Li, Peijun, Sun, Tieheng, Stagnitti, Frank, Zhang, Chungui, Zhang, Hairong, Xiong, Xianzhe, Allinson, Graeme, Ma, Xuejun and Allinson, Mayumi 2002, Field-scale bioremediation of soil contaminated with crude oil, *Environmental engineering science*, vol. 19, no. 5, pp. 277-289.

ENVIRONMENTAL ENGINEERING SCIENCE
Volume 19, Number 5, 2002

Field-Scale Bioremediation of Soil Contaminated with Crude Oil

**Peijun Li,[1] Tieheng Sun,[1] Frank Stagnitti,[2*] Chungui Zhang,[1] Hairong Zhang,[1]
Xianzhe Xiong,[2] Graeme Allinson,[2] Xuejun Ma,[1] and Mayumi Allinson[2]**

[1]*Institute of Applied Ecology*
Chinese Academy of Sciences
Shenyang 110015, People's Republic of China
[2]*School of Ecology and Environment*
Deakin University
Warrnambool Campus
Warrnambool
Vic. 3280, Australia

ABSTRACT

Field-scale remediation of oil-contaminated soils from the Liaohe Oil Fields in China was examined using composting biopiles in windrow technology. Micronutrient-enriched chicken excrement and rice husk were applied as nutrition and a bulking agent. The lipase activities of indigenous micro-organisms were analyzed, and three indigenous fungi with high lipase activities was identified. An inoculum consisting of the three indigenous fungi and one introduced (exotic) fungus was applied to four different types of oil-contaminated soils. The results showed that the inoculum of indigenous fungi increased both the total colony-forming units (TCFU) and increased the rate of degradation of total petroleum hydrocarbons (TPH) in all contaminated soils but at different rates. In sharp contrast to other studies, the introduction of exotic micro-organisms did not improve the remediation, and suggests that inoculation of oil-contaminated sites with nonindigenous species is likely to fail. On the other hand, indigenous genera of microbes were found to be very effective in increasing the rate of degradation of TPH. The degradation of TPH was mainly controlled by the compositions of aromatic hydrocarbons and asphaltene and resin. Between 38 to 57% degradation of crude oils (with densities ranging from 25,800 to 77,200 mg/kg dry weight) in contaminated soils was achieved after 53 days of operation. The degradation patterns followed typical first-order reactions. We demonstrate that the construction and operation of field-scale composting biopiles in windrows with passive aeration is a cost-effective bioremediation technology.

Key words: oil-contaminated soil; composting; windrow pile; Liaohe oil field; bioremediation; inoculation

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*Corresponding author: School of Ecology and Environment, Deakin University, P.O. Box 423, Warrnambool, Victoria, Australia 3280. *Phone*: 61 3 5563 3535; *Fax*: 61 3 5563 3143; *E-mail*: frankst@deakin.edu.au

INTRODUCTION

A NUMBER OF DIFFERENT TECHNOLOGIES can be applied for the direct remediation of oil-contaminated soils, and among them bioremediation may be the most cost-effective process for treating lightly or moderately contaminated soils. Since the Gulf War in 1991, numerous remediation investigations have been applied to desert soils in Kuwait contaminated by oil. For example, Al-Daher *et al.* (2001) treated both lightly and heavily oil-contaminated soils using windrow soil pile systems; Balba *et al.* (1998) used landfarming, windrow composting piles and static bioventing piles. Yateem *et al.* (1998) investigated the role of white rot fungi on remediating oil-contaminated soil in the Gulf area.

Composting biopiles is one of the most common and effective *ex situ* bioremediation technologies, and has been shown to be effective in cleaning soils contaminated with petroleum hydrocarbons (Samson *et al.*, 1994; Puustinen *et al.*, 1995; Filauro *et al.*, 1998; Jorgensen *et al.*, 2000). Composting biopiles is a process of excavating contaminated soils, amending the soils with a bulking agent (usually straw, hay, saw dust, wood chips, or similar material), placing the soil in piles or windrows and treating the piles with organic materials, nutrients, and air. The biopiles may be static and incorporate installed aeration systems or turned frequently by mechanical means. Bioaugmentation with micro-organisms also occurs to accelerate the remediation. A large range of commonly occurring soil microbial communities are capable of degrading a wide variety of compounds under aerobic conditions, including the aliphatic and aromatic hydrocarbons found in petroleum fuels (Cerniglia, 1984; Rosenberg, 1992). Further, a broad spectrum of microbial communities can be isolated from hydrocarbon-contaminated soils (Jorgensen *et al.*, 2000).

The addition of organics to contaminated soil enhances the activity of specific degraders. To further enhance biotreatment, commercial inocula have been developed to specifically degrade petroleum hydrocarbon-contaminated soils (Morrison, 1997; Neralla and Weaver, 1997). However, the addition of commercially available inocula may not always be effective in accelerating the bioremediation process. For example, Jorgensen *et al.* (2000) reported that no significant improvement in remediation of biopiles containing petroleum hydrocarbon-contaminated soils occurred when augmented with two commercial inocula than compared with piles containing indigenous microbial communities. Their study led to the conclusion that it is more important to create suitable conditions for growth of indigenous bacteria than to introduce exotic species. In most cases, commercial petroleum hydrocarbons inocula only add an additional cost and provide no additional benefit (Jorgensen *et al.*, 2000).

The Liaohe Oil Field, situated in the Liaohe River estuary area, is the third largest oil field in China, and since 1990, supplies an annual crude oil production of 14 to 15 million tons, one-tenth of the national production. In 1998, more than 30,000 tons of soil contaminated with crude oil was reported in this region (Jiang, 2000). The average oil content of the soil was 30%, which means about 10,000 tons of crude oil entered the environment during that year (Jiang, 2000). The Liaohe River estuary area is an important environmental resource for China. It contains the largest reed wetland system in Asia, serves as an important wildlife habitat, and supports agricultural cultivation and extensive shrimp and crab aquaculture. Soil contaminated with crude oil, either by spillage or dumping, causes sustained and serious problems to agriculture and the natural environment in this region.

The crude oil from Liaohe Oil field is uniquely characterized by its high density, high wax content, and high viscosity. These characteristics make soil remediation more difficult than usual. To identify a cost-effective and environmentally acceptable technology, a combined scheme of chemical extraction and biodegradation has been investigated since 1995. This scheme is applied to soil with oil contents exceeding 10%. The remediation technique as currently applied in this region involves two phases: first, the soils are chemically treated to recover as much oil as possible. Chemical recovery reduces the oil content to below 10%. Then *ex situ* biopile composting technology is used to further remediate the soil.

To develop optimal conditions for biopile composting in the Liaohe Oil field, a laboratory-scale experimental program was first developed and reported by Ding *et al.* (1999) and Li *et al.* (1999). In their study, different temperatures, pile moisture contents, pH, and C:N:P ratios were tested using orthogonal statistical designs on contaminated soils with oil contents less than 10% to determine optimal biopile parameter values. Using the results from the laboratory-scale experiments, a larger outdoor experiment consisting of windrow composting biopiles was recently developed (Ding *et al.*, 2000) and specific results from this experiment are reported herein.

Other Chinese researchers have also recently reported laboratory-scale experimental bioremediation experiments e.g., Wei and Qui, 1997; Chang *et al.*, 1998; Sun *et al.*, 1999; Zhang *et al.*, 1999), but our study is the first to report results from an operating, field-scale, windrow, composting biopile. Specifically we report on the effectiveness of composting biopiles for remediation of postchemically treated, crude-oil contaminated soils of the Liaohe Oil Field and examine the contribution of inocula with indigenous and introduced fungi on the re-

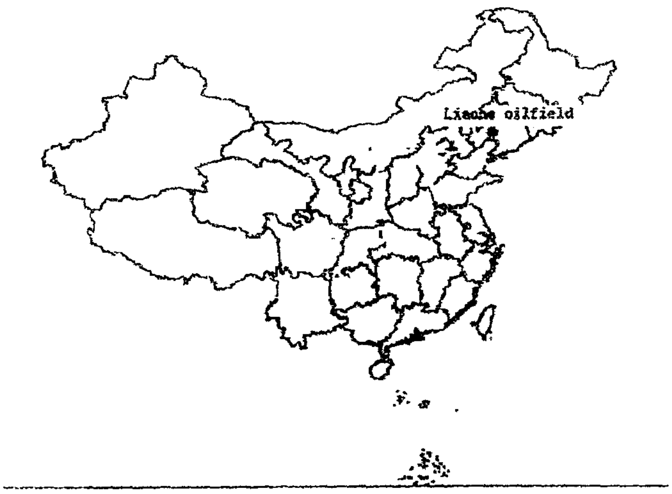

Figure 1. Location of Liaohe Oil Field study site.

mediation process. Four different crude oil compositions were examined, and we also compare and contrast the degradation of these different oil compositions under field conditions.

MATERIALS AND METHODS

Site description

The experimental site is situated at a treatment facility in the Shuguang oil recovery region, which is located centrally in the Liaohe Oil Field (see Fig. 1). Contaminated soils from this region and another 10 oil recovery regions surrounding the Shuguang region can be conveniently transported to this treatment facility.

The composting material treatment area is 20 m long by 10 m wide (200 m^2 in area) and the designed treatment capacity of soils for a biopile windrow is 16 cubic meters. The floor of this area is a 5-mm steel plate, which is raised 0.5 m above the ground surface. Under the floor (steel plate), lies a leachate collection system. Contaminated soils, amended with fertilizer, fungal inocula, and bulking agent were heaped on the treatment area in windrows. Ten biopile windrows (8 × 2 × 0.5 m) were constructed, but only eight windrows were used in this research. The eight windrows contained crude oil-contaminated soils from four different sites labeled A, B, C, and D (see Table 1). Four windrows (one each containing crude oil contaminated soil type, A, B, C, or D) were subjected to inoculation with fungi and bacteria, and another four were kept as a control (no inoculation). Each windrow biopile can treat 8 m^3 soil.

A passive aeration system is used. Venting of each windrow biopile is achieved by insertion of nine PVC pipes of 10 cm diameter, with 10 mm-diameter holes bored on the walls of the pipes and separated every 20 cm. These venting pipes are inserted vertically into the biopile and extend to beneath the composting bed floor. The treatment facility is housed in a waterproof enclosure to prevent the impacts of rain and wind. The operation of the composting biopile experiment commenced on September 2, 1999, and finished on October 25, 1999.

Table 1. Physical and chemical properties of four different crude oils used in this study.

Oil type	*Density g/cm³*	*Asphaltenet + resin %*	*Wax %*	*Condensation point °C*	*Viscosity 50°C mpa·s*
A (Thin)	0.90–0.94	21.99	10.8	nd	93.3
B (High condensation)	0.87–0.88	11.5–24.3	32.1–34.3	nd	6.3[a]
C (Thick-high viscosity)	0.95	31–38	4.0–9.2	8.0–15.0	3680.0–11052.0
D (Thick)	0.93–0.94	21.5–33.91	4.2–7.4	13.0–19.0	566.6

[a] Viscosity at 100°C; nd—not determined.

Table 2. Typical composition and organic content of the soil used in this study.

TN %	*TP* %	*pH*	*Org-C* %	*Water content* %
0.14	0.047	7.4	1.04	9.65

Soil description and preparation

Soils contaminated with crude oil were excavated from different sites around Shuguang. The typical composition, for example, pH, total nitrogen (TN), total phosphorous (TP), organic and water contents of the soils, are summarized in Table 2. These values are supplied from the Liaohe Oil Administration Bureau. In accord with the objectives of the experiment outlined in the introduction, soils containing total petroleum hydrocarbon (TPH) content less than 10% were selected for study. Soils contaminated with four different compositions of crude oil were examined. The physical and chemical characteristics of the four crude oil compositions are presented in Table 1.

Amendment of nutrients and bulking agents

A special complex fertilizer, the product of the Organic Pellet Fertilizer Plant, Panjin City, was applied as nutrient amendment for composting biopiles. This fertilizer is produced using chicken excrement and micronutrients through mixing, heating, sterilization, desiccation, and formation. The typical composition of this fertilizer: organic matter: 39.3% (by wt.); nitrogen: 1.82%; P_2O_5: 5.96%; and K_2O: 2.62%. The pH was 8.2. Approximately 500 kg of fertilizer were mixed with 8 ton of crude oil-contaminated soil. Rice husk from a nearby farm and sawdust from a local timber factory were applied as bulking agents, the ratio of soil to bulking agent was approximately 10:1 on the volume basis. Wood or bark chips are usually recommended as bulking agents due to their high xylogen contents and high resistance to degradation. However, they were not easy to obtain, and rice husks were readily available at minimal cost. The application of the rice husk increases the BOD of the composting material. However, the degradation products of the rice husks are harmless to the soil microbial population, and the heat produced from its degradation enhances TPH degradation. The nutritional properties of the four contaminated soils after amendment with fertilizer and bulking agent are summarized in Table 3. The application of N in this research is higher than other field experiments (Al-Daher *et al.*, 2001), where 50:1 for the C:N ratio was reported.

Preparation and application of inoculum

Soils contaminated by crude oils from different oil recovery regions of the Liaohe Oil Field were collected for the determination of dominant fungi, bacteria, and actinomycete. Separation and identification of fungi, bacteria, and actinomycete were achieved. Lipase activities of different micro-organisms were detected using Tween 80 and neutral red methods as described by Fan (1989). Three indigenous fungi with high lipase activities, *Mucor* sp., *Cunninghamella* sp., and *Fusarium* sp. were detected and consequently used in the inoculum. The white rot fungus, *Phanerochaete chrysosporium*, supplied from the Chinese Academy of Forestry, was also used in our inoculum. The fungi were first cultured in potato dextrose broth (PDB) for 5 to 7 days; when the spore was formed, cultures were propagated in PDB media for another 3 to 4 days in conical flasks on a shaker. All cultures were incubated at 25°C, with pH held constant at 6. Finally, the fungi were immobilized on solid culture medium, a mixture of rice husk and wheat bran in the ratio of 3:1. Potato juice was used to adjust the moisture of solid cultural medium to 20% and then further propagated for 3 to 5 days at 25°C. Before application to the biopiles, the fungal inocula (consisting of four different species) were thoroughly mixed and equally applied to each of the four different crude oil-composting biopiles.

Table 3. Nutritional and physical properties of four crude oil-contaminated soils after amendment of fertilizer and bulking agents.

Oil type	*Moisture* %	*K-N* %	*TP* %	*TK* %	*Org-matter* %	*pH*	*TPH*[a]	*C:N*
A	14.40	0.31	0.24	0.08	12.10	7.60	5.50	23:1
B	18.10	0.37	0.35	0.11	14.39	6.80	7.72	23:1
C	17.61	0.59	0.40	0.13	10.47	7.80	2.58	10:1
D	16.80	0.42	0.32	0.15	13.08	8.00	4.16	18:1

[a] TPH: g/100 g dry soils.

Table 4. First-order degradation regression equations of TPH residues in soils g/kg.

Oils		R^2	n	*Significance*
A	$y_a = 48.89\ e^{-0.0152\ t}$	0.833	5	[a]
B	$y_b = 70.91\ e^{-0.0166\ t}$	0.788	5	[a]
C	$y_c = 25.56\ e^{-0.0097\ t}$	0.911	5	[a]
D	$y_d = 40.33\ e^{-0.0113\ t}$	0.978	5	[b]

Key: [a]5% level of significance ($p < 0.05$); [b]1% level of significance ($p < 0.01$).

Enumeration of microbial populations

Microbial densities of total fungi and bacteria in soil were measured as the number of colony-forming units (CFU) per gram of dry soil using the agar-plate technique (Xu, 1983; Yateem *et al.*, 1998). Colonies of microbes from contaminated soils and composting material were first isolated from tryptone-glucose-yeast (TGY) plates at high dilutions to represent the more numerous fungi and bacteria and then purified by three times on TGY solid medium to enumerate others. The identification of microbes was performed according to the methods of the Institute of Microbiology (1978a,b).

Other methods

Total petroleum hydrocarbons (TPH) was determined by gravimetric measurement (National EPB, China, 1986). A portable gas analyzer, (CYES-II, Xuelian Instrument Manufacture, Shanghai), was used to measure O_2 and CO_2 concentrations. The probes were placed in the compost 15 cm beneath the surface of the biopile. The compost temperature was also placed 15 cm below the soil surface. The temperature probe was encased in a protective cast. Soil samples (100 g) were collected weekly at random locations to determine moisture content. The samples were oven dried at 65°C until a predetermined weight was reached. Analyses of oil compositions were performed using the methods of Xie (1987).

RESULTS AND DISCUSSION

Kinetics

The degradation pattern of TPH in the composting biopiles inoculated with indigenous fungi followed a typical first-order degradation process. Regression was applied to four composting biopiles each containing a different composition of crude oil (see Table 1). The following model $y = a\ e^{bt}$, where a and b are constants

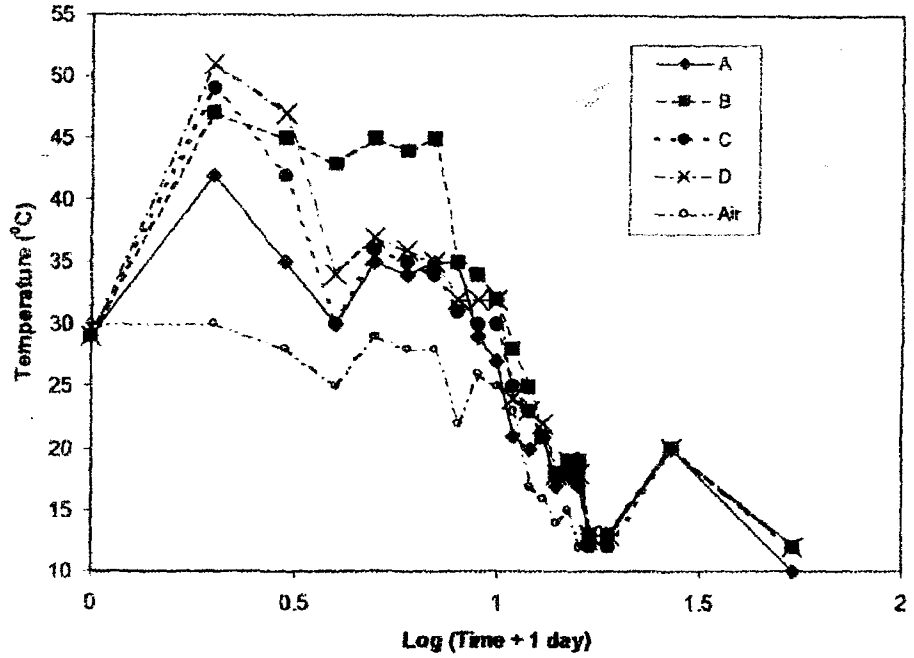

Figure 2. Changes in compost temperature for each of the four different crude oil-contaminated soils over 53 days of operation.

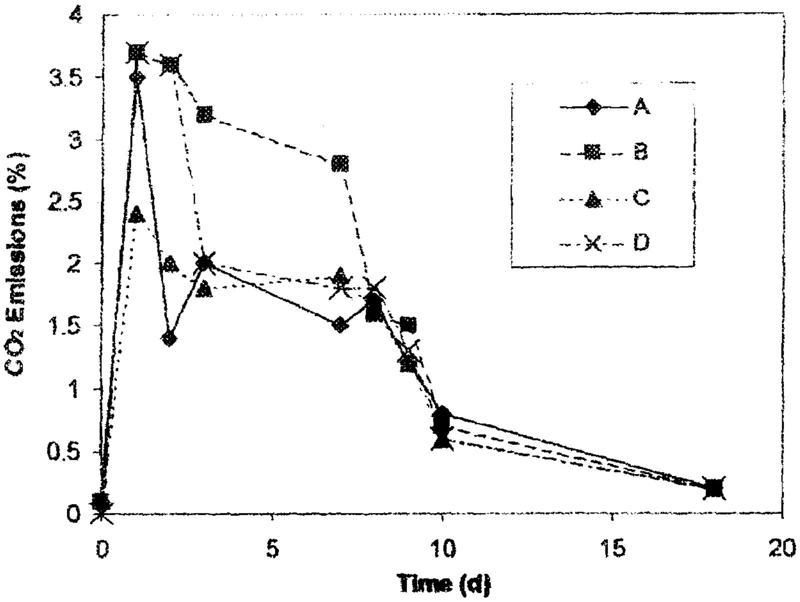

Figure 3. Changes in CO_2 emissions for each of the four different crude oil-contaminated soils for the first 18 days of operation.

fit by the least-squares regression, was applied to the degradation data collected from each biopile. The initial value of TPH residues in soils (*a*), the degradation coefficient (*b*), and the statistical summaries for each composting biopile is summarized in Table 4. These values were determined by least-squares regression. All degradation partial regression coefficients (*b*) ranged from -0.0097 to -0.0166, indicating a very similar rate of decay between different crude oil contaminated soils. This pattern is similar with other reported studies (e.g., Dott *et al.*, 1989; Jorgensen *et al.*, 2000). The coefficients of determination (R^2) ranged from 0.788 to 0.978, and they were all statistically significant, indicating strong first-order degradation kinetics.

Changes in temperatures and CO_2 contents inside each of the four inoculated composting biopiles are presented in Figs. 2 and 3, respectively. The temperatures and CO_2 contents of the composting biopiles reached the highest values on the second day. The temperature of composting biopiles increased by as much as 10 to 15°C in one night without any heat source or thermal isolation. The temperature of the biopiles approached air temperatures around 20 days later. Degradation of TPH was still observed at 10°C air temperature. The patterns of change in CO_2 contents were very similar to the changes in biopile temperatures. For the first 10 days, a very high concentration of CO_2, which reached as high as 3.7%, occurs. To avoid possible poisoning of microbes in the biopiles, the compost was turned and mixed every day in the first week to replenish oxygen.

The treatment cycle, as indicated by the rapid degradation of TPH in the biopiles, is important in a bioremediation process because it is closely related to the operating costs. The faster the degradation, the cheaper the overall cost. The optimization of operating parameters, that is, the degradation coefficient and peak values of temperature and CO_2 emission, are useful to evaluate the conditions of operation. Jorgensen *et al.* (2000) reported 70% degradation of petroleum hydrocarbon with initial concentration of 700 to 2,400 mg (kg dry wt.)$^{-1}$ over 150 days; Filauro *et al.* (1998) reported 48% degradation from an initial TPH of 10,000 mg kg^{-1} and 60% degradation from initial TPH of 16,000 mg kg^{-1}. These were all field-scale biopiles with wood chips as bulking agent. In this study, 38.4 to 56.7% degradation of initial crude oils 25,800 to 77,200 mg (kg dry wt.)$^{-1}$, were obtained using rice husk as bulking agent after 53 days operation.

Characteristics of crude oils on their degradation

The physical and chemical characteristics of the four crude oils and soils as reported from the Liaohe Oil Administration Bureau were presented in Tables 1 and 3. Typical crude oil has a density of 0.85 g cm^{-3} (Dutta and Harayama, 2000). Oils from the Liaohe Oil Field are famously characterized with higher density, high wax con-

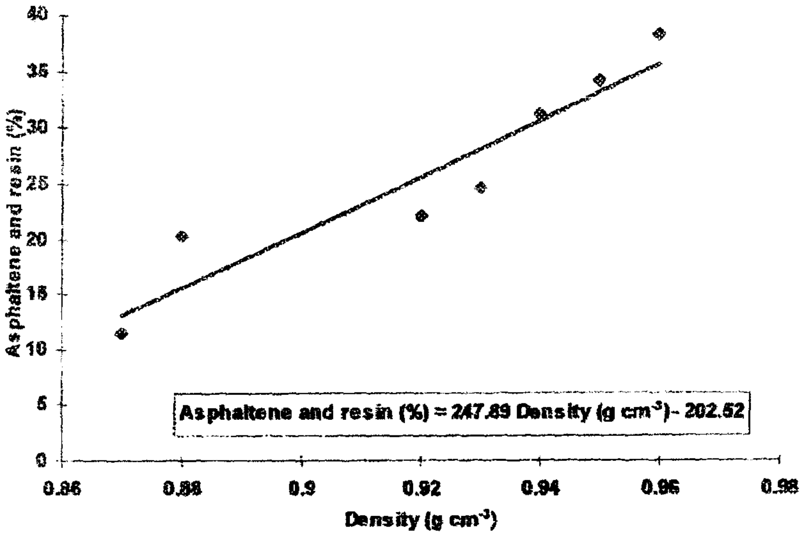

Figure 4. Relationship between densities of oils and asphaltene and resin.

tents, and high viscosity. We found that the contents of asphaltene and resin increased linearly with the increase of oil density (see Fig. 4). A good linear regression between the densities of crude oils and the contents of asphaltene and resin was found ($y = 247.89$ x -202.52; $R^2 = 0.884$, $n = 7$, $p < 0.01$). The data in Fig. 4 were measured, and are slightly different to the data supplied by the Liaohe Oil Administration Bureau (Table 1).

It is normally believed that the degradation of crude oil is mainly related to the degradation of aromatic fraction. However, we found that asphaltene and resin composition has a more important effect on oil degradation rates (see Fig. 5). A statistically significant linear regression between the asphaltine and resin content for each of the four different crude oil contaminated soils and the degradation of TPH was found ($y = -1.18 \times +79.31$; $R^2 = 0.968$, $n = 4$, $p < 0.05$). Wax is considered to be difficult for remediation because of its low solubility, but interestingly, we found that the high concentration oil (B) (see Table 1) had the fastest degradation rate (see Fig. 5) and, therefore, wax was not a limiting factor for remediation in this case. The wax is an orthoalkane hydrocarbon with C_9 and C_{16}.

These relationships are very useful to predict the degra-

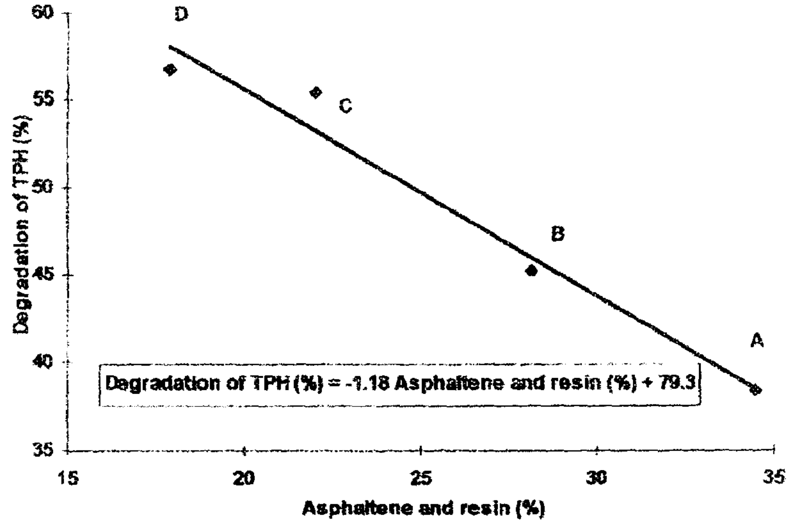

Figure 5. Relationship between asphaltene and resin with the degradation of TPH.

Table 5. Spectrum of major microbial genera found in the four crude oil-contaminated soils.

Microbial communities	*Genera*	*Sources*[a]
Fungi	*Mucor* sp.	A, B, C, D
	Cunninghamella sp.	B
	Fusarium sp.	C, D
	Saccharomyces sp.	C
	Penicillium sp.	C
	Aspergillus sp.	D
Bacteria	*Zoogloea* sp.	A, B, C, D
	Flavobacterium sp.	A, B, C, D
	Bacillus sp.	A, B, C, D
Actinomycete	*Streptomyces* sp.	A, B, C, D

[a] Contaminated soils described in Table 2.

dation patterns of crude oil in soil, and may determine if further technical countermeasures should be considered to increase the degradation rate of asphaltene and resin.

Effects of inocula with indigenous fungi on TPH degradation

Some researchers reported that polycyclic aromatic hydrocarbons can be rapidly oxidized by lignin-degrading white rot fungi (Barr and Aust, 1994; Bezalel *et al.*, 1996; Bogan and Lamar, 1996; Kotterman *et al.*, 1997). Zheng and Obbard (2000) investigated the removal of PAHs from soil using white rot fungus *Phanerochaete chrysosporium*. Yateem *et al.* (1998) studied the roles of three white rot fungi including *P. chrysosporium* in remediating oil-contaminated soil. Three indigenous fungi with high lipase activities, *Mucor* sp., *Cunninghamella* sp., and *Fusarium* sp. were detected and consequently used in the inoculum. The white rot fungus, *P. chrysosporium*, supplied from the Chinese Academy of Forestry, was also used in our inoculum.

The indigenous microbes commonly found in the four crude oil-contaminated soils were isolated using the dilute plate method and identified using lipase activities tests. The genera for the commonly found microbial communities are summarized in Table 5. The genera with the highest enumeration and lipase activity were described as the dominant genera. Bacteria and actinomycete have extensive distribution in each of the four different crude oil contaminated soils. However, except for *Mucor* sp., fungi have quite a narrow spectrum in the contaminated soils we examined. This may be partly due to the sensitivity of fungi to some composition of oils or adaptability to a specific contaminated environment.

Lipase activities are a qualitative measure of the capability of the microbes to degrade the oil. Lipase activities of dominant microbial communities isolated from the four contaminated soils are presented in Table 6. Most microbial communities isolated from the contaminated soils recorded lipase activity, and therefore a potential for crude oil degradation. Fungi generally have higher lipase activities especially for *Mucor* sp. and *Cunninghamella* sp., and then followed by *Fusarium* sp., *Penicillium* sp., and *Aspergillus* sp. Bacteria and actinomycete recorded similar lipase activities; *Zoogloea* sp. was the highest in this group. The neutral red method detected higher activities of fungi than Tween 80, and is therefore recommended for lipase activity analyses of fungi. Tween 80 is more suitable for bacterial analyses because of its high sensitivity. On the basis of lipase activity analyses, the indigenous genera *Mucor* sp., *Cunninghamella* sp., and *Fusarium* sp. were selected for inclusion in the inoculum used for TPH degradation and the introduced fungus *P. chrysosporium*, was also selected for contrast.

Table 6. Lipase activities of superior microbial communities isolated from the oil contaminated soils.

Community	*Genera*	*Tween 80*	*Neutral red*
Fungi	*Mucor* sp.	+	+++
	Cunninghamella sp.	++	+++
	Fusarium sp.	++	++
	Saccharomyces sp.	+	+
	Penicillium sp.	++	++
	Aspergillus sp.	++	++
Bacteria	*Zoogloea* sp.	++	++
	Flavobacterium sp.	−	+
	Bacillus sp.	+	+
Actinomycete	*Streptomyces* sp.	++	+

−: no activity; +: low activity; ++: strong activity; +++: very strong activity.

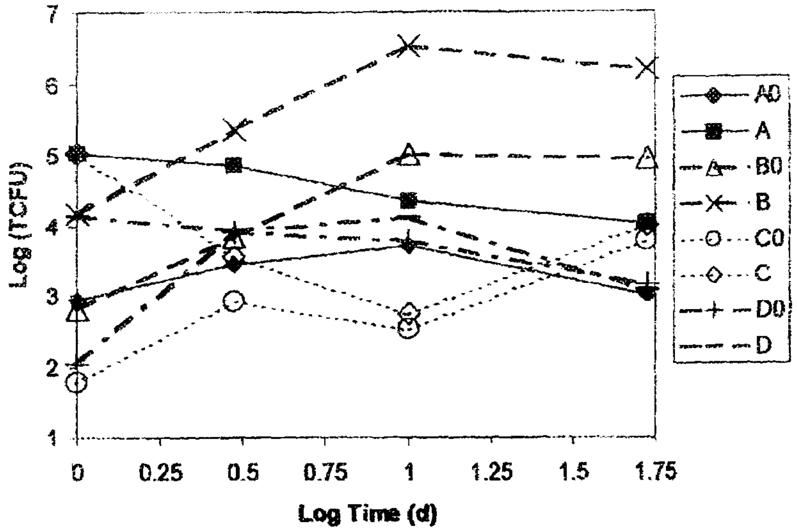

Figure 6. Changes in total CFU of fungi for crude oil-contaminated soils inoculated with bacteria and fungi (A, B, C, D) compared to those not inoculated (A0, B0, C0, and D0).

Effects of inocula on the distribution and CFU of fungi in the composting soils

The total fungi counts expressed as colony-forming units (CFU) per gram of dry soil were assayed in both inoculated soils (termed A, B, C, and D) and in control soils without inoculation (termed A0, B0, C0, and D0). The comparisons of two treatments (inoculated and control) for four contaminated soil types with time is presented in Fig. 6. The inoculation of indigenous fungi have obviously increased the CFU in soils A, B, and C, but only slightly increased the total CFU in soil D. For soil A and B, the CFUs in inoculated soils are usually higher by an order of 10-fold when compared to the control soils. The number of micro-organism in soil will obviously be closely correlated with the degradation rate of organic pollutants. Crude oil-contaminated soil type B recorded the highest total CFU. The composition of this soil obviously provides more carbon active surfaces that promotes microbial metabolism of hydrocarbons.

From the results of reisolation and reidentification of fungi genera, we found that fungi genera only persisted in the soils where they were isolated. For example, *Cunninghamella* sp. was found only in soil type B, and should not be isolated from other soils after inoculation and composting. The introduced fungi *P. chrysosporium* could not be isolated from any of the four soil types after inoculation and composting, which strongly suggests that inoculation of oil-contaminated sites with nonindigenous species is likely to fail. Our results confirm the results of other recent studies (e.g., Jorgensen *et al.*, 2000). The culture of indigenous genera is more likely to raise the TPH degradation rate than the introduction of exotic genera.

After 53 days (marking the end of the experiment) decreases of total CFU of fungi were observed in both inoculated and contrast soils A, B, and D. However, an increase of CFU for soil C was recorded after 53 days. Decreases of CFU are caused by a deficiency of metabolic substrates, by aging microbial communities, or by preferential leaching and transport (Stagnitti, 1999). In

Table 7. Effects of inoculation with indigenous fungi on the TPH degradation (%).

Time (day)	*A0*[a]	*A*	*B0*[a]	*B*	*C0*[a]	*C*	*D0*[a]	*D*
0	0	0	0	0	0	0	0	0
10	7.45	19.09	14.41	17.49	11.21	13.10	10.68	12.74
18	27.38	39.45	34.66	29.53	18.19	20.93	22.44	22.36
26	44.19	50.00	51.78	55.96	24.14	28.29	34.83	31.25
53	53.73	55.45	55.92	56.74	33.19	38.37	41.24	45.19

[a] A0, B0, C0, D0 are control soils with no inoculation.

Table 8. The residues of composition of TPH in soil (g/kg).

Oil		*TPH*	*Aromatic*	*Saturated*	*Asphaltene & resin*
A	Day 0	54.28	10.67	26.09	8.54
	Day 53	24.54	5.98	8.87	5.35
B	Day 0	75.84	10.82	44.13	8.07
	Day 53	33.40	6.12	14.18	6.26
C	Day 0	25.58	4.89	6.46	8.12
	Day 53	15.90	3.31	3.66	7.31
D	Day 0	40.93	7.91	20.14	7.16
	Day 53	22.79	5.30	8.28	5.93

our experiments, given the time frame, the homogenization of the compost, and uniformity of compost moisture content, we can eliminate the later two possible causes and conclude that the reason for the decrease in CFU was mainly caused by the shortage of effective carbon and energy sources, which were exhausted by the rapid degradation. To increase total TPH degradation rates, the addition of effective carbon and energy sources as substrate is recommended. In soil type C, the high concentrations of asphaltene and resin obviously increased the retention time of micro-organisms. The total CFU after 53 days was still in the growth stage, but obviously would eventually decrease in a manner similar to the other soil types.

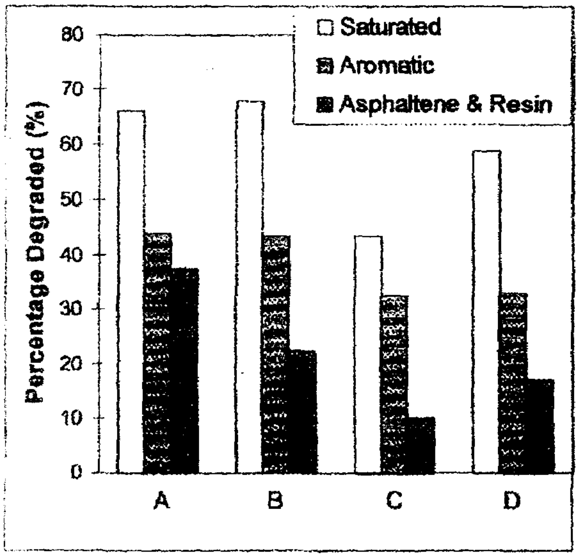

Figure 7. Percent degradation of different compositions of THP in soils after 53 days.

Effects of inoculation on TPH degradation

The effects of inoculation on the rate of TPH degradation for each of the four crude oil-contaminated soils are presented in Table 7. Soils that were inoculated (A, B, C, D) always recorded a higher percentage increase in TPH degradation than the control soils (A0, B0, C0, D0). After 53 days the TPH degradation was 3.2, 1.5, 15.6, and 9.6% higher for inoculated soils A, B, C, and D, respectively, compared to their control. For soil types A and B, the inoculation only slightly increased the percentage degradation of TPH after 53 days. For these soil types, however, the inoculation did accelerate the TPH degradation substantially from day 10 to 26, when compared to the control soils. Much higher percentages were recorded for soil types C and D. The early period of rapid degradation of TPH in inoculated soils, particularly experienced in soil types A and B suggests that inoculation may substantially reduce the treatment time.

The degradation of different compositions of oil

The residues and degradation amounts of total petroleum hydrocarbons, their aromatic and saturated fractions, and asphaltene and resin content for each of the contaminated soil types are shown in Table 8. The percentage degradation rates are presented in Fig. 7. The initial values in Table 8 are actual values measured at day 0, and are therefore slightly different to the least-squares fitted values presented in Table 4. The TPH values in Table 8 are also slightly different to the values presented in Table 3. The values in Table 3 are average values from two different samples taken at random locations in the compost. The values in Table 8 are a single value measured at the monitor point. After 53 days of treatment, the order of the remediation efficiency of different composition of TPH are: saturated hydrocarbons > aromatic > asphaltene and resin. The removal rates of saturated hydrocarbons were from 43.43 to 67.87%. Soils containing high condensation and thin oils had the highest degradation rates, from 66.0 to 67.87%. The degradation rate of saturated hydrocarbons in thick oil-contaminated soils was 43.34%. The range of degradation rates for aromatic hydrocarbons were from 32.31 to 43.96%, and as with saturated hydrocarbons, the soils containing high condensation and thin oils had higher rates than soils contaminated with thick oils. Asphaltene and resin were the most difficult to remediate with removal rates from 9.98 to 37.35%. The key problem for TPH remediation is to create optimal conditions for degrading asphaltene and resin.

Effects of nutrient and carbon addition on TPH degradation

A complex fertilizer containing N, P, K, and organic matter was added to the composting biopiles to accelerate the bioremediation. The rate of application was determined from a previous small-scale laboratory experiment (Ding *et al.*, 2000). In this experiment, the C:N ratios were controlled to levels between 10:1 to 23:1.

Some studies showed that the degradation of xenobiotic compounds by fungi was enhanced under nutrient limited conditions (Leatham and Kirk, 1983; Katayama *et al.*, 1992). However, for soils with very high concentrations of TPH, the application of N and P, under a controlled constant C:N:P ratio, was also suggested to induce peroxidases enzymes production in some fungi strains, and thus can positively reduce the degradation rate (Kaal *et al.*, 1995). In this research, TPH degradation was obviously accelerated by N, P, and K application. Our results demonstrate that under controlled ratios of 10:1 to 23:1 of C:N, nutrition is not the key factor controlling TPH degradation. For example, oil C had the highest C:N ratio but lowest TPH degradation rate.

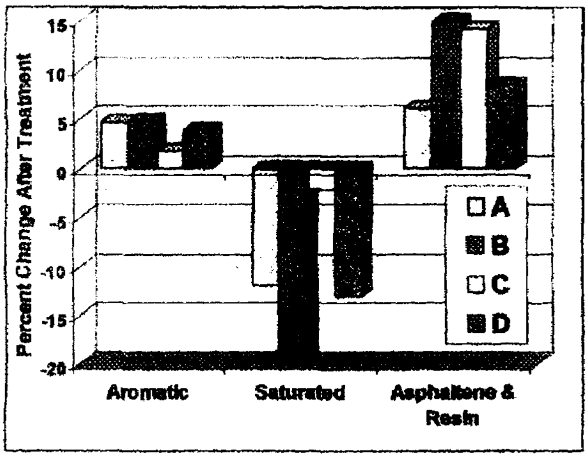

Figure 8. Percent change in different compositions of TPH after treatment with complex fertilizer.

On the other hand, the supply of effective carbon sources may be an important factor influencing TPH degradation. The biological degradation of most aromatic compounds with high molecular weight are generally initiated by cometabolic forms (Heitkamp and Cerniglia, 1989), and some compositions of mineral oil can act as cometabolic substrates for degradation of aromatic compounds (Raymond, 1972). In other words, the degradation of some aromatic compounds requires some consumption of saturated hydrocarbons. In this study, the aromatic hydrocarbons are degraded together with the saturated hydrocarbons (see Fig. 8). We found a strong correlation between rates of degradation for aromatic and saturated hydrocarbons for the four contaminated soil types. The correlation coefficients ranged from 0.56 to 0.76. After 53 days of operation, the proportions of aromatic hydrocarbons, asphaltene, and resin increased in the residues of TPH and that of saturated hydrocarbons decreased (Fig. 8).

After exhausting the saturated hydrocarbons and other cometabolic substrates in the soil, new carbon sources should be added to soils containing oils with high aromatic proportions to ensure a continuous high level of degradation of aromatic hydrocarbons. In this study, the concentration of asphaltene and resin is as high as 31.75% of TPH (for soil type C), and the degradation of asphaltene and resin is more difficult than aromatic hydrocarbons. Our experiment showed that some organic matter such as high molecular aromatic hydrocarbons are not easily degraded by micro-organisms. The degradation mechanism of these compounds is usually by cometabolism, that is, the degradation requires the existence of some compounds that is easier to be utilized as carbon sources. Consequently, the purposes of the carbon addition are to maintain a satisfactory C, N, and P ratio and simultaneously enhance enzymatic activity. The degradation of aromatic hydrocarbon continued with the degradation of saturated hydrocarbons. At the end of degradation, the ratio of aromatic hydrocarbons to saturated hydrocarbons increased, indicating that the consumption of saturated hydrocarbons is higher than that of aromatic hydrocarbons. For further degradation of aromatic hydrocarbon and asphaltene and risen, it is therefore essential to add new carbon sources.

The composting biopiles are still being continuously operated today to the further remediate persistent compounds. This process may be expected to last more than 1 year (Balba *et al.*, 1998). Because aged TPH is strongly adsorbed to clay and organic fractions of the soil, the biodegradability and bioavailability of TPH will decrease rapidly, the replenishment of carbon sources as cometabolism substrate and the application of surfactant will therefore be necessary to further enhance the remediation. We are also currently investigating the eco-toxicologic impacts of the remediation. A combination of concentration control of pollutants and toxicologic tests will be useful to determine the acceptable level of TPH residue in soil.

CONCLUSIONS

We reported the results of a full-scale study of bioremediation of oil-contaminated soils from the Liaohe Oil Fields in China using composting biopile windrows technology. Inoculation of the crude-oil contaminated soils with indigenous fungi was found to accelerate the degradation of TPH in all soils types. Lipase activity is useful for the screening of indigenous microorganisms with high TPH degrading ability. For nonsterilized soil, the exotic fungus had no significant acceleration on degradation of TPH, and it could not be isolated from any of the four soil types after composting.

The TPH degradation patterns followed typical first-order reactions, with degradation rates ranging from 38 to 57% of THP after 53 days operation of composting biopiles in windrows. Thick oils tended to have the lowest rates of degradation. Asphaltene and resin were found to have the lowest rates of degradation when compared to saturated and aromatic fractions of THP. A strong correlation between the degradation of saturated and aromatic hydrocarbons and crude oil densities was found. The degradation of TPH is a result of degradation of four components in which saturated hydrocarbons had the highest degradation rate and asphaltene and risen had the lowest. In the latter case, the degradation was as low as 10% after 53 days of operation. The degradation of TPH is closely related with the proportion of different components. We found a positive correlation between asphaltene and resin percent and density, and a negative correlation between degradation rate of TPH and asphaltene and resin percent. This indicates that the degradation rate of TPH will be slower with higher density crude oils.

Amendment using enriched chicken excrement as nutrition and rice husk as a bulking agent was successfully applied, and consequently, N and P were not limiting factors in this study; however, the addition of new carbon sources should be considered at the late stage in the bioremediation process to accelerate the degradation of aromatic hydrocarbons and asphaltene and resin.

ACKNOWLEDGMENTS

This work was supported by the Chinese Academy of Sciences, National Natural Scientific Research Foundation of China, the Administrative Bureau of the Liaohe Oil Field, the Australian Research Council Linkage-International Grant #LX0211202, and the Deakin University Research Grant Scheme. The authors are also grateful for careful and useful comments from two anonymous referees.

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