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Aerobic degradation of methyl *tert*-butyl ether in a closed symbiotic system containing a mixed culture of *Chlorella ellipsoidea* and *Methylibium petroleiphilum* PM1

Weihong Zhong[a,*], Yixiao Li[a], Kedan Sun[a], Jing Jin[a], Xuanzhen Li[a], Fuming Zhang[b], Jianmeng Chen[a,**]

[a] College of Biological and Environmental Engineering, Zhejiang University of Technology, 18 Chaowang Road, Hangzhou, Zhejiang Province 310032, China
[b] Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

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ABSTRACT

The contamination of groundwater by methyl *tert*-butyl ether (MTBE) is one of the most serious environmental problems around the world. MTBE degradation in a closed algal–bacterial symbiotic system, containing a mixed culture of *Methylibium petroleiphilum* PM1 and *Chlorella ellipsoidea*, was investigated. The algal–bacterial symbiotic system showed increased MTBE degradation. The MTBE-degradation rate in the mixed culture ($8.808 \pm 0.007\ mg\ l^{-1}\ d^{-1}$) was higher than that in the pure bacterial culture ($5.664 \pm 0.017\ mg\ l^{-1}\ d^{-1}$). The level of dissolved oxygen was also higher in the mixed culture than that in the pure bacterial culture. However, the improved efficiency of MTBE degradation was not in proportional to the biomass of the alga. The optimal ratio of initial cell population of bacteria to algae was 100:1. An immobilized culture of mixed bacteria and algae also showed higher MTBE degradation rate than the immobilized pure bacterial culture. A mixed culture with algae and PM1 immobilized separately in different gel beads showed higher degradation rate ($8.496 \pm 0.636\ mg\ l^{-1}\ d^{-1}$) than that obtained with algae and PM1 immobilized in the same gel beads ($5.424 \pm 0.010\ mg\ l^{-1}\ d^{-1}$).

1. Introduction

Methyl *tert*-butyl ether (MTBE) is one of the organic chemicals with the highest worldwide production volumes. The occurrence and fate of MTBE in the environment is a major scientific and political issue because MTBE has been listed as a possible human carcinogen by the U.S. Environmental Protection Agency (EPA). In only a few years of extensive use, MTBE has become one of the most frequently detected groundwater pollutants in the United States and Europe [1,2]. The poor condition of fuel-storage systems has led to the leakage of more than 400,000 underground storage tanks since 1988 in the United States as identified by the EPA [3]. In Europe, although some countries have implemented stricter storage-facility regulations as early as the 1970s, the number of reports about point-source leakage of MTBE-containing gasoline is increasing [4]. Similar contamination sites have also been found in China because of the country's rapidly increasing petrol usage. In our previous local investigation, about $18\ \mu g\ l^{-1}$ of MTBE was detected in samples of well-water obtained from places near highway gasoline stations in East China.

There is widespread contamination of groundwater by MTBE. As a result, the bioremediation of MTBE-impacted aquifers has become an active area of research. As reviewed by Deeb et al. [5], a number of organisms that can either partially degrade or completely mineralize MTBE have been isolated from diverse environments. MTBE could be utilized as the sole source of carbon and energy or degraded co-metabolically with alkanes by these organisms. Monooxygenase is considered as the first enzyme that initiates and cleaves ether bonds in the MTBE-biodegradation pathway [6]. Therefore, aerobic biodegradation should be the favored process. Although anaerobic biodegradation of MTBE could be achieved using SO_4, Fe (III), Mn (IV), and NO as the terminal electron acceptors, the MTBE-mineralization efficiency of anaerobic biodegradation is lower than that of aerobic biodegradation [7,8]. Native aerobic MTBE-degrading microorganisms could be stimulated and their growth promoted by dissolved oxygen (DO) released into the anaerobic MTBE plume from oxygen-pressurized polymeric tubing [9]. Although microbial communities indigenous to groundwater can degrade MTBE under both aerobic and anaerobic conditions, both MTBE degradation activity and the growth of MTBE-degrading bacteria can be improved by oxygen enrichment [10]. Therefore, oxygen is necessary for degradation of MTBE by bacteria, especially in a closed system or subsurface.

Several bioreactors, including biotrickling filter, biomass concentrator reactor, laboratory-scale biofilter made of a natural

* Corresponding author. Tel.: +86 571 88320658; fax: +86 571 88320057.
** Corresponding author. Tel.: +86 571 88320386; fax: +86 571 88320387.
E-mail addresses: whzhong@zjut.edu.cn (W. Zhong), jchen@zjut.edu.cn (J. Chen).

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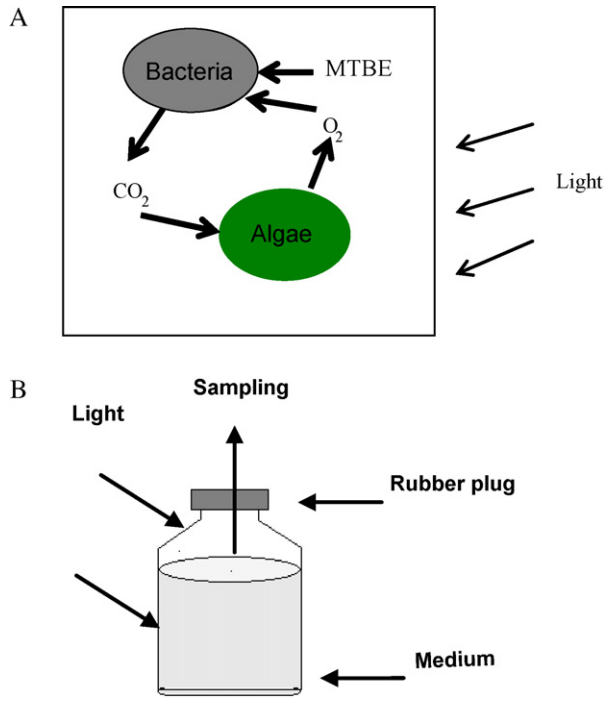

Fig. 1. Diagram showing the possible mechanism of a closed algal–bacterial symbiotic system (A) and a simple closed system for MTBE degradation in this study (B).

fiber (kenaf) mat, aerobic fluidized bed reactor, batch- and continuous-upflow fixed-biofilm reactors, continuously stirred tank reactor, and upflow fixed-bed reactor, have been used for MTBE removal from MTBE-contaminated effluents under aerobic conditions [11–18]. In our previous work, a "cannula" was introduced to study MTBE biodegradation in a closed system [19]. A high level of DO in the broth within the inner tube was achieved by the addition of H_2O_2 in the outer tube of the cannula. However, the cannula method has a limitation. For instance, H_2O_2 can supply oxygen only for a short period. Therefore, a method that ensures the permanent supply of oxygen in a closed system is necessitated. To date, several types of photobioreactors with an algal–bacterial mix have been used for wastewater treatment [20,21]. The possible mechanism of such photobioreactors for MTBE degradation is illustrated in Fig. 1A, in which oxygen is presumably continuously supplied by algal photosynthesis, which utilizes the carbon dioxide produced from the complete mineralization of organic chemicals by bacteria [22,23]. The objective of this study is to investigate MTBE degradation using a simple symbiotic system containing a mixed culture of *Methylibium petroleiphilum* PM1 and *Chlorella ellipsoidea* in a closed bottle (Fig. 1B).

2. Materials and methods

2.1. Materials

MTBE (HPLC grade) was purchased from Merck Schuchardt OHG (Hohenbrrunn, Germany). Glucose, peptone, yeast extract, and agar were purchased from Jonya Marine Biological Engineering Co. Ltd. (Shanghai, China).

2.2. Microorganisms and incubation conditions

C. ellipsoidea was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. *M. petroleiphilum* PM1 was generously supplied by Dr. Kate M. Scow (University of California, Davis, United States), it was originally isolated from a biofilter in southern California, USA [24].

C. ellipsoidea was cultured in the medium Chu #10 [25] under room temperature and with natural lighting. The composition of Chu #10 was as follows ($g\,l^{-1}$): $Ca(NO_3)_2$ 0.04, K_2HPO_4 0.01, $MgSO_4{\cdot}7H_2O$ 0.025, Na_2CO_3 0.02, Na_2SiO_3 0.025, and $FeCl_3$ 0.008, the pH of the medium was 7.0. Algal cells were harvested by centrifugation at 4000 rpm for 10 min. The cells were suspended in different medium as inoculum for different experiments.

PM1 was cultivated on trypticase soy agar (TSA) slants for 48 h at 28 °C to activate the cells. The composition of TSA is as follows ($g\,l^{-1}$): tryptone 17, yeast extract 6, NaCl 5, K_2HPO_4 2.5, glucose 2.5, and agar 20, the pH was 7.2. One loop of PM1 cells from a slant was inoculated into 30 ml of TSA liquid medium without agar (trypticase soy broth, TSB) in a 250 ml flask. The culture was incubated overnight at 30 °C in a shaking incubator (ZHWY-2112B, Shanghai, China) at 150 rpm. Cells were harvested from the medium by centrifugation at 4000 rpm for 20 min (Labfuge 400R, Heraeus, Germany). Then, the cells were washed thrice with sterile saline to prepare the cell suspension. The cell density in the suspension was calculated from the optical absorption at 600 nm ($OD_{600\,nm}$) according to the relationship between $OD_{600\,nm}$ and the cell density determined by TSA-plate cell counting method.

The minimum salty medium (MSM) [26] for MTBE degradation was designed as follows ($g\,l^{-1}$): KH_2PO_4 0.7, K_2HPO_4 0.85, $(NH_4)_2SO_4$ 1.2, $MgSO_4{\cdot}7H_2O$ 0.1, $CaCl_2{\cdot}2H_2O$ 0.01, $FeSO_4{\cdot}7H_2O$ 0.001, and 5 ml original stock solution containing the following trace elements in 10 l of de-ionized water: 0.6 g of H_3BO_3, 0.4 g of $CoCl{\cdot}6H_2O$, 0.2 g of $ZnSO_4{\cdot}7H_2O$, 0.6 g of $MnCl_2$, 0.06 g of $NaMoO_4{\cdot}2H_2O$, 0.04 g of $NiCl_2{\cdot}6H_2O$, and 0.02 g of $CuCl_2{\cdot}2H_2O$.

2.3. Growth medium for algal–bacterial mixed culture

To select the optimal medium for MTBE degradation by the mixed culture of algae and bacteria, a medium containing a mixture of MSM and Chu #10 (1:1) was evaluated in a 100 ml sealed bottle. Cells (2.5×10^8 for PM1, 5.0×10^6 for algae) were inoculated into 25 ml each of MSM, Chu #10, and mixed medium, and incubated in an illuminated shaking incubator (with four 8-W daylight lamps) at 150 rpm and 30 °C. The changes in MTBE concentration and biomass at 24, 32, 48, and 56 h were recorded.

2.4. MTBE degradation by a mixed culture of free algae and bacteria

After the optimal medium was selected, a 5-ml cell suspension of PM1 (2.7×10^8 cells ml^{-1}) and a 5-ml cell suspension of *C. ellipsoidea* (1.3×10^6 cells ml^{-1}) were inoculated into 200 ml of MTBE-containing MSM in a 250-ml sealed bottle used as a closed system. The mixed culture was then incubated in the illuminated shaking incubator.

The effect of the ratio of the cell population of algae to that of PM1 on MTBE degradation was investigated in the closed system by adjusting the sizes of the inocula of PM1 and alga. To prepare the inocula with different cell ratios (1:1, 10:1, 100:1, and 1000:1), 1-ml cell suspensions of PM1 (9.3×10^7 cells ml^{-1}) were mixed with different volumes and dilutions of the cell suspension of *C. ellipsoidea* (1.8×10^7 cells ml^{-1}) to form a total 10 ml inoculum.

2.5. MTBE degradation by a mixed culture of immobilized algae and bacteria

To evaluate the MTBE degradation by a mixed culture of immobilized algae and PM1, three different immobilization strategies were applied.

(1) Algae and PM1 were immobilized separately in different gel beads.
(2) Algae and PM1 were immobilized in the same gel beads.
(3) Only PM1 was immobilized as a control.

The immobilization of cells was carried out as follows: cells of PM1 and Chlorella were harvested separately by centrifugation and washed twice with saline. Equal volumes of cell suspension and 4% sodium alginate were mixed to make a cell-alginate suspension, which was dropped into a solution of 4% $CaCl_2$. After 2 h of solidification, the gel beads were collected and stored in saline at 4 °C.

Cells entrapped in gel beads were inoculated into 200 ml of MTBE-containing MSM to evaluate MTBE degradation using the culture conditions mentioned in Section 2.4. Gel beads without PM1 and algae were also evaluated as controls.

2.6. Assays of MTBE and DO

All the experiments were carried out in sealed bottles that served as the closed system (Fig. 1). Each experiment was conducted in triplicate. About 1.2 ml of culture was sampled from each sealed bottle using a needle injector. The culture was centrifuged in sealed Eppendorf tubes for 10 min under 1000 rpm, 4 °C to remove the PM and algal cells. Then supernatant was transformed to a sealed sample bottle and stored at a 4 °C refrigerator until gas chromatography (GC) analysis.

The MTBE concentration in the supernatant was detected by GC analysis (DAI-GC/FID, GC-14B, Shimadazu, Japan). The conditions for the GC analysis were as follows: pure nitrogen as the carrier gas with a flow rate of 1.0 ml min^{-1}; a flame ionization detector (FID); a polyethylene glycol capillary column (PEG-20M; 30 mm × 0.32 mm × 0.33 μm); polyethylene as the immobilized phase; and temperatures of the column, FID, and gasifying chamber being 55, 150, and 140 °C, respectively. The sampling quantity was 1 ml of supernatant each time.

The Do level in the closed system was measured using the DO portable meter Lovibond® SensoDirect Oxi200 (Tintometer GmbH, Germany). Repeated sampling performing in one bottle might result in DO change due to the pores produced by the needle during sampling. To avoid such errors, three bottles were prepared for each sampling time point, and the sampled bottles were not put back into the incubator. A control was accordingly set for each experiment with a bottle containing only the medium with MTBE.

2.7. Cell-density measurement

The cell density of *M. petroleiphilum* PM1 in pure culture was directly determined from the optical density at 600 nm using an ultraviolet-visible single-beam spectrophotometer (Model 752, Shanghai Spectrum Instruments Co., Ltd., Shanghai, China), whereas the cell density of *C. ellipsoidea* was calculated by direct microscopic counting with a Petroff-Hausser counting chamber. In the mixed culture, the cell densities of *M. petroleiphilum* PM1 and *C. ellipsoidea* were determined by the colony-counting method on agar plate and by direct microscopic counting with a cell counter, respectively.

2.8. Statistical analysis

All computer-based statistical analyses were performed with the SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA), and one-way ANOVA was used to determine the differences between different treatments at a significance level of 0.01 or 0.05.

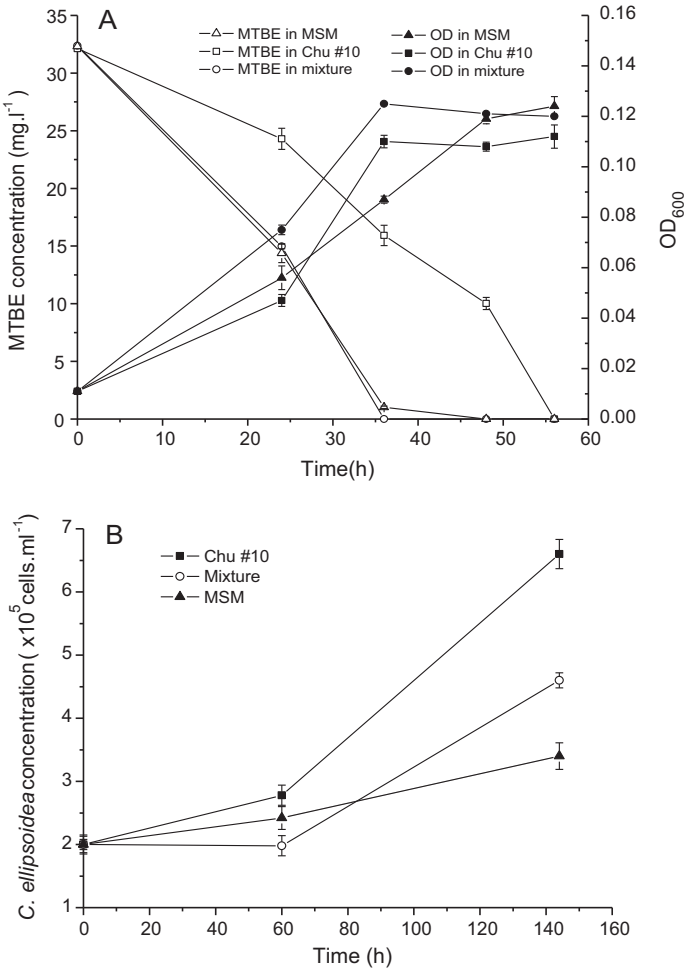

Fig. 2. Selection of medium for an algal–bacterial mixed culture in a closed system. (A) Growth of and MTBE degradation by *M. petroleiphilum* PM1 in MSM, Chu #10, and the mixture of MSM and Chu #10; (B) growth of *C. ellipsoidea* in MSM, Chu #10, and the mixture of MSM and Chu #10.

3. Results and discussion

3.1. Selection of the medium for algal–bacterial mixed culture in a closed system

To select an optimal medium for the degradation of MTBE in a symbiotic system, the MTBE-degradation rate, the OD_{600} value, and the algal-cell density in three media (MSM, Chu #10, and their mixture) were compared. The results (Fig. 2A) showed no significant differences between the different media based on the growth trends of the microorganisms, which were derived from the slopes of the log phase of bacterial growth. However, the biomass of PM1 in (1) MSM and (2) the MSM/Chu #10 mixture was higher than that in Chu #10 after 56 h culturing. In addition, the MTBE-degradation rate in MSM and the MSM/Chu #10 mixture was higher than that in Chu #10 during the entire culture period. Nevertheless, the rates of both PM1 growth and MTBE degradation appeared to be the same in MSM and the MSM/Chu #10 mixture. The Chu #10 medium contains inorganic sources of carbon (Na_2CO_3), which may compete with CO_2. However, CO_2 is the sole carbon source in MSM and hence MSM is considered the optimal medium for the mixed culture of *C. ellipsoidea* and *M. petroleiphilum* PM1 in a closed symbiotic system.

A significant increase in the cell density of *C. ellipsoidea* was observed after 144 h of incubation in all media. The cell density after 144 h of incubation was 3.3, 2.3 and 1.7 times higher than

Table 1
Comparison of degradation rates between pure and mixed cultures.

Parameters conditions	Algae only	PM1 only	Mixed culture
MTBE-degradation rate ($mg\,l^{-1}\,d^{-1}$)	1.824 ± 0.072^{aA}	5.664 ± 0.017^{bA}	8.808 ± 0.007^{cA}

[A] The mean values in same line with the same superscript letters are not significantly different ($P > 0.05$), whereas those with different superscript letters are significantly different ($P < 0.01$) after statistical analysis.

the initial values in Chu #10, MSM/Chu #10 mixture, and MSM, respectively (Fig. 2B), which suggested that MSM is not the optimal medium for algal growth. However, as the purpose of this study was to investigate MTBE degradation, we selected MSM as the medium for our experiments.

3.2. MTBE degradation by a mixed culture of free algae and bacteria

The MTBE-degradation rate of the mixed culture reached $8.808 \pm 0.007\,mg\,l^{-1}\,d^{-1}$, which was very much higher than that of the pure culture of PM1 ($5.664 \pm 0.017\,mg\,l^{-1}\,d^{-1}$) and *C. ellipsoidea* ($1.824 \pm 0.072\,mg\,l^{-1}\,d^{-1}$; $P < 0.01$; Fig. 3A and Table 1). These results coincided with the DO levels shown in Fig. 3B. The DO level in the pure PM1 culture decreased gradually to $6.54\,mg\,l^{-1}$ after 7 days, whereas in both pure *C. ellipsoidea* culture and the mixed culture, it remained at a high level ($8.27\,mg\,l^{-1}$ after 7 days). More DO was present in the mixed culture than in the pure culture of PM1, with DO levels being higher by 0.25, 1.37, 0.58, 1.33, and $1.73\,mg\,l^{-1}$ at 48, 72, 96, 144, and 168 h, respectively; That is, on an average, the level of DO was higher by $1.05\,mg\,l^{-1}$, which can be attributed to the algal photosynthesis. MTBE degradation has been reported to be initially catalyzed by monooxygenase, one of the key enzymes in the process of MTBE degradation [27–29]. Thus, both oxygen supply and high DO levels play important roles in MTBE degradation. In addition, MTBE could be degraded by PM1 alone (without *C. ellipsoidea*), although at a lower rate, in the closed system (Fig. 3A), suggesting that the amount of oxygen in the closed system containing only PM1 might be at the threshold level for MTBE biodegradation. However, a higher residual oxygen level might improve the MTBE-degrading monooxygenase activity of PM1 and result in a higher MTBE-degrading rate. *C. ellipsoidea* grew better in the mixed culture than in a pure algal culture (Fig. 3C), which suggests that the synergistic relationship in algal–bacterial microcosms improves both DO level and algal growth.

According to the COD of MTBE ($2.73\,g\,COD\,g^{-1}$ MTBE), approximately $27\,mg\,l^{-1}$ of O_2 are required to degrade $10\,mg\,l^{-1}$ MTBE. A comparison between the DO levels and decrease in MTBE concentrations in the individual pure cultures and the mixed culture after 168 h is shown in Table 2. The decrease in DO levels was not equal to the calculated value of DO demand value. All three cultures, only alga (A), only PM1 (B) and PM1 and alga mixture (C) showed a lower decrease in DO levels in the broth than the calculated DO demand for MTBE degradation. This discrepancy might have resulted from the oxygen supply from the air phase (DO = $310\,mg\,l^{-1}$, standard state, 298 K, 25 °C, 101,325 Pa) inside the bottle. However, cultures A and C showed lower DO decreases than the culture B, which might have resulted from the supply of oxygen supply by the alga. Among all cultures, the culture C showed the highest decrease in MTBE combined with the lowest decrease in DO, suggesting that the mixing of PM1 and *Chlorella* improved MTBE degradation in a closed system due to the prevalent higher DO levels.

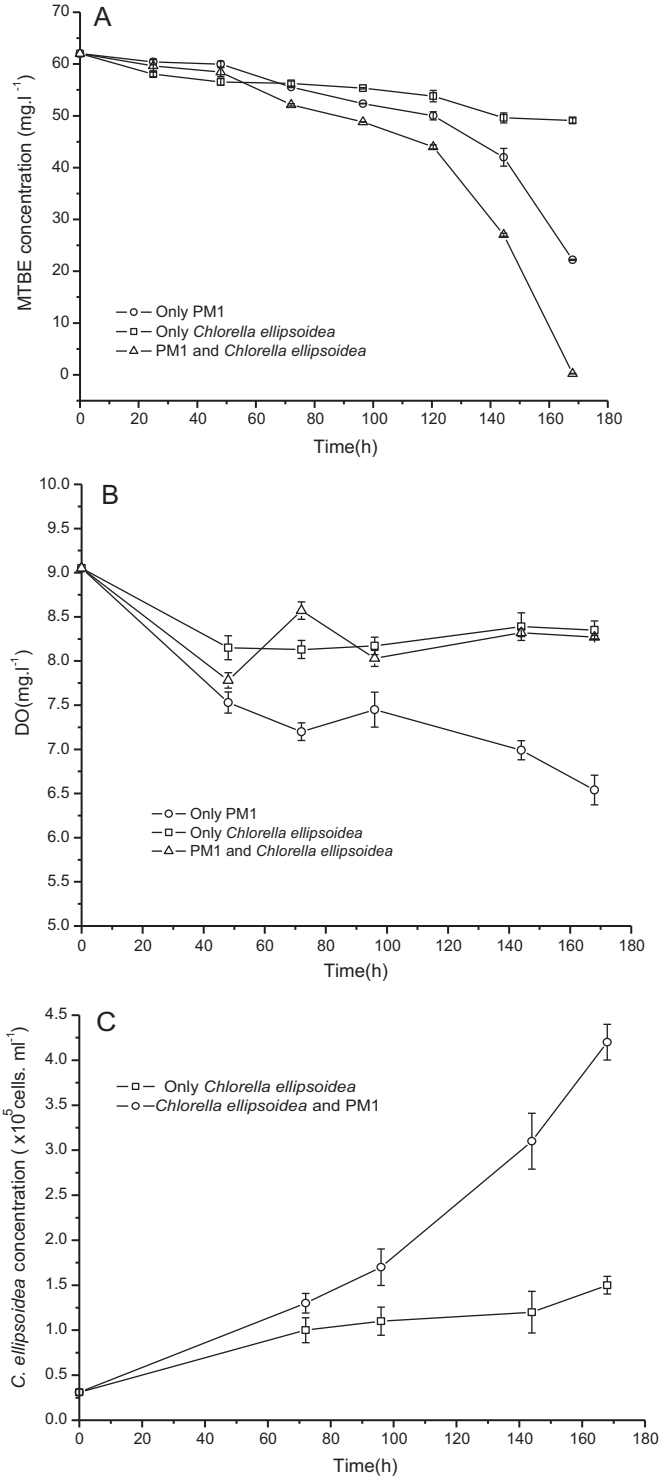

Fig. 3. Comparison of (A) MTBE degradation and (B) change in DO levels in MSM containing only PM1, only alga, and the mixture of PM1 and alga; (C) growth of *C. ellipsoidea* in MSM when cultured alone and in association with PM1.

Table 2
Comparison between decreases in DO and MTBE levels in pure and mixed cultures after 168 h.

	DO decrease (calculated demand) ($mg\,l^{-1}$)	MTBE decrease ($mg\,l^{-1}$)
Only alga (A)	0.70 (035.22)	12.90
Only PM1 (B)	2.51 (108.65)	39.80
Mixed culture of PM1 and alga (C)	0.78 (168.60)	61.76

Table 3
Effect of the ratio of PM1 to alga on MTBE-degradation rate after 120 h.

Ratio of PM1 to alga	1:1	10:1	100:1	1000:1
MTBE-degradation rate ($mg\,l^{-1}\,d^{-1}$)	3.600 ± 0.264^{bA}	3.096 ± 0.475^{bA}	6.384 ± 0.615^{cA}	2.280 ± 0.366^{aA}

[A] The mean values in same line with the same superscript letters are not significantly different ($P > 0.05$), whereas those with different superscript letters are significantly different ($P < 0.01$) after statistical analysis.

We thus designed a batch culture in a bottle which mimics a closed symbiotic system to evaluate the possibility for its application in the treatment of MTBE-contaminated underground waters (Fig. 1). In practice, a continuous photoreactor is required to undergo long-term ex-situ treatment, for which an immobilized cell technology is more suitable. Therefore, the activity of gel-entrapped cells in terms of MTBE removal in a closed symbiotic system was further evaluated.

3.3. Effect of ratio of the cell populations of PM1 to alga on MTBE degradation

The effect the ratio of the cell populations of PM1 to algae on MTBE biodegradation was evaluated before conducting the study on gel entrapped cells. Different cell-population ratios of PM1 versus alga, that is, 1:1, 10:1, 100:1, and 1000:1, were designed by adjusting the amount of inoculum of the alga while maintaining a constant amount of PM1. The results (Table 3) showed that a cell population ratio (PM1 versus algae) of 100:1 is optimal for the biodegradation of MTBE by a mixed free-cell culture in the closed system. Very high (1:1 and 10:1) or very low (1000:1) algal cell density in the mixed culture resulted in lower MTBE-degradation rates (Table 3).

There have been a few previous reports about the effect of the ratio of algal cells to bacterial cells on the ecological mutualism. For instance, Wang et al. [30] reported that the ratio of algae to bacteria in an alginate gel has a role in NH_3–N removal due to its effect on the ecological structure. Guerrini et al. [31] described bacterial-algal interactions in polysaccharide production under phosphate limitation and increasing N/P ratios in the medium. Miyamoto et al. [32] reported that maximum hydrogen evolution by a mixed culture of *Chlamydomonas reinhardtii* and *Rhodospirillum rubrum* was observed at a ratio of 8:2 and that the highest amount of hydrogen per algal dry weight was observed at a ratio of 2:8. However, the mechanism of this phenomenon is still not fully clear. For further understanding the effect of the proportion between alga and bacteria on MTBE biodegradation, more researches on the mechanism of the synergetic effect (such as the effect of metabolites on each other) in this symbiotic system are proposed.

3.4. MTBE degradation by a mixed culture of immobilized algae and bacteria

An immobilized mixed culture with a 100:1 ratio of PM1 to algae was first evaluated. However, the cells immobilized in this ratio showed no significant improvement in MTBE degradation. The quantity of algae was probably too low to carry out sufficient photosynthesis in the immobilized mixture (data not shown).

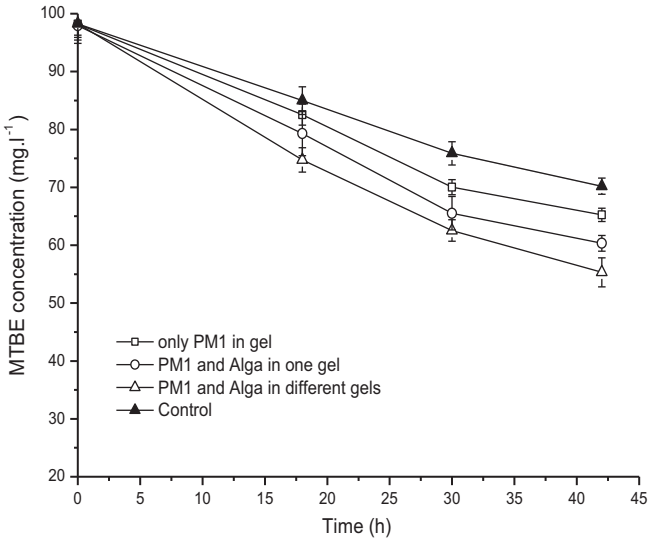

Fig. 4. Closed-system MTBE degradation by gel-immobilized bacteria and a mixed culture of PM1 and alga in one gel and in different gels (PM1: alga = 1:1 = 1.5×10^8:1.5×10^8 cells as the initial inoculum in gel beads).

Thus, a 1:1 ratio of PM1 to algae was finally selected to prepare the immobilized cells using different immobilization strategies. As shown in Fig. 4, the control cultures (gel beads without PM1 and alga) could absorb MTBE and resulted in an MTBE decrease in the system. Accordingly, for evaluation of the immobilization strategies, the decrease in MTBE levels caused by gel absorption was subtracted (Table 4). The MTBE-degradation rate of the mixed culture with algae and PM1 immobilized separately in different gel beads was higher ($8.496 \pm 0.636\,mg\,l^{-1}\,d^{-1}$) than that of the mixed culture with algae and PM1 immobilized in the same gel beads ($5.424 \pm 0.010\,mg\,l^{-1}\,d^{-1}$). Both the above mixed-culture gel beads showed higher MTBE-degradation rate than the pure PM1-entrapped gel beads ($2.784 \pm 0.168\,mg\,l^{-1}\,d^{-1}$). Furthermore, the color of the beads turned greener after 42 h of incubation, indicating that algae grew well in the gel beads. Although absorption of MTBE by the beads occurred, the gel-entrapped cells, especially the mixed culture with algae and PM1 immobilized separately in different gel beads, still achieved the same level of degradation-rate as the free cells (Table 1). Thus, the application of entrapped cells is feasible for treatment of MTBE contamination.

A comparison of the MTBE-degradation rates reported in previous studies is listed in Table 5. Aerobic bioreactors containing mixture of cultures [15] showed higher degradation rates than other processes. The removal rate of MTBE under anaerobic con-

Table 4
Comparison of rates of degradation of MTBE by gel-entrapped cells under different conditions.

Conditions	Only gel beads	Only PM1 in gel beads	Alga and PM1 in the same gel bead	Alga and PM1 in different gel beads
MTBE-removal rate ($mg\,l^{-1}\,d^{-1}$)	16.032 ± 0.792	18.816 ± 0.624	21.456 ± 0.782	24.528 ± 1.428
MTBE-degradation rate[A] ($mg\,l^{-1}\,d^{-1}$)	–	2.784 ± 0.168^{aB}	5.424 ± 0.010^{bB}	8.496 ± 0.636^{cB}

[A] MTBE-degradation rate is equal to the total MTBE-removal rate of the system minus the MTBE-removal rate of only the gel beads.
[B] The mean values in same line with the same superscript letters are not significantly different ($P > 0.05$), whereas those with different superscript letters are significantly different ($P < 0.01$) after statistical analysis.

Table 5
Comparison of the MTBE-degradation rates reported by different authors.

Inoculum	Inoculum size	Initial MTBE level ($mg\,l^{-1}$)	Conditions	Process volume	Degradation rate ($mg\,l^{-1}\,d^{-1}$)	Ref.
F-consortium	$286\,mg\,l^{-1}$	100	Aerobic	Batch, 1.7 l fixed biofilm reactor	12.0–67.2	[35]
Microbial consortium from gasoline-polluted soil	Biofilm on sintered glass rings	10–100	Aerobic	Continuous-upflow fixed-bed reactor	132 ± 2	[18]
Flavobacteria-cytophaga and PM1-like organisms	Biofilm on granular activated carbon	Influent: 7.8; flow rate: $22.7\,l\,d^{-1}$	Aerobic	Fluidized bed reactor	176	[15]
Petroleum-impacted aquifer	30-g sediments	50	Anaerobic	Batch, 60-ml serum bottles, headspace: 28 ml	1.13	[8]
Free PM1	$0.5\,mg\,l^{-1}$	50	Closed batch system	50 ml in 250 ml bottle	17.04–30.96	[33]
Gel-entrapped PM1	2.4×10^9 cells/g-bead;	50	Closed batch system	20 ml in 250 ml bottle (4 g of beads)	171.4	[34]
Mixed culture of free PM1 and algae	1.35×10^9 PM1 cells; 6.5×10^6 algal cells	60	Closed batch system	200 ml in 250 ml bottle	8.808 ± 0.007	This study
Mixed gel-entrapped PM1 and algae	1.5×10^8 PM1 cells; 1.5×10^8 algal cells	50	Closed batch system	80 ml in 100 ml bottle (10 g of beads)	8.496 ± 0.636	This study

ditions in a batch process was mostly $\leq 1\,mg\,l^{-1}\,d^{-1}$ and was very low compared to the mineralization rates by aerobic degradation in reactors [36]. The degradation rate of the closed symbiotic system in this study was higher than that of anaerobic processes and lower than that of aerobic bioreactor. However, compared with aerobic bioreactors, the present closed symbiotic system has two advantages. First, there is a lower potential risk of MTBE leakage during the treatment period; and second, there is no need for external oxygen supply, which means that the cost of the process is low. Therefore, the present closed symbiotic system and its potential photobioreactor could be considered favorably for the removal of MTBE contaminants from groundwater. However, optimization of the conditions, such as the inoculum size and the volume ratio (cell-entrapped gel beads versus MTBE-containing solution), is required to improve MTBE degradation in the closed symbiotic system. In addition, an in-depth understanding of the synergetic effect of PM1 and alga in this symbiotic system is imperative for further applications.

4. Conclusions

A symbiotic system containing a mixed culture of *C. ellipsoidea* and PM1 could improve MTBE degradation due to the achievement of higher DO levels during the whole process. The presence of algae in the culture contributed to the higher DO level in the system by photosynthesis. Similar to the free-cell culture, the immobilized mixed culture of algae and PM1 showed higher efficiency of MTBE degradation than the pure culture of immobilized PM1 alone. The use of an algal–bacterial symbiotic system is a potential strategy for application in degradation of aqueous MTBE.

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