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Degradation of methyl *tert*-butyl ether by gel immobilized *Methylibium petroleiphilum* PM1

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Abstract

Cells of *Methylibium petroleiphilum* PM1 were immobilized in gel beads to degrade methyl *tert*-butyl ether (MTBE). Calcium alginate, agar, polyacrylamide and polyvinvyl alcohol were screened as suitable immobilization matrices, with calcium alginate demonstrating the fastest MTBE-degradation rate. The rate was accelerated by 1.8-fold when the beads had been treated in physiological saline for 24 h at 28 °C. MTBE degradation in mineral salts medium (MSM) was accompanied by the increase of biomass. The half-life of MTBE-degradation activity for the encapsulated cells stored at 28 °C was about 120 h, which was obviously longer than that of free cells (approximately 36 h). Efficient reusability of the beads up to 30 batches was achieved in poor nutrition solution as compared to only 6 batches in MSM. The immobilized cells could be operated in a packed-bed reactor for degradation of 10 mg L^{-1} MTBE in groundwater with more than 99% removal efficiency at hydraulic retention time of 20 min. These results suggested that immobilized cells of PM1 in bioreactor might be applicable to a groundwater treatment system for the removal of MTBE.

Keywords: Methyl *tert*-butyl ether; Immobilization; Alginate; Groundwater; *Methylibium petroleiphilum* PM1

1. Introduction

Methyl *tert*-butyl ether (MTBE), a widely used gasoline additive, has become a widespread contaminant over the past 30 years. Due to its low cost and easy blending with gasoline, MTBE has been the dominant fuel oxygenate to enhance octane index compared to other oxygenates, such as ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME) (Korte et al., 1997). The frequent occurrence of leakage of fuel tanks leads to serious MTBE-contamination, and its low biodegradablity makes MTBE the second most commonly detected contaminant in urban groundwater in USA (Squillace et al., 1996). Now MTBE is no longer used in the USA, but has contaminated many groundwater drinking wells where the costs of cleanup are estimated to be very high.

MTBE is recalcitrant to biodegradation due to its structure bearing an ether bond and a short branched chain. The half-life of MTBE in groundwater was estimated to be at least 2 years, and it was reported as a persistent and non-biodegradable compound in early 1990s (Suflita and Mormile, 1993). MTBE degradation was first reported by Salanitro et al. (1994), who isolated a mixed bacterial culture capable of degrading MTBE. To date, MTBE can be degraded by direct metabolizers (Francois et al., 2002; Hanson et al., 1999; Hatzinger et al., 2001) and cometabolizers (Hardison et al., 1997; Hernandez-Perez et al., 2001; Steffan et al., 1997). Among these isolates, *Methylibium petroleiphilum* PM1 exhibits relatively high activity for MTBE degradation under aerobic condition (Hanson et al., 1999). It can use MTBE as the sole source of carbon and energy, although the cell yield (0.18 mg of cells mg^{-1} of MTBE) is low even under optimal conditions (Hanson et al., 1999). If microorganisms are employed for MTBE-contaminated water treatment, one major problem is that of separating small cells from the treated effluents for repeated use.

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Cell immobilization, which has been widely applied in specific pollutant removal (Chang et al., 1999; González et al., 2001; Wang et al., 2001), may be an effective and economical technique for solving such problem. Immobilization can provide high cell concentrations and promising cell reuse, eliminate the costly processes of cell recovery, and moreover, offer stability advantages over free cells for varied applications (Zouari et al., 2002; Quan et al., 2003). In the case of MTBE degradation, previous reports have focused on the use of cells adherence to granular activated carbon, sintered glass, or rocks during microorganism growth, which requires long period of start-up to degrade MTBE efficiently due to the nature of cells and the character of matrixes (Fortin and Deshusses, 1999; Kharoune et al., 2001; Vainberg et al., 2002). Encapsulation is an important technique used to immobilize cells because such immobilization process can be performed immediately, and encapsulated cells often have higher operational stability owing to protection from direct exposure to toxic compounds in wastewater by immobilization agents (Naito et al., 2001). Thus the application of encapsulated cells for MTBE degradation might be advantageous.

To develop an efficient bioprocess suitable for MTBE removal in groundwater, immobilized cells of *M. petroleiphilum* PM1 was employed in this study where the feasibility and character of MTBE biodegradation by immobilized cells were also investigated.

2. Methods

2.1. Microorganism and cultivation conditions

The microorganism, *M. petroleiphilum* PM1 (ATCC BAA-1232), was isolated from a biofilter in southern California by the research group headed by Dr. Kate M. Scow (University of California at Davis, USA) (Hanson et al., 1999). Mineral salts medium (MSM), composed of KH_2PO_4 0.25 g L^{-1}, K_2HPO_4 0.4 g L^{-1}, $(NH_4)_2SO_4$ 0.3 g L^{-1}, $MgSO_4 \cdot H_2O$ 0.1 g L^{-1}, $CaCl_2 \cdot 2H_2O$ 0.01 g L^{-1}, $FeSO_4 \cdot 7H_2O$ 0.001 g L^{-1} (pH 7.2), was supplemented with 0.2 g L^{-1} MTBE in MSM as the sole carbon source for cell culture. After growth for 3 d (28 °C), 2% (v/v) inoculum was transferred to fresh medium with 0.5 g L^{-1} MTBE. When cells were in late exponential growth state, they were harvested by centrifugation at 9000 rpm (4 °C) and washed with 50 mM phosphate buffer (pH 7.0).

2.2. Immobilization of whole cells

For immobilization in alginate, the cell paste (0.216 g dry weight) was suspended in 100 mL of physiological saline (0.85% NaCl, w/v) and then mixed with 100 mL of 4% (w/v) sodium alginate. The mixture was extruded dropwise from a syringe to a stirred solution of calcium chloride (1%, w/v). After stirring for 2 h, beads were harvested and suspended in fresh calcium chloride solution of the same composition until used.

In the case of agar immobilization, the cell suspension (0.216 g dry weight) was added to 100 mL of agar solution (4%, w/v) at approximately 50 °C and mixed by stirring. The agar/cell mixture was then cooled by itself, and the resulting gel was cut into cubes (about 0.5 cm × 0.5 cm × 0.2 cm).

For immobilization in polyacrylamide (0.216 g dry weight), the cell suspension was added into 100 mL of physiological saline containing 12% (w/v) acrylamide and 0.2% (w/v) bis-acrylamide. Then 1.8% (w/v) ammonium persulfate were added to produce polymerization, and the produced gel was cut into cubes (about 0.5 cm × 0.5 cm × 0.2 cm).

In the case of polyvinyl alcohol (PVA) entrapment, PVA was diluted with deionized water, and heated in a water bath at 95 °C for about 30 min until dissolved. The cell suspension was mixed thoroughly with 50 mL of PVA solution, and then the mixture was extruded as drops into saturated boric acid solution. The beads were solidified for 16 h at room temperature, then washed twice with physiological saline and stored at 4 °C for further use.

2.3. MTBE-degradation reaction by immobilized PM1

Immobilized cells were added into 250 mL sealed serum bottles containing 20 mL MSM. And the MTBE concentration was 50 mg L^{-1} if not specifically mentioned in this paper. Degradation was carried out at 28 °C in a rotary shaker at 150 rpm. For the measurement of MTBE concentrations, a portion of the reaction mixture was withdrawn and centrifuged at 9000 rpm for 10 min at 4 °C, and the resulting supernatant was subjected to the analysis. Effectiveness factor (η) was defined as the ratio of actual reaction rate to that without diffusion limitation (free cells).

2.4. Repeated batch MTBE degradation by immobilized PM1

To evaluate the reuse potential of the alginate immobilized cells, two kinds of solution, MSM and poor nutrition solution (PNS) composed of 0.04 g L^{-1} K_2HPO_4, 0.05 g L^{-1} $(NH_4)_2SO_4$, 0.05 g L^{-1} $MgSO_4$, and 0.001 g L^{-1} $FeSO_4 \cdot 7H_2O$, were supplemented by 50 mg L^{-1} MTBE for batch degradation. After complete degradation, the beads were washed by physiological saline and transferred to fresh MSM and PNS. Repeated batch MTBE degradation was carried out under the same condition as the first cycle.

2.5. Continuous MTBE degradation by immobilized PM1 in a packed-bed reactor

MTBE degradation was performed by the groundwater supplemented with varying concentration substrate and in the packed-bed reactor, a cylindrical perspex column (35.0 × 4 cm) which was packed with 140 g wet alginate-immobilized beads to a height of 25 cm with a working volume of 105 mL. The groundwater (from one site of

Hangzhou, China), without any pretreatment, was supplemented with MTBE and other compounds to achieve the same composition as PNS, and passed through the column in an upflow mode using a peristaltic pump. Aeration through the bottom of column was maintained at 20 mL min^{-1}. The residual concentration of MTBE in the effluent, which was taken out from the outlet of the above packed-bed, was measured by gas chromatography/mass spectrometry.

2.6. Enumeration of viable cells

Entrapped cells were quantified as follows: the beads of known weight were washed twice in sterile physiological saline and suspended in 0.1 M phosphate buffer (pH 7.0). Then the suspension was stirred in a rotary shaker at 28 °C until the gel beads were thoroughly dissolved. The cell suspension was diluted and spread on R_2A plates, whose composition was as follows (g/L): yeast extract 0.5, proteose peptone 0.5, casein hydrolysate 0.5, glucose 0.5, soluble starch 0.5, sodium pyruvate 0.3, K_2HPO_4 0.3, $MgSO_4 \cdot 7H_2O$ 0.05, agar 12.0, pH 7.2. The plates in triplicate were inoculated with 0.1 mL of the 10^{-3}, 10^{-4} and 10^{-5} dilutions, and spread with a sterile glass rod. The appropriate dilution (30–300 colonies) was selected and the average colony forming was determined after 4 d incubation at 28 °C.

2.7. Analytical methods

MTBE concentration was determined by using direct-liquid injection gas chromatography (GC) with flame ionization detector as previously described by Fortin and Deshusses (1999). MTBE of low concentrations and its intermediate products were identified by gas chromatography/mass spectrometry (Agilent 6890N/MS5975), which was coupled with purge-and-trap concentrator. A 30 m × 0.25 mm × 0.25 μm HP-5MS capillary column (J&W Scientific, USA), held at 45 °C for 3 min and programmed at 10 °C min^{-1} to 160 °C, was employed for separation. The mass spectrometer was operated in electron impact (EI) mode at 70 eV, with the interface and ion source temperatures respectively at 280 °C and 230 °C.

3. Results

3.1. Effects of immobilization matrixes on MTBE degradation

Entrapment matrix was considered to affect degradation capability of whole cells. Fig. 1 exhibits the time course of residual MTBE degraded by PM1 cells immobilized with agar, calcium alginate, polyacrylamide and PVA. The degradation activity greatly varied when the cells were immobilized by different supports. Calcium alginate immobilized cells showed the highest activity for MTBE degradation, and agar was also a good support as cells immobilized with

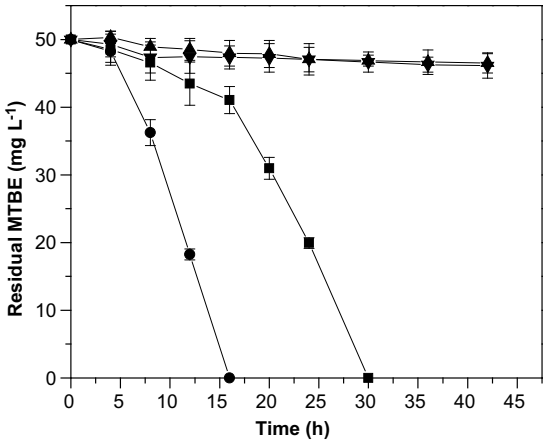

Fig. 1. MTBE degradation by cells encapsulated in different matrixes. Equivalent amounts of cells were immobilized by each material and exposed to 20 mL of MSM containing 50 mg L^{-1} MTBE in 250-mL bottles at 28 °C. The experiments were done in triplicate and the error bars indicate standard deviation between replicates. Symbols: (■) agar; (●) Ca-alginate; (▲) PVA; (▼) polyacrylamide.

it degraded MTBE completely after 35 h. On the other hand, the synthetic compounds—polyacrylamide and PVA, showed rather negative effects, with which immobilized cells could not degrade MTBE at all even after 42 h. Although the mechanical strength of the above materials was better than that of the natural materials studied in this research, the optimum alternative was still calcium alginate with respect to degradation activity.

3.2. Effect of sodium alginate concentration

Solutions of sodium alginate at various concentrations were mixed with cell suspension to make gel beads for MTBE degradation. The experimental results were shown in Table 1. The optimum concentration of alginate was 4% as evidenced by its highest MTBE-degradation rate (3.69 mg L^{-1} h^{-1}). It is evident that when alginate concentration exceeded 5%, the degradation activity of capsulated cells decreased obviously. And lower alginate concentrations also resulted in decreased degradation rate (e.g. 3.19 mg L^{-1} h^{-1} for 2% concentration) as well as lower mechanical strength of the beads.

It was interestingly found that the degradation activity increased greatly when the gel beads had been immersed

Table 1
Effect of sodium alginate concentration on degradation rate

Concentration of sodium alginate (%)	Degradation rate (mg L^{-1} h^{-1})	
	Untreated	Treated
2	3.19 ± 0.18	4.53 ± 0.22
3	3.31 ± 0.20	5.06 ± 0.25
4	3.69 ± 0.16	6.86 ± 0.32
5	3.40 ± 0.21	6.18 ± 0.27
6	2.92 ± 0.17	4.52 ± 0.21

The beads that had been immersed in physiological saline (28 °C) for 24 h were also investigated. Results are the average standard deviation of three replicates.

(28 °C) in physiological saline for 24 h before MTBE degradation (Table 1). The effectiveness factor (η) of the beads formed with 4% alginate was 0.40, whereas it reached 0.75 when the beads had been treated by the above method. This trend was similar to the beads with other considered alginate concentrations, and the increases in bead weight and volume were also observed during the process.

3.3. Effects of cell density and bead size

Cell loading in gel beads may affect degradation rate (Kaul et al., 2006). Cells of different dry weight (0.12–0.86 g) were mixed with 100 mL sodium alginate and extruded to calcium chloride solution. The effect of cell loading on MTBE degradation was shown in Fig. 2. The increase of degrading-rate by immobilized cells was observed with the increase of cell loading. Beads with a cell concentration of 0.65 g dry weight/100 mL gel displayed high degradation activity and resulted in a MTBE-degradation rate of 6.21 mg L^{-1} h^{-1}, which was similar to that with 0.86 g dry weight/100 mL gel.

The biomass concentration was determined during MTBE degradation, as shown in Fig. 3. And PM1 cells inside alginate beads at two different initial concentrations both exhibited some growth when MTBE degradation occurred. With larger initial cell density (6.1×10^8 cells g^{-1} of bead), biomass was increased by 1.3×10^8 cells g^{-1} of bead after MTBE at 53.5 mg L^{-1} was degraded completely. However, when lower cell density (2.1×10^8 cells g^{-1} of bead) was used, the biomass concentration increased to 4.0×10^8 cells g^{-1} of bead with the complete degradation of 52.3 mg L^{-1} MTBE. The calculated cell yields were 1.1 mg cells mg^{-1} of MTBE and 1.6 mg cells mg^{-1} of MTBE, respectively. The majority of cell growth appeared to occur at or close to the bead surface after 6 cycles of MTBE degradation due to larger cell densities in these regions compared with that in the core of the bead observed by scanning electron microscopy (data not shown).

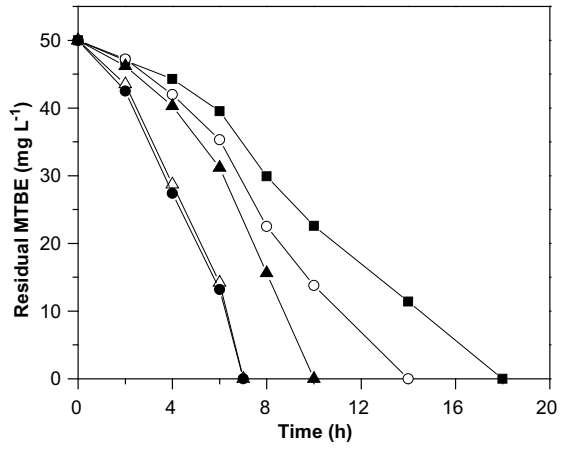

Fig. 2. Effects of cell density in the gel beads on MTBE-degradation rate. Cells of different dry weight were mixed with 100 mL of sodium alginate and then extruded to calcium chloride solution. Symbols: (■) 0.12 g; (○) 0.21 g; (▲) 0.43 g; (△) 0.65 g; (●) 0.86 g.

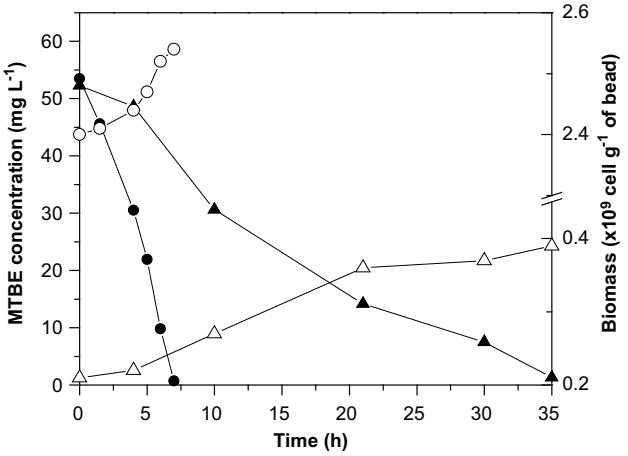

Fig. 3. Microbial growth in immobilized beads harboring initial biomass of 6.1×10^8 (△) and 2.4×10^9 (○) cells g^{-1} of bead. MTBE degradation by lower (▲) and higher (●) biomass was also determined.

To examine the effect of bead size on biodegradation rate, beads of different diameters (2.0, 2.5, 3.0, 4.0 and 5.0 mm) were prepared by different kinds of syringe. The degradation efficiency was enhanced, though not greatly, with the decrease of bead diameter (data not shown). Maximum degradation rate (7.16 mg L^{-1} h^{-1}) was attained with the bead diameter at 2 mm. A further increase in bead diameter to 5 mm led to a decrease of degradation rate to 5.64 mg L^{-1} h^{-1}.

3.4. Storage stability of MTBE-degradation activity

The storage stability affects MTBE degradation by immobilized cells in repeated use. To estimate their storage stability in comparison with that of free cells, the immobilized cells were stored at 28 °C in physiological saline for regular determined intervals and then degraded 50 mg L^{-1} MTBE. The storage stability of MTBE-degradation activity was evaluated from its half-life, which was defined as the time when MTBE degradation ratio reached 50% of the initial ratio. As shown in Fig. 4, the storage stability of MTBE-degradation activity was increased by immobilization. In the case of free cells, the half-life of MTBE-degradation activity was about 36 h, in comparison with 120 h of immobilized cells. The free cells exhibited sharply decreased degradation activity when they had been stored at 28 °C more than 48 h, and became almost inactive after 96 h. Although the half-life of PM1 was increased to 120 h by immobilization with alginate, it still appeared low with respect to the real MTBE remediation. However, to our satisfaction, the addition of MTBE into the solution enhanced the half-life to approximately 500 h (data not shown).

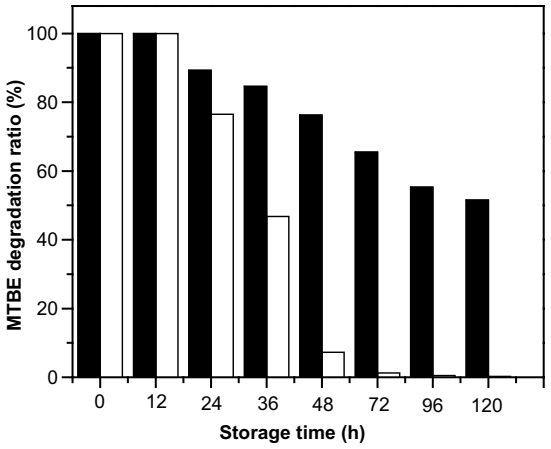

Fig. 4. Storage stability of the immobilized beads (■) and free cells (□) at 28 °C.

3.5. Repeated batch degradation by immobilized cells

The beads immobilized with PM1 cells were used for repeated MTBE degradation in MSM. As shown in Fig. 5, the repetitive degradation of MTBE by alginate immobilized cells could proceed for only 6 cycles when carried out in MSM which was used for PM1 cell cultivation. In the 6th batch degradation, the beads started to break and cell leakage occurred, which in turn led to a significant decrease in the number of cell after the wash step by physiological saline, and consequently a sharp reduction of reaction rate was observed in the 7th batch. Reaction solution PNS was also employed, in which immobilized cells were loaded and examined for repeated degradation. The form of bead remained intact intact, and the degradation activity decreased insignificantly even after 30 cycles when carried out in PNS, which indicated the feasibility of the beads for consecutive MTBE degradation. It was also found that Ca-alginate immobilized cells degraded MTBE in MSM and PNS with similar rate in the first 6 cycles.

3.6. Continuous degradation of MTBE in a packed-bed reactor

Groundwater supplemented with MTBE was passed through the column at the hydraulic retention time (HRT) of 20 min. MTBE concentration respectively in the influent and effluent was monitored each day. The concentration of MTBE in the effluent was less than 0.1 mg L^{-1}. However, the performance with 99% removal efficiency could only keep up for 28 d, when MTBE-degradation rate started to decrease and the removal efficiency was 85% after 40 d (data not shown). Biomass increased by 4.6×10^8 cells g^{-1} of bead after 10 d. The frequently detected intermediate of MTBE degradation, *tert*-butyl alcohol (TBA), was not observed even during the decrease of removal efficiency. Isopropanol, *tert*-butyl formate (TBF), and acetone were also monitored in the effluent, and turned out to be consistently below detection limits. Cells immobilized in alginate gel could even grow in groundwater without any supplement but 10 mg L^{-1} MTBE. Effect of HRT (range from 0.17 to 0.42 h) on

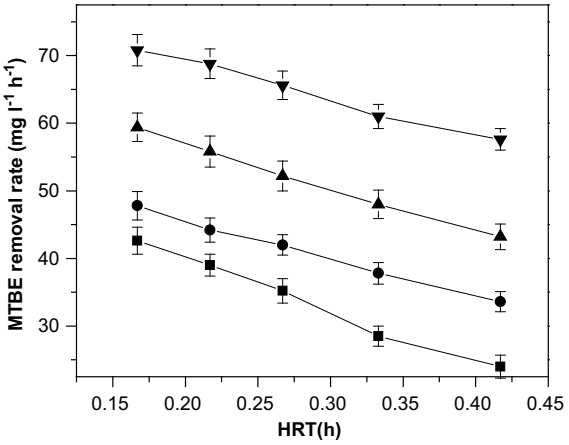

Fig. 6. Effect of HRT on MTBE removal rate with substrate loading at concentration of 10 (■), 15 (●), 20 (▲), 25 mg L^{-1} (▼). Error bars indicate standard deviation between replicates ($n = 3$).

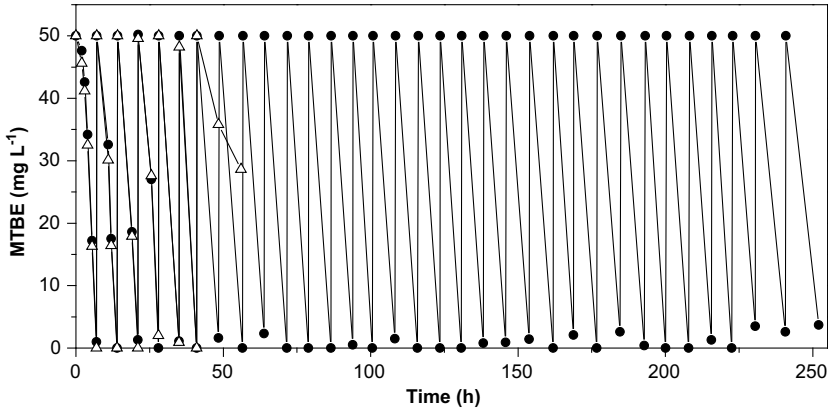

Fig. 5. Repetitive batch degradation of MTBE in MSM (△) and PNS (●).

removal rate with varying influent MTBE concentrations is illustrated in Fig. 6. MTBE-degradation rate was accelerated with the decrease of HRT and influent concentrations.

4. Discussion

Natural polymers may provide improved bacterial survival during immobilization and degradation processes (Cassidy et al., 1996), which is also confirmed by the result of this study where alginate and agar were more suitable than polyacrylamide and PVA. High tightness of the beads by synthetic compounds, which caused great mass transfer resistance of oxygen and substrate through the gel bead, might account for such phenomenon. However, it is strange that no degradation activities of cells immobilized with polyacrylamide and PVA was detected in this experiment, because if the cells were active some degradation should gradually take place even if transfer rates were supposed to be low. The strong inhibitory effect on PVA immobilized cells was presumably ascribed to the agglomeration of PVA gel beads and the toxicity of saturated boric acid, based on the report by Lozinsky and Plieva (1998). In the case of polyacrylamide entrapment, besides the toxicity of polyacrylamide, living cells suffered a more severe toxicity from its monomer, and moreover, cell integrity and activity could be impaired during polymerization which generated heat and free radicals (Cheetham et al., 1979). The finding that cells immobilized with agar at 50 °C had lower degrading capability than that with alginate might be ascribed to the thermal inactivation of enzymes in cells during immobilization.

It is rather interesting that the gel beads provided much faster rate (about by 1.8-fold) of MTBE degradation when pretreated in physiological saline at 28 °C for 24 h in this experiment. The beads swelled with intact form during such process, leading to the increase of pore sizes inside, the decrease of mass transfer resistance, and subsequently the increase in reaction rate. Under such conditions, not only the treated substrate MTBE but also the products were easier to diffuse through the gel beads. Moreover, it was reported that oxygen was quite necessary and used as electronic acceptor in MTBE biodegradation by PM1 (Wilson et al., 2002; Zhong et al., 2007). Oxygen might be the major substrate which appeared to be limited during MTBE degradation by PM1 cells (Zhong et al., 2007), so the enhanced diffusion of oxygen and MTBE into the beads brought about increased activity.

The growth of encapsulated cells might depend on the immobilization material, cell loading and type of cells used. In this study, more cell growth in beads with lower initial density was obtained compared to that with the higher density. Such about 50% decrease in cell yield when simply changing the initial cell concentration, might be due to the following reason: gel beads immobilized with more cells lessened oxygen diffusion and provided less space in the support for cell growth, and consequently hindered cell multiplication; thus more portion of substrate worked as the energy source for cells to maintain metabolism in the beads which loaded with more biomass, though finally the same amount of MTBE was completely degraded. Such phenomenon was also reported by Freeman and Lilly, who suggested that the cell growth depended on diffusion limitations imposed by the porosity of gel matrix and the impact of accumulating biomass (Freeman and Lilly, 1998).

The immobilized cells exhibited much better storage stability than free cells in this study, indicating the promising feasibility of MTBE degradation by encapsulated cells in future application. Moreover, the fact that supplement of MTBE into the storage solution was helpful for sustaining degradation activity made MTBE remediation by immobilized cells more feasible. Medium composition for MTBE degradation may affect operational stability due to its influence on cell growth, and even affect immobilization matrix interactions by swelling of gels. Calcium alginate immobilized cells could degrade MTBE only for 6 cycles in MSM before the beads dissolved, which made immobilization hard to be applied in MTBE degradation. The main reason for such problem may be the presence of phosphates and certain cations, such as K^+, NH_4^+ and Mg^{2+}, which can be chelated with calcium ions and make calcium alginate gel be unstable. In order to overcome the difficulty, we devised an operating solution PNS, which contains low-concentration minerals than that of MSM. The beads could perform more than 30 batches in PNS, with comparable activity and intact form, which further confirmed our assumption and achieved promising results. It appeared that the poor nutrition in PNS was enough for the immobilized cells to degrade 50 mg L^{-1} MTBE completely and to sustain such high activity.

To date, several studies have reported the use of attached microbial cells for removal of MTBE (Kharoune et al., 2001; Stringfellow and Oh, 2002; Vainberg et al., 2002). Kharoune et al. (2001) achieved 99% removal efficiency with initial MTBE concentration of 80 mg L^{-1} by microbial consortium attached to sintered glass rings in an upflow fixed-bed reactor after a long period. Stringfellow and Oh (2002) examined the treatment of MTBE contaminated water in fluidized bed reactor with GAC as bed materials. It required 80 d for cultures to attach and adapt before 98% removal efficiency was achieved. Such immobilizations demonstrated high efficiency when the systems performed steadily. However, it required longer time for cultures to enrich, acclimate, and achieve desired biomass in such above reactors, and moreover, it was hard for some microorganisms to attach to the surface of solid matrixes. The thickness of cell film was not enough for high degradation efficiency under certain conditions. *Hydrogenophaga flava* ENV735, an important MTBE-degrading culture with extremely fast degradation rate, could not attach to GAC for scale-up application (Steffan et al., 2000). Because MTBE migrates in groundwater at a rather rapid rate, the time for microorganisms to grow and attach to solid matrixes appears too long to wait when downstream receptors are of concern. The efficiency of immobilization we

explored was better than previously reported work. However, the period of sustaining high removal activity was shorter than some reports (Fortin and Deshusses, 1999; Kharoune et al., 2001). Because it can be preceded immediately and restart up in short period to deal with the resistant compounds, Ca-alginate entrapped cells provides a new choice for MTBE remediation in ground water, and may be the preferred alternative in some cases. The exploration of some new technologies that can modify beads and enhance its stability may make cell encapsulation with alginate more valuable in further large-scale application, which is also a research subject of our research group.

The possible intermediates of MTBE degradation, including TBA, TBF, isopropanol and acetone, were not detected to accumulate even during the decrease of reactor performance. This suggested that the major limiting step in MTBE degradation can be the accessibility and cleavage of the ether bond, instead of the accumulation of intermediates. It has been reported that alginate encapsulated cells can be used not only in bioreactors, but also in *in situ* soil or groundwater for contaminants biodegradation (Cassidy et al., 1996). Therefore, the application of alginate immobilized *M. petroleiphilum* PM1 for *in situ* MTBE bioremediation may be an alternative in future application.

Acknowledgements

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