



## Bioremediation of oil-contaminated soil using *Candida catenulata* and food waste

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Enhancement on degradation ability of petroleum hydrocarbon by the microbial strain in the composting process with food waste.

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### ABSTRACT

Even though petroleum-degrading microorganisms are widely distributed in soil and water, they may not be present in sufficient numbers to achieve contaminant remediation. In such cases, it may be useful to inoculate the polluted area with highly effective petroleum-degrading microbial strains to augment the existing ones. In order to identify a microbial strain for bioaugmentation of oil-contaminated soil, we isolated a microbial strain with high emulsification and petroleum hydrocarbon degradation efficiency of diesel fuel in culture. The efficacy of the isolated microbial strain, identified as *Candida catenulata* CM1, was further evaluated during composting of a mixture containing 23% food waste and 77% diesel-contaminated soil including 2% (w/w) diesel. After 13 days of composting, 84% of the initial petroleum hydrocarbon was degraded in composting mixes containing a powdered form of CM1 (CM1-solid), compared with 48% of removal ratio in control reactor without inoculum. This finding suggests that CM1 is a viable microbial strain for bioremediation of oil-contaminated soil with food waste through composting processes.

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### 1. Introduction

Bioremediation of petroleum hydrocarbon-polluted soil relies on the petroleum-degradation ability of the microbial consortium resident in the soil (Braddock et al., 1997; Franzmann et al., 2002; Lee and Merlin, 1999; Marquez-Rocha et al., 2001; Venkateswaran and Harayama, 1995). Although petroleum-degrading microorganisms are widely distributed in both soil and water, they may not be present in sufficient numbers at a given polluted site. In such cases, it may be useful to inoculate the polluted area with highly effective petroleum-degrading microbial strains in a process called bioaugmentation (Supaphol et al., 2006; Yerushalmi et al., 2003).

In general, some isolated organisms can effectively degrade single pollutant in lab-conditions but when introduced into actual field conditions with multiple pollutants, they cease to function as anticipated (Singer et al., 2005; Quan et al., 2003). In addition, the introduced strains may not compete well with the indigenous microorganism in the soil to remain dominant or viable (Bouchez et al., 2000; Das and Mukherjee, 2007; Mohanty and Mukherji,

2008; Supaphol et al., 2006). Bioremediation efficiency is thus a function of the ability of the inoculated microbial degraders to remain active in the natural environment (Alexander, 1999). From this standpoint, bioaugmentation is still experimental although it has been practiced in agriculture and in wastewater treatment for years (Agathos and El Fantroussi, 2005). To test viability of bioaugmentation of contaminated soils, a series of laboratory evaluations must be conducted to particularly characterize microbial populations and microcosm to exhaustively evaluate the selected strains capacity to colonize and to degrade target soil pollutant (Sabaté et al., 2004).

There are approximately 70 genera of known oil-degrading microorganisms, including bacteria such as *Achromobacter*, *Acinetobacter*, *Actinomyces*, *Bacillus*, *Burkholderia*, *Exiguobacterium*, *Klebsiella*, *Microbacterium*, *Nocardia*, *Pseudomonas*, *Spirillum*, *Streptomyces* and *Vibrio*, and fungi or yeast such as *Allescheria*, *Aspergillus*, *Candida*, *Debaryomyces*, *Mucor*, *Penicillium*, *Saccharomyces* and *Trichoderma*. Under natural conditions, these microorganisms in most areas comprise very few, compared with the total number of identified microorganisms. However, at petroleum hydrocarbon-polluted sites, these populations may grow and increase because they use petroleum hydrocarbon as a carbon source (Ahn et al., 1999; Aldrett et al., 1997; Altas, 1981; Bento et al., 2005; Chaerun et al., 2004; Das and Mukherjee, 2007; Gallego et al., 2001;

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Hua et al., 2003; Mohanty and Mukherji, 2008; Palittapongarnpim et al., 1998; Supaphol et al., 2006).

During microbial degradation of petroleum hydrocarbons, the *n*-alkane chain length is one of the most important factors because shorter-chain petroleum hydrocarbons are generally degraded more rapidly than longer-chain hydrocarbons (Mohanty and Mukherji, 2008; Seklemova et al., 2001; Jonge et al., 1997). In addition to chain length, petroleum hydrocarbon decomposition efficiency is also determined by structures of oil hydrocarbons (for example, polycyclic structures) as well as by the basic soil system characteristics including water, pH, temperature, mineral nutrients, nitrogen, phosphorus, and organic compounds. Although petroleum is a source of abundant carbon, its lack of nitrogen and phosphate makes it far less biodegradable than other biowastes such as food waste, sewage sludge and livestock manure and so on (Altas, 1981; Beaudin et al., 1999; Head and Swannell, 1999; Michel et al., 1993; Namkoong et al., 2002; Supaphol et al., 2006). In contrast, food waste is rich in carbon, organic nitrogen, phosphorous and mineral compounds required for growth of microorganisms (Joo et al., 2001, 2007; Nakasaki et al., 1992). Therefore, addition of food waste provides required nutrients for enhanced biodegradation of petroleum hydrocarbon (Joo et al., 2001; Nakasaki et al., 1992). Composting has long been a popular treatment method for food waste, and relatively recent studies have suggested that soil composting may be a useful method for bioremediation of petroleum-contaminated soil (Beaudin et al., 1996; van Gestel et al., 2003; Joo et al., 2007; Jorgensen et al., 2000; Kirchman and Ewnetu, 1998; Namkoong et al., 2002; Rojas-Avelizapa et al., 2007). In a recent study we elucidated the efficacy of using food waste for supplementing deficient nutrients for enhanced microbial composting during bioremediation of 1% (w/w) petroleum hydrocarbon contaminated soil (Joo et al., 2007). The study reported in this article is an extension of this previous study and challenged the effectiveness of the inoculants at a much higher 2% petroleum hydrocarbon contaminated soil. In this study, we isolated a number of hydrocarbon-degrading microbial strains from various sources and compared their effectiveness in diesel fuel-amended medium. We then inoculated the most effective microorganism into a composting reactor containing a mixture of hydrocarbon-contaminated soil and food waste to test the enhancement of this inocula petroleum-degradation ability against a control (no inoculums but under the same environment but the different oil concentration with the previous study).

## 2. Materials and methods

### 2.1. Isolation of oil-degrading microorganisms

Oil-degrading microbial strains were isolated from oil-contaminated soil samples taken from six different sites (gas stations and auto repair shops), and from the composts of food waste composting process as well as from sewers, leaf molds, and livestock manure piles. About 1 g sample was inoculated in 100 mL 2% diesel fuel-amended medium and cultivated at 120 rpm for 3 days. Three times acclimation of 1% (v/v) inoculation of samples was carried out from which 2 to 6 different colonies for each sample were isolated from the agar plates. The basic medium was a nutrient broth (3 g bacto-beef extract, 5 g bacto-peptone and 1 L water, adjusted to pH 7.0). For diesel fuel-amended medium, the nutrient broth was supplemented with 2% (w/w) diesel oil (LG Co., Ltd., Korea). For the agar plates used to examine colonies of microbial strains, NB (the nutrient broth supplemented with 1.5% agar) and GPY agar plate (10 g glucose, 5 g peptone, 5 g yeast extract, 1 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>·5H<sub>2</sub>O, 15 g agar and 1 L water, adjust to pH 6.8) were used. The confirmation of pure culture in every liquid- and solid-cultivation was performed with colony observation on these agar plates.

The isolated microorganisms were cultivated in 2% diesel fuel-amended medium and seven microbial strains exhibiting the highest growth were selected for further examination of their oil-degrading efficacies. The most efficient microbial strain was identified using a BIOLOG KIT (Biolog Technologies Ltd., Israel) based on biological characteristics such as carbon sources and several other characteristics summarized in Table 2.

### 2.2. Measurement of growth and emulsification activity

To test growth in culture medium, the seven selected microorganisms were individually cultured (three replications). In our case, 1 mL inoculums of optical density of 1 was inoculated into 75 mL fresh 2% diesel fuel-amended medium, and the microbial strains were grown at 30 °C at 120 rpm for 3 days. The cell density in each culture was measured at a wavelength of 660 nm with a spectrophotometer.

For analysis of emulsification effectiveness, 1 mL inoculum of the seven selected microorganisms were individually inoculated into a 50 mL 2% diesel fuel-amended medium and incubated at 30 °C at 120 rpm for 3 days (three replications). Exactly 1 mL of each culture broth was then inoculated into a 50 mL fresh 2% diesel fuel-amended medium, and samples were cultured for 1 day. Each culture was then filtered through a polytetrafluoroethylene (PTFE) filter (pore size = 0.2 μm), and 1 mL of the resulting supernatant was mixed with 5.2 mL potassium phosphate buffer (pH 7.0) containing 0.2 mL *n*-hexadecane. The mixture was shaken for 2 min and allowed to settle for 10 min. Absorbance of the suspension at 540 nm was then measured using a spectrophotometer.

### 2.3. Evaluation of oil-degrading ability in diesel fuel-amended medium

Each of the seven microbial strains selected were cultured as above, and 1 mL of each inoculum (optical density of 1) was inoculated into a 100 mL 2% diesel fuel-amended medium and incubated at 30 °C at 120 rpm for 3 days (three replications). Each culture was mixed with 50 mL methylene chloride and centrifuged at 10,000 rpm for 10 min. The supernatants were introduced into a separating funnel and oil component were extracted two to three times via shaking and settling (Hwang et al., 2000). Petroleum hydrocarbon contents were analyzed using a gas chromatograph with a flame ionization detector (GC/FID, HP-6890 PLUS, Hewlett-Packard, USA) and a HP-5 column (crosslinked 5% PHME siloxane, 0.32 mm × 30 m, Hewlett-Packard, USA), after filtration through a PTFE filter (pore size = 0.45 μm). Oil-degradation ratio was calculated as the concentration of removed petroleum hydrocarbon against a control test (without inoculums of microorganisms).

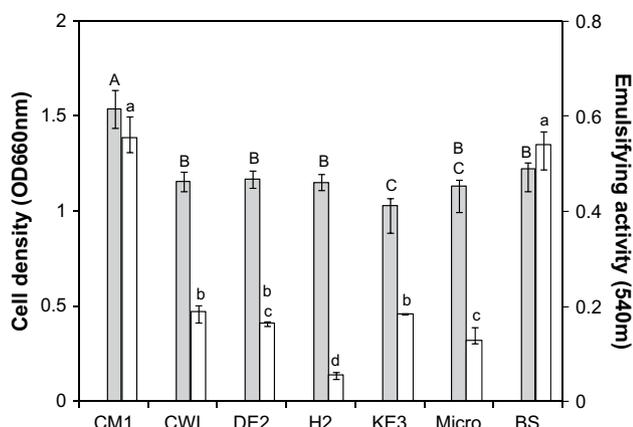
### 2.4. Evaluation of oil-degrading ability in a contaminated soil

To test the petroleum-degrading ability of the selected best-performing microbial strain (based on the previous experiments) composting was performed in soil mixed with food waste and diesel oil. The composting reactor used was cylindrical with insulated walls and a working volume of 6 L and total volume of 8 L. The lid held a temperature sensor/recorder, and could be tightly sealed to prevent leakage of the generated gas. To maintain aerobic conditions, compressed air was input at 0.5 L/min through a flow meter located near the bottom of the reactor. A multi-gas analyzer (Gas Data LMSx, Gas Data Ltd., UK) was used to measure generated CO<sub>2</sub> gas and consumed O<sub>2</sub> gas during the composting reaction.

Contaminated soil composting was conducted under three experimental conditions (see Table 1): control (no CM1, CM1 is selected the best strain of seven), CM1-liquid (augmented with CM1 cultivated in nutrient broth) and CM1-solid (augmented with powder-like CM1). The CM1-liquid was the culture liquid after CM1 was inoculated and cultivated in nutrient broth. For generation of powder-like CM1 (CM1-solid), 0.5% CM1-liquid was inoculated with 70% rice bran and 30% okara (the edible residue left after the production of bean curd), and composting process was conducted under aerobic condition to manufacture a pure CM1 composting-microbial agent for 3 days. Water content of this material was about 15% after sun-drying, and 110 g of this was added as a seed in the CM1-solid and minor in a carbon sources. Under each experimental condition, soil [77% (w/w)] and food waste [23% (w/w)] were combined on a wet basis, mixed to homogeneity with 2% diesel oil, and equally fed to three reactors. A 110 g of CM1-liquid and CM1-solid, culture broth and microbial agent were, respectively, added to two of the three reactors, while the third reactor was used as the control. The soil used was an organic-poor (4.5% volatile solids) sandy soil with a water content of 13% (Joo et al., 2007). The food waste consisted of 32% grain, 51% vegetable, 15% meat and fish, and 2% other ingredients with the following overall characteristics: 5.1% nitrogen, 1.1% phosphorus, 1% potassium, and a C/N ratio of 11. Oil-degradation ratio was calculated as the amount of the removed petroleum hydrocarbon against the initial concentration of the hydrocarbon in soil sample.

**Table 1**  
Mixing ratios during various remediation experiments

Mixing ratios	
Control	Soil 5 kg (77%) + food waste 1.5 kg (23%) + diesel oil 200 ml (2% w/w)
CM1-solid	Control + <i>C. catenulata</i> CM1 microbial agent 110 g (powder form)
CM1-liquid	Control + <i>C. catenulata</i> CM1 culture broth 100 ml (110 g)



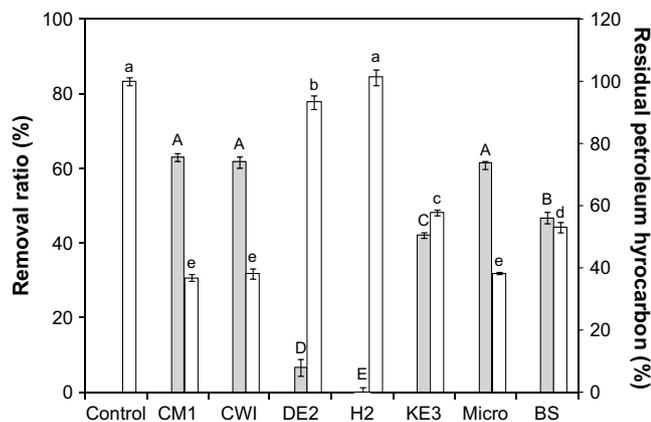
**Fig. 1.** Growth (shaded) and emulsifying activity (unshaded) of the seven selected microorganisms grown in diesel fuel-amended medium for 3 days. Means ( $n = 3$ ) with the same letter (capital for cell density and lower case for emulsifying activity) were not significantly different at  $\alpha = 0.05$ .

### 2.5. Petroleum hydrocarbon (pH) analysis

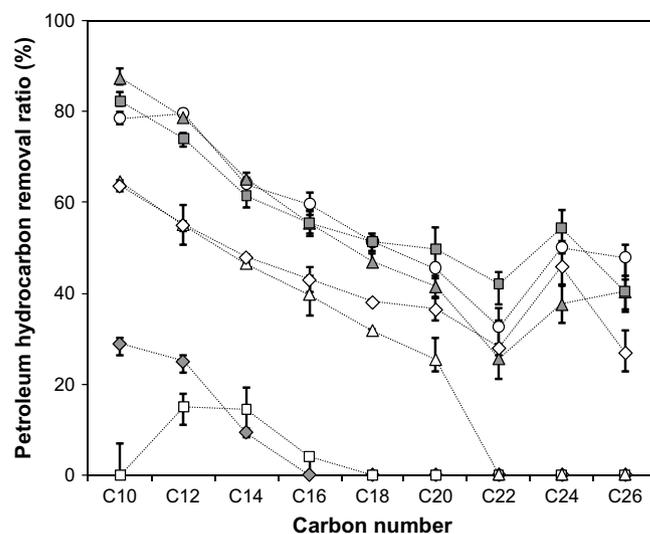
A composite soil compost was taken by mixing samples taken from three points (top, middle and bottom) in the reactor; three time-analysis for each sample performed to get accurate value. Although Soxhlet extraction is a common method for extracting oil from soil composting mixture, we used shaking-extraction in this study, because this method is more convenient and had shown equivalent high extraction efficiency (Hwang et al., 2000). For each shaking-extraction, 10 g of soil composting mixture was mixed with 5 g anhydrous sodium sulfate. The samples were then mixed with 50 mL methylene chloride and shaken at 200 cycles per min for 2 h. The methylene chloride layer was filtered with PTFE filter paper (pore size = 0.45  $\mu\text{m}$ ), and the filtrate was analyzed using a GC/FID, on a HP-5 column. The injection temperature was 250  $^{\circ}\text{C}$  and the detector temperature was 280  $^{\circ}\text{C}$ . The oven temperature was programmed from 40 to 280  $^{\circ}\text{C}$  at 12  $^{\circ}\text{C}/\text{min}$  and held for 20 min and finally maintained for 8 min at 280  $^{\circ}\text{C}$ . The carrier and make up gases were nitrogen and helium, respectively, and the gas ratio was 1:10 (purge:total). The calibration curve was prepared by analyzing blanks containing 200 ppm, 50 ppm, 10 ppm and 2 ppm diesel range organics (DRO-diesel fuel 31064, Thames Restek UK Ltd., UK), which consisted of C10, C12, C14, C16, C18, C20, C22, C24, C26 and C28 petroleum hydrocarbon components. Linear regression for the calibration of the respective concentrations against the chromatograph areas was statistically good ( $y = 10.86x + 4.525$  ( $y$ : area,  $x$ : mg/kg, with an  $R^2$  of 0.998)).

### 2.6. Statistical analysis

Analyses of variances (ANOVAs) were performed to statistically select microorganisms based on growth and oil-emulsification, their ability to degrade



**Fig. 2.** Removal ratios and the residual petroleum hydrocarbon levels (unshaded: residual petroleum hydrocarbon; shaded: removal ratio) in diesel fuel-amended medium treated with seven selected isolates for 3 days. Means ( $n = 3$ ) with the same letter (capital for cell density and lower case for emulsifying activity) were not significantly different at  $\alpha = 0.05$ .



**Fig. 3.** Removal ratio of petroleum hydrocarbons with even carbon numbers versus carbon number following three-day culture with each of the seven selected isolates in diesel fuel-amended medium. Symbols: (○) CM1, (■) Micro, (□) H2, (◆) DE2, (◇) BS, (△) KE3 and (▲) CWI.

long-chain hydrocarbons, and their ability to actually degrade these hydrocarbons in the soil. When significant difference was indicated by ANOVA on any of these indices, the least significant difference (LSD) method was used for pair-wise separation of the means. A standard statistical software (SAS) was used for statistical analyses in this study, and test for significance between means is implied at  $\alpha = 0.05$  unless stated otherwise.

## 3. Results and discussion

### 3.1. Isolation, growth and oil-emulsification

We initially isolated 54 microorganisms from oil-contaminated soils and 20 microbial strains from composts. The classification of 74 strains was not carried out in this step since it is not necessary

**Table 2**  
Basic characteristics of CM1 in identification

Carbon source	Result	Carbon source	Result
Fumaric acid	v	D-Glucosamine	v
L-Malic acid	–	$\alpha$ -D-Glucose	v
Methyl succinate	v	D-Galactose	+
Bromo succinic acid	–	D- Psicose	–
L-Glutamic acid	v	L-Rhamnose	–
$\gamma$ -Amino-butyric acid	v	Sorbose	–
$\alpha$ -Keto-glutaric acid	–	$\alpha$ -Methyl-D-glucoside	–
2-Keto-D-gluconic acid	–	$\beta$ -Methyl-D-glucoside	–
D-Gluconic acid	v	Amygdalin	–
Dextrin	v	Arbutin	–
Inulin	–	Salicin	–
Cellobiose	–	Maltitol	–
Gentiobiose	–	D-Mannitol	+
Maltose	–	D-Sorbitol	–
Maltotriose	–	Adonitol	–
D-Melezitose	–	Arabitrol	–
D-Melibiose	–	Xylitol	–
Palatinose	–	<i>i</i> -Erythritol	–
D-Raffinose	–	Glycerol	v
Stachyose	–	Tween 80	–
Sucrose	–	L-Arabinose	v
D-Trehalose	–	D-Arabinose	v
Turanose	–	D-Ribose	+
N-Acetyl-D-glucosamine	–	D-Xylose	+

+, Positive; –, negative; v, weak reaction. The result of identification is *Candida catenulata*.

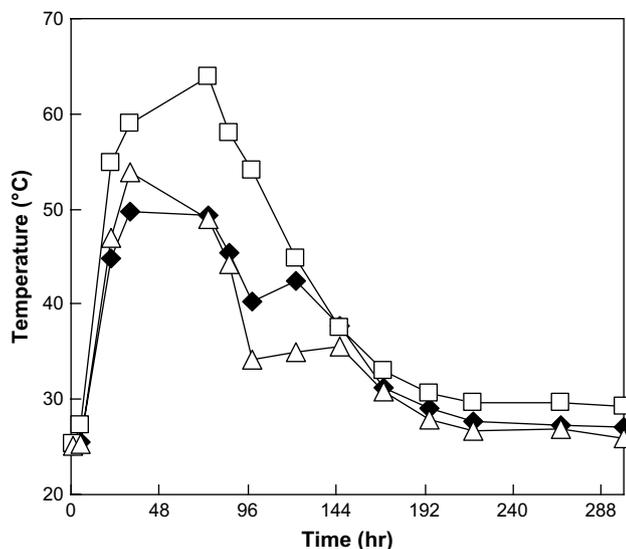


Fig. 4. Temperature changes during composting of food waste plus oil-contaminated soil mixtures (◆ – control; □ – seeded with CM1-solid; and △ – seeded with CM1-liquid).

because the aim of this experiment was to select the effective oil-degrading microbes of them. We tested these microbial strains for growth in 2% diesel fuel-amended medium as a pure culture by each colony-inoculation, and seven of the fastest growing microorganisms were finally selected. Other strains mostly indicated no or lower than 0.5 increase in cell density (optical density; OD660 nm). In microscopic observations based on the size and shape, CM1 was identified as a yeast, CW1 as a fungus, and DE2, H2, KE3, Micro and BS as bacteria.

The selected microbes were freshly cultivated in diesel fuel-amended medium, and growth (cell density) was measured using absorbance. The CM1, which was originally isolated as the dominant strain obtained from composting material, showed a significantly higher growth rate in diesel fuel-amended medium (Fig. 1).

We then examined the ability of each selected microbial strain to emulsify *n*-hexadecane. The microorganisms were individually inoculated into a diesel fuel-amended medium containing 2% diesel oil, and emulsification activity was measured. As shown in Fig. 1, CM1 showed a significantly higher tendency to emulsify than the other strains.

### 3.2. Analysis of oil-degradation ability in diesel fuel-amended medium

The seven selected microorganisms were cultivated in diesel fuel-amended media, and the residual oil was extracted from each culture and measured with GC/FID. As shown in Figs. 2 and 3, isolated CM1, CW1, Micro and BS showed significantly higher effectiveness of degradation of long-chain hydrocarbons. The removal ratio of C16 (*n*-hexadecane), the most abundant component in petroleum hydrocarbon was more than 60% in cultures of CM1, CW1 and Micro, and more than 40% in those containing BS and KE3. The least degraded component was C22, which showed a maximum removal of 45%. The overall removal of petroleum hydrocarbon for CM1, Micro and CW1 were 63%, 62% and 62%, respectively (Fig. 2). Although BS showed a high emulsifying activity (Fig. 1), this activity did not translate into a high removal ratio of the oil. This observation suggests that the strain indicating high emulsifying activity does not necessarily indicate that it has a correspondingly high oil-degrading ability.

Consistent with previous findings (Seklemova et al., 2001), we observed that the shorter-chain petroleum hydrocarbons were degraded more effectively than the longer chains. Our results revealed that 14- to 20-hydrocarbon chains predominated in our diesel oil sample, suggesting that it is important to develop a more efficient method for degrading longer-chain petroleum hydrocarbons.

### 3.3. Identification of CM1

After observing that the oil-degrading ability of CM1 was the highest amongst the seven isolated strains, this was then used in subsequent contaminated soil composting experiments. The identification was, however, carried out after finishing this study, indicating that the strain of CM1 was *Candida catenulata*. The basic characteristics of the strain conducted by a reputable and professional identification-institute are shown as Table 2. *C. catenulata* is usually found and used at the dairy process for milk, cheese and dry-cured ham (Rosaria Corbo et al., 2001; Simoncini et al., 2007; Vasdinyei and Deak, 2003). No effort was made to identify the other strains.

### 3.4. Petroleum hydrocarbon removal in contaminated soil using CM1

We tested the performance of composting of oil-contaminated soil bioaugmentation using two different methods of delivery of

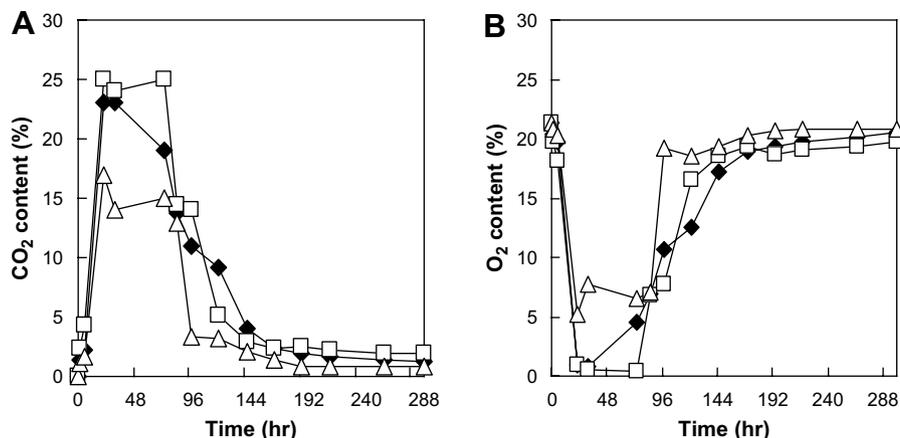


Fig. 5. Changes in CO<sub>2</sub> (A) and O<sub>2</sub> contents (B) in exhaust gas during composting (◆ – control; □ – seeded with CM1-solid; and △ – seeded with CM1-liquid).

CM1: a powder form – CM1 microbial agent (CM1-solid), and liquid culture agent (CM1-liquid). Petroleum hydrocarbon removal was measured in compost mix augmented with CM1-solid or CM1-liquid and a control (containing no CM1). The composting mixes themselves contained 77% soil and 23% food waste; this mixing ratio was chosen because mixing ratios with more than 25% food waste resulted in excess water content and insufficient air supply, in previous experiments.

As shown in Fig. 4, the hydrocarbon composting reactor inoculated with CM1-solid reached a maximum temperature of 65 °C, whereas those inoculated with CM1-liquid and the control reactors realized high of only 50 °C. An examination of the carbon dioxide and oxygen contents in the exhaust gas shown in Fig. 5, indicated the emitted carbon dioxide peaked on days 1–3 in all three reactors. During this time, the oxygen concentration was almost zero, due to active microbial oxidation. On day 4, the temperature and carbon dioxide emission decreased, while oxygen concentration increased to the level in ambient air. Comparison of the emission levels among the three reactors revealed that the CM1-solid-augmented composting generated the most carbon dioxide (Fig. 5A). To evaluate the effect of petroleum hydrocarbon volatilization at high temperature and aeration, a control test was additionally carried out by comparing the initial petroleum hydrocarbon concentrations (1% and 3% diesel oil) with samples composted for 10 days. The concentration of petroleum hydrocarbons in the samples after 10 days of composting was about 3–4% lower than that of the initial sample immediately after mixing materials (6% for C10–C16, 2% for C18–C28). It is probable that the decrease was caused by aeration or the biodegradation by indigenous microorganisms present in the

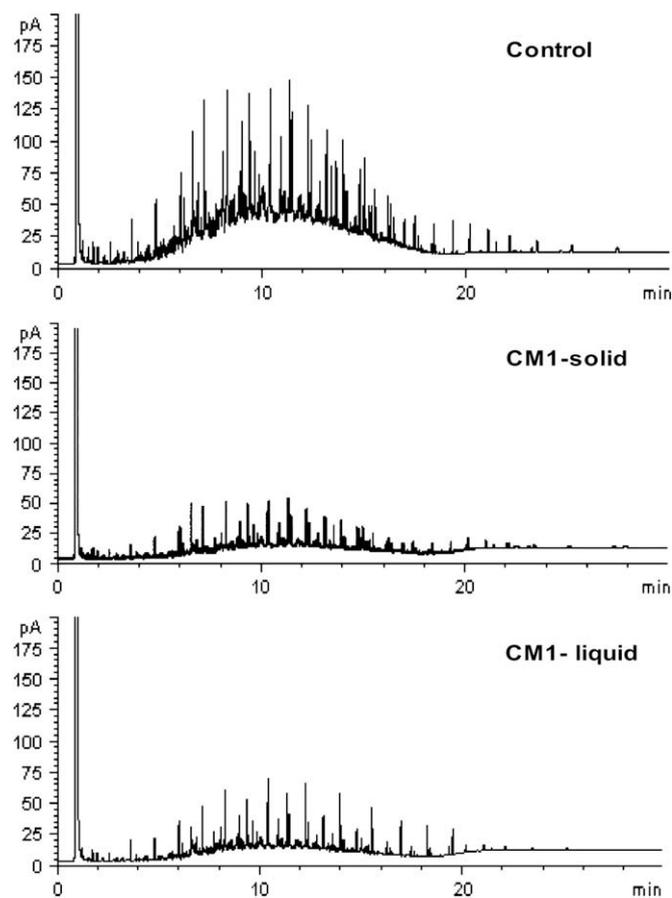


Fig. 6. Gas chromatogram of residual petroleum hydrocarbon present after 13 days of treatment. The retention time in minutes for each components is as follows: C10 (4), C12 (7), C14 (9), C16 (11), C18 (13), C20 (15), C22 (16), C24 (18), C26 (19) and C28 (21).

Table 3

Concentrations of respective petroleum hydrocarbons before and after 13 days of treatment

	Removal ratio <sup>a</sup>	Total residual petroleum hydrocarbon <sup>b</sup>
Initial	–	10,189 ± 5 <sup>a</sup>
Control	48 ± 0.003	5341 ± 16 <sup>b</sup>
CM1-liquid	84 ± 0.005	1628 ± 8.5 <sup>c</sup>
CM1-solid	78 ± 0.005	2232 ± 12 <sup>c</sup>

<sup>a</sup> Removal ratio, RR =  $(IC - RC/IC) \times 100\%$ , where IC and RC are initial residual concentration of hydrocarbons, respectively.

<sup>b</sup> The sum of the residual concentrations of C10, C12, C14, C16, C18, C20, C22, C24, C26 and C28. Means ( $n = 3$ ) with the same letter were not significantly different at  $\alpha = 0.05$ .

original soils. However, this 3–4% loss was minor compared to 78–84% of the initial oil removed.

Lastly, we used GC/FID to directly examine the residual petroleum hydrocarbons in each reactor after 13 days of composting. Our results revealed that samples from reactors containing CM1-solid and CM1-liquid-inoculated compost mixes had significantly lower petroleum hydrocarbon peaks than the control sample (Fig. 6). Further analysis indicated that 84% and 78% of the input petroleum hydrocarbons were degraded in the CM1-solid and CM-liquid-inoculated compost mixes, respectively, compared to only 48% in the control reactor without inocula (Table 3). In the colony observations on the agar plates, CM1 colonies in final samples of CM1-liquid and CM1-solid reactor were observed as the main microbes and they were not detected in the sample of control reactor. These observations indicate that the addition of CM1 strain increased the efficiency of petroleum-degradation. However, the CM1-solid and CM1-liquid were not significantly different at 84% and 78%, respectively, but addition of CM1-solid will be slightly better than that of CM1-liquid when it is applied in the field. The CM1 was fixed in the mixture of rice bran and okara by aerobic composting reaction for 3 days and CM1 composting-microbial agent (CM1 microbial agent) was produced. Organics in the CM1 microbial agent of 110 g were not much and CM1 degraded the mixture, with food waste and diesel oil. This result suggests that CM1-solid was more appropriate in composting process than CM1-liquid because CM1-solid adheres more easily in solid state reactions.

If only nutrients (such those provided by food waste) were added to the field, the intrinsic microorganisms will still flourish but biodegradation of petroleum hydrocarbon will be similar to that observed in our control reactors without any inoculants. In this study, approximately 1.5% CM1-solid (of total soil mixture) was used. This amount of inoculant is still enormous and perhaps not practical for field application. In addition, even if similar amount was inoculated to the field, it is doubtful that the same level of biodegradation of oil will be achieved in actual field conditions. Although bioaugmentation has greatly advanced in recent years, there are still a lot of challenges when it comes to applying laboratory isolated oil-degrading microorganisms to field conditions, most important being competition from other microbial consortia in the field (Das and Mukherjee, 2007; Mohanty and Mukherji, 2008; Supaphol et al., 2006). Future work should be directed at mechanisms of introducing highly effective lab-isolated microorganism into natural environments so that they compete favorably within the consortium of intrinsic populations.

#### 4. Summary and conclusions

In this study, we evaluated the decomposition patterns of petroleum hydrocarbons having different carbon numbers. In CM1-inoculated liquid cultures grown for 3 days, the removal ratio decreased as the carbon number increased suggesting that

shorter-chain hydrocarbons underwent decomposition more readily than the longer chains. In contrast, samples from CM1-solid-inoculated composting reactor showed similarly high levels (~80%) of petroleum hydrocarbon degradation, regardless of carbon number. These results collectively indicate that CM1-augmented soil composting in the presence of food waste improve the degradation of longer-chain petroleum hydrocarbons. For the soil composted in our reactor system for 13 days in control reactor without inoculum, 48% of the petroleum hydrocarbons were degraded, which was considerably higher than the previously reported removal rates. The CM1-solid-augmented composting mix had a peak temperature approximately 10 °C higher than the CM1-liquid-augmented and control mixes. The former degraded 84% while the latter degraded 78% of the input petroleum hydrocarbons over the 13-day composting period. These findings indicate that bioremediation of oil-contaminated soil added food waste with CM1 improves oil-degradation in contaminated soil during composting and that CM1-solid is expected to be a better oil-degrading microbial agent in the polluted soil.

In terms of direct application of CM1 to an oil-contaminated site, the composting reactors used in this study are somewhat impractical. Thus, future studies will be required to address the efficacy of CM1-solid augmentation of biopiles and windrow composting of contaminated soil. However, the present results suggest that the use of CM1-solid augmentation may be beneficial in these as well as other composting-based bioremediation systems.

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