Biodegradation (2008) 19:259–268
DOI 10.1007/s10532-007-9132-8

ORIGINAL PAPER

Aerobic biodegradation of *tert*-butyl alcohol (TBA) by psychro- and thermo-tolerant cultures derived from granular activated carbon (GAC)

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Received: 21 February 2007 / Accepted: 7 May 2007 / Published online: 31 May 2007

Abstract *Tert*-butyl alcohol (TBA) is a metabolite of methyl *tert*-butyl ether and is itself possibly a fuel oxygenate. The goals of this study were to enrich and characterize TBA-degrading micro-organism(s) from a granular activated carbon (GAC) unit currently treating TBA. The results reported herein describe the first aerobic, TBA-degrading cultures derived from GAC. Strains *KR1* and *YZ1* were enriched from a GAC sample in a bicarbonate-buffered freshwater medium. TBA was degraded to 10% of the initial concentration (2–5 mM) within 5 days after initial inoculation and was continuously degraded within 1 day of each re-amendment. Resting cell suspensions mineralized 70 and 60% of the TBA within 24 h for *KR1* and *YZ1*, respectively. Performance optimization with resting cells was conducted to investigate kinetics and the extent of TBA degradation as influenced by oxygen, pH and temperature. The most favorable temperature was 37°C; however, TBA was degraded from 4 to 60°C, indicating that the culture will sufficiently treat groundwater without heating. This is also the first report of psychrotolerant or thermotolerant TBA biodegradation. The pH range for TBA degradation ran from 5.0 to 9.0. Phylogenetic data using a partial 16S rRNA gene sequence (570 bases) suggest that the primary members of *KR1* and *YZ1* include uncharacterized organisms within the genera *Hydrogenophaga*, *Caulobacter*, and *Pannonibacter*.

Keywords Biodegradation · Bio-GAC · Mixed microbial culture · Oxygenate · Psychrotolerant · Thermotolerant · *Tert*-butyl alcohol

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Introduction

Reports have shown that *tert*-butyl alcohol (TBA) is a key metabolite in the methyl *tert*-butyl ether (MTBE) biodegradation pathway and can be present as impurities in MTBE; it may also be added directly to fuels (Salanitro et al. 1994; Steffan et al. 1997) TBA is used in some manufacturing processes for plastics, resin polymers, perfumes, paint removers, insecticides, and pharmaceutical products (Tay et al. 2005), and therefore may enter the environment. Currently there are no federal regulatory limits for TBA in drinking water; however, some states have set their own limits. For example, California's drinking water action level for TBA is 12 parts per billion (ppb) (Kane et al. 2001).

Tert-butyl alcohol is miscible in water and adsorbs poorly to organic matter within subsurface solids, which can cause the contaminant to travel long distances. Additionally, due to its physical properties (low-Henry's constant and log K_{oc}), traditional adsorption and air-stripping technologies are less effective than biological remediation (Zhuang et al. 2005). MTBE and BTEX compounds, often co-contaminants with TBA, are also highly soluble in water and can travel from a spill site. The Henry's Law constant for TBA (1.4×10^{-5} atm m^3 mol^{-1}) is 40 times lower than MTBE (Schmidt et al. 2004) making air stripping less effective for TBA. BTEX compounds have been reported to degrade more readily in the subsurface (Feris et al. 2004), and therefore may be degraded before impinging on sensitive receptors. Biological treatment is also a feasible option for TBA remediation when site conditions are well understood or when ex situ systems are utilized (Wilson et al. 1986; Deeb et al. 2000; Wilson and Kolhatkar 2002).

Aerobic degradation and anaerobic degradation have both been reported; anaerobic degradation is generally slower and more site-specific than aerobic TBA metabolism (Deeb et al. 2000; Finneran and Lovely 2001; Somsamak et al. 2001). Several pure cultures capable of aerobic MTBE degradation are also capable of degrading TBA, including PM1 (Hanson et al. 1999), IFP2012 (Francois et al. 2002), and ENV735 (Hatzinger et al. 2001). Several aerobic mixed cultures capable of aerobic TBA degradation have been reported (Salanitro et al. 1994; Eweis et al. 1997; Fortin et al. 2001). These cultures were enriched from activated sludge, either directly or from bioreactors seeded with activated sludge (Salanitro et al. 1994; Eweis et al. 1997; Hanson et al. 1999; Francois et al. 2002), or from environmental samples (Fortin et al. 2001).

The cultures presented here are the first that have been derived from a GAC system, to the best of our knowledge, which may make these cultures well suited for bioaugmentation into existing GAC systems. The previously described cultures also have limited metabolic flexibility with respect to temperature (Salanitro et al. 1994) or pH (Eweis et al. 1997), which may lead to additional operational costs for ex situ treatment. Temperatures cited in the literature range from 25 to 30°C and cells did not grow at 37°C (Nakatsu et al. 2006). Propane-oxidizing bacteria strains ENV425 and ENV421 had between four and sevenfold decreases in MTBE degradation rates and two to threefold decreases in TBA degradation rates when the temperature was dropped from 28 to 13°C (Steffan et al. 1997). A pH range from 5 to 10 was investigated for PM1 but only an optimum pH of 6.5 was reported which indicates that growth is limited to a narrow pH range (Nakatsu et al. 2006). The cultures described below are less sensitive to the key operational parameters (pH, temperature, and substrate starvation) relative to the previously described aerobic TBA degraders (Eweis et al. 1997; Hanson et al. 1999).

Although in situ treatment may be feasible, when TBA plumes impinge on sensitive receptors such as drinking water wells, pump and treat may be the most frequently requested strategy from a regulatory standpoint. One developing technology is using biologically activated carbon or ''bio-GAC'' systems. These systems differ from traditional GAC systems by allowing biofilms to form on the surfaces and pores of the GAC; this allows TBA to be transformed to innocuous end-products and can prolong the life of the carbon. The biological activity can form naturally from native bacteria or known TBA degraders can be introduced to the system. Both are valid approaches but the former may result in longer lag times before treatment.

The purpose of this project was to characterize the degradation capabilities of GAC-derived cultures enriched from an existing GAC system. The cultures will ultimately be used to bioaugment systems where native TBA degraders are not present or to shorten the acclimation period. Microbial characterization and TBA degradation optimization were investigated for each of the enrichments. *KR1* has been selected as the primary culture for the remainder of the (broader) project due to its generally faster degradation kinetics under different conditions (pH and temperature); it is therefore the focus for this report. However, *YZ1* data have also been presented within the text when most relevant, as this basic microbial physiology is unique amongst TBA degraders.

Materials and methods

Site conditions and sample

The bio-GAC sample was received from a site located at Fountain Valley, California, which was

employing a bio-GAC system to specifically treat TBA. Prior to the carbon system the groundwater was treated by air stripping to remove MTBE and other fuel components. The groundwater treated had TBA levels as high as 350 ppm. The TBA was being removed to sufficient levels with the bio-GAC. The system initially required more than a year to stabilize.

Culture enrichment and maintenance

Two different aerobic bacterial consortia (*KR1* and *YZ1*) were enriched from a sample of GAC taken from the site described above. GAC material was shipped from the site to the University of Illinois on blue-ice and was processed immediately upon arrival. Approximately 10 g of wet GAC were placed in a total volume of 50 mL of fresh water (FW) medium (in 125 mL conical flasks with 103 mL headspace volume) containing the following components (g L^{-1} unless specified otherwise): $NaHCO_3$, 2.5; NH_4Cl, 0.25; $NaH_2PO_4{\cdot}H_2O$, 0.6; KCl, 0.1; modified Wolfe's vitamin and mineral mixtures (each 10 mL L^{-1}) and 1 mL of 1 mM Na_2SeO_4 (Lovley et al. 1993). TBA was added as the sole carbon and energy source to an initial concentration of 2 and 5 mM for *KR1* and *YZ1*, respectively, from a sterile 100 mM stock solution. Initial enrichment conditions were based on a range of TBA concentrations and these values (2 mM for *KR1* and 5 mM for *YZ1*) represent the concentration that was degraded most rapidly following initial GAC inoculation; they were therefore used for the different cultures in the remaining experiments. Transfers were made (2% vol : vol) into new FW media when turbidity demonstrated cell growth. Once active cultures were obtained and TBA degradation was confirmed, the cultures were maintained by transferring weekly into new TBA amended FW media. Cultures were kept at 30°C in the dark on a shaker table at 100 revolutions per minute (RPM). All transfers were made using standard aseptic techniques.

Growth experiments

Experiments were performed using 2% transfers of cultures into a total liquid volume of 50 mL, in 125 mL bottles with Teflon-lined Mininert valve screw tops (Supelco, St. Louis, MO, USA). Cultures were incubated at 30°C in the dark on a shaker table. TBA was added as the sole carbon and energy source at an initial concentration of 2 and 5 mM for *KR1* and *YZ1*, respectively. To minimize oxygen limitation, cultures were either supplied excess oxygen via the Mininert gas-lock (*KR1*) or a sterile Styrofoam stopper was used in place of the screw top until 2 h before sampling (this method was only used for the *YZ1* growth experiments, which are represented in Fig. 1; control data were generated in the same manner to account for headspace volatility losses; all remaining *YZ1* experiments were performed with Mininert gas-locks). The concentration of TBA was quantified over time using a GC-FID as described below. Uninoculated bottles (pH = 7.0 and 30°C) were used as a control. All experiments and controls were performed in triplicate.

Nutrient experiments

Nutrient experiments were performed as the growth experiments above with the following differences: these bottles were run at 2 mM TBA for both cultures and varying concentrations of nitrogen, as NH_4Cl (1, 5, 7.5, and 10 mM), and phosphorus, as $NaH_2PO_4{\cdot}H_2O$ (1, 5, and 10 mM), were amended. Activity was quantified by TBA degradation.

Starvation experiments

Starvation experiments were conducted as described above for growth experiments. After the TBA was initially degraded, the cultures were incubated for a starvation period of 7, 14 or 21 days without any carbon substrates or alternate electron donors. After the specific starvation period the bottles were re-amended with TBA and mass loss was measured with a GC-FID.

Resting cell suspensions

Cultures were harvested (0.5–1 L) during logarithmic growth phase by centrifugation (5,000 × *g*, 15 min, 4°C) to form a dense cell pellet. The pellets were washed and resuspended in 30 mL of 250 μM bicarbonate buffer and centrifuged again (5,000 × *g*, 15 min, 4°C). Final pellets were resuspended in 1.0 mL of bicarbonate buffer and combined. Aliquots

from the harvesting procedure were then used in mineralization experiments.

Mineralization experiments

Bicarbonate buffer (250 μM) was added to 60 mL serum bottles with 0.1 mL [U-^{14}C]-TBA (4 mCi mmol^{-1}, Moravek Biochemicals, La Brea, CA, USA, >97% radiochemical purity) to provide 0.3 μCi per bottle (equating to 5.8 μM TBA) and aliquots from the cell suspension (1.3 mL) were added to reach a total volume of 13 mL. Bottles were sealed with a butyl rubber stopper fastened with an aluminum crimp with significant headspace to prevent oxygen limitation. Typical non-growth conditions were pH 7, ambient air, incubated at 30°C, unless otherwise noted below. All experiments were performed in triplicate. $H^{14}CO_3^-$ was used to determine the partitioning coefficient for $^{14}CO_2$. Uninoculated bottles were used as a control.

Temperature experiments

Temperature range experiments were performed as described above for the mineralization experiments. Incubations were performed in sealed tubes with oxygen added to the headspace via syringe and uniformly radiolabeled [^{14}C]-TBA added as the sole electron donor. Temperature was adjusted by placing bottles in incubators, or temperature controlled rooms. Incubators were set at 18, 37, and 60°C. Temperature controlled rooms were set at 4 and 30°C. Controls were at 4°C (low), 30°C (active control), and 60°C (high).

pH experiments

pH experiments were performed as described above for the mineralization experiments. Hydrogen chloride and sodium hydroxide were used to adjust the pH of the bicarbonate buffer (approximately pH 7.0) from pH 5.0 to 9.0 with increments of 1 pH unit.

Oxygen experiments

Oxygen experiments were performed as described above for the mineralization experiments. Oxygen content was varied in the headspace by flushing with pure oxygen (100%) or by flushing with nitrogen and then amending with 1% (vol : vol) oxygen.

Analytical methods

Tert-butyl alcohol degradation was monitored by sampling 0.1 mL of headspace with a gas lock syringe and analyzed by gas chromatography (Hewlett Packard Series 6890A) with an HP-1 capillary column (Hewlett Packard, Palo Alto, CA, USA) connected to a flame ionization detector (FID). The temperature program was at 40°C for 4 min with a temperature ramp of 40°C min^{-1} up to 160°C.

The production of $^{14}CO_2$ and $^{14}CH_4$ from [U-^{14}C]-TBA was monitored by sampling 1 mL headspace using a gas proportional radiochromatography detector (IN/US Systems Inc., Tampa, FL, USA, GC-Ram) connected to a gas chromatograph (Hewlett Packard Series 6890A).

Molecular analysis

DNA was extracted from single colonies that were grown on solid (agar) freshwater medium with TBA as the sole carbon source. *KR1* consistently developed three distinct colonies; *YZ1* developed several colonies but only one was consistently recovered with each liquid plating; this single colony was used for *YZ1* analysis. DNA was extracted from individual colonies using a fast DNA spin kit (Bio101 Systems). Partial 16S rDNA sequences were amplified with universal Eubacterial primers 338 forward (338F) and 907 reverse (907R). Each PCR mixture consisted of 10 μL of DNA template, 10 μL of 10× buffer (Qiagen, Valencia, CA, USA), 5 μL of buffer Q (Qiagen), 2 μL of deoxynucleotide triphosphate mixture (Qiagen), 2 μL of forward and reverse primers, 0.5 μL of Taq polymerase (5 U μL^{-1}; Qiagen), and the balance was UV sterile distilled water. Each reaction was 100 μL. PCR amplification was performed in a thermal cycler (Bio-RAD, Hercules, CA, USA) with an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 94 (30), 50 (30), and 72°C (45 s) with a final extension at 72°C for 7 min. The PCR product was purified using a Qiagen DNA purification kit and sequenced at the University of Illinois Core Sequencing Facility. Sequence results were uploaded to BLAST (NCBI) and compared to known 16S rDNA sequences.

Results

KR1 and *YZ1* degraded 2 and 5 mM TBA, respectively, when incubated at standard conditions (30°C, pH 7, and ambient air) after a 5-day acclimation period. When further re-amended with TBA, *KR1* continuously degraded 2 mM TBA within 1 day (Fig. 1) and *YZ1* degraded 5 mM TBA within 3 days (data not shown). Under non-growth conditions, *KR1* and *YZ1* mineralized 70 and 50%, respectively (Fig. 2) within 24 h. However, later experiments demonstrate that nearly 100% TBA was mineralized at higher pH values.

Temperature influenced TBA degradation. *KR1* and *YZ1* both mineralized TBA most effectively at 37°C. TBA was also mineralized at the extremes of the temperature test: 4°C for *YZ1* and *KR1* and 60°C for *YZ1* only (Fig. 3a, b). Experiments were conducted using sealed tubes, with uniformly radiolabeled [^{14}C]-TBA as the sole electron donor. Although the temperature will influence the aqueous partitioning of TBA, the only pathway for $^{14}CO_2$ is via microbial mineralization. Both cultures mineralized TBA at all pH-values between 5.0 and 9.0; although the degradation rate and extent varied for each culture (Fig. 4a, b). The rate constant increased with increasing pH for both cultures and TBA was degraded most effectively at pH 8.0–9.0. TBA was mineralized with 100% O_2 and ambient air; however, TBA did not degrade, except for one replicate sample of *YZ1*, with 1% oxygen. The cell protein normalized

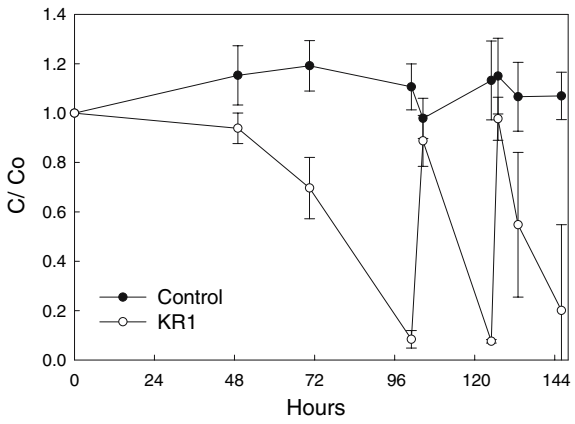

Fig. 1 TBA degradation by growing cells of strain *KR1*; controls were uninoculated. Initial TBA concentration was 2 mM. Results are the means of triplicate analyses; *bars* indicate one standard deviation

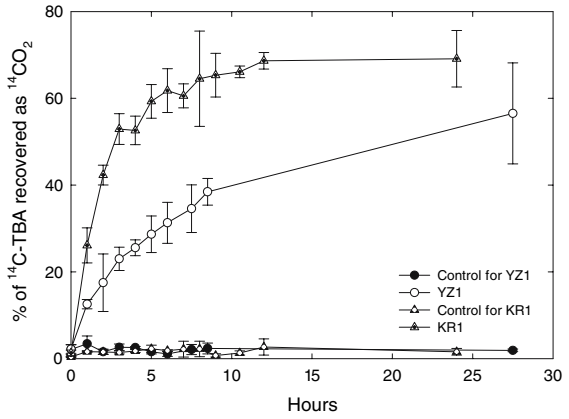

Fig. 2 TBA mineralization by resting cells of strains *KR1* and *YZ1*. Results are the means of triplicate analyses; *bars* indicate one standard deviation

degradation kinetics for each parameter investigated are summarized in Table 1.

Tert-butyl alcohol degradation was monitored under growth conditions with varying nutrient concentrations. Results for *KR1* are presented in Fig. 5a, b. Both *KR1* and *YZ1* degraded TBA fastest at 1 mM nitrogen (as ammonium nitrate). Nitrogen concentrations above 7.5 mM inhibited TBA degradation in the time frame investigated. In further investigations with *YZ1*, TBA was continuously degraded with 5 mM nitrogen (data not shown) suggesting a threshold somewhere between 5 and 7.5 mM nitrogen. Increasing nitrogen concentrations (between 1 and 5 mM) was not an impediment to TBA degradation, but increasing phosphate concentrations increased the degradation rates.

Starvation experiments were conducted in which TBA was withheld following complete degradation to test the longevity of the culture in the absence of its sole carbon and energy source. The results demonstrated that *KR1* completely degraded 2 mM TBA within 3 days after being subjected to a 7-day starvation period. *KR1* degraded 2 mM TBA within 5 days after a 14-day starvation period. *KR1* degraded 2 mM TBA within 6 days following a twenty-one (21) day starvation period (Fig. 6). Longer starvation periods were tested and at each point longer than 21 days the culture had a 48-h lag time before degradation resumed. *YZ1* degraded 5 mM TBA completely within 6 days after a 7-day starvation (data not shown).

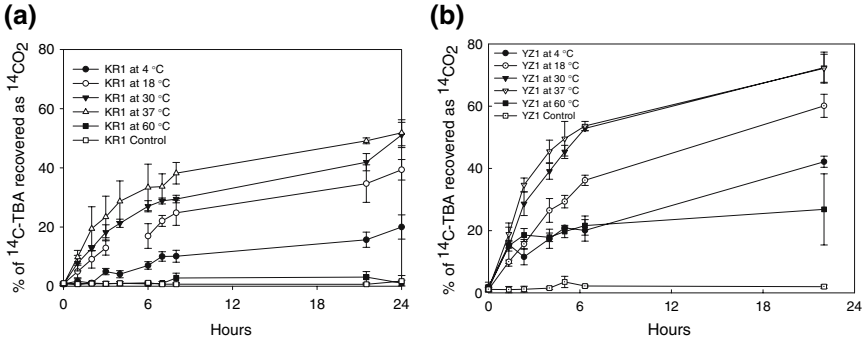

Fig. 3 Plots of TBA mineralization by resting cells of strains *KR1* (**a**) and *YZ1* (**b**) for varying temperatures. Results are the means of triplicate analyses; *bars* indicate one standard deviation. Uninoculated controls were at pH 7 and 30°C

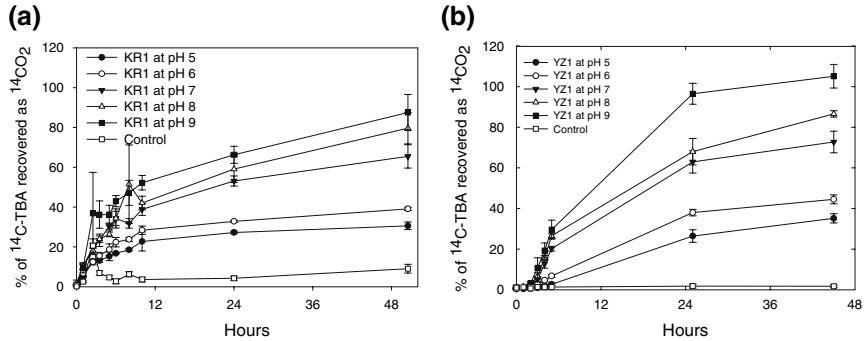

Fig. 4 Plots of TBA mineralization by resting cells of strains *KR1* (**a**) and *YZ1* (**b**) for varying pH. Results are the means of triplicate analyses; *bars* indicate one standard deviation. Uninoculated controls were at pH 7 and 30°C

Table 1 TBA mineralization kinetics for strains *KR1* and *YZ1*

	First order degradation rate constant (k) mg cell protein^{-1} h^{-1}	
	KR1	*YZ1*
Temperature		
4°C	0.008	0.004
18°C	0.021	0.008
30°C	0.029	0.014
37°C	0.037	0.015
60°C	0.000	0.004
Oxygen		
100%	0.030	0.023
Ambient air	0.023	0.019
1%	0.000	0.0002
pH		
pH5	0.012	0.001
pH6	0.016	0.004
pH7	0.024	0.014
pH8	0.033	0.019
pH9	0.035	0.021

Phylogenetic data based on analysis of a partial fragment (570 bases within the 338–907 region) of the 16S rRNA gene extracted from individual colonies indicated that the cultures were mixed and had at least three dominant genera represented. *KR1* consistently plated three distinct colonies, of which one was operationally defined as dominant with respect to growth timeframe and plate coverage. *YZ1* plated multiple colonies; however, one was consistent and defined as dominant with respect to plate coverage. These colonies were selected for DNA analysis. The mixed cultures *KR1* and *YZ1* both contained dominant phylotypes that were most closely related to organisms in the genera *Hydrogenophaga*. The nearest relative (98% related) was *Hydrogenophaga* strain D11-24b2 (BLAST accession no. AM402226; unpublished) for both *KR1* and *YZ1*. This nearest phylogenetic relative is not identical to known *Hydrogenophaga* species that are reported to degrade MTBE or TBA (*Hydrogenophaga flava* ENV735) (Hatzinger et al. 2001). Other phylotypes present in *KR1* came back most closely related to *Caulobacter*

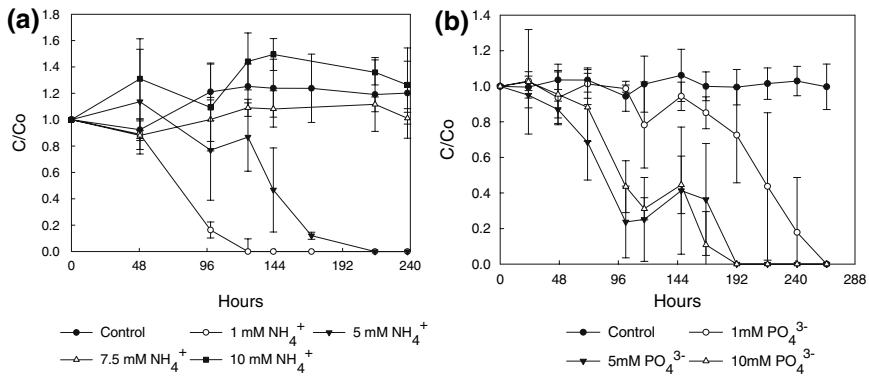

Fig. 5 TBA degradation by strain *KR1* for varying nitrogen (**a**) and phosphorus (**b**) concentrations. Initial TBA concentration was 2 mM. Results are the means of triplicate analyses; *bars* indicate one standard deviation

(98% related, strain MBIC1405, BLAST accession no. AB016847) and *Pannonibacter* (98% related, *P. phragmititus* strain LMG 5421, BLAST accession no. AM269447); however, neither was defined as the dominant phylotype. The physiological differences between the enrichments (*YZ1* and *KR1*) were used to define them as distinct from each other.

Discussion

These results demonstrate that TBA can be degraded by cultures enriched from granular activated carbon (GAC) that has not been amended in the field to specifically promote biological activity. The cultures enriched as part of this project actively degraded TBA within 2 days after a short acclimation period, and TBA degradation has been maintained for ~1 year. While the purpose of the investigation was to provide an inoculum culture to decrease the lag time associated with bio-GAC mediated TBA degradation, the cultures enriched are unique with respect to several physiological properties and are relatively robust when compared to other MTBE and TBA degrading, aerobic micro-organisms (Deeb et al. 2000; Schmidt et al. 2004).

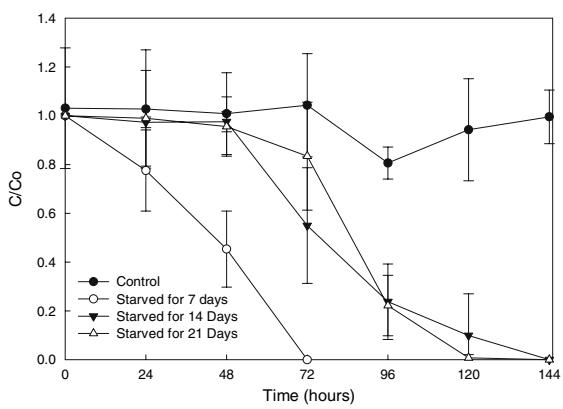

Fig. 6 TBA (2 mM) degradation by strain *KR1* after starvation periods of 7, 14, and 21 days. A representative control is shown for 21-day starvation, other controls are similar. Results are the means of triplicate analyses; *bars* indicate one standard deviation

The mineralization data demonstrate that TBA is being transformed to innocuous end products. In addition, TBA is the sole carbon and energy source for these cultures, which has only been identified in three other liquid microbial enrichments and possibly one stable column reactor community (which has not been recovered in liquid culture) (Hanson et al. 1999; Hatzinger et al. 2001; Francois et al. 2002; Wang and Deshusses 2007). Further work is being done to determine if the degradation pathway is similar to the previously reported (Steffan et al. 1997) pathway for TBA with intermediates including 2-hydroxy isobutyric acid, 2-propanol, and acetone; all of which *KR1* can use as a growth substrate. Initial data (not presented) demonstrated that 2-hydroxy isobutyric acid (HIBA) is part of the degradation pathway; other metabolites have not been identified.

The optimum temperature for TBA degradation in both cultures was 37°C; however, both cultures degraded TBA over a larger range of temperatures than has been previously reported for aerobic TBA degradation. Both cultures were able to mineralize TBA at 4°C, which is the first reported evidence of psychrotolerant TBA degradation. Low-temperature MTBE mineralization has been reported for aquifer sediments at temperatures as low as 4°C, but TBA degradation was not investigated (Bradley and Landmeyer 2006). In situ microbial communities are likely more robust at temperature extremes due to commu-

nity diversity. Enrichment cultures generally tolerate fewer environmental stresses. However, *YZ1* and *KR1* withstood colder temperatures without loss of TBA degradation, which has implications for the eventual ex situ remediation strategy. Groundwater heating is an operational cost that may not be necessary with this culture. However, the degradation rates outside of the normal temperature range (30–37°C) were slower, which will affect performance once brought to the field. Even if heating is necessary for typically cold groundwater, these cultures will be better suited to tolerate fluctuations or periods of low temperature.

YZ1 also degraded TBA at 60°C, which is the first evidence of thermotolerant TBA degradation. Although these cultures will be used to treat groundwater that most likely has a mean temperature of 14–24°C, the results suggest that temperature fluctuations will not interfere with TBA mineralization. Even if the biomass is not growing (non-dividing cells, as in the experiments), TBA will be mineralized by the resting cell mass. To the best of our knowledge this is the first report of either psychro- or thermo-tolerant, aerobic TBA biodegradation by an enriched culture.

Both cultures mineralized TBA to a greater extent under alkaline conditions, and groundwater is usually circumneutral to alkaline. Recent data suggest that *KR1* also grows better at alkaline conditions (data not shown). TBA being mineralized significantly faster and to a greater extent at the higher pH-values for both cultures may be explained by the metabolite chemistry. A higher proportion of the intermediate 2-hydroxy isobutyric acid (HIBA, $pK_a = 4.9$) will be present as hydroxy isobutyrate (the carboxylate anion) at higher pH, and may be more biologically available. HIBA is a intermediate in the degradation pathway and can be the rate limiting step in TBA degradation (Steffan et al. 1997). The pH influence may increase the rate of HIBA transformation, and subsequently complete mineralization.

Adding 100% oxygen did not significantly increase the rate or extent of mineralization relative to using ambient air in batch studies, which is unique when compared to some MTBE degraders (Steffan et al. 1997). Although in reactor and field scale applications oxygen delivery to the system will be important, supplying ambient air instead of pure oxygen will be sufficient for these cultures, which decreases costs. Limiting oxygen content to 1% inhibited TBA mineralization, and neither of the enrichments will grow with nitrate, which is different than other TBA degrading micro-organisms used for bioaugmentation (Nakatsu et al. 2006).

Nitrogen was a critical parameter with respect to culture activity, as cultures thrived at lower concentrations while higher concentrations inhibited TBA degradation. About 7.5 mM nitrogen was the maximal level that sustained TBA biodegradation for *YZ1*. Neither culture could degrade TBA at a nitrogen concentration of 10 mM, indicating an inhibitory level between 7.5 and 10 mM. *KR1* was not able to degrade TBA with 7.5 mM nitrogen within 10 days and the culture formed granules indicative of stress; however the culture was able to degrade TBA after 16 days, which is markedly different from the healthy growing *KR1* cells. Nutrients are sometimes overlooked in process development because cells generally have enough to maintain activity, but nitrogen may be critical to field success. In addition, nitrogen as a co-contaminant may influence TBA degradation. In California or other agricultural states, nitrogen may be one of the highest concentration contaminants in groundwater (Stepak 2006). Higher nitrogen concentration can inhibit TBA degradation; therefore, it is an operational parameter that must be monitored to ensure effective biodegradation.

Phosphorus did not have similar inhibitory effects and each culture behaved differently to varying concentration. TBA degradation decreased in *KR1* at 1 mM, although doubling the phosphate concentration from 5 to 10 mM increased the degradation rate slightly. *YZ1* activity (TBA degradation) was inhibited at 10 mM phosphate, but activity increased when phosphate was scaled from 1 to 5 mM.

Culture longevity is important in field scale applications as TBA concentration in the groundwater may fluctuate. Previous data showed that maintaining degradation activity was difficult in cultures when starved of the contaminant or presented with alternate substrates (Kane et al. 2001; Sedran et al. 2002). Starvation results demonstrate that the cultures were able to recover after periods of zero TBA loading. In field systems there may be additional carbon sources in the groundwater, which may extend starvation periods or may shorten lag periods shown after 14- and 21-day starvation periods. However, the data demonstrated that TBA degradation was not irreversibly lost once the cultures had been starved for several weeks.

The results demonstrate that cultures derived from GAC units can be optimized to degrade TBA and that the two cultures generated in this project are more robust than previously described cultures. The initial data with temperature, pH, oxygen, and substrate starvation demonstrate that *KR1* and *YZ1* adapt easily to reactor systems with constantly shifting geochemical conditions. The work presented here has already been transitioned into the next phase of the project, which is investigating TBA degradation in a packed-bed GAC reactor with strain *KR1*.

Conclusions

Tert-butyl alcohol was degraded by two enrichment cultures from biologically activated GAC; these cultures have been designated *KR1* and *YZ1*. The enriched cultures thrive in alkaline conditions and mineralize TBA over a wide range of temperatures. The data presented are the first describing psychro- and thermo-tolerant TBA biodegradation in enriched, aerobic cultures. The culture *KR1* has been selected for further investigation as an inoculum for a bio-GAC reactor and the work presented here will help optimize operating conditions for rapid TBA degradation. These data demonstrate that biomass that grows naturally on GAC units can be further enriched for TBA (and possibly alternate contaminant) biodegradation.

Acknowledgments This project was supported by Atlantic Richfield Company, a BP affiliated company. We thank Patrick McLoughlin of Microseeps for suggestions regarding TBA analysis. We thank Julie Avanto of SECOR International Inc. for providing the GAC samples from California.

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