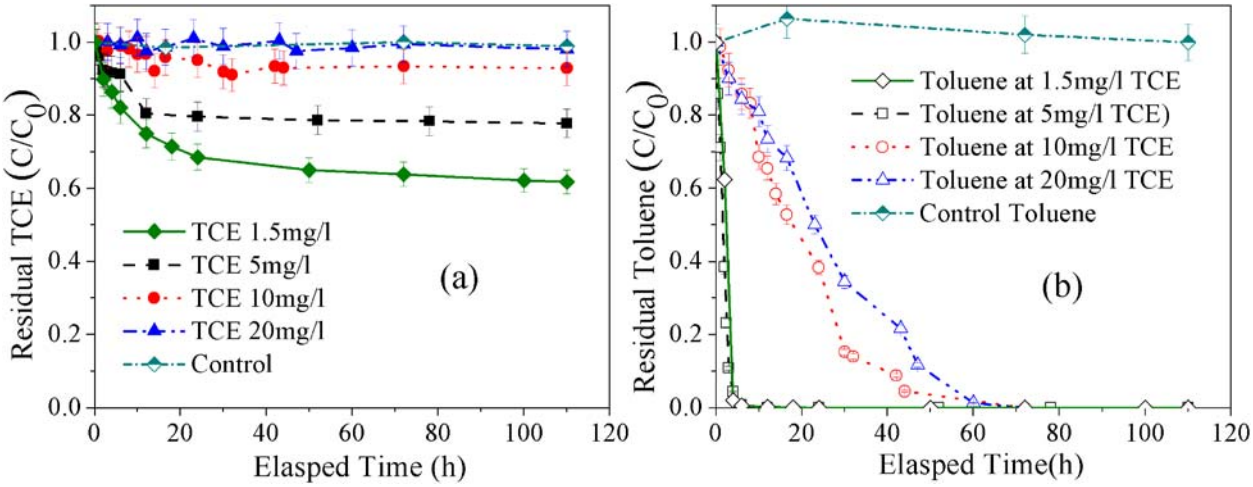

Figure 2. TCE and toluene (10 mg/L) degradation by suspended cultures. (a) TCE and (b) toluene.

At 10 and 20 mg/L TCE with 10 mg/L toluene (Fig. 2a), the suspended cultures displayed insignificant TCE removal and very slow degradation of toluene. Toluene was degraded completely in 60–70 h, but there was <8% TCE degradation at 10 mg/L TCE, and no degradation was observed at 20 mg/L TCE.

As shown in Fig. 3, encapsulated systems displayed higher and faster TCE and toluene removal compared with the suspended cultures (Fig. 2). It was anticipated that biofilms would increase diffusion resistance compared with suspended cells, causing a lag in the degradation profiles. However, there was no lag observed in all cases for toluene and TCE degradation. Toluene was degraded in approximately 20 h, and simultaneous TCE degradation was observed.

3.2 Simultaneous degradation of TCE and toluene at high toluene (60 mg/L)

Insignificant TCE removal at 10 and 20 mg/L TCE in suspended cultures and a relatively superior but still incomplete degradation (approximately 25–60% at 1.5–20 mg/L TCE) in encapsulated systems could be attributed to the low toluene supply (10 mg/L), as discussed previously. Hence, a toluene concentration of 60 mg/L was supplied by keeping the same TCE concentrations to obtain *t* a comprehensive response from both the suspended and encapsulated *B. cepacia* G4. Figure 4 shows the degradation of TCE (1.5, 5, 10, 20 mg/L) and toluene (60 mg/L) for the suspended culture, and Fig. 5 shows the degradation for the encapsulated cultures.

Figure 4a reveals that TCE degradation occurred at all concentrations of TCE with the suspended cultures when 60 mg/L toluene was used, unlike at 10 mg/L toluene (Fig. 2a). The toluene, however, followed similar degradation trends in both cases. The toluene was degraded in 19, 20, 30, and 50 h for 1.5, 5, 10, and 20 mg/L TCE, respectively. The different toluene degradation times may be either due to higher values of TCE or toluene or both. It has been reported that toluene beyond 30 mg/L is toxic to microorganisms.

TCE was simultaneously degraded in suspended culture as soon as toluene degradation started, resulting in no lag time. In addition, the TCE conversions were higher than in the previous case (1.5, 5, 10, 20 mg/L TCE and 10 mg/L toluene) in which little degradation occurred (Fig. 2a). This shift may have been due to the higher

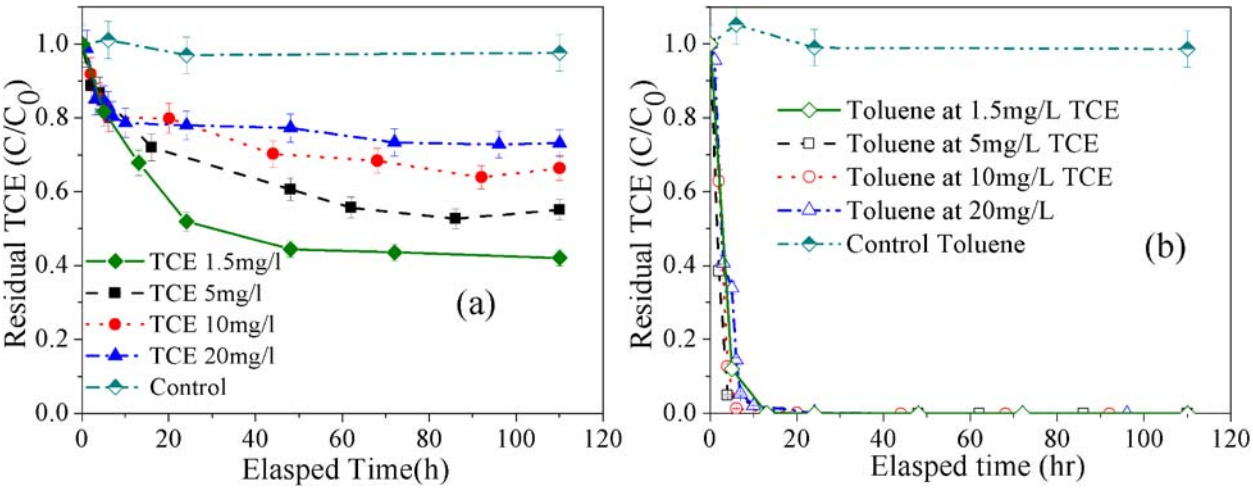

Figure 3. TCE and toluene (10 mg/L) degradation by encapsulated cultures. (a) TCE and (b) toluene.

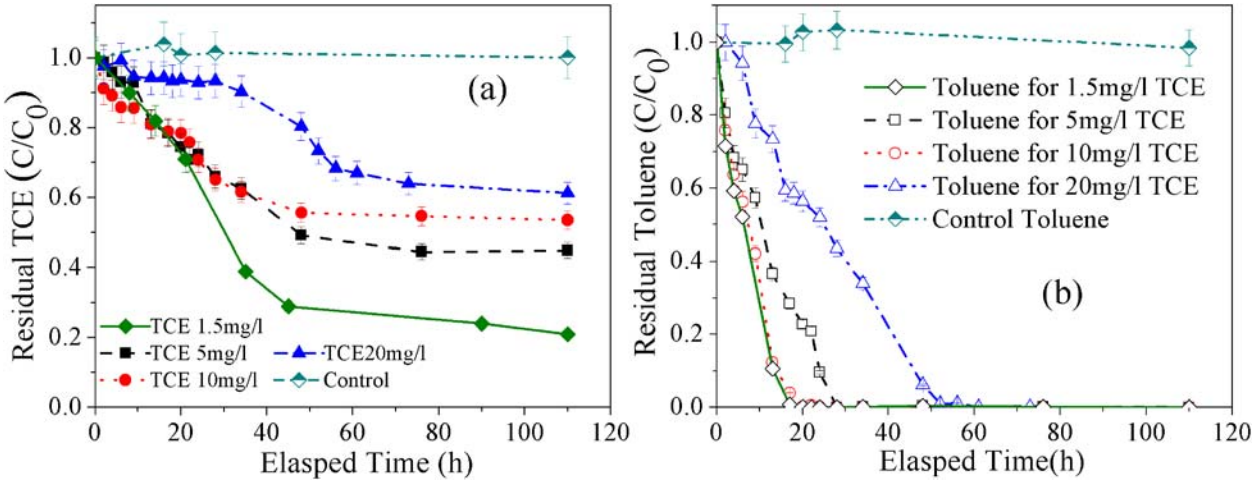

Figure 4. TCE and toluene (60 mg/L) degradation by suspended cultures. (a) TCE and (b) toluene.

substrate availability. It was reported that at low toluene concentrations, cytotoxicity from TCE to TCE-converting microorganisms is a selective disadvantage [18]. These findings imply that at higher toluene concentrations, *B. cepacia* G4 (a well-known tolerant of high concentrations of TCE) can use available toluene for growth and maintenance energy, and surplus toluene can be used to express toluene 2-monooxygenase. Thus, TCE degradation is possible at high concentrations of TCE, but high toluene is also required in such scenarios. The results from this study are different from those of Chen et al. [8], who reported that no degradation by suspended cultures *Pseudomonas putida* F1 occurred at 20 mg/L TCE when using 100 mg/L phenol as the substrate.

Figure 5 shows the degradation of TCE and toluene by encapsulated cultures. TCE degradation (Fig. 5a) was higher compared with the suspended cultures (Fig. 4a). Similarly, toluene (60 mg/L) was degraded at much faster rates than suspended cells. Toluene was degraded at approximately similar rates regardless of the TCE concentration used (Fig. 5b). The TCE and toluene degradation rates were also superior when compared with the encapsulated cultures at 10 mg/L toluene (Fig. 3). A lag time of approximately 9 h, however, was observed in the encapsulated culture for 5 and 10 mg/L TCE (Fig. 5a). TCE degraded rapidly from 9 to ~15 h (for TCE 5 mg/L), which corresponds to the time span in which the toluene degradation was completed (~98%).

At a toluene concentration of 60 mg/L, the encapsulated systems had 100, 72, 54, and 44% efficiencies at 1.5, 5, 10, and 20 mg/L of TCE, respectively (Fig. 5a). The overall removal efficiencies in the suspension cultures were 79, 55, 50, and 25% at the same TCE concentrations and under the same conditions.

3.3 Transformation capacities

The specific removal, which is also known as the transformation capacity, is defined as "the amount of TCE that the microorganism can transform under given conditions before they are killed (due to toxicity of TCE or its byproducts)" [7].

Figure 6 shows the comparison of transformation capacities for suspended and encapsulated *B. cepacia* G4 at 10 and 60 mg/L toluene. The highest transformation capacity was observed at 60 mg/L toluene for both the encapsulated and suspended cultures, and

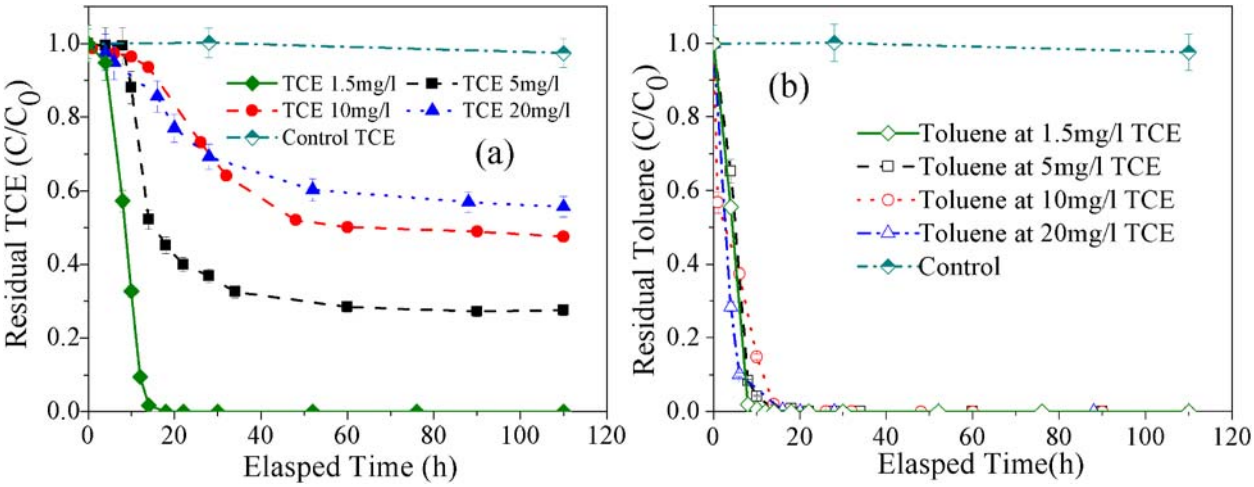

Figure 5. TCE and toluene (60 mg/L) degradation by encapsulated cultures. (a) TCE and (b) toluene.

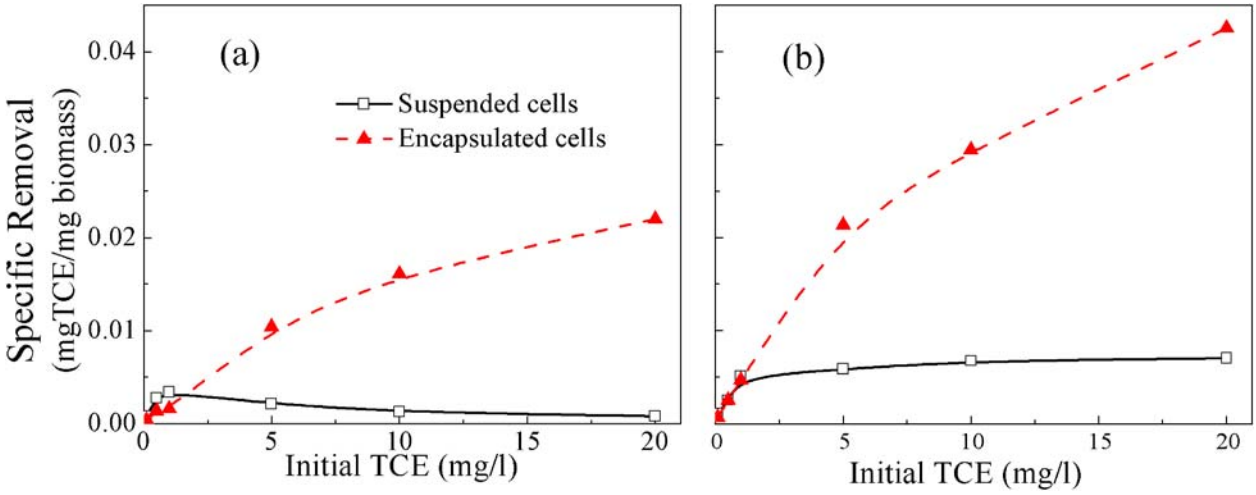

Figure 6. Specific removal transformation capacity at (a) 10 mg/L toluene and (b) 60 mg/L toluene.

the highest observed values were 46.98 and 5.94 μg TCE/mg biomass. Lower T_c values were observed with suspended cultures. In addition, 10 mg/L toluene resulted in a lower T_c compared with 60 mg/L toluene for the suspended cultures (Fig. 6a).

3.4 Kinetic analysis

The comparative values of the two best fitted kinetic parameters of toluene degradation are listed in Table 2 for the suspended and encapsulated cultures. The maximum rate of substrate utilization (k) for the suspended cultures was calculated as 0.0783 ± 0.0104 and 0.1302 ± 0.0141 mg toluene/mg cells/h for the encapsulated cultures with a 90% confidence interval (CI).

The half-saturation constant (K_s) was 22.6 ± 0.8 and 14.81 ± 0.4 mg/L for the suspended and encapsulated cultures with a 90% CI, respectively. The first-order reaction rate constant (k/K_s) for the encapsulated cultures (Table 2) was 2.3-fold higher than that of the suspended cultures.

The values of K_{sc} are 0.248 ± 0.11 and 0.202 ± 0.09 mg/L for the suspended and encapsulated cultures, respectively. The value of the inhibition constant for the suspended cultures was estimated to be 4.76 mg/L for the suspended cultures and was >300 for the encapsulated cultures. The higher value of K_i indicates a low inhibition by TCE [17, 19]. This finding indicates that the encapsulated cultures tolerated higher TCE concentrations than the suspended cultures. In fact, the first-order degradation rate constant (k_c/K_{sc}) for the encapsulated cultures was 4.3-fold higher than that of the suspended cultures. The higher values of k_c/K_{sc} for the encapsulated system demonstrates the that degradation of TCE was higher compared with the suspended system, which is also evident from the degradation profiles shown in Figs. 2–5.

4 Discussion

The higher removal by encapsulated cultures indicates that the encapsulation system provided better protection to microorganisms compared with the suspended culture. The control experiments demonstrated an almost negligible loss of toluene and TCE (Figs. 2 and 3). It has been reported that bacterial activity is either completely stopped or greatly suppressed as TCE concentration increases due to the toxicity related to TCE and its transformation byproducts [8].

It has also been reported that the toxicity related with TCE oxidation in *B. cepacia* G4 is not due to general structural destruction to the cell membrane [20]. There may be several reasons for the reduction in the activity of toluene 2-monooxygenase subjected to elevated TCE concentrations, for example, some particular component of the oxygenase may be inactivated, electron flow to the terminal oxygenase component may have declined, damaged di-iron center of α subunit of the hydroxylase or similar inhibitory effect [20]. Other reasons may also include the limitation of oxygenases at high TCE concentrations [8] and increases in the maintenance energy demand [20].

The results indicate that encapsulation protects the cells from the toxicity of TCE and its byproducts. The TCE transformation efficiencies of encapsulated systems were approximately 60, 45,

Table 2. Kinetic constants for TCE and toluene degradation

Microorganism	Toluene			TCE			
	k (mg toluene/mg cells per h)	K_s (mg/L)	k/K_s (L/mg cells per h)	K_c (mg TCE/mg cells per h)	K_{sc} (mg/L)	k_c/K_{sc} (L/mg cells per h)	K_i (mg/L)
B. cepacia G4 suspended	0.0783 ± 0.0104	22.577 ± 0.798	0.0038051	0.000337 ± 0.0000748	0.248 ± 0.112	0.000581	4.7549
B. cepacia G4 encapsulated in PEG polymer	0.1302 ± 0.0141	14.8125 ± 0.487	0.0087899	0.000327 ± 0.000202	0.202 ± 0.0908	0.0025154	>300

35, and 25% at 1.5, 5, 10, and 20 mg/L TCE, respectively, for substrate concentration as low as 10 mg/L. Superior results were observed for encapsulated systems compared with suspended cultures. These results also indicate that PEG encapsulation pellets not only regulated TCE, toluene and oxygen but also enhanced the utilization of toluene. This finding also indicates that the diffusion of the target pollutant and substrate may not be a significant factor at these concentrations when using PEG encapsulation.

From OD_{540} profiles (data not shown), we observed that biomass was not affected. Instead, the concentration of microbes increased. In contrast, at 10 mg/L of toluene, a great reduction in OD_{540} was observed. The OD_{540} dropped almost 60% from the initial value over a period of 110 h. This reduction could be attributed to the fact that the available toluene was not sufficient to maintain such a high biomass while it was also exposed to high concentrations of TCE, which required increased maintenance energy demands as well. Consequently, there was no or little expression of toluene 2-monooxygenase, which was insufficient to handle the high amount of TCE. The inactivation of low-expressed enzyme (due to high TCE concentrations) may be another reason for the low TCE removal.

Referring to Fig. 5, it can be concluded that TCE degradation started when most of the toluene had been degraded. This trend is different from the behavior of the encapsulated cultures at 10 mg/L toluene (Fig. 3a). In that scenario, no lag time was observed in any case. It appears that this lag time was not due to the diffusion resistance, as it would have occurred for all cases of encapsulation in this study (e.g., Fig. 3). This lag time may have been due to the competition between TCE and toluene to diffuse into the encapsulation pellets, which can be reflected by different water partition coefficients, densities and water solubility for both TCE and toluene [7]. In the encapsulated system, TCE has to compete for active sites inside the PEG pellet, that is, to diffuse into the pellet. The failure of TCE to diffuse into the pellet in the first stage might be due to the high concentration of toluene (60 mg/L) in addition to the higher diffusion rate of toluene. Both of these factors can cause the saturation of the PEG pellet with toluene prior to the diffusion of TCE. The TCE diffusion starts, however, with toluene degradation by encapsulated *B. cepacia* G4, and the diffusion increases as the concentration of toluene decreases with time, making it easier for TCE to diffuse into pellets. This situation is only magnified as the TCE concentration increases from 5 to 10 and 20 mg/L. However, a lag time was observed at 10 mg/L of TCE, but there was also slight removal of TCE. This phenomenon was not observed at 20 mg/L of TCE, and TCE degraded simultaneously with toluene at that concentration.

The TCE transformation rates were higher in the encapsulated cultures as compared with the same TCE concentrations (1.5, 5, 10, and 20 mg/L) with lower toluene concentrations (10 mg/L), as shown in Fig. 3a. Hence, as the toluene concentration increases, the expression level of the enzyme increases, and the cells are protected from direct contact with toxic concentrations of TCE. This process can be considered an extra benefit of the encapsulation along with the many other benefits, such as the better protection of cells, reuse, long life and maintaining high cell biomass in the system.

Referring to Fig. 6, transformation capacity is an indicator of the toxicity of TCE and its products, these results indicates that as TCE concentration increases, the toxicity also increases, thus increasing the maintenance energy requirements. However, if plenty of primary substrate is available, the microorganism can adequately maintain its growth requirements, as observed for 60 mg/L of toluene. In contrast, if the primary substrate or growth substrate is not sufficient, the microorganism cannot maintain itself against the high toxicity of TCE. Consequently, the amount of biomass in the environment drops, as was observed with 10 mg/L of toluene and with 1.5–20 mg/L TCE. Similar trends were described by Chen et al. [8]. The values of T_c were consistent with previously reported values in the literature [21]. A breakthrough point after which a decreasing trend in T_c was recorded in previous studies [8] was not observed in our case except for suspended cultures at 10 mg/L toluene. This shows that PEG encapsulation can better protect the cometabolic degraders at high TCE concentrations as compared to other encapsulated materials used in previous researches.

The higher value of k/K_s for the encapsulated cultures indicates that *B. cepacia* G4 performed better in PEG encapsulation than in suspension. These results are contrary to those found by Chen et al. [8], who reported slower kinetics for chitosan-encapsulated *P. putida* compared with suspended cultures when phenol was used as a growth substrate at 100 mg/L TCE and the degradation rate was simulated using Haldane's equation (Eq. (5)). The best fitted parameters (at 90% CI) are listed in Table 2. The values of k_c are 0.000337 ± 0.0000748 mg TCE/mg cells/h and 0.000327 ± 0.000202 mg/L for the suspended and encapsulated cultures, respectively.

The slightly different values of k_c and K_{sc} for both cultures show that both systems had almost similar performance towards overall TCE degradation. For the same amount of toluene, the suspended cultures, however, degraded lesser amount of TCE as compared to the encapsulated cultures. The extra toluene in suspended mode may have been utilized to support the repair mechanism in response to the toxicity (due to direct contact) of high TCE concentrations. The faster kinetics also show that PEG polymer presented negligible diffusion resistance for TCE and toluene as have been reported by Chen et al. [8] for phenol and chitosan. They observed the slower kinetics due to the diffusion resistance when *P. putida* F1 were encapsulated in chitosan beads to degrade the phenol and TCE.

These results also demonstrate that PEG encapsulation did not harm the encapsulated microbial community, which is in contrast to the conclusions of Radway et al. [22] who stated that the reaction of an immobilization material (polyurethane foam) suppressed the efficiency of *B. cepacia* G4.

5 Conclusions

A toluene-degrading microorganism, *B. cepacia* G4 (ATCC 53617), was encapsulated in PEG degrader pellets to degrade TCE and toluene, and the degradation efficacy of the encapsulated system was compared with the efficiency of a suspended system. The experimental results of this study indicate that the primary substrate, toluene (10 and 60 mg/L), and the cometabolic secondary substrate, TCE (1.5, 5, 10, and 20 mg/L), were degraded simultaneously at all concentrations. No lag time was observed due to diffusion resistance in the PEG encapsulation system. Higher transformation capacities were observed for the encapsulated cultures at both toluene concentrations (10 and 60 mg/L), which indicates that the encapsulated cultures not only provided protection from TCE toxicity but also resulted in the efficient utilization of the available toluene. The highest transformation capacity was observed for the encapsulated cultures with a toluene concentration of 60 mg/L (42.9 μg TCE/mg biomass).

The first-order reaction rate constant (k/K_s) for toluene degradation in encapsulated cultures was 2.3-fold higher than that of the suspended cultures, whereas (k_c/K_{sc}) for TCE, the value was 4.3-fold higher than the value of the suspended cultures. The higher kinetics values observed with the encapsulated cultures demonstrate that the efficiency and capability of *B. cepacia* G4 was enhanced. The higher K_i (>300 mg/L) value for the encapsulated *B. cepacia* G4 as compared with the suspended cultures (4.75 mg/L) demonstrate that PEG-encapsulated *B. cepacia* G4 can tolerate and degrade much higher TCE concentrations.

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