ELSEVIER

Journal of Hazardous Materials B124 (2005) 217–223

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Assessment of microbial respiratory activity of a manufactured gas plant soil after remediation using sunflower oil

Zongqiang Gong [a,*], Kassem Alef [b], Berndt-Michael Wilke [b], Maike Mai [b], Peijun Li [a]

[a] *Institute of Applied Ecology, Chinese Academy of Sciences, P.O. Box 417, Wenhua Road 72, Shenyang 110016, PR China*
[b] *Institute of Ecology, Berlin University of Technology, Franklinstrasse 29, D-10587 Berlin, Germany*

Received 18 January 2005; received in revised form 7 May 2005; accepted 12 May 2005
Available online 27 June 2005

Abstract

Microbial activity of a manufactured gas plant (MGP) soil, as well as remaining oil degradability, before and after remediation using sunflower oil was assessed. A sandy soil contaminated with polycyclic aromatic hydrocarbons (PAHs) was collected from an MGP site in Berlin, Germany. Column solubilizations of PAHs from the field-moist soil and air-dried soil using sunflower oil as an extractant at an oil/soil ratio of 2:1 (v/m) were carried out to compare PAH removals from the soil under these two conditions. After column solubilizations, portions of untreated soil (UTS), solubilized field-moist soil (SFMS), and solubilized air-dried soil (SADS) were amended with nutrients. Both nutrient amended and unamended soil samples were subjected to soil respiratory measurement. Soil respiration parameters, such as basal respiration rate, nutrient-induced respiration rate, lag time, exponential growth rate, respiratory activation quotient, peak maximum time, and cumulative CO_2 evolution were calculated from the soil respiration curves. The parameters were compared using analysis of variance (ANOVA) and least-significance difference (LSD). Results showed that the impact of soil moisture on the PAH removals was quite significant, with the SADS showing higher PAH removals and the SFMS showing lower ones. There were significant differences between the respiration parameters with respect to the UTS, SFMS, and SADS. Basal respiration rate, nutrient-induced respiration rate, and exponential growth rate were lower for the SFMS and SADS relative to the UTS. Lag time and peak maximum time were higher for the SFMS and SADS relative to the UTS. Exponential growth rate was higher for the SFMS relative to the SADS. These parameters demonstrated that soil microbial activity was reduced at the onset of the test, because a lot of bioavailable materials for microbial growth were removed by sunflower oil. On the other hand, cumulative CO_2 evolutions in the SFMS and SADS were higher than that in the UTS, indicating that soil respiration was activated after soil microorganisms got acclimatized to the remaining sunflower oil, and remaining sunflower oil was biodegradable.

Keywords: Soil respiration; Microbial activity; Vegetable oil; Soil remediation; Polycyclic aromatic hydrocarbons (PAHs)

1. Introduction

Manufactured gas plant (MGP) sites exist commonly in many countries, many of which are contaminated with process residues that include tars, sludges, lampblack, and other hydrocarbons. The primary components of concern in these process residues are PAHs [1,2]. PAHs are persistent pollutants and cause concern because of their toxic, carcinogenic, and mutagenic effects [3–5]. Conventional and ecological bioremediation of soils in MGP sites is not very effective due to the recalcitrant nature of PAHs, especially PAHs with high molecular weights [6–8].

There has been an increasing awareness that field-specific, efficient, and cost effective remediation schemes are quite necessary to remove as much PAHs as possible from the MGP soils. In our previous results, we found sunflower oil can be used as a promising agent to remove more than 90% of PAHs from the MGP soils, and soil moisture had a significant effect on PAH removals. After percolating through soil column, some of sunflower oil remained in the soils [9,10]. Some chemical and biological effects of vegetable oil in soils have

* Corresponding author. Tel.: +86 24 8397 0367; fax: +86 24 8397 0300.
E-mail address: zgong@iae.ac.cn (Z. Gong).

0304-3894/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2005.05.008

Nomenclature

C_R	cumulative CO_2 evolution: total CO_2 released in the respiration process
R_B	basal respiration rate: constant mass of CO_2 released per hour per gram of soil without nutrient addition
R_n	nutrient-induced respiration rate: constant mass of CO_2 released per hour per gram of soil after nutrient addition
Q_R	respiratory activation quotient: basal respiration rate divided by nutrient-induced respiration rate, namely, $Q_R = R_B/R_n$
t_{lag}	lag time: the time from addition of nutrients until exponential growth started
$t_{peak\,max}$	peak maximum time: the time from addition of nutrients to the maximum respiration rate

Greek letter

μ	growth rate: rate constant during exponential increase of the respiration rate

been demonstrated in the literatures. For instance, vegetable oil amendment increased the biodegradation of various PAHs by 15–80% with a mixed bacterial culture and a pure culture of *Comamonas testosteroni* in a PAH-contaminated soil slurry system [11]. Vegetable oil in soil was rapidly degraded if suitable microbial conditions had been well maintained [12].

Sunflower oil was used in the previous studies because it is edible, causes less harmful effects on soil microflora, and is said to be an environmental-friendly solvent [9,10]. However, remaining oil in the soils does add organic contents to the soils, and so may still have substantial effects on soil quality. For instance, some literatures disclosed that some of aromatic plants produced essential oils, which were mixtures of low-molecular weight isoprenoids with antimicrobial activity to soil microorganisms [13,14]. If the antimicrobial activity does exist, decomposition of oils in soil would be critically affected. However, other contrary results showed soil respiration was activated in the presence of essential oils. The increase of soil respiration was found to be a primary rather than secondary effect. The oil did not inhibit the soil microorganisms, thereby providing substrate easily decomposable to others [15,16]. It is interesting that some constituents of the essential oils were immediately effective, while others required a time lag [17]. Given these effects, it is also necessary to examine the influence of remaining oil in our results on soil microbial activity.

Soil respiration is a widely used method for assessment of microbial activities of soils [18–21]. Thus, an international standard organization (ISO) method for the determination of soil quality was established using kinetics of soil respiration rates combined with the following microbial parameters: basal respiration rate, nutrient-induced respiration rate, lag time after addition of nutrients, growth rate during the exponential phase, and cumulative oxygen consumption or CO_2 evolution [22]. It is also useful in assessing decomposition processes of organic compounds in soils [23,24].

The aims of the present work are (1) to determine the effects of the remaining sunflower oil in the remediated soil on soil microbial activities; (2) to test the biodegradability of the remaining oil; (3) to compare the microbial effects of the remaining oil in the solubilized field-moist and air-dried soils. Soil respiration test was performed on the untreated and sunflower oil solubilized soils. In spite of different conditions, sunflower oil activated soil respiration after a lag time.

2. Materials and methods

2.1. Soil

A sandy soil was collected from a former MGP site, in Berlin, Germany, historically contaminated with PAHs. The soil was sieved through a 2-mm sieve to break soil clumps and to remove rocks, and was then mechanically mixed to ensure homogeneity. After this, the soil was divided into two portions. One portion of the soil was stored at 4 °C as a field-moist soil, and the other was air-dried in a fume hood for 5 days. Water contents of the field-moist and air-dried soils were 17.1 and 0.3%, respectively. Representative physical and chemical characteristics of the soil are shown in Table 1.

2.2. Soil column

Each soil column was made by cutting off the bottom of a glass reagent bottle, inverting it, and fitting the neck with a screen to support the soil sample. The column had a height of 33 cm and an inner diameter of 21 cm; the outlet at the bottom of the column was 6 cm in diameter. The design of the soil column is shown in Fig. 1.

Table 1
Chemical and physical characteristics of the MGP soil[a]

pH	Corg (%)	EC (μS/cm)	Sum of PAH (mg/kg)	Water content (%)	Texture (%)			Heavy metals (mg/kg)					
					Clay	Silt	Sand	Cd	Cr	Cu	Ni	Pb	Zn
6.6	4.6	151	1254.8	17.1	3.8	0.6	95.6	<0.2	<3	3.6	2.7	<3	8.2

[a] $n = 2$.

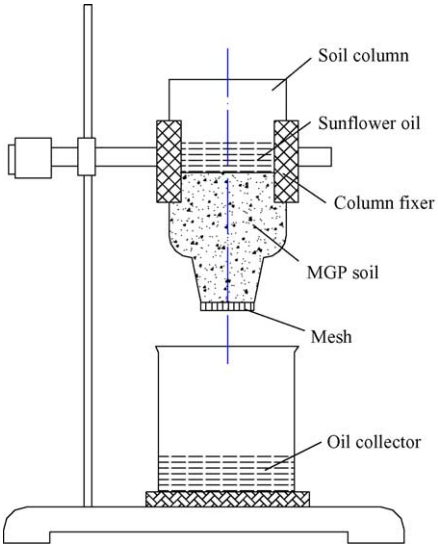

Fig. 1. The design of the column experiment.

2.3. Column solubilizations of PAHs from the field-moist and air-dried soils

Each soil column was packed uniformly with 1 kg of soil based on dry weight (1.2 kg of field-moist soil or 1 kg of air-dried soil), which occupied about 10 cm of the column in depth. One liter of sunflower oil was poured into the soil column; when this oil had percolated out of the soil column, a second liter of sunflower oil was added to the column. After the two percolations, about 4–5% of oil remained in the soils. Samples were taken from these oily soils and centrifuged to remove as much residual oil as possible before PAH analysis. A mass balance test in a previous experiment verified this oily soil analysis [10].

2.4. Soil sample extraction and cleanup

The soil samples were extracted and cleaned using a German VDLUFA method (Verband Deutscher Landswirtschaftlicher Untersuchungs- und Forschungsanstalten) for determination of PAHs in soils [9,10]. Ten grams of soil sample were extracted in a glass bottle filled with 15 g of sodium chloride, 100 ml of deionized water, 200 ml of acetone, and 150 ml of dichloromethane. The bottle was then sealed and shaken for 16 h at 200 rounds per minute on a shaker. A 5-ml aliquot of organic phase extract was concentrated by a rotary evaporator, and was cleaned by a silica gel column filled with 3 g of deactivated aluminum oxide (activated by oven heating in a oven at 130 °C for 16 h, and deactivated by 15% water addition), 5 g of activated silica gel (70–230 mesh, activated by heating in a oven at 130 °C for 16 h, and cooled in a desiccator at least for 10 min before use), and 10 g of anhydrous sodium sulphate. The silica gel column was eluted with 100 ml of 4:1 hexane/dichloromethane (v/v). The eluate collected from the silica gel column during the cleanup was concentrated by evaporator and dried by nitrogen gas, and was finally dissolved in 1 ml of acetonitrile before HPLC analysis.

2.5. HPLC analysis

Quantitative analysis of the soil extracts was done by an HPLC equipped with a gradient pump (KNAUER K1001), an autosampler (TSP AS100), and a reverse-phase C-18 column. Indenopyrene (IP) was quantified by UV detector, while phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benz[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DahA), and benzo[*g,h,i*]perylene (BghiP) were measured by fluorescence detector (TSP FL2000) at specific excitation/emission wavelengths. Elution conditions were as follows: for the first 5 min, a 1:1 (v:v) mixture of water and acetonitrile was used as the solvent; during the next 15 min, acetonitrile in the mixture was linearly increased to 100%; and that composition was maintained for 15 min; finally, the solvent composition was returned to the initial condition over the next 2.5 min. In all cases, 8 μl of sample was injected into the HPLC by the autosampler. The concentrations of PAHs were calculated using HPLC software CHEM EURO 2000. An external standard mixture was used for quantification of PAHs.

2.6. Soil respiration test

The water contents of solubilized field-moist soil (SFMS) and solubilized air-fried soil (SADS) were measured again before soil respiration test, those were 1 and 0.1%, respectively. The SFMS, SADS, and untreated soil (UTS) were divided into two parts, respectively. One part of the each soil was supplemented with 0.35 g of NH_4Cl, 0.5 g of $NaNO_3$, 1 g of K_2HPO_4, 0.3 g of $MgSO_4{\cdot}7H_2O$, 0.1 g of $CaCl_2$, and 0.05 g of $FeCl_2$ per kilogram of soil for nutrient amendment. The other part of each soil was not amended with the nutrients. To equalize the soil water contents and distribute nutrients homogeneously in the soils, those soils were moisturized using a sprinkler and mechanically stirred for at least 10 min. Soil respirations were measured by monitoring CO_2 evolutions with an interval time of 0.5 h. In the soil respiration test, 20 g (dry weight, calculated out using moist weight and water content) of each soil sample was transferred to a 250-ml beaker, which was then placed into a soil respirometer. The CO_2 released was captured with KOH solution placed on the top of the beaker. The CO_2 evolution was obtained by measuring electrical conductivity of the KOH solution. Each soil sample was measured in four replicates.

2.7. Statistical analyses

The respiration parameters of each replicate of the UTS, SFMS, and SADS were determined according to the ISO method [22]. Means of individual parameters were compared using one-way analysis of variance (ANOVA) and least-

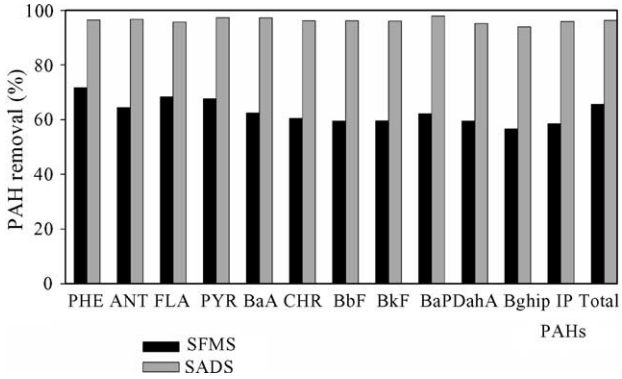

Fig. 2. Comparison of PAH removals from the soil samples, SFMS and SADS.

significance difference (LSD) of a statistical software, SPSS 11.5, at 95% confidence. The homogeneity of the variances was tested with Levene's test.

3. Results

3.1. Soil characterization

The chemical and physical characteristics of the MGP soil are summarized in Table 1. The soil was typically sandy, proportion of the sand was 95.6%. Soil pH was 6.6, organic carbon content was 4.6%, and water content was 17.1%. The soil was highly contaminated with PAHs. Low levels of heavy metals were detected in this soil sample.

3.2. Column solubilizations of PAH from the field-moist and air-dried soils

Results of column solubilizations of PAHs from the field-moist and air-dried soils are shown in Fig. 2. It can be clearly seen that the impact of soil moisture on the PAH removals was quite significant, with the SFMS showing relative lower PAH removals, which were in the range of 56.6–71.6%, and the SADS exhibiting relative higher PAH removals, which varied between 93.9 and 97.9%. The total 12 PAH removals of the

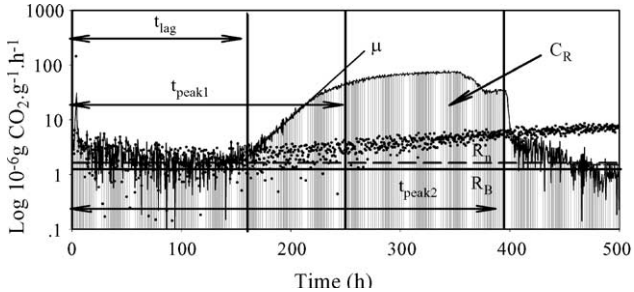

Fig. 3. Some relevant parameters for the interpretation of soil respiration curves (solid curve with shade: nutrient amended soil sample SADS, scattered dot curve: unamended soil sample SADS).

SFMS and SADS were 65.6 and 96.3%, respectively. In summary, higher soil moisture inhibited solubilization efficiency of sunflower oil. It is hypothesized that drying the soil may result in soil structure change which is more conducive to the extraction [25,26]. Note that in this study, water content of the field-moist soil decreased after sunflower oil solubilization, because the field-moist soil in the column was also open to air in the nearly 7 day solubilization process. However, the water content of the air-dried soil was not affected so much, as it had already been air dried before.

3.3. Soil respiration test

In the soil respiration test, the following parameters were obtained from the soil respiration curves: basal respiration rate (R_B), nutrient-induced respiration rate (R_n), respiratory activation quotient (Q_R), lag time (t_{lag}), growth rate (μ), peak maximum time ($t_{peak\,max}$), cumulative CO_2 evolution (C_R). A typical data set, along with graphic representation of the various parameters, is shown in Fig. 3, and nomenclatures are appended.

Results of the soil respiration measurements are presented as the rate of average CO_2 evolution (Figs. 4–6). Tables 2 and 3 exhibit statistical values (means, standard deviations, and significant values) of the parameters of the UTS, SFMS, and SADS. In the soils with organic content >0.1%, hydrophobic organic pollutants always partition into soil bound organic materials [27]. Removals of PAHs from

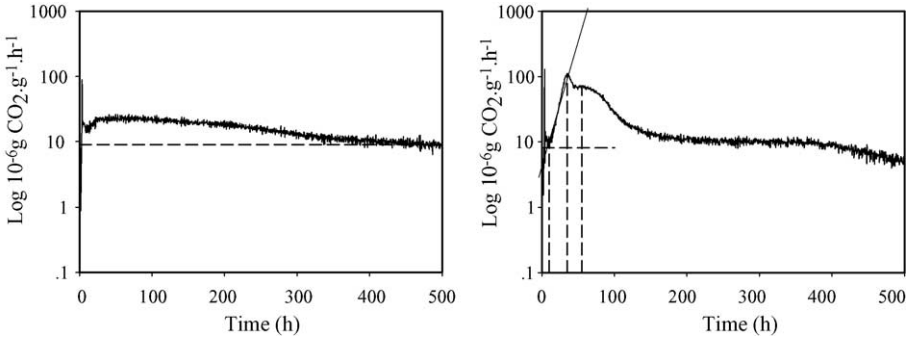

Fig. 4. CO_2 evolution rate of soil sample UTS (left: unamended soil; right: nutrient amended soil; the solid curves are the means of respiration, and the dash lines are used for parameter references).

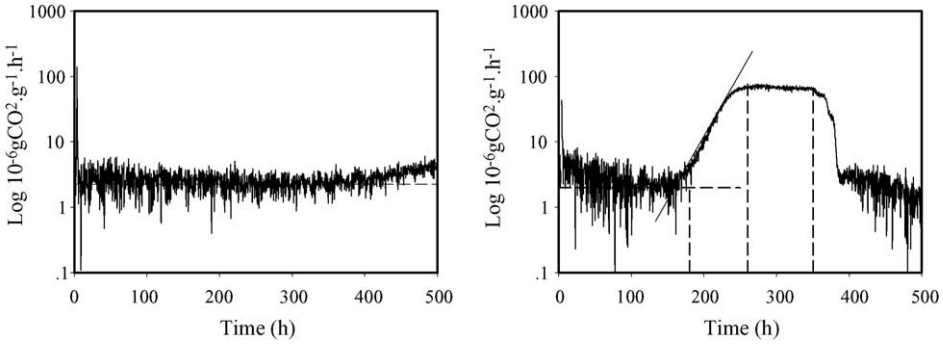

Fig. 5. CO_2 evolution rate of soil sample SFMS (left: unamended soil; right: nutrient amended soil; the solid curves are the means of respiration, and the dash lines are used for parameter references).

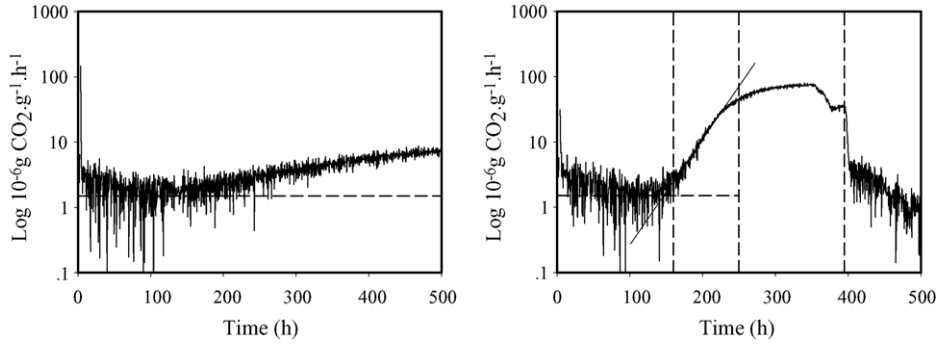

Fig. 6. CO_2 evolution rate of soil sample SADS (left: unamended soil; right: nutrient amended soil; the solid curves are the means of respiration, and the dash lines are used for parameter references).

the soils inevitably resulted in that bioavailable organic materials for microbial growth were somewhat stripped out during the oil solubilization. Thus, the SFMS and SADS exhibited statistically lower basal respirations (R_B) than the UTS did (Table 2). Even after nutrient amendment, soil respirations were not activated at once shown by respiratory activation quotient (Q_R) (Table 2), indicating that remaining oil in the soils was not metabolized by autochthonous bacteria significantly in the initial period. On the other hand, lag time, peak maximum time, and exponential growth rate also proved that the microbial activities were lower at the onset of the test for the SFMS and SADS relative to the UTS. As more PAHs were removed from the SADS than those from the SFMS by the sunflower oil solubilization, it can be assumed that more bioavailable organic materials were also solubilzed by the sunflower oil, which resulted in a lower exponential growth rate for the SADS relative to the SFMS (Table 2). Cumulative CO_2 evolution (C_R) measured in the soil respiration process

Table 2
Comparison of means of parameters using one-way ANOVA and LSD[a]

Soil	R_B (μg CO_2/g h)		R_n (μg CO_2/g h)		Q_R	
	Mean ± S.D.[b]	p[c]	Mean ± S.D.	p	Mean ± S.D.	p
UTS	9.0 ± 1.1 A	<0.001	9.3 ± 2.2 A	<0.001	1.0 ± 0.4 A	0.984
SFMS	2.3 ± 0.9 B		2.2 ± 0.5 B		1.0 ± 0.4 A	
SADS	1.6 ± 0.6 B		1.7 ± 0.3 B		1.0 ± 0.3 A	
	t_{lag} (h)		$t_{peak\ max1}$ (h)		μ	
	Mean ± S.D.	p	Mean ± S.D.	p	Mean ± S.D.	p
UTS	12.9 ± 1.7 A	<0.001	31.4 ± 0.9 A	<0.001	0.055 ± 0.002 A	<0.001
SFMS	178.5 ± 19.5 B		256.8 ± 20.6 B		0.026 ± 0.001 B	
SADS	159.5 ± 14.1 B		245.3 ± 21.6 B		0.018 ± 0.003 C	

[a] $n = 4$, 95% confidence interval, C_R is not included in this table.
[b] Indicated by LSD that values in each column followed with different letters are statistically different.
[c] Significance values indicated by one-way ANOVA.

Table 3
Comparison of cumulative CO_2 (C_R) evolutions of the soils using one-way ANOVA and LSD[a]

Soil	Without nutrient amendment (μg CO_2/g)[b]	With nutrient amendment (μg CO_2/g)	p[c]
UTS	16427 ± 1136 Aa	19729 ± 734 Ab	0.003
SFMS	2839 ± 790 Ba	21031 ± 657 Bb	<0.001
SADS	3662 ± 1596 Ba	21050 ± 304 Bb	<0.001
p[d]	<0.001	0.018	

[a] $n = 4$, 95% confidence interval.
[b] Indicated by LSD that values in each column followed with different capital letters, and in each row followed with different lowercase letters are statistically different.
[c] Significance values indicated by one-way ANOVA between soils with and without nutrient amendment.
[d] Significance values indicated by one-way ANOVA among UTS, SFMS, and SADS.

should also be taken into account as an important parameter, which is reflected by the total area beneath the curve down to the time axis (see Fig. 3). Calculated C_R values are shown in Table 3, and the means were compared using ANOVA and LSD. Cumulative CO_2 evolutions were much lower in the non-nutrient-amended SFMS and SADS than it was in the corresponding UTS case, the reason for that was considered to be a combination of three factors: (1) biodegradable PAH and organic materials had been stripped out during the oil solubilization, so soil microorganisms had nothing to grow on; (2) oil-degrading bacteria were present at very low initial numbers; (3) nutrients were low. After nutrient amendment, cumulative CO_2 evolutions became statistically higher for the SFMS and SADS relative to the US (Table 3), so the nutrient was considered to be a crucial factor for microbial activities of the SFMS and SADS. Maybe in the nutrient amended SFMS and SADS, oil-degrading bacteria began to grow quickly after acclimation to the oily soil, and remaining oil, served as an introduced carbon source, provided enough organic carbon for the soil respiration. Thus, we can conclude that the remaining sunflower oil could be degraded after the soil microorganisms got acclimatized to the oil.

4. Conclusion

The results presented in this study clearly revealed the usefulness of soil respiration measurement for the description of soil microbial activity and remaining oil degradability before and after remediation using sunflower oil. On the one hand, soil microbial activities were reduced at the onset of the test, shown by the increasing lag time, peak maximum time, and decreasing exponential growth rate, because a lot of bioavailable materials for microbial growth were removed by sunflower oil. This effect was much more significant for the SADS relative to the SFMS, due to that more bioavailable materials, together with more PAHs, were removed by sunflower oil solubilization from the SADS than those from the SFMS. On the other hand, after a lag period, at least some of the autochthonous bacteria of the soils began to mineralize the remaining oil, and their growth was promoted and demonstrated by the increasing cumulative CO_2 evolutions.

Acknowledgements

A scholarship from BMBF, Germany (CHN 093) to Z. Gong is gratefully acknowledged. We thank Liane Kapitzki and Ruth See for technical assistance. Z. Gong and P. Li also acknowledge financial supports from National Key Basic Research Program of China (No. 2004CB418506) and National Science Foundation of China (20337010, 20277040). However, any opinions, findings, or conclusions expressed herein are those of the authors and do not reflect the views of supports.

References

[1] T.D. Hayes, D.G. Linz, D.V. Nakles, A.P. Leuschner, Management of Manufactured Gas Plant Sites, vol. 1, Amherst Scientific Publishers, Amherst, MA, 1996, p. 20.
[2] S.B. Hawthorne, D.G. Poppendieck, C.B. Grabanski, R.C. Loehr, Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction, Environ. Sci. Technol. 36 (2002) 4795–4803.
[3] S. Tao, Y.H. Cui, F.L. Xu, B.G. Li, J. Cao, W.X. Liu, G. Schmitt, X.J. Wang, W.R. Shen, B.P. Qing, R. Sun, Polycyclic aromatic hydrocarbons (PAHs) in agricultural soil and vegetables from Tianjin, Sci. Total Environ. 320 (2004) 11–24.
[4] F. Haeseler, D. Blanchet, V. Druelle, P. Werner, J.P. Vandecasteele, Ecotoxicological assessment of soils of former manufactured gas plant sites: bioremediation potential and pollutant mobility, Environ. Sci. Technol. 33 (1999) 4379–4384.
[5] F. Haeseler, D. Blanchet, V. Druelle, P. Werner, J.P. Vandecasteele, Analytical characterization of contaminated soils from former manufactured gas plants, Environ. Sci. Technol. 33 (1999) 825–830.
[6] A.J.M. Lagadec, D.J. Miller, A.V. Lilke, S.B. Hawthorne, Pilot-scale subcritical water remediation of polycyclic aromatic hydrocarbon- and pesticide- contaminated soil, Environ. Sci. Technol. 34 (2000) 1542–1548.
[7] A. Tiehm, M. Stieber, P. Werner, F.H. Frimmel, Surfactant-enhanced mobilization and biodegradation of polycyclic aromatic hydrocarbons in manufactured gas plant soil, Environ. Sci. Technol. 31 (1997) 2570–2576.
[8] I.T. Yeom, M.M. Ghosh, C.D. Cox, Kinetic aspects of surfactant solubilization of soil-bound polycyclic aromatic hydrocarbons, Environ. Sci. Technol. 30 (1996) 1589–1595.
[9] Z. Gong, K. Alef, B.M. Wilke, P. Li, Dissolution and removal of PAHs from a contaminated soil using sunflower oil, Chemosphere 58 (2005) 291–298.

[10] Z. Gong, B.M. Wilke, K. Alef, P. Li, Influence of soil moisture on sunflower oil extraction of polycyclic aromatic hydrocarbons from a manufactured gas plant soil, Sci. Total Environ. 343 (2005) 51–59.
[11] J.K. Pannu, A. Singh, O.P. Ward, Influence of peanut oil on microbial degradation of polycyclic aromatic hydrocarbons, Can. J. Microbiol. 49 (2003) 508–513.
[12] Y. Mihara, D. Sugimori, F. Hasumi, Rapid degradation of vegetable oil at the high concentration and improvement of oil degradation rate by mixed culture, J. Jpn. Petro. Inst. (in Japanese) 43 (2000) 392–395.
[13] J.A. Amaral, R. Knowles, Inhibition of methane consumption in forest soils by monoterpenes, J. Chem. Ecol. 24 (1998) 723–734.
[14] K. Karamanoli, D. Vokou, H. Menkissoglu, H.I. Constantinidou, Bacterial colonization of the phyllosphere of mediterranean aromatic plants, J. Chem. Ecol. 26 (2000) 2035–2048.
[15] D. Vokou, D. Chalkos, G. Karamanlidou, M. Yiangou, Activation of soil respiration and shift of the microbial population balance in soil as a response to Lavandula stoechas essential oil, J. Chem. Ecol. 28 (2002) 755–768.
[16] D. Vokou, N.S. Margaris, J.M. Lynch, Effects of volatile oils from aromatic shrubs on soil microorganisms, Soil Biol. Biochem. 5 (1984) 509–513.
[17] D. Vokou, N.S. Margaris, Decomposition of terpenes by soil microorganisms, Pedobiologia 31 (1988) 413–419.
[18] G.D. Bending, M.K. Turner, F. Rayns, M.C. Marx, M. Wood, Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes, Soil Biol. Biochem. 36 (2004) 1785–1792.
[19] B.M. Wilke, Effects of single and successive additions of cadmium, nickel, and zinc on carbon dioxide evolution and dehydrogenase activity in a sandy luvisol, Biol. Fertil. Soils 11 (1991) 34–37.
[20] T.H. Anderson, K.H. Domsch, Application of eco-physiological quotients (q CO_2 and q D) on microbial biomass from soils of different cropping histories, Soil Biol. Biochem. 22 (1990) 251–255.
[21] K. Vig, M. Megharaj, N. Sethunathan, R. Naidu, Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review, Adv. Environ. Res. 8 (2003) 121–135.
[22] ISO/FDIS 17155, Soil quality-determination of abundance and activity of the soil microflora using respiration curves, 2002.
[23] A. Eisentraeger, G. Maxam, J.P. Rila, W. Dott, A stepwise procedure for assessment of the microbial respiratory activity of soil samples contaminated with organic compounds, Ecotox. Environ. Safe. 47 (2000) 65–73.
[24] K. Hund, B. Schenk, The microbial respiration quotient as indicator for bioremediation process, Chemosphere 28 (1994) 477–490.
[25] T.J. Smyth, R.G. Zytner, W.H. Stiver, Influence of water on the supercritical fluid extraction of naphthalene from soil, J. Hazard. Mater. 67 (1999) 183–196.
[26] Y. Zhang, W. Frankenberg Jr., Effect of soil moisture on dimethylselenide transport and transformation to nonvolatile selenium, Environ. Sci. Technol. 33 (1999) 3415–3420.
[27] C.J.A. Magleod, K.T. Semple, Influence of contact time on extractability and degradation of pyrene in soils, Environ. Sci. Technol. 34 (2000) 4952–4957.