JOURNAL OF PURE AND APPLIED MICROBIOLOGY, September 2013. Vol. 7(3), p. 1979-1987

Degradation of Petroleum Hydrocarbons in Composting Process of Cattle Manure under Different Conditions

Rugang Wang, Qinglong Liu and Jingchun Tang*

Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), College of Environmental Science and Engineering, Nankai University, Tianjin - 300 071, China.

(Received: 02 June 2013; accepted: 20 September 2013)

Decomposition of petroleum hydrocarbon is thought to be important for disposal and management of waste containing petroleum hydrocarbons. Composting process of cattle manure spiked with petroleum hydrocarbons was characterized under different temperatures and pollutant contents. The pH of compost samples decreased after composting in most treatments except that with higher oil content. The highest Total Petroleum Hydrocarbons (TPH) degradation rate 42.2% was found at 1% TPH concentration and temperature of 50 °C after 21 d. The contents of saturated hydrocarbons (SH) fraction declined, and aromatic hydrocarbons (AH) decreased first and increased at the end of composting. The polar fraction (PF) behaved the same as that of AH at 20 °C, but decreased in the end at 50 °C. The 3-ring and 5-ring Polycyclic Aromatic Hydrocar-bons (PAHs) decreased and 6-ring PAHs increased at 20 °C, but 6-ring PAHs increased first and decreased at 50 °C. The catalase activity (CA) and polyphenol oxidase activity (POA) were inhibited under higher temperature and pollutant content, while dehydro-genase activity (DA) increased. The microbial diversity increased with higher incuba-tion temperature as shown by denaturing gradient gel electrophoresis (DGGE) analysis.

Keywords: Composting; Total Petroleum Hydrocarbons (TPH); Polycyclic Aromatic Hydrocarbons (PAHs); Enzymatic activity; Microbial diversity.

The release of crude oil into the environment by oil spills is receiving worldwide attention. Many accidents can cause soil pollution (Zhou *et al*., 2005) and, for this reason, techniques have been developed to clean up petroleum pollution. Biological treatments are more efficient and cheaper than chemical and physical ones. In relation to biological treatment, the bioremediation technology is being employed for the degradation of crude oil in soil matrix through transforming petroleum hydrocarbons into less toxic compounds by microorganisms (Schinner *et al*., 1996a). Nutrient concentration played a very important role on the biodegradation of petroleum hydrocarbons in soil (Chaineau *et al*., 2005). Several studies have reported on the potentials of composted materials in the biodegradation of chemical pollutants (Meyer and Steinhart, 2000; Trejo-Hernandez *et al*., 2007; Wischmann and Steinhart, 1997). The degradation of oil was significantly enhanced by the addition of organic amendments relative to straight soil in the soil-composting system with different compost: soil ratios (Namkoong *et al*., 2002).

High degradation rate of petroleum hydrocarbons can be achieved during composting process. Petroleum hydrocarbons were degraded

* To whom all correspondence should be addressed.
Tel.: +86-22-83614117; Fax: +86-22-83614117;
E-mail: tangjch@nankai.edu.cn

by 80% after only 15 d of composting in the presence of food waste (Joo *et al*., 2007). Composting process can be affected by many influencing factors. Temperature is an important factor that affected the degradation rate of organic substances during composting (Tang *et al*., 2007). This suggested that degradation of petroleum hydrocarbons in compost can also be affected by temperature. On the other hand, petroleum is toxic (Sun and Zhou, 2007) and the toxicity of petroleum hydrocarbons is strongly correlated with the lower boiling-point fractions and especially with those within the C10-C19 range (Brils *et al*., 2002; Jonker *et al*., 2006). Higher TPH content resulted in a lower degradation rate due to the toxicity of petroleum hydrocarbons on organisms in the bioremediation process (Cai *et al*., 2010; Peng *et al*., 2009). However, the effect of TPH content on the composting microorganisms is still unknown to us.

Microbial degradation plays a major role in the removal of petroleum hydrocarbons in composting process. The enzymatic activity can be used to describe the effects of poisonous compounds on the soil microbiological population (Ratsep, 1991). At the same time, PAHs is an important component and can also be degraded extensively during composting process within a shorter time (Jorgensen *et al*., 2000; Lu *et al*., 2010). Petroleum hydrocarbons are complex mixture of hundreds of components that can be separated into saturates, aromatics, resins and asphaltenes. The saturated hydrocarbons are usually the most abundant, while PAHs cause the greatest concern because of their toxic and genotoxic potentials (Vila and Grifoll, 2009). Some high ring PAHs is toxic and resistance to biodegradation even after a relatively long time span of 370 d (Kriipsalu *et al*., 2008). The fluctuation of PAHs is an important factor in determining the effectiveness of composting to decrease the ecological toxicity of petroleum hydrocarbons.

The aim of this work was to investigate the effect of different TPH concentrations and different temperatures on the degradation of TPH fractions and different PAHs. The change of microbial activity and diversity during the composting process was also characterized for a better control of microbial community in TPH contaminated composting process.

MATERIALS AND METHODS

Composting materials and experimental condition

Six plastic flower pots marked treatments 1 to 6 were loaded respectively with 300 g cow manure from a cattle farm in Tianjin, China, which had been pre-sieved through a 1 mm sieve. The petroleum content of composting materials was set to 0, 5%, 0, 1%, 5%, 10% (of dry mass) in the six treatments by spiking crude oil obtained from Shengli oil field in Shandong province, China. The moisture content was adjusted to 55% with distilled water. Covered with plastic wrap, the treatments 1 and 2 were put into incubator at 20 °C, and the treatments 3, 4, 5 and 6 were incubated at 50 °C. Water was replenished to keep the 55% moisture content every 3 d.

Analysis of water content and pH

Appropriately 100 g sample were taken at the beginning of composting, the 7th d, the 14th d and the 21st d and saved at -20 °C in refrigerator for the analysis of microbial properties. The moisture content for each sample was determined by weighing 5.0 g sample in an aluminum container and drying it at 105 °C for 12 h. The container was weighed and the moisture content was quantified. For pH analysis, 10 g dry sample and 25 ml distilled water were mixed and shaken for 30 min in a flask. After filtration, the pH value was determined by a pH meter.

Determination of the TPH, fractional components (SH, AH and PF) and the PAHs contents

5 g sample with an amount of Na_2SO_4 (pre-baked at 400 °C for 6 h) was wrapped with filter paper pre-soaked in dichloromethane. The paper parcel was put into 50 ml centrifuge tube and 20 ml dichloromethane was added. The tube was ultrasonically extracted for 10 min. Then the paper parcel was transferred to Soxlet extractor and the liquid was removed into a round-bottomed flask pre-baked to constant weight. The tube was washed 3 times by total 130 ml dichloromethane and the washings were collected into a round-bottomed flask. Soxlet extraction was carried out at 54 °C for 12 h and then the round-bottomed flask was bathed under 54 °C to evaporate dichloromethane. The amount of residual TPH was determined gravimetrically (Mishra *et al*., 2001).

Further fractionation of TPH containing SH, AH and PF was practiced by the silica gel

column chromatography followed by the gravimetric analysis (Mills *et al*., 1999; Mishra *et al*., 2001; Peng *et al*., 2009). A glass column ($ 10 mm×300 mm) was filled with 12 cm activated silica gel (pre-baked at 300 °C for 4 h) and 1 cm dry sodium sulfate (pre-baked at 400 °C for 6 h) on the upper layer. TPH extracts were dissolved in n-hexane and separated into soluble and insoluble fractions (asphaltene). Asphaltene was removed by n-hexane precipitation. The soluble fractions were loaded on the top of the silica gel column, and eluted by solvents with different polarities. The saturated hydrocarbons were eluted by n-hexane, followed by an n-hexane/dichloromethane (1:1) mixture to obtain aromatic hydrocarbons. Finally, methanol was employed to elute polar (resin) fraction.

Sixteen PAHs were analyzed: Naphthalene (Nap); Acenaphthylene (AcPy); Acenaphthene (Acp); Fluorene (Flu); Phenanthrene (PA); Anthracene (Ant); Fluoranthene (FL); Pyrene (Pyr); Benzo (a) anthracene (BaA); Chrysene (CHR); Benzo (b) fluoranthene (BbF); Benzo (k) fluoranthene (BkF); Benzo (a) pyrene (BaP); Dibenzo (a,h) anthracene (DBA); Benzo (g,h,i) perylene (BghiP); Indeno (1,2,3-cd) pyrene (IND). The contents of PAHs were analyzed by GC-MS D5975 (Agilent Technologies, the U.S.) (Antizar-Ladislao *et al*., 2006). The method to obtain the AH had been described above. The GC inlet was operated in pulsed (0.90 min, 30.0 psi) splitless mode at 270 °C with helium as carrier gas. The injection volume was 1 ul and the inlet purged at 50 ul min^{-1} in 1 min after injection; the column flow was maintained at 1 ml min^{-1}. Separation was achieved using an DP-5MS column (19091S-433 30 m ×0.25 mm ×0.25 mm).The temperature program comprised 70 °C for 2 min, 10 °C min^{-1} to 300 °C where it was held until the end of the analysis (35 min). The MS transfer line was 280 °C providing conductive heating of the MS source to about 230 °C. The instrument was tuned using perfluorotributylamine. The MS was operated in selective ion monitoring (SIM) mode. The 16 US EPA PAHs (quantification ion/confirmation ions), internal standards (quantification ion/confirmation ion) and surrogates (quantification ion/confirmation ions) for SIM GC-MS mode were: naphthalene (128/127, 129, 102), naphthalene-d8 (136/137, 134, 108), 1-fluoronaphthalene (146/120, 125), 2-fluorobiphenyl (172/171, 170), acenaphthylene (152/151, 153, 76), acenaphthene (154/153, 152), acenaphthene-d10 (164/162,160, 163), fluorene (166/139, 165), phenanthrene (178/165, 163, 82,176), anthracene (178/179, 176, 89), fluoranthene (202/200, 101, 203), pyrene (202/200, 201, 101, 203), benzo[a]anthracene (228/226, 229), chrysene (228/226, 230, 113), benzo[b]fluoranthene (252/250, 253, 126), benzo[k]fluoranthene (252/253, 250, 126), benzo[a]pyrene (252/207, 253, 250, 126), indeno[1,2,3-c,d] pyrene (276/276, 279, 138), dibenzo[a,h]anthracene (278/279, 139, 276), benzo[g,h,i]perylene (276/138, 137, 277). The GC-MS system was calibrated prior to the analysis of samples using calibration standards.

Measuring methods of enzymatic activity

Catalase activity (CA) was determined by $KMnO_4$-titration. 5 g dry sample was put into a conical flask respectively, adding 40 ml distilled water and 5 ml hydrogen peroxide (0.3%). There was no sample in the CK. Covered with plugs, the flasks were shaken with the speed of 120 r min^{-1} for 30 min. Then 5 ml H_2SO_4 (3 N) was added to end the reaction. After filtration, 25 ml filtrate was titrated to be red with $KMnO_4$ solution (0.1 N). Values of CA were expressed as ml of $KMnO_4$ solution (0.1 N) used g^{-1} and were presented as an average of three replicates.

Dehydrogenase activity (DA) was determined according to standard procedures (Method 05.04-B) provided by the US Department of Agriculture and US Composting Council. Values of DA were expressed as mg of triphenyl formazan (TPF) released g^{-1} $24h^{-1}$ and were presented as an average of three replicates.

The polyphenol oxidase activity (POA) was determined according to the method of Jiang et a1.(Jiang *et al*., 2002), by adding 0.5 ml of enzyme preparation to 3 ml of 500 mmol L^{-1} catechol (100 mmol L^{-1} sodium phosphate, pH 6.4) as a substrate. An absorbance was measured at 420 nm. The POA was expressed in mg g^{-1} $3h^{-1}$.

PCR-DGGE analysis

Total DNA was extracted from the compost samples using the ZR Soil Microbe DNA Kit™ (Zymo cor., Beijing). The amount of DNA was estimated visually after electrophoresis in 1.0% agarose gels by ethidium bromide staining of 2 ug ml^{-1}. Compost DNA was amplified in a PCR thermocycler (Gene Amp PCR system2700, Applied

Bio system) with the universal bacterial primer set for 16S rDNA: 357f-GC and 518r, to obtain products of about 230 bp for denaturing gradient gel electrophoresis (DGGE). Each polymerase chain reaction (PCR) mixture contained 1 ul extracted DNA as a template, 0.5 ul each primer, 2.5 ul DNA polymerase with Mg^{2+}, 2 ul dNTPs, 0.1 ul TAQ and 18.4 ul ddH_2O in a final volume of 25 ul. The PCR included an initial 5 min denaturation at 94 °C, followed by 30 thermal cycles of 1 min at 94 °C, 1 min at 56 °C, and 1 min at 72 °C. Thermal cycling was completed with an extension step at 72 °C for 7 min. Products were checked by electrophoresis in 1.5% (w/v) agarose gels and ethidium bromide staining (2 ug ml^{-1}) (Ros *et al*., 2006).

DGGE was performed with the Bio-Rad DCode System. Twenty microliter of PCR product was loaded on polyacrylamide gels with denaturing gradient of 30% (7%(w/v) acrylamide–bisacrylamide (37.5:1), 2.55 M urea, 14.68% formamide) to 60% (7%(w/v) acrylamide–bisacrylamide (37.5:1), 3.57 M urea, 20.56% formamide) with 1×Tris–acetate EDTA (EDTA) as buffer at 60 °C and 160 V for 3.5 h. After silver staining of the gels in an automated gel stainer, gels were scanned. Scanned gels were analyzed after normalization with the Quantity One software (version 4.31, Bio-Rad).

Statistical analyses

The data from the experiment were statistically processed on a computer using the Excel XP and Origin 8.0. Sampling and chemical analyses were carried out in triplicate in order to decrease the experimental errors and to increase the experimental reproducibility. The confidence of data generated in the present investigations has been analyzed by standard statistical methods to determine the mean values and standard deviation (S.D.). The values in figures were expressed as (mean ± S.D.) of the three replicates.

RESULTS AND DISCUSSION

Changes in pH during composting

The pH values had a downtrend tendency during composting except treatment 6 with higher petroleum content after 21 d of composting (Fig. 1). The mean pH values of the original manure, the manure after spiking with petroleum hydrocarbons and the compost products (21 d) were 8.97, 9.03 and 8.72, respectively. Before composting, treatment 4 showed the highest pH value of 9.24, which implies the change of pH with the petroleum addition. After composting the pH value increased in treatment 6, indicating different effects of high TPH content during composting process.

Changes in TPH, three components (SH, AH and PF) and PAHs

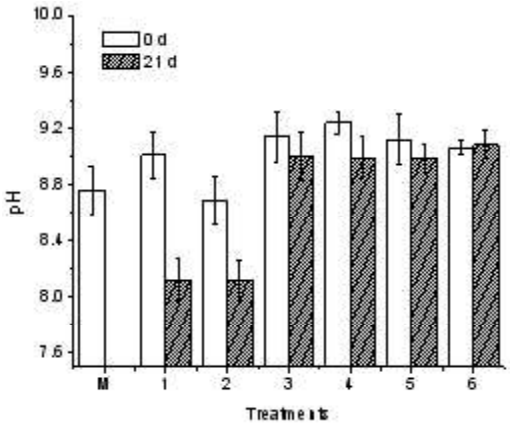


Fig. 1. The pH values of treatments at initial and end of composting. Values are expressed as means ± SD(n=3)

During 21 d of composting, the TPH content in each sample decreased. The TPH degradation percentage in 21 d was approximately 10%, and the highest value was 42.21% in the treatment 4 (Fig. 2). It can be inferred that 1% TPH concentration was appropriate for microbial metabolism, while higher TPH concentration of 5% and 10% restrained microbial degradation.

Fig. 3 shows the variation of three components of SH, AH and PF in treatments 2 and 5. The SH was nearly declined in all the two treatments. In treatment 2 (Fig. 3A), the AH and the PF contents decreased in first 14 d and then increased in the last 7 d. In treatment 5 (Fig. 3B), the PF content increased in the first 7 d and then decreased in the last 14 d, but the change of the AH content was opposite. The result suggests that SH fraction degraded more easily than AH and PF fractions. The increase of AH and PF content during composting maybe caused by the relative decomposition of organic matter in the compost. The results are in agreement with the previous findings (Chaineau *et al*., 2005; Vega *et al*., 2009).

Fig. 4 shows the changes of PAHs content

during composting process under different temperatures. Under 20 °C (Fig. 4A), the five and three ring PAHs content decreased but the four and six ring PAHs content increased, and the two ring PAHs content was very low during composting process. Under 50 °C (Fig. 4B), the six ring PAHs content decreased especially during the last 7 d, but the three and four ring PAHs content increased. From the result of the treatments 2 and 5, it indicated that PAHs content had different changes under different composting temperatures. The reduction of PAHs content indicated the biodegradation of PAHs by the microorganism in the composting process, while the increase of PAHs content may be due to the relative decomposition of the cow manure. It is known that four, five and six ring PAHs have greater carcinogenic potential than two, three or seven ring PAHs (Kanaly and Harayama, 2000). PAHs are often difficult to degrade by conventional treatment method as they are recalcitrant and non-reactive in environment (Gao *et al*., 2006). Some studies demonstrated that biological oxidation using microorganisms can achieve PAHs degradation (Juhasz and Naidu, 2000; Romero *et*

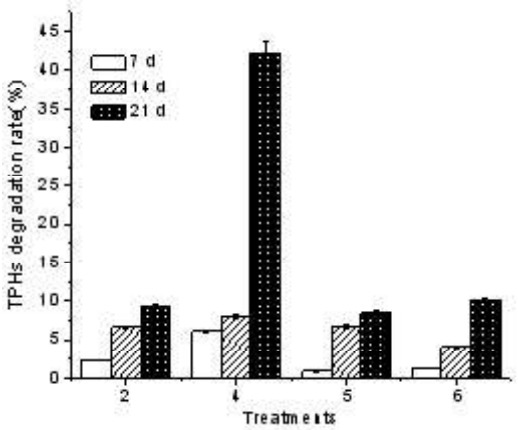


Fig. 2. TPHs degradation rate (%) at different time of composting under different incubation temperatures Values are expressed as means±SD(*n*=3)

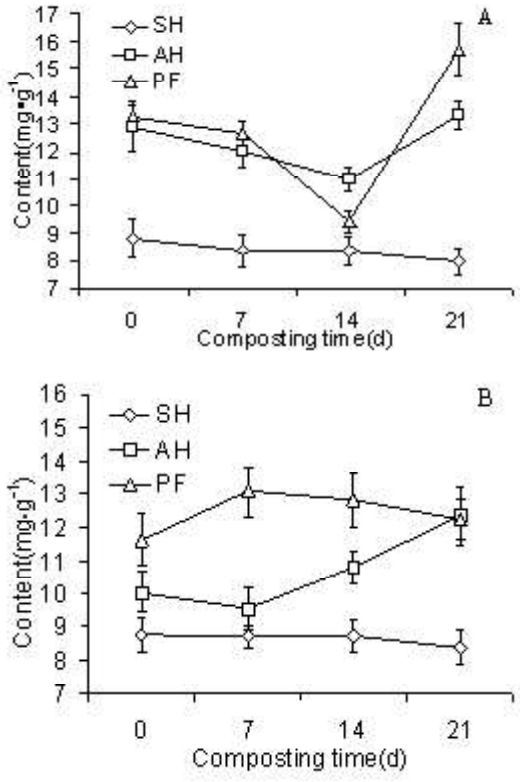
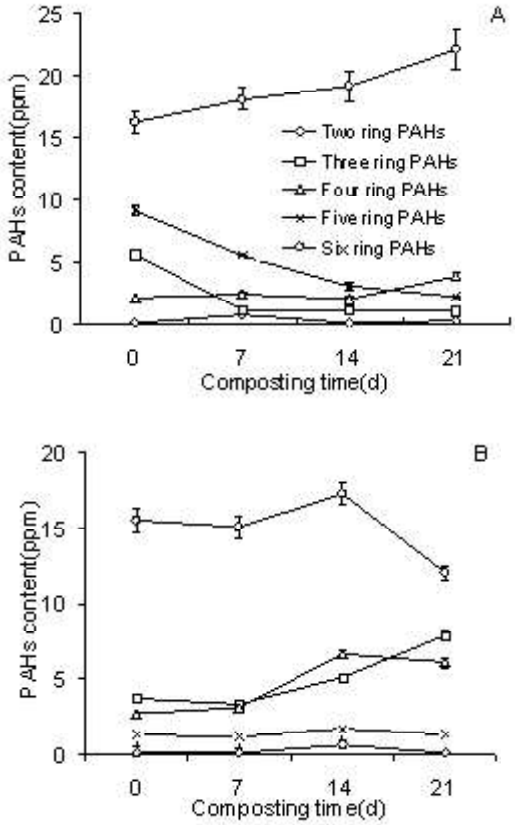


Fig. 3. Changes of different components contents in compost over time. A: treatment 2. B: treatment 5. SH: saturated hydrocarbon; AH: aromatic hydrocarbon; PF: polar fraction. Values are expressed as means±SD(*n*=3)



A: treatment 2. B: treatment 5. Values are expressed as means±SD(*n*=3)

Fig. 4. Changes of PAHs content during composting.

al., 1998), and the same conclusion can be achieved in this research.

Enzymatic activity

Microbial enzymes are important factors for microbial metabolic activity. Catalase uses hydrogen peroxide to oxidise toxins including phenols, formic acid, formaldehyde and alcohols, and the reactions are crucial to life (Zhou *et al*., 2004). During the composting period, the addition of petroleum in the compost resulted in a higher initial CA. The CA of all samples increased at the late period of composting with the maximum of 2.15 ml g^{-1} in treatment 3 under 50 °C and with no TPH addition but decreased at the 7 d with the minimum value of 0.95 ml g^{-1} in treatment 6 with highest TPH addition (Fig. 5A). It inferred that microbial activity might be inhibited during the early days of composting. The inhabitation increased with higher TPH content indication that microbial oxidation of TPH was inhibited by high contents of petroleum hydrocarbons. Ma *et al*. had basically the same result (Ma *et al*., 2003).

The DA was increased first and then decreased in treatment 5. The maximum value is 5.77 mg TPF g^{-1} $24h^{-1}$ at the 14 d (Fig. 5B). There is a similar result that maximum values of DA are observed at the end of thermophilic stage or at the beginning of mesophilic stage, however, the maximum DA values are higher (Barrena *et al*., 2008). DA can reflect changes in the respiratory activity of a given population size in response to changes in the soil environment (Schinner *et al*., 1996b). Active dehydrogenase is considered to exist in the soil as an integral part of intact cells. Dehydrogenase conducts a board range of oxidative activities that are responsible for degradation of organic matter (Margesin *et al*., 2000).

It was reported that oxidase especially polyphenol oxidase played a role in the process of conversion of aromatic organic compounds to humus in soil, and polyphenol oxidase was negatively correlated to the level of humification (Zhou *et al*., 1981). POA during 21 d composting decreased until the 14th d and then increased at time of 21 d of composting (Fig. 5C). The change range of the values is 6.51-12.69 mg g^{-1} $3h^{-1}$, and the minimum value (6.51±0.29) mg g^{-1} $3h^{-1}$ appeared at the 14 d, inferring that the inhibition of POA during composting process. This result also coincided with the conclusion that polyphenol oxidase may be active during the first mesophilic stage of composting or after the thermophilic stage

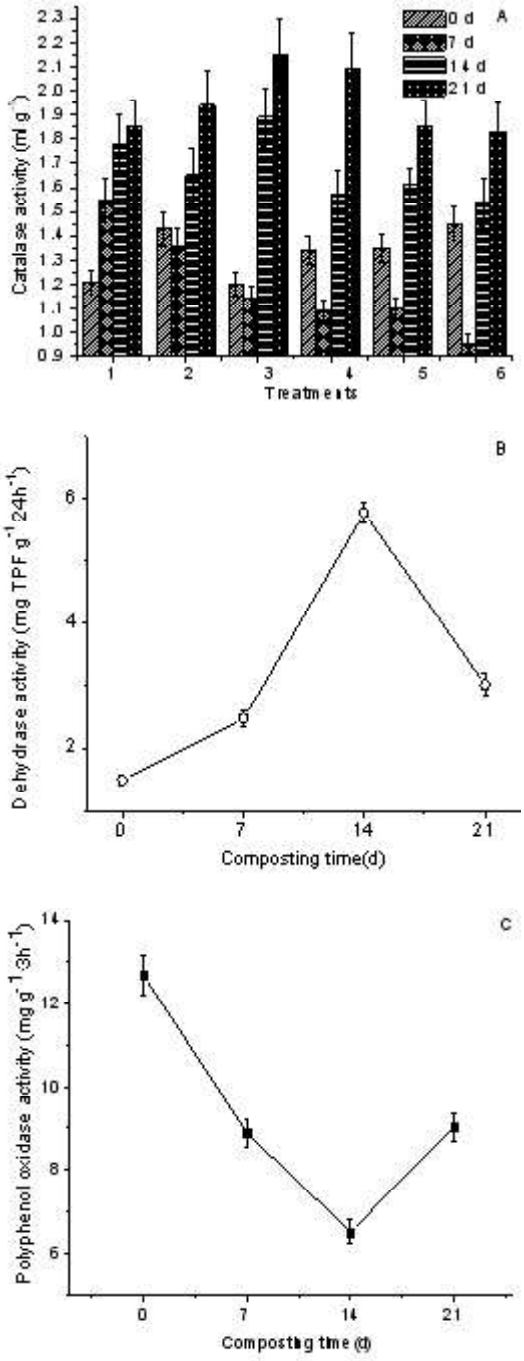


Fig. 5. Changes in enzymatic activity in during composting. A: CA of all treatments. B: DA of treatment 5. C: POA of treatment 5. Values are expressed as means±SD(n=3)

(Dees and Ghiorse, 2001). The result suggests that different enzymes behave different during TPH contaminated composting process.

PCR-DGGE analysis result

Fig. 6 showed that the diversity of microbial community increased in composting process with higher temperature of 50 °C as compared to 20 °C. The same conclusion was also confirmed in previous studies (Ma *et al*., 2003). With less bands, a low diversity was found among treatment 2 indicating small changes of microbial diversity during composting process at low temperature of 20 °C incubation. The cluster analysis on DGGE band showed great differences among four samples in treatment 5, which suggested great change of microbial community during composting process with higher incubation temperature. The four samples in treatment 2 concentrated closely as shown in Fig. 6b. However, the four samples in treatments 3 and 5 showed a larger distance in cluster analysis result which indicated more intensive shift of microbial community. There also exists difference among samples of treatments 3 and 5 indicating changes in microbial community caused by different contents of petroleum pollutants. Treatment 3 with 1% crude oil content has more bands than treatment 5 with 5% crude oil content after 21 d composting. It can be inferred that there is more species of microbes under lower petroleum content. Some special bands appeared after high temperature composting was marked in Fig. 6a.

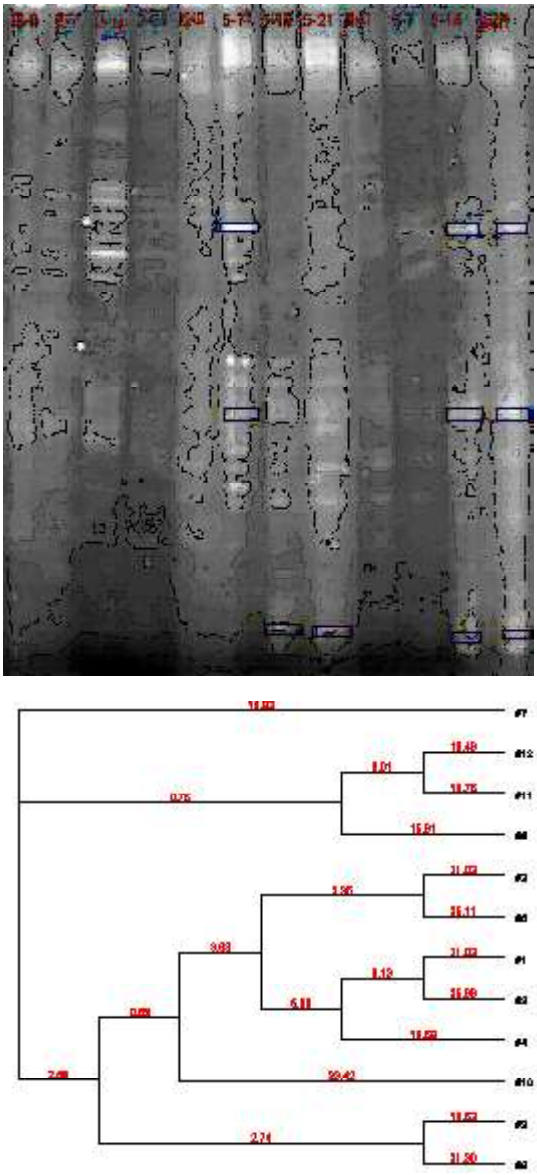


Fig. 6. PCR–DGGE analysis result (a): DGGE profiles of treatments 2, 5 and 3; (b): Cluster analysis on the DGGE profiles of treatments 2(#1,2,3,4), 5(#5,6,7,8) and 3(#9,10,11,12)

This paper aims to characterize the composting conditions for petroleum hydrocarbon degradation. It was suggested that temperature and TPH content affected the biodegradation of hydrocarbons greatly. The microbial community structure and enzyme also changed accordingly. Thus, proper temperature and TPH content should be controlled properly in a field composting process for petroleum waste disposal.

ACKNOWLEDGEMENTS

This work was supported by (1) National Natural Science Foundation of China (31270544), (2) 863 Major Program (2013AA06A205), (3) Tianjin Hi-tech Support Program Key Projects (11ZCGYSF01400), (4) State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (MARC2011D050).

REFERENCES

1. Antizar-Ladislao, B., Lopez-Real, J. and Beck, A.J., Degradation of polycyclic aromatic hydrocarbons (PAHs) in an aged coal tar contaminated soil under in-vessel composting

conditions. *Environ Pollut,* 2006; **141**(3): 459-68.
2. Barrena, R., Vazquez, F. and Sanchez, A., Dehydrogenase activity as a method for monitoring the composting process. *Bioresource Technol*, 2008; **99**(4): 905-908.
3. Brils, J.M. *et al*., Oil effect in freshly spiked marine sediment on Vibrio fischeri, Corophium volutator, and Echinocardium cordatum. *Environ Toxicol Chem*, 2002; **21**(10): 2242-2251.
4. Cai, Z., Zhou, Q.X., Peng, S.W. and Li, K.N., Promoted biodegradation and microbiological effects of petroleum hydrocarbons by Impatiens balsamina L. with strong endurance. *J Hazard Mater*, 2010; **183**(1-3): 731-737.
5. Chaineau, C.H., Rougeux, G., Yepremian, C. and Oudot, J., Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. *Soil Biol Biochem,* 2005; **37**(8): 1490-1497.
6. Dees, P.M. and Ghiorse, W.C., Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. *Fems Microbiol Ecol*, 2001; **35**(2): 207-216.
7. Gao, Y.Z., Ling, W.T. and Wong, M.H., Plant-accelerated dissipation of phenanthrene and pyrene from water in the presence of a nonionic-surfactant. *Chemosphere,* 2006; **63**(9): 1560-1567.
8. Jiang, A.L., Tian, S.P. and Xu, Y., Effects of controlled atmospheres with high-O-2 or high-CO2 concentrations on postharvest physiology and storability of "Napoleon" sweet cherry. *Acta Bot Sin*, 2002; **44**(8): 925-930.
9. Jonker, M.T.O., Brils, J.M., Sinke, A.J.C., Murk, A.J. and Koelmans, A.A., Weathering and toxicity of marine sediments contaminated with oils and polycyclic aromatic hydrocarbons. *Environ Toxicol Chem*, 2006; **25**(5): 1345-1353.
10. Joo, H.S., Shoda, M. and Phae, C.G., Degradation of diesel oil in soil using a food waste composting process. *Biodegradation,* 2007; **18**(5): 597-605.
11. Jorgensen, K.S., Puustinen, J. and Suortti, A.M., Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. *Environ Pollut*, 2000; **107**(2): 245-54.
12. Juhasz, A.L. and Naidu, R., Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. *Int Biodeter Biodegr*, 2000; **45**(1-2): 57-88.
13. Kanaly, R.A. and Harayama, S., 2000. Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. *J Bacteriol*, **182**(8): 2059-67.
14. Kriipsalu, M., Marques, M., Hogland, W. and Nammari, D.R., Fate of polycyclic aromatic hydrocarbons during composting of oily sludge. *Environ Technol*, 2008; **29**(1): 43-53.
15. Lu, M. *et al*., Remediation of petroleum-contaminated soil after composting by sequential treatment with Fenton-like oxidation and biodegradation. *Bioresource Technol,* 2010; **101**(7): 2106-2113.
16. Ma, Y., Zhang, J.Y. and Wong, M.H., Microbial activity during composting of anthracene-contaminated soil. *Chemosphere*, 2003; **52**(9): 1505-13.
17. Margesin, R., Walder, G. and Schinner, F., The impact of hydrocarbon remediation (diesel oil and polycyclic aromatic hydrocarbons) on enzyme activities and microbial properties of soil. *Acta Biotechnol*, 2000; **20**(3-4): 313-333.
18. Meyer, S. and Steinhart, H., 9Effects of heterocyclic PAHs (N, S, O) on the biodegradation of typical tar oil PAHs in a soil/compost mixture. *Chemosphere*, 2000; **40**(4): 359-367.
19. Mills, M.A., McDonald, T.J., Bonner, J.S., Simon, M.A. and Autenrieth, R.L., Method for quantifying the fate of petroleum in the environment. *Chemosphere,* 1999; **39**(14): 2563-2582.
20. Mishra, S., Jyot, J., Kuhad, R.C. and Lal, B., In situ bioremediation potential of an oily sludge-degrading bacterial consortium. *Curr Microbiol,* 2001; **43**(5): 328-35.
21. Namkoong, W., Hwang, E.Y., Park, J.S. and Choi, J.Y., Bioremediation of diesel-contaminated soil with composting. *Environ Pollut,* 2002; **119**(1): 23-31.
22. Peng, S.W., Zhou, Q.X., Cai, Z. and Zhang, Z.N., Phytoremediation of petroleum contaminated soils by Mirabilis Jalapa L. in a greenhouse plot experiment. *J Hazard Mater*, 2009; **168**(2-3): 1490-1496.
23. Ratsep, R., Biological variables for monitoring the effects of pollution in a small catchments areas. A Literature survey, *Environmental Monitory Nord*, 1991; **8**: 6-21.
24. Romero, M.C., Cazau, M.C., Giorgieri, S. and Arambarri, A.M., Phenanthrene degradation by microorganisms isolated from a contaminated stream. *Environmental Pollution*, 1998; **101**(3): 355-359.
25. Ros, M., Pascual, J.A., Garcia, C., Hernandez, M.T. and Insam, H., Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. *Soil Biol Biochem*, 2006; **38**(12):

3443-3452.
26. Schinner, F., Ohlinger, R. and Margesin, R., Methods in Soil Biology. Springer Press Berlin, Germany. 1996a.
27. Schinner, F., Ohlinger, R. and Margesin, R., Methods in Soil Biology. Springer Press, Berlin, Germany 1996b.
28. Sun, F.H. and Zhou, Q.X., Metal accumulation in the polychaete Hediste japonica with emphasis on interaction between heavy metals and petroleum hydrocarbons. *Environmental Pollution*, 2007; **149**(1): 92-98.
29. Tang, J.C., Shibata, A., Zhou, Q.X. and Katayama, A., Effect of temperature on reaction rate and microbial community in composting of cattle manure with rice straw. *J Biosci Bioeng,* 2007; **104**(4): 321-328.
30. Trejo-Hernandez, M.R., Ortiz, A., Okoh, A.I., Morales, D. and Quintero, R., Biodegradation of heavy crude oil Maya using spent compost and sugar cane bagasse wastes. *Chemosphere,* 2007; **68**(5): 848-855.
31. Vega, F.A., Covelo, E.F., Reigosa, M.J. and Andrade, M.L., Degradation of fuel oil in salt marsh soils affected by the Prestige oil spill. *J Hazard Mater*, 2009; **166**(2-3): 1020-1029.
32. Vila, J. and Grifoll, M., Actions of Mycobacterium sp. strain AP1 on the saturated- and aromatic-hydrocarbon fractions of fuel oil in a marine medium. *Appl Environ Microbiol,* 2009; **75**(19): 6232-9.
33. Wischmann, H. and Steinhart, H., The formation of PAH oxidation products in soils and soil/ compost mixtures. *Chemosphere*, 1997; **35**(8): 1681-1698.
34. Zhou, L.K., Zhang, Z.M. and Chen, E.F., Enzymatic activities in black soils. *Acta Pedologic Simica*, 1981; **18**: 158-166.
35. Zhou, Q., Cheng, Y., Zhang, Q. and Liang, J., Quantitative analyses of relationships between ecotoxicological effects and combined pollution. *Sci China C Life Sci*, 2004; 47(4): 332-9.
36. Zhou, Q., Sun, F. and Liu, R., Joint chemical flushing of soils contaminated with petroleum hydrocarbons. *Environ Int,* 2005; **31**(6): 835-9.