Removal of Methyl Parathion from Artificial Off-Gas Using a Bioreactor Containing a Constructed Microbial Consortium

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Methyl parathion (MP), a highly toxic organophosphorus pesticide, was widely used for agriculture crop protection. During the production of MP and the process of MP-containing wastewater treatment, MP can release into the atmosphere and will do great harm to adjacent communities. A consortium comprised of an engineered microorganism and a natural *p*-nitrophenol (PNP) degrader was assembled for complete mineralization of MP. We genetically engineered *Escherichia coli* BL21 (DE3) enabling the overexpression of methyl parathion hydrolase (MPH). In addition, we isolated *Ochrobactrum* sp. strain LL-1 that utilized PNP, a product of MP hydrolysis, as the sole carbon, nitrogen, and energy source. The coculture effectively hydrolyzed 0.2 mM MP and prevented the accumulation of PNP in suspended culture. A laboratory-scale bioreactor containing the dual-species consortium was developed for the treatment of artificial off-gas containing MP. The bioreactor maintained over 98% of average MP removal efficiency over a 75 day period, and PNP produced from hydrolysis of MP was degraded completely, indicating that complete mineralization of MP was achieved. The strategy of linking degrading consortium to a bioreactor may provide an alternative to physicochemical abatement technologies for the treatment of waste-gas streams containing MP as well as other PNP-substituted organophosphates.

Introduction

Synthetic organophosphates (OPs) are widely used to control various pests for agriculture and for public health protection. In the U.S. alone, over 40 million kilograms of organophosphorus pesticides are consumed annually (1). OPs are acute neurotoxins by virtue of their potent inhibition of acetyl-

cholinesterase. These compounds cause enormous damage to nontarget organisms because the acetylcholinesterase is present in all vertebrates (2).

Enzymatic detoxification of OPs by organophosphorus hydrolase (OPH) has attracted considerable interest because it is economical and effective (3, 4). Hydrolysis of OPs by OPH reduces their toxicity by several orders of magnitude (1). Identical *opd* genes coding for OPH were found in two soil microorganisms, *Pseudomonas diminuta* (5) and *Flavobacterium* sp (6). A *Pseudomonas* sp. was isolated that can cometabolically degrade MP, and a gene similar to *opd* gene was found (7). Utilization of MP by *Flavobacterium balustinum* as the sole carbon source was observed and the *opd* gene was found to be linked with a novel PNP degradation gene (8). However, MP is hydrolyzed by the OPH 30-fold slower than the other PNP-substituted OP, paraoxon (9). Another gene with identical function is *mpd*, first isolated from methyl parathion-degrading *Plesiomonas* sp., but it showed no homology to the known *opd* genes (10). The highest similarity with predicted protein sequence was found to be 31% with β -lactamase, suggesting significant novelty of the gene-enzyme system. We recently cloned *mpd* gene (GenBank accession no. DQ677027) from chlorpyrifos-degrading *Stenotrophomonas* sp. strain YC-1 (11). To date, the *mpd* genes found in bacteria from different genera were highly conserved (2).

PNP, produced from hydrolysis of MP, is a stable and toxic intermediate. To avoid the generation of toxic hydrolytic product, a natural PNP degrader, *P. putida* JS444, was genetically engineered by targeting MPH onto the outer membrane, resulting in strain with both MPH activity and PNP mineralization capability (12). Although the engineered strain was endowed with MP mineralizing activity, the use of displayed MPH may increase the metabolic burden of host strain.

An alternative strategy to constructing a complete degradative pathway in a single microbe is to combine bacteria with complementary metabolic pathways into functional assemblages (13–15). This approach has been used for biodegradation of several xenobiotic pollutants, including 4-chlorodibenzofuran (13), parathion (14), and chlorpyrifos (15). Complete mineralization of 4-chlorodibenzofuran or chlorpyrifos was achieved by coculture of two natural degraders (13, 15). In another study, a consortium composed of two engineered strains was assembled for complete mineralization of parathion (14).

The removal of residual OPs from soil and water using degrading microorganisms has been well documented (16, 17). For some pesticides, up to 90% of the application amount may volatilize from agricultural fields into the air, which constitutes a large source of potential human exposure (18). Once in the air, the OP phosphorothionates (e.g., MP, parathion, and chlorpyrifos) are converted from thion ($P = S$) to oxon ($P = O$) compounds by reacting with photochemically produced hydroxy radicals (19). The oxon compounds are more reactive, and are more potent inhibitors of acetylcholinesterase than are the parent compounds (1). Marklund et al. (20) reported that several OPs were detected in indoor air from domestic and occupational environments. Although trace amounts of OPs resided in the air tend to be ignored, some attempts to degrade OPs from artificial off-gas have been made. An OPH-containing gas phase reactor, developed by Yang and co-workers (21), was applied to degrade paraoxon from gas. However, there are no reports of treating waste gases containing OPs based on a constructed consortium to date.

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MP, classified by the World Health Organization as a Category Ia (extremely toxic) and by the U.S. Environmental Protection Agency as a Toxicity Category I (most toxic) insecticide (22), was chosen as a model compound for the study. We genetically engineered *Escherichia coli* to endow it with methyl parathion hydrolase activity and isolated PNP-degrading *Ochrobactrum* sp. Moreover, a consortium with MP mineralizing activity was constructed by coculture of the two organisms. Furthermore, a laboratory-scale bioreactor inoculated with the consortium was developed for the treatment of artificial off-gas containing MP. Compared to the previously established bioreactors (21), the bioreactor containing the specific degrading consortium possesses the potential of complete mineralization of PNP-substituted OPs from artificial off-gas. Considering the cost of purification and stability of the enzyme, the bioreactor using multiple strains with distinct metabolic capabilities seems to be more suitable to field-scale remediation than that with purified or crude enzymes.

Materials and Methods

Construction of Engineered *E. coli* for Overexpression of Recombinant MPH. *E. coli* BL21 (DE3) ($F^- ompT hsdS_B (r_B^- m_B^-)$ *gal dcm* (DE3), Novagen) was used. For construction of a fusion protein between mature MPH and His₆ tag, the regions encoding MPH without a signal sequence were PCR amplified from plasmid pMDQ containing an intact *mpd* gene from strain YC-1 (11) with forward primer 5'-CATATG-GCCGCACCGCAGGTGCG-3' and reverse primer 5'-CTC-GAGCTTGGGGTTGACGACCG-3' (the *Nde*I and *Xho*I sites, respectively, are underlined). PCR was performed with the following cycling profile: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and elongation at 72 °C for 1 min, final extension at 72 °C for 8 min. The PCR products were digested with *Nde*I and *Xho*I and ligated with similarly digested pET30a (Novagen). The resulting plasmid mixture was transformed into *E. coli* BL21 (DE3) competent cells, which were plated on the LB agar plates containing 50 µg/mL kanamycin. The plasmid extracted from the transformants was digested with *Nde*I and *Xho*I, and then run on 0.7% (w/v) agarose gel to confirm the size of the inserted fragment. A positive recombinant plasmid (named pETM) was sequenced to ensure that no mutation had been incorporated during the PCR.

E. coli BL21 (DE3) cells harboring pETM were induced with 1 mM IPTG for 3 h at 30 °C when cells were grown to an OD₆₀₀ = 0.6. Harvested cells were disrupted by sonication, and the soluble fraction was loaded onto a Ni-NTA column. Recombinant His₆-tagged MPH was purified using Ni-NTA affinity chromatography, following the procedure provided by the manufacturer (Novagen). The purity of the enzyme was examined by 12% SDS-PAGE (23).

Isolation and Identification of a PNP-Degrading Bacterium. Enrichment was performed by successive subculturing of sludge samples in minimal salt medium (MSM) (24) with increasing concentration of PNP up to 300 mg/L at 30 °C on a shaker at 200 rpm. The enrichment culture of the fourth transfer was plated onto MSM agar plates containing 100 mg/L PNP. Colonies appearing on the agar plates after 3 days of incubation at 30 °C were picked and restreaked to ensure purity. The isolates were then inoculated into MSM containing 100 mg/L PNP to test degradation ability. An isolate, designated LL-1, capable of degrading PNP rapidly was selected for further investigation.

Strain LL-1 was initially identified using standard methods (25). The 16S rRNA gene of strain LL-1 was amplified by PCR and sequenced as described previously (11). The determined sequence was compared with those of type strains available in the GenBank database using the BlastN program. The 16S

rRNA gene sequence has been deposited in the GenBank database under accession no. EU098001.

PNP Biodegradation Studies. A total of 100 µL of cell suspension (10⁶ cells/mL, quantified by the dilution plate count technique) of strain LL-1 was inoculated into MSM with PNP (0.8 mM), and uninoculated medium with PNP (0.8 mM) was used as a control. Samples were incubated at 30 °C on a shaker at 200 rpm. Cultures were regularly checked for bacterial growth, PNP degradation, and nitrite release. Bacterial growth was monitored by measuring the OD₆₀₀ using a Beckman DU800 spectrophotometer. Nitrite ion was quantitatively determined by the method of Montgomery and Dymock (26). PNP was quantified by HPLC (Agilent 1100), and its metabolites were identified by GC-MS (6890N GC-5973N MSD, Agilent Technologies, Palo Alto, CA) (27). Hydroquinone oxidation was monitored spectrophotometrically by measuring the increase at 320 nm (28). The enzyme assay was carried out in 20 mM phosphate buffer (pH 7.2) containing 0.05 mM hydroquinone and 0.6 mg of total cellular protein.

To determine whether the enzyme system responsible for PNP degradation was induced or constitutively expressed, PNP-induced and noninduced resting cells were prepared as described by Rappert et al. (29). Induced cells were grown in MSM containing 100 mg/L PNP at 30 °C until the yellow color of PNP disappeared. Noninduced cells were grown in MSM without PNP. The cells were tested for their ability to degrade PNP in the presence of chloramphenicol. The OD₆₀₀ of the resting-cell suspensions was adjusted to 1.0. Chloramphenicol (100 µg/mL) was used to inhibit de novo protein synthesis of the resting cells.

Coculture Study. Flasks containing LB medium supplemented with 50 µg/mL kanamycin, 1 mM IPTG, and 0.2 mM MP were inoculated with *E. coli* BL21 (pETM) only or with both *E. coli* BL21 (pETM) and strain LL-1. The initial OD₆₀₀ of both strains inoculated was 0.03. All cultures were grown with shaking at 30 °C. At each time point, a 1 mL aliquot was withdrawn, centrifuged, and the supernatant was used for PNP analysis by HPLC (27).

Bioreactor Structure, Operating Conditions, and Analytical Methods. The artificial off-gas containing MP was continuously passed through a bioreactor comprising a 35 cm vertical Plexiglas column (i.d. 6 cm) in which there was a 20 cm high zone filled with foam cubes (Qingdao Jili Chemical Co. Ltd., China) providing a porous and inert packing media. Four sampling ports designed for the bioreactor were used to monitor the concentration of MP in the inlet and outlet gas as well as relative humidity (RH), pH, and temperature. Besides the inlet (sampling port 1) and outlet gas (sampling port 2) in correspondence to compound concentration before and after the treatment, sampling ports 3 and 4 were assigned for sampling the liquor and packing media, respectively. A 1000 mL Muencks gas-washing bottle contained 500 mL of 50 mg/L MP in methanol/water (80/20, v/v). An air stream supplied by an air blower was passed through the Muencks gas-washing bottle. Subsequently, the artificial off-gas with 0.062–5.11 mg/m³ MP and more than 95% of RH was passed through the bioreactor in an upflow mode. The flow rate was controlled by valve and metered by calibrated flowmeter. A schematic diagram of the treatment process is shown in Figure 1.

Prior to inoculation, *E. coli* BL21 cells (pETM) grown to an OD₆₀₀ = 0.6 were induced with 1 mM IPTG for 3 h at 30 °C. PNP-induced cells of strain LL-1 were prepared as described previously (11). Subsequently, 5 g cells of each strain were suspended in 80 mL nutrient solution containing 1 g/L NH₄Cl, 1.5 g/L K₂HPO₄, 0.5 g/L KH₂PO₄, 0.1 g/L MgSO₄, and 1 g/L glucose, supplemented with 10% LB medium and then inoculated into packing media. The bioreactor was continuously operated for 75 days in a laboratory where the

FIGURE 1. Schematic diagram of the treatment process. (1) bioreactor, (2) packing media, (3) Muencks gas-washing bottle, (4) mix chamber, (5) air pump, (6) flowmeter, (7) air pump, (8) flowmeter, (9) flowmeter, (10) Temp/RH meter, (11) Temp/RH meter, (12) meter pump, (13) phosphate buffer tank, (14) sampling port 1, (15) sampling port 2, (16) sampling port 3, (17) sampling port 4.

temperature varied between 23 and 30 °C. To maintain the desired pH and water content of packing media at 7.2 and 60–70%, respectively, the phosphate buffer was added into packing media once every two weeks during bioreactor operation. Liquor samples (1 mL) were withdrawn from sampling port 3 at regular intervals and filtered through 0.22 μm membrane filter and kept at $-20\text{ }^{\circ}\text{C}$ until analyzed. Remaining PNP in packing media was extracted twice with diethyl ether. The extracts were evaporated to dryness at 30 °C and redissolved in 0.5 mL methanol for HPLC analysis.

The bioreactor performance was evaluated by measuring the concentration of MP in the inlet and outlet gas. The pH, RH, and temperature were continuously monitored during bioreactor operation. MP-containing artificial off-gas was collected by a glass gas sampling bulb (Agilent 10370-00) from sampling port 2 and then injected by a gastight syringe (Agilent 5182-9604) into a HP 5890 II GC (Hewlett-Packard, Wilmington, DE) equipped with an electronic capture detector (ECD) and a HP-1 capillary column. PNP and its metabolites were determined using an Agilent 1100 series HPLC equipped with a diode array detector (DAD) and a Zorbax 300SB-C18 column. Details for GC and HPLC analyses are described by Qiu et al. (27). The pH was determined using a digital pH meter (PH-3C, Shanghai, China). The Dewpoint Thermohygrometer (WD-35612, OAKTON, Germany) was used to measure the RH and temperature.

Results and Discussion

Functional Expression of *mpd* Gene in *E. coli*. The *E. coli* BL21 cells harboring pETM were spread on LB agar plates containing 50 $\mu\text{g}/\text{mL}$ kanamycin and 100 mg/L MP as indicator. The recombinants produced yellow transparent halos after incubation at 37 °C for no more than 12 h. The cell suspensions of the recombinants degraded 100 mg/L MP completely in 20 min as shown by HPLC analysis, which was 12-fold faster than that of *mpd*-containing strain YC-1 (11). The engineered *E. coli* BL21 exhibited enhanced degradative capabilities by overexpressing recombinant MPH compared to natural degrader YC-1, suggesting potential use of the engineered strain in detoxification of OPs.

Recombinant MPH was purified from 1 L expression cultures by metal affinity chromatography using a Ni-NTA column. His₆-tagged MPH tightly bound to the Ni-NTA matrix and fusion proteins were eluted with elution buffer containing 300 mM imidazole, thereby allowing their one-step purification to electrophoretic homogeneity. The data on the purification are summarized in Supporting Information (SI) Table S1. From 1 L of *E. coli* culture we routinely obtain 15 mg of purified MPH with a purification of 23 fold, 82% final yield and a specific activity of 257 U/mg. The purified His₆-

tagged MPH gave a single band appeared at the position of 32 kDa in SDS-PAGE (data not shown), which is in good agreement with that calculated from the protein sequence of 303 amino acids.

Isolation and Identification of PNP-Degrading Strain LL-1. Several different isolates capable of degrading PNP were obtained by enrichment procedure. One of them was named strain LL-1; it showed good growth and the highest degrading capability. Strain LL-1 was rod shaped, Gram negative, oxidase and catalase positive, and resistant to kanamycin. The 16S rRNA gene sequence of strain LL-1 was very similar to that of the *Ochrobactrum* strains. All the sequence similarity values between strain LL-1 and other *Ochrobactrum* species were above 98%, suggesting that strain LL-1 was affiliated to genus *Ochrobactrum*.

Degradation of PNP by *Ochrobactrum* sp. Strain LL-1. Strain LL-1 could utilize PNP as the sole carbon, nitrogen, and energy source. PNP (0.8 mM) was completely degraded in 28 h concomitant with bacterial growth and nitrite release (SI Figure S1). No degradation and bacterial growth were observed in uninoculated controls. Due to the utilization of nitrite by the strain as the nitrogen source, the amounts of PNP depletion and nitrite release were not stoichiometric. The addition of extra carbon sources (e.g., 1 g/L glucose) accelerated the rate of PNP degradation (data not shown), which suggested that strain LL-1 could steadily degrade PNP in contaminated sites where carbon sources usually occur. Strain LL-1 could degrade PNP up to 500 mg/L within 96 h and no significant inhibition was observed below 600 mg/L. PNP degradation by strain LL-1 was very rapid at temperatures ranging from 18 to 30 °C, with the most rapid degradation rates at 28 °C. Strain LL-1 rapidly degraded PNP in MSM at pHs ranging from 7.0 to 9.0, but the degradation was very slow at above pH 10 or below pH 6.0.

In resting-cell assays, PNP-induced resting cells degraded 0.8 mM PNP in 6 h. Contrarily, no decrease of PNP was observed with noninduced resting cells, which indicated that PNP was metabolized by an induced enzyme system. The involvement of catabolic plasmids in the degradation of xenobiotic compounds has been extensively documented (30). In the study, no plasmid DNA was detected from strain LL-1. Additionally, successive subculturing of strain LL-1 in nutrient-rich media still maintained the PNP-degrading capability. These observations suggest that the PNP-degrading gene may be chromosome based.

Identification of Intermediates of PNP Degradation by *Ochrobactrum* sp. Strain LL-1. During degradation of PNP by strain LL-1, a new chromatography peak with retention time (RT) 22.08 min appeared while the original peak of PNP with RT 29.09 min decreased in GC-MS analysis. The new peak was identified as hydroquinone based on mass spectral properties (data not shown). However, no other putative intermediates (4-nitrocatechol or 1,2,4-benzenetriol) were detected. In spectrophotometric assays, the original peak of hydroquinone at 288 nm disappeared gradually and a transient peak around 320 nm appeared, indicating the formation of γ -hydroxymuconic semialdehyde (28). In addition, strain LL-1 was able to utilize 0.8 mM hydroquinone as the sole carbon source. The results make us propose that PNP could be degraded by strain LL-1 via the hydroquinone pathway (SI Figure S2).

Coculture Study. MP hydrolysis by *E. coli* BL21 harboring pETM led to steady PNP accumulation during growth as a monoculture, reaching a concentration of 178 μM in 60 h (SI Figure S3). When *E. coli* BL21 grew in coculture with *Ochrobactrum* sp. strain LL-1, 36 μM PNP accumulated during the first 30 h, which was subsequently degraded over the remainder of the experiment. PNP is toxic to microbes; it inhibits the growth of microbes (28). The growth rate of strain BL21 decreased gradually at PNP concentrations greater than

FIGURE 2. Removal of methyl parathion (MP) from artificial off-gas using a bioreactor containing a constructed microbial consortium. (●) inlet gas, (▲) outlet gas, and (■) removal efficiency.

0.2 mM; and growth was completely inhibited by 0.6 mM PNP. Because strain LL-1 could rapidly degrade the PNP formed during MP hydrolysis, the inhibitory effect of PNP on the growth of *E. coli* BL21 could be eliminated by coculture with strain LL-1.

Bioreactor Performance for Removal of MP from Artificial Off-Gas. The MP removal efficiency was examined over a 75 day period by changing the inlet gas concentration of the bioreactor. The inlet gas concentration ranged between 0.062 and 5.11 mg/m³, and the gas flow rate was 0.06 m³/h. The maximum MP elimination capacity was 950 mg/m³h, corresponding to empty bed residence time (EBRT) of 35 s and inlet gas concentration of 5.11 mg/m³. As shown in Figure 2, the overall MP removal capability was 91.4–100%. MP was not detected in the outlet gas when the inlet gas concentration ranged between 0.18 and 1.6 mg/m³. MP was completely removed from artificial off-gas within the first 14 days. Subsequently, the inlet gas concentration significantly changed, resulting in the fluctuation of the removal efficiency curve. During the overall operation, average over 98% of MP was removed from artificial off-gas as judged by the outlet gas concentration. In contrast, the outlet gas concentration did not reduce in uninoculated bioreactor under the same operating conditions. The results indicated that MP could be effectively removed from artificial off-gas by introducing the engineered *E. coli* BL21 into the bioreactor.

The fact that the bioreactor maintained over 98% of average MP removal efficiency during the overall operation (Figure 2) indicated that the initial amounts of MPH synthesized from IPTG-induced *E. coli* BL21 cells were sufficient to enable degradation of low concentrations of MP from artificial off-gases. Enzyme assays with purified MPH have also demonstrated that MPH is an effective catalyst for degradation of MP (SI Table S1), and that MPH is extremely stable in a wide range of pH and temperature. In the temperature range from 25 to 35 °C and in the pH range from 7 to 9, the MPH activities were more than 90% of the maximum activity. Lyophilized MPH powder still maintained 80% of the original activity over a 3 month period. A concern raised by the study is that induction of MPH synthesis with IPTG will be greatly limited by the high cost of IPTG in future large-scale treatment of waste gases containing OPs. To reduce the operating cost, no IPTG was added to continuously induce the protein expression over the 75 day period.

The bioreactor performance for treatment of artificial off-gas containing MP is shown in Figure 3 where the elimination capacity is plotted versus the MP load. A linear relationship is obtained between the elimination capacity and the MP load which indicates that MP was almost completely removed. In the study, the bioreactor containing the con-

FIGURE 3. The bioreactor performance for treatment of artificial off-gases with various concentrations of methyl parathion.

FIGURE 4. Concentration of *p*-nitrophenol (PNP) in the liquor (▲) and packing media (■) during bioreactor operation.

sortium could effectively remove MP from artificial off-gases with various concentrations of MP. However, further experiments with an inlet gas of a higher MP concentration are required to assess the elimination capacity of the bioreactor against MP-containing artificial off-gas.

PNP Degradation in the Bioreactor. Because the PNP formed from hydrolysis of MP is toxic, the concentration of PNP is needed to be monitored during bioreactor operation. Initially, there were 0.27 and 0.02 mg/L PNP, respectively, in the liquor and packing media. Subsequently, PNP reduced gradually and disappeared completely after 14 days of operation (Figure 4). While the inlet gas with increasing concentration of MP was passed through the bioreactor, PNP appeared again and reached a maximum (0.62 mg/L) in the liquor. Subsequently, PNP reduced gradually and no PNP was found after 75 days of operation. In contrast, no degradation of PNP occurred in the bioreactor inoculated with recombinant *E. coli* BL21 only. The results indicated that the inoculated PNP-degrading strain LL-1 was truly responsible for the degradation of PNP.

Identification of Metabolites of MP Degradation in the Bioreactor. MP is hydrolyzed by MPH to PNP (10), which is converted to 1,2,4-benzenetriol or hydroquinone (28, 31). 4-Nitrocatechol and 1,2,4-benzenetriol are reported to be the major degradation products when PNP is degraded by Gram-positive bacteria (31), and hydroquinone is found to be the key catabolic intermediate when PNP is degraded by Gram-negative bacteria (28). PNP and hydroquinone were detected by HPLC during bioreactor operation but not 4-nitrocatechol and 1,2,4-benzenetriol (data not shown), which demonstrated that MP could be degraded by the dual-species consortium via MP → PNP → hydroquinone → → TCA cycle (SI Figure S2). The data support the conclusion that the process of mineralization of MP is initiated by hydrolysis leading to the generation of PNP and dimeth-

ylthiophosphoric acid, and PNP degradation then proceeds through formation of hydroquinone.

Isolation of the Dual-Species Consortium from the Bioreactor. After operation for 75 days, samples from the bioreactor were spread on LB agar plates containing 50 µg/mL kanamycin and 100 mg/L MP (designated LKM). Bacterial colonies that developed yellow zones because of the degradation of MP were isolated and purified. The isolates were identified as *E. coli* by sequencing their 16S rRNA genes. We isolated the same size of plasmid as pETM from the isolates, and *mpd* gene was PCR amplified from the plasmids. Similarly, samples from the bioreactor were spread on LB agar plates containing 50 µg/mL kanamycin and 100 mg/L PNP (designated LKP). Bacterial colonies that turned the medium from yellow to colorless were isolated and purified. The isolates were identified as *Ochrobactrum* sp. by 16S rRNA gene sequence analysis. Moreover, all isolates degraded PNP at the same rate as strain LL-1. The evidence that the two organisms inoculated could be isolated from the bioreactor again suggested that the dual-species consortium inoculated was truly responsible for the observed degradation of MP and PNP.

To date, thousands of homologous and heterologous proteins were successfully expressed to high levels in *E. coli* BL21 (DE3). Moreover, some attempts to use *E. coli* in a field-scale remediation have also been made. Strong et al. (32) reported that a recombinant *E. coli* overexpressing atrazine chlorohydrolase significantly increased degradation in soil heavily contaminated with atrazine. However, *E. coli* was generally thought to survive for a long time neither in the environment nor in technical system. Bacteria with high biofilm-forming capabilities have attracted much attention in biodegradation of pollutants. It has been found that some of these bacteria coaggregate with other microorganisms to develop biofilms, and that these biofilms are capable of holding other microorganisms (33). In the study, we have demonstrated that strain LL-1 possesses either high biofilm-forming capability or the potential to be cocultured with *E. coli* BL21 to form biofilm. The biofilm biomass formed by the mixed culture was higher than that of each alone (data not shown). The two organisms could be cultivated together in a biofilm which may be beneficial for the long-term survival of the consortium (14). A recent study showed that *E. coli* bacteria were able to survive and grow in porous media coated with *P. aeruginosa* biofilm with a relatively low nutrient supply (34). By the end of operation, viable cell counts were estimated on LKM agar at 37 °C for *E. coli* BL21 and on LKP agar at 30 °C for strain LL-1. The BL21 CFU and the LL-1 CFU were 5.6×10^7 and 1.1×10^8 CFU, respectively. *E. coli* BL21 retained high numbers of cells, which indicated that *E. coli* could be well maintained in the dual-species consortium over the 75 day period.

Potential of the Bioreactor for Treatment of Waste Gases Containing OPs. To date, the *opd*-like sequences were identified from American, Philippine, and Australia isolates (5, 6, 35) while strains with *mpd*-like sequences were only reported from China (2, 10). They have little sequence similarity. Changes in substrate specificity have been reported for MPH by Cui et al. (10). Currently, 71 organophosphorus pesticides are available commercially (36), of which 26 contain dimethyl alkyl groups. Unfortunately, OPH has been shown to lack any hydrolytic activity toward numorous dimethyl OPs (35). In contrast, the recombinant MPH exhibited high k_{cat}/K_m values for dimethyl OPs (data not shown), which make it an attractive candidate for the remediation of pollution caused by dimethyl OPs.

Bioremediation is now accepted as a safe and economical alternative to physicochemical methods that are expensive and less efficient (37). At present, several chemicals have been successfully removed from contaminated sites by

inoculation of degrading consortium (16, 17, 38). In the present study, a consortium comprised of two metabolically complementary bacteria, *E. coli* BL21 with MPH activity and *Ochrobactrum* sp. strain LL-1 with PNP mineralizing capability, was assembled for treatment of artificial off-gas containing OPs. The dual-species consortium combined with an artificial off-gas treating bioreactor can function cooperatively to perform complete mineralization of the target compound MP from artificial off-gas. While the applicability of the artificial off-gas treating bioreactor has been illustrated for MP, it will also be valid for other PNP-substituted OPs, paraoxon, and parathion. Additionally, the strategy of linking degrading consortium to a bioreactor may be useful in developing a field-scale bioreactor for the treatment of waste-gas streams containing OPs.

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Supporting Information Available

Three figures and 1 table show additional details of our study. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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