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Enhancing co-metabolic degradation of trichloroethylene with toluene using *Burkholderia vietnamiensis* G4 encapsulated in polyethylene glycol polymer

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The biodegradation potential of *Burkholderia vietnamiensis* G4 (*B. vietnamiensis* G4) was evaluated under encapsulation in comparison with direct exposure to trichloroethylene (TCE) (0.1, 0.5, 1 and 5 mg/L) and toluene (10 and 50 mg/L), maintaining aerobic conditions. *B. vietnamiensis* G4 was encapsulated in polyethylene glycol (PEG) polymer. Under suspended conditions, the degradation rate decreased as the initial TCE concentration increased, even with a higher amount of substrate available. However, the encapsulated systems were less suppressed, presumably by mitigated toxicity, and completely removed TCE with 50 mg/L of toluene. The transformation yield (*Ty*) was as high as 0.427 mg-TCE/mg-toluene for the encapsulated cultures and 0.1007 mg-TCE/mg-toluene for the suspended cultures. The *Ty* value for the encapsulated cultures was one to two orders higher than what has been reported in the literature. The higher *Ty* values in the encapsulated cultures compared with those from suspended cultures showed that the PEG encapsulation provided more a favourable environment for efficient substrate use.

**Keywords:** *B. vietnamiensis* G4; co-metabolism; PEG encapsulation; TCE; toluene; transformation yield

**Introduction**

Trichloroethylene (TCE) is a priority pollutant that potentially causes several types of cancer and numerous other human health effects.[1] Due to its vast industrial application as a degreasing agent, it is discharged into aquatic systems and soil by industry. It has also been reported that TCE is one of the most frequent groundwater contaminants in the Republic of Korea.[2] TCE is biologically persistent via normal aerobic metabolism, but the co-metabolic degradation of TCE by aerobic bacteria is well known and has been well documented.[3] However, the local biodiversity of contaminated sites (surface and subsurface) may not contain the proper microorganisms that can induce co-metabolic removal of such persistent compounds, in such cases; bio-augmentation can be used.[4]

Through bio-augmentation, bacterial strains that can execute biotransformation using one or more contaminants as a substrate are introduced. There is always a large possibility that the introduced bare or suspended cells may be exposed to external hazardous conditions. For example, physical (changes in pH, temperature, organic solvent poisons, flushing, etc.), chemical or biological (competition, enzymes, bacteria and viruses) conditions may pose serious threats to desired process efficiency.[5] A protection line or barrier may be employed which can isolate the cells from the surrounding environment. The ideal protection line or barrier should not affect the metabolic ability of cells to degrade pollutants, their viability and access to target pollutant as well as to the substrates. This potential solution can be achieved by encapsulation of suitable microorganisms.

The subjected species should not only be resistant to high loading, but also to the toxicity of the metabolites. *Burkholderia vietnamiensis* (formerly *cepacia*) G4 (*B. vietnamiensis* G4) is one of the most extensively studied bacterial strains to degrade TCE via co-metabolism. *B. vietnamiensis* G4 shows poor adhesive characteristics towards biofilm formation.[6,7] This characteristic of *B. vietnamiensis* G4 has resulted in its limited use in bioreactors, although it has the ability to co-metabolize TCE at relatively high concentrations compared with other co-metabolic degraders.[7,8] The best alternative to this problem for advantageous use of *B. vietnamiensis* G4 is its encapsulation to treat wastewater effluents and [*in situ* bioremediation of contaminated sites. Encapsulation offers several advantages, e.g. microorganisms are well protected from harsh environmental conditions, they can be recovered easily from the subject solution/environment, higher cell density can be maintained over longer periods of time to achieve high substrate conversion efficiency, reduced reactor volumes, good operational stability of engineered systems, low substrate inhibition at relatively...
high concentrations and avoidance of lag time for the development of biofilm.[5,9,10]

Stability, long-term repeated use, inertness of encapsulation material towards the microbial community and cell entrapment efficiency are the key features in the selection and success of a cell encapsulation technique.[11] This means that selection of the encapsulation material holds prime importance. Numerous studies have been reported using different encapsulation materials to encapsulate cells, e.g. polyvinyl alcohol, boric acid, calcium alginate, agarose, polyurethane, chitosan, biolite, polyacrylamide gel, peat and glass.[6] The components of a gel matrix, including polyacrylamide and polyurethane, are toxic to the encapsulated cells. Calcium alginate is very mild and, when gelled by an ionic bond swells and dissolves in a solution containing a chelating agent such as phosphate.[5] Polyvinyl alcohol, which is inexpensive and non-toxic to cells, is not mechanically strong. Cross-linking the bead of polyvinyl alcohol with boric acid solution increases the bead strength and durability but damages cells due to the lengthy cross-linking process. The addition of some alginate or phosphate to the boronic solution during the cross-linking procedure prevents the polyvinyl alcohol beads from aggregating.[9] Cells encapsulated in chitosan beads are affected by the surface properties of such beads,[9] while B. vietnamiensis G4 embedded on polyurethane foam was not protected from the toxicity of TCE, as reported by Radway et al. [7].

Encapsulation using polyethylene glycol (PEG) polymer has been successfully tested in the treatment of textile industry wastewater, organic wastewater, swine wastewater and refractory organics found in textile wastewater and has been found to be stable for prolonged continuous operation.[12,13] PEG is stable and inert towards chemical reactions in aqueous solutions and is biologically safe.[14] Also, PEG can offer solutions to most of the remaining problems pertaining to other encapsulation materials. Although PEG encapsulation has been tested by a number of researchers, it has not yet been investigated for the removal of TCE or for the encapsulation of B. vietnamiensis G4 for co-metabolism. The aim of this study was to investigate the suitability of PEG to encapsulate B. vietnamiensis G4 for aerobic co-metabolic removal of TCE using toluene as the primary substrate.

Materials and methods

Biomass and culture growth conditions

Burkholderia vietnamiensis G4 (ATCC 53617) was purchased from the American Type Culture Collection (ATCC). The microorganisms were grown in sterilized nutrient broth (NB) containing (per litre) peptone 5 g; meat extract 1 g; yeast extract 2 g; sodium chloride 5 g; agar 15 g, and the final pH was 7.0 ± 0.2. The nutrient solution containing the high biomass was centrifuged and rinsed with sterile phosphate-buffered saline (PBS) for 5–10 min. The composition of PBS per litre was: NaCl, 8.1 g; Na2HPO4, 2.302 g; and Na2H2PO4, 0.194 g. The microorganisms were then cultured in a phosphate-buffered mineral medium. The composition of the medium was: (NH4)2SO4, 1.234 g; K2HPO4, 0.854 g; KH2PO4, 0.694 g; MgSO4·H2O, 0.460 g; CaCl2·2H2O, 0.176 g; and FeSO4·7H2O, 0.001 g per litre along with 5 ml of trace metal solution. The trace metal solution contained ZnSO4·7H2O, 20 mg; H3BO3, 60 mg; CuCl2·2H2O, 2 mg; CoCl2·6H2O, 40 mg; MnCl2·4H2O, 6 mg; NiCl2·6H2O, 4 mg and Na2MoO4·2H2O, 6 mg per litre.[15] During routine culture growth and acclimation, toluene was fed as the sole energy and carbon source. The medium solution was changed on every other day. Once the cells attained steady removal of toluene, the pellets were sent for visual analysis (described in detail in the cell density section). The pH of the medium was 7.2, and the temperature was maintained at 28°C on a shaking incubator at 150 rpm. Pure oxygen was supplied to maintain super saturation conditions, i.e. greater than 30 mg/L.

Synthesis of cell-encapsulated PEG pellets

For encapsulation, the centrifuged cells were washed twice with sterile PBS and were suspended in 80.8 ml of the medium. Then, 15 ml PEG pre-polymer (PEG-diacylate 600), 0.25 g initiator (K2S2O8) and 80 ml of cells (approximately 3200 mg/L) were mixed together for 3 min; then 0.9 g of sorbitol (C6H12O6) was added into this mixture as a cross-linker and stirred for 1 min. A 2.5 ml borax solution (6 g sodium tetraborate + 96 ml of water) was then added as an additive. After 1 min 0.5 ml Promoter (N’N’N’-tetramethylethylenediamine) was injected into the mixture. After mixing for 10 s, 1 ml of acetic acid (diluted 1:10) was injected. After mixing for a further 15–20 s, the final mixture was extruded into a 4 mm diameter tube using a sterile polyvinyl chloride (PVC) syringe to produce PEG cords. These cords were then cut into 4-mm-long pellets using a specially designed cutting arrangement. The specific surface area of each pellet was 1500 m2/m3. 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.[10]

Cell density in encapsulation pellets

To inspect the distribution and growth of cells inside the pellets, two groups of pellets, freshly synthesized pellets and pellets which were acclimated to (the steady removal of) toluene for about 3 months, were sent to the Department of Microbiology, Chungbuk University, Republic of Korea, where two visual techniques were employed. Fluorescence microscopy was used to obtain high-resolution images of sliced pellets. To identify the cell distribution pattern in the pellets, a confocal laser scanning microscope (CLSM) was used.
Analytical methods

For TCE and toluene analysis, a gas chromatograph (GC) (Varian GC3400CX, 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°C and 230°C, respectively. The column temperature was maintained at 40°C for 3 min and then increased by 8°C/min until reaching 120°C.[16] The detection limit of the equipment used for both TCE and toluene was 1 μg/L. The concentration in liquid phase was calculated by the dimensionless form of Henry’s law constant ($H_c$) at 28°C. $H_c$ for TCE and toluene was 0.445 and 0.298, respectively.[10] The TCE and toluene concentrations were calculated by the ratio of the chromatogram peak area. Standard curves for TCE (up to 20 mg/L) and toluene (up to 50 mg/L) were prepared with five bottles containing both the chemicals at an appropriate range of concentrations against the range of expected sample concentrations. The $r^2$ values for the curves were over 99% mostly.

For the low ranges of TCE and toluene, an aliquot of 100 μl from headspace was collected from the serum bottle with the help of a gas tight syringe (Hamilton, USA) and injected into the GC. For the high ranges of TCE and toluene, a 10 μl aliquot was collected, and final values were multiplied by a factor of 10.

Results and discussion

Physical observation of pellets

Figure 1(a) and 1(b) shows the images of the encapsulation pellets obtained using a fluorescence microscope. It was observed that fresh pellets had a relatively high cell density, but the density and size of the cells in the pellets diminished with time. During the period of acclimation and routine growth, the encapsulated cells were fed with toluene as a sole carbon source. The samples for visual analysis were taken when a steady removal of toluene was achieved. On the other hand, the cells in fresh PEG pellets had been grown in the NB solution. The smaller size of the cells in the PEG pellet might have reflected a potential influence of the restricted space for growth, which needed further investigation for clarification. Images by CLSM (Figure 1(c)) showed the distribution of cells close to the surface of a pellet. The cell density looked greater towards the surface compared with internal areas. Lower densities were observed deep inside the pellets. This is a typical pattern of bacterial growth in biofilms where limited substrates and oxygen have to diffuse into the biofilm to react with cell enzymes.[17] The PEG polymer pellets remained intact for the whole experimental span; however, a colour change was observed from white to pale yellow. The colour change did not affect the microbial activity (on repeated use). The colour of the pellets was restored to the original when resuspended in a fresh mineral solution.

Abiotic removal of TCE and toluene in the encapsulation pellets

To determine whether the PEG pellets experienced abiotic removal due to adsorption onto or into the pellet, a sorption test was performed for both toluene and TCE in separate reactors, along with controls.[10] PEG pellets without any encapsulated cells were sampled using the same experimental design described earlier. There was no adsorption observed either for toluene or TCE compared with the controls (data not shown). No removal was observed for TCE or toluene along the full span of experiments. This shows that neither toluene nor TCE was adsorbed onto the PEG pellets, which is contrary to the findings of Radway et al.[7] who reported about 16% removal of TCE due to adsorption when B. vietnamiensis G4 was embedded onto polyurethane foam.

Simultaneous degradation of TCE and toluene

Figure 2(a)–(c) shows the degradation of 0.1, 0.5 and 1 mg/L of TCE, respectively, along with 10 mg/L of toluene in suspended and encapsulated modes. Toluene was degraded almost completely and quickly (within ~5 h) in the encapsulated cases, regardless of the input.
concentrations of the TCE. Also was the removal in the suspended cases, although it took a little longer when the input TCE concentration was higher. The TCE degradation occurred simultaneously with toluene removal; however, it continued even after toluene was completely degraded. The extent of TCE removal in the suspended cultures was slightly superior to that in the encapsulated cultures, although the encapsulated was faster in toluene removal. Control vials showed no decrease in concentration, indicating that the removal in both systems was due to biodegradation, most probably via co-metabolism.

It is important to note that there was no observable lag time in the suspended or encapsulated cultures, as other researchers have reported such for both modes. TCE started degrading along with toluene removal as shown in Figure 2(a)–(c). Chen et al. [9] reported a lag period in TCE degradation against phenol using *Pseudomonas putida* F1 for both suspended and encapsulated cells in chitosan beads. TCE was degraded only when phenol was degraded completely. It was speculated that competition between TCE and phenol existed for active sites of enzymes in the suspended cells, while a diffusion resistance might have been responsible for the lag in the encapsulated cultures. Similar trends were reported by Futama et al. [18]. Since Kim et al. [10] observed only slight lag time in *P. putida* F1 cultures with toluene and TCE; it appears that toluene is less competitive than phenol against TCE co-metabolism.

As TCE concentration increased, the per cent removal of TCE (Figure 2(a)–(c)) decreased in both systems (i.e. suspended and encapsulated cells), showing that the provided TCE to toluene ratios are very important in the overall removal process. This is in accordance with a previous report. [15] The decrease in TCE removal in this study might have been due to insufficient primary substrate that could have caused lower production of enzymes and internal electron donors (e.g. NADH). The removal rate of toluene in the suspended cultures slowed when the TCE concentration was greater than 0.5 mg/L, indicating a toxic effect of TCE at that concentration. As there was no observable delay of toluene removal in the encapsulated cultures, it appears that the concentration gradient into the pellets (thus, lowered TCE concentration inside the pellet) acted favourably for toluene degradation.

**Degradation of TCE and toluene with high substrate availability**

To verify the behaviour of the encapsulated cultures with respect to high substrate availability, toluene was fed at 50 mg/L with the same experimental conditions noted above. Figure 3(a)–(c) shows the degradation of TCE (0.1, 0.5 and 1 mg/L, respectively) under high concentrations of toluene, i.e. 50 mg/L. Toluene in the suspended cultures was degraded in about 15 h for 0.1 mg/L of TCE, while
it took about 38 h for the other concentrations (for TCE concentrations of 0.5 and 1 mg/L), indicating TCE and/or toluene toxicity. The trend is similar to a previous experiment with 10 mg/L of toluene, where 0.5 and 1 mg/L of TCE caused a reduction in the toluene degradation rate in 5–13 h. This result may also imply that the toxicity trend is similar from 0.5 to 1 mg/L or higher. However, the removal of toluene in the encapsulated cultures was completed in a similar fashion within 6–8 h in all three TCE concentrations, as seen in Figure 1. Toluene or TCE was not lost abiotically during the experiment, as demonstrated by the control experiment results.

The removal efficiency of TCE dramatically increased compared with the removal shown in Figure 2, indicating the importance of a proper supply of the primary substrate. More important is that TCE was removed much faster in the encapsulated cultures and completely removed (100%) within 10–12 h in all cases. This is a reversal from the observation seen in Figure 2, in which the suspended cultures gave slightly better results. The cause for this reversal was unclear, though it was suspected that the toxicity/inhibition from the elevated initial toluene concentration inhibited the removal of TCE in the suspended cultures; the encapsulated cultures might have been less affected due to the concentration gradient in the PEG pellets for both TCE and toluene.

The toxicity of higher concentrations of toluene has been reported in the literature. A ratio of 1:50 seems to work well for 1 mg/L of TCE; however, a ratio of 1:100 in the last experiment (TCE 0.1 mg/L and toluene 10 mg/L in Figure 2(a)) did not result in complete degradation of TCE at 0.1 mg/L. The reason could be that 10 mg/L of toluene was not sufficient to perform cell growth, energy and enzymatic reactions and repair to damaged cells. On the other hand, 50 mg/L of toluene was sufficient to perform all these functions. Hence, to achieve good results, both ratios and sufficient supplies should be given equal importance.

As reported in the literature, the removal efficiencies of TCE by different encapsulated cultures have varied between 30% and 90% under various conditions and growth substrates. About 81% removal of TCE (initial concentration, 3.1 mg/L) was reported by Radway et al. [7] when B. vietnamiensis G4 was embedded in polyurethane foam using benzene (2 mg/L) as a growth substrate. However, they did not evaluate their system for high concentrations of TCE and benzene or toluene.

**Degradation trends of TCE and toluene at higher concentration of TCE**

Figure 4 shows the degradation of TCE (5 mg/L) against 10 and 50 mg/L of toluene. In Figure 4(a), toluene (10 mg/L) was almost degraded in about 5–8 h in both the suspended and encapsulated cultures in a similar fashion to the degradation of 0.1 mg/L of TCE (Figure 2(a)). From this experiment, it appears that 5 mg/L of TCE does not significantly inhibit toluene degradation, although TCE concentration appears to affect toluene removal in the cases illustrated in Figures 2 and 3. The TCE removal efficiency of encapsulated cultures was almost two times higher than the suspended cultures in Figure 4(a). This shows that an encapsulated system gave better protection from TCE toxicity compared with the suspended cultures at a higher TCE concentration (5 mg/L) and relatively low available substrate (toluene, 10 mg/L). For suspended cultures, it has been reported that bacterial activity is either stopped completely or greatly suppressed as the TCE concentration increases, due to toxicity related to TCE or its transformation by-products. The same trend was reported by Mu and Scow [15] in which biodegradation kinetics was greatly inhibited by the toxicity of TCE and its transformation by-products.

Figure 4(b) shows the results for testing in a TCE concentration of 5 mg/L and toluene concentration of 50 mg/L. The overall trend of removal was similar to that in Figure 3(c), in spite of the much higher TCE concentration. TCE was simultaneously degraded in the suspended culture as soon as toluene degradation started. But a lag time of about 9 h was observed in the encapsulated culture. Then, TCE degraded rapidly at 9–15 h (Figure 4(b)). This time span is almost exactly the same as for the degradation
of toluene. The reason for this delayed trend of TCE degradation was unclear, though it is suspected that the lag time may be due to the excess of toluene to TCE. In addition, toluene may have influenced diffusion kinetics for the PEG material, occasionally causing a delayed initial degradation process, as Kim,[16] observed that toluene replaced previously adsorbed TCE in activated carbon adsorption. Although a lag time was observed in TCE degradation, the removal rate of PEG-encapsulated cells was much faster, once TCE began to degrade, than that of the suspended cells.

On the other hand, TCE degradation was greatly suppressed in both cultures in comparison with Figure 3, perhaps due to TCE toxicity. TCE conversions, however, were higher compared with the previous case in Figure 4(a) (5 mg/L of TCE and 10 mg/L of toluene). This might be due to greater substrate availability. Mars et al.[20] reported that, at low toluene concentrations, cytotoxicity by TCE to TCE converting microorganisms is a selective disadvantage. This implies that, at higher toluene concentrations, B. vietnamiensis G4, which is well known to withstand higher concentrations of TCE, can use available toluene for maintenance energy, and surplus toluene can be used to express toluene 2-monoxygenase. Thus, TCE degradation is possible even at a relatively high concentration if sufficient toluene is available.

**Transformation yields**

To quantify the effectiveness of bacteria with regard to the co-metabolic degradation of TCE, transfer yield (Ty), as proposed by Alvarze-Cohen and McCarty,[21] is commonly used and is defined as the maximum mass of TCE degraded per unit mass of substrate (in this study, toluene) used to grow co-metabolic degraders (i.e. mg TCE removed/mg toluene utilized). In a biological degradation process, it is quite common that sudden higher concentrations of the substrate and pollutant (shock loading) can disturb the efficiency and stability of the process. To verify the shock loading mitigation strength, both cultures were subjected to high TCE concentrations. In order to evaluate the boundaries of toxicity for this encapsulation system, both suspended and encapsulated cultures were used to degrade a wide range of TCE, i.e. 0.1–20 mg/L. Figure 5 shows the comparison of suspended and encapsulated cultures for the Ty at toluene concentrations of 10 and 50 mg/L, respectively, for a TCE range of 0.1–20 mg/L. The Ty of encapsulated cultures was higher than that of the suspended cultures for both toluene concentrations (i.e. 10 and 50 mg/L). These results indicate that the encapsulated cultures used the given substrate more efficiently to co-metabolize TCE. This could be due to the fact that suspended cultures have to use the given substrate to cope with the toxicity, resulting in a low Ty. The highest Ty was observed at a toluene concentration of 10 mg/L in the encapsulated system, while the lowest was observed in suspended cultures for the same concentration (Figure 5(a)). Although the Ty for the encapsulated cultures with 50 mg/L of toluene was lower than those at 10 mg/L, they were higher than the suspended cultures at the same concentrations. It has been reported that, as TCE concentration increases, the Ty also increases until strong toxicity and enzyme inhibition become factors.[21]

The Ty of the encapsulated system continued to increase until 20 mg/L of toluene for both concentrations of toluene (Figure 5), although the rate was lower at higher TCE concentrations. It is interesting to note that there was no decrease observed in encapsulated cultures even at the highest TCE concentration (i.e. 20 mg/L), unlike Chen et al.[9] who reported a sharp decrease in the Ty for TCE values greater than 15 mg/L with a higher phenol supply (100 mg/L). This result indicates that PEG encapsulation of B. vietnamiensis G4 can protect the biomass up to 20 mg/L of TCE or maybe even higher. Although overall removal decreased, an increase in the Ty value shows the extent to which the TCE degrader activity was suppressed. This factor can help to design strategies to improve TCE degradation.

The Ty values for 10 mg/L of toluene were higher than those reported in the literature but were within the range for 50 mg/L of toluene (0.014 mg TCE/mg toluene).
These values, however, are similar to the values reported in different studies using phenol as the primary substrate (0.002−0.11 mg TCE/mg phenol). In the suspended cultures, the $T_v$ decreased at TCE concentrations greater than 5 mg/L. This is in accordance with Chen et al.,[9] who reported that TCE concentration of 10 mg/L or higher is toxic and inhibitory to microorganisms.

Figure 5 shows the given and resulting $T_v$ (mg TCE/mg toluene). For 10 mg/L toluene, the highest $T_v$ (0.10) occurred at a ratio of 0.21 mg-TCE/mg-toluene in suspended cultures while this ratio was 1.83 for encapsulated cultures giving a $T_v$ value of 0.427 mg-TCE-mg-toluene. Similarly for 50-mg/L toluene, the $T_v$ for encapsulated cultures was also much higher than that for suspended cultures. The highest $T_v$ value was 0.145 mg-TCE/mg-toluene, which was observed when the TCE/toluene ratio was 0.33 mg-TCE/mg-toluene. At same toluene supply (i.e. 50 mg/L), the value of ($T_v$) for suspended cultures was 0.09 mg-TCE/mg-toluene which occurred at 0.18 mg-TCE/mg-toluene. The higher $T_v$ values observed by encapsulated cultures even at higher TCE/toluene ratios show that encapsulated cultures observed less toxicity of TCE due to PEG encapsulation.

These values are higher than what has been reported in the literature, even with a lower TCE to toluene ratio. A large range of $T_v$ has been reported with different cultures and growth substrates for TCE mineralization. Numerous studies have been conducted with auxiliary non-aromatic substrates to increase the transformation efficiency of many microorganisms. For pure culture B. vietnamiensis G4, the $T_v$ reported is 0.014 mg TCE/mg toluene using toluene as the substrate.[8] With mixed cultures and chemostat set-ups, $T_v$ values ranging from 0.0021 to 0.003 mg TCE/mg toluene have been reported using toluene as the substrate.[22] While 0.052–0.222 mg TCE/mg phenol have been reported using phenol as the substrate.[23] A range of $T_v$ (0.002–0.106 μmol of TCE/μmol of substrate) values was reported by Tejasen,[24] when a variety of aromatic and non-aromatic substrates were used. A $T_v$ value of 0.106 μmol/μmol tetraphenoxysilane as a growth-inducing substrate was reported in the same study. Our value of 0.427 mg TCE/mg toluene (0.30 μmol of TCE/μmol toluene) for $T_v$ is the highest to date, according to the authors’ knowledge. The higher transformation yields observed from this study show a success with the PEG-encapsulated system.

Conclusions

Burkholderia vietnamiensis G4 was encapsulated in PEG polymer and used to degrade TCE (0.1, 0.5, 1 and 5 mg/L) via co-metabolism. Toluene was used as a growth substrate (10 and 50 mg/L). The experiments concluded that:

1. The encapsulated cultures were less suppressed by the toxicity of TCE, resulting in less of a decrease in efficiency as the TCE concentration increased.
2. Experiments with 50 mg/L of toluene produced higher overall removal efficiencies in both (suspended and encapsulated) cultures as compared with 10 mg/L of toluene. However, the degradation rates of toluene and TCE were higher in the encapsulated cultures.
3. The encapsulated cultures used the available substrate very efficiently, resulting in a high transformation yield that was one to two orders higher than previously reported values for toluene as substrate.

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