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Effect of applying biosolids on the biodegradation of toluene and naphthalene contaminated soils

Hung Yueh Chang[1], Jui Min Hung*[1], Yeong Shing Wu[2], Yi Ru Lin[1], Hung Yu Lai[3] and Chih Jen Lu[1, 3]

[1]Department of Environmental Engineering, [3]Center for Environmental Restoration and Disaster Reduction, National Chung Shing University, No. 250, Kuo Kuang Road, Taichung 402, Taiwan, Republic of China
[2]Sinotech Engineering Consultants, Inc. Environmental Engineering Research Center, Taiwan, No. 171, Sec. 5, Nanking E. Road, Taipei 105, Taiwan, Republic of China

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Abstract: *Biosolids contain nutrients, organic matters and micro-organisms that can provide soil benefits. In this study, toluene and naphthalene-contaminated soils were used to investigate the effect of applying biosolids on the enhancement of the biodegradation rate. The oxygen uptake rate (OUR) was determined with a respirometer and was used to calculate the oxygen uptake percentage of substrate in biosolids (α value) using a two-phase respirogram. Experimental result showed that the application of biosolids had positive effect on the enhancement of the biodegradation rate of toluene and naphthalene in the contaminated soils. The biodegradation rates of toluene and naphthalene were 15% and 20% in soils without applying biosolids, respectively. With the biosolids, its biodegradation rate for the two contaminants was about 4-fold higher in relative to control and the maximum value occurred in a soil to biosolids ratio as 1: 0.5. The α value for toluene and naphthalene was in the levels of 10-20% which revealed that the biosolids used in this study was mainly composed by micro-organism.*

Key word: *Toluene, Naphthalene, Biosolids, Bubble respirometer, 2-phases respirogram*
PDF of full length paper is available online

Introduction

Toluene and naphthalene are two important components of petroleum products and are hazardous to the health of human beings, plants, and animals (Zhou and Crawford, 1995; Vasudevan and Rajaram, 2001; Sarkar *et al.*, 2005). Soils contaminated with these contaminants can be treated by physical, chemical, and biological methods to decrease their concentrations (Ting *et al.*, 1999). Among these methods, bioremediation is the use of exotic or indigenous micro-organism to utilize the contaminants, such as the bioaugmentation and biostimulation (Sarkar *et al.*, 2005). To reduce the period for bioremediation, different amendments could be added to the contaminated soils to increase the microbial population and the biodegradation rate. The degradation of pollutants by micro-organism was thus increased due to an increase in nutrients resulted from the application of C, N and P. Among these nutrients, the effect of C supply on the increase in the biodegradation rate has been studied with the use of biosolids and composts (Namkoong *et al.*, 2002).

Bio-solids are the organic solids produced from the wastewater treatment system after drying and composting (USEPA, 2000). They have the benefits of low cost, high availability, and slow release of the nutrients (McBride, 2003). The application of legal biosolids has positive effect on increasing the organic carbon content, soil fertility, and biological activity (Sastre *et al.*, 1996; Ros *et al.*, 2003; Kao *et al.*, 2006; Chen *et al.,* 2005). The release of various nutrients from biosolids after applying into soils was able to enhance the biodegradation rate of pollutants because the microbial biomass increased (Margesin and Schinner, 2001; Duncan *et al.*, 2003). Sarkar *et al*. (2005) reported that the application of biosolids was efficient to increase the microbial population and the biodegradation of total petroleum hydrocarbons (TPH) in diesel-contaminated soil. In Taiwan, most of the biosolids produced by the wastewater treatment plants were incinerated and land-filled. The reuse of biosolids is able to decrease the landfill area and also provide essential nutrients for the growth of plants. Many researchers also showed that the application of legal biosolids to the contaminated soil was able to reinforce the biodegradation of contaminants. The objective of this study is to assess the effect of applying biosolids on the enhancement of the biodegradation rate of toluene and naphthalene in artificially contaminated soils.

Materials and Methods

Soils used in this study were sampled from central Taiwan and artificially spiked with toluene and naphthalene. The biosolids used in this study were taken from a domestic-sewage treatment plant of Taichung city and then air-dried, ground, and passed through a 10 mesh sieve. Artificially toluene or naphthalene-contaminated soils and biosolids were mixed uniformly and then added into 125 mL brown vessels for the batch experiment *i.e.* mixture of soil and biosolid (MSB). Four different ratios of soil/biosolids (w/w) were employed to study the effect of biosolids on the biodegradation rate

* **Corresponding author:** m15kado@cpami.gov.tw

which included 1: 0 (control) (MSB_{ck}), 1: 0.2 (MSB_{5x}), 1: 0.5 (MSB_{2x}), and 1: 1 (MSB_{1x}), respectively. At various period after added into brown vessels, toluene and naphthalene were analyzed (EPA Taiwan, 2002 with headspace gas; NIEA M157.00C) or supersonic extraction method; NIEA M167.00C) with GC-FID (HP 5890A). The bubble respirometer (AER-200 Type, CES company, USA) was used to determine the change of oxygen uptake of different treatments in continuously. Wu *et al.* (2003) reported that the non-homogenous between soils and biosolids has influence on the determination of oxygen uptake by bubble respirometer. To avoid the interference, extra-pure water was added into brown vessels and stirred (200-250 rpm) to keep the system in a suspending state. The oxygen uptake rate (OUR) and accumulated oxygen uptake (O_u) were used to plot the 2-phases respirogram. Based on the result of Wu *et al.* (2003) and Wu *et al.* (2004), there are two respiration phases in a typical 2-phases respirogram and their relationships are hyperbolic (1st phase) and linear functions (2nd phase) respectively (Fig. 1 and Table 1). The 1st phase is associated with exogenous respiration (substrate utilization) and the endogenous respiration (biomass decay), while the 2nd phase associated only with the endogenous respiration.

According to Wu *et al*. (2003), we can calculate the oxygen uptake in the separating point of the two phases ($O_{u,sp}$) and extending intercept of O_u ($O_{u,ex}$) by using the 2-phases respirogram. $O_{u,sp}$ and $O_{u,ex}$ are the oxygen uptake of microorganism degradable substrate and the maximum value in the respirometer system. Equation (1) and (2) were employed to illustrate the relationship between $O_{u,sp}$, $O_{u,ex}$, initial BOD of substrate (S_0), initial BOD of micro-organism (X_0), and the observed growth yield (Y_0).

$$O_{u,\,sp} = S_0\,(1 - Y_0) \qquad \text{..........(1)}$$

$$O_{u,\,ex} = S_0 + X_0 \qquad \text{..........(2)}$$

The above two equations can be rearranged as equations (3) and (4) if we use biodegradability ($\beta_{BOD/ThOD}$) to demonstrate the relationship between initial BOD of substrate (S_0) and its theoretical COD ($S_{0,\,ThOD}$).

$$O_{u,\,sp} = \beta_{BOD/ThOD} \times (1 - Y_0) \times S_{0,\,ThOD} \qquad \text{..........(3)}$$

$$O_{u,\,ex} = \beta_{BOD/ThOD} \times S_{0,\,ThOD} + X_0 \qquad \text{..........(4)}$$

We can calculate the value of S_0 by using equations (3) and (4) and the result of linear regression between $O_{u,ex}$ and $S_{0,\,ThOD}$ under different concentrations of substrate ($\beta_{BOD/ThOD}$: slope; X_0: intercept of O_u axis). The $O_{u,sp}$ and $O_{u,ex}$ in equations (3) and (4) were re-expressed as equations (5) and (6) to get the amounts of oxygen uptake contributed by microorganism and the substrate in biosolids. The characteristics and unit of each code used in equations (5) and (6) was illustrated in Table 1. If we assumed that the applied biosolid is the only contributor of microorganism, the S_0 in equations (3) and (4) include the oxygen consumed by the biodegradation of pollutants and the organic materials of soils and biosolids (sum of β_{S+C} × (S+C) and B × β_B × α). X_0 in equation (4) can be replaced by

Table - 1: The definition and unit of various parameters used in this study

Symbol	Definition	Unit
S	Oxygen uptake of soil organic matters	mg COD
C	Oxygen uptake of substrate	mg COD
B	Amounts of biosolids added	mg
β_B	Biodegradation amounts of biosolids	mg BOD mg^{-1}-*B*
β_{S+C}	Biodegradation amounts of soil and pollutants	mg BOD mg^{-1}-COD of *S* + *C*
α	Oxygen uptake percentage of substrate in biosolids	mg BOD mg^{-1}-BOD of *B*

Table - 2: The concentrations of heavy metal in the tested biosolids

Heavy metal	TCLP* extractable concentration (mg l^{-1})	TCLP standards of Taiwan (mg l^{-1})
Arsenic	0.02	5
Cadmium	<0.0046	1
Copper	<0.013	15
Lead	<0.054	5
Murcury	<0.00031	0.2
Selemium	<0.016	1
Chromium	<0.018	5
$Chromium^{6+}$	<0.006	2.5

* = Toxicity characteristic leaching procedure

Table - 3: The basic characteristics of the biosolids and soils used in this study

Basic characteristics	Soil	Biosolids
pH value	7.3	6.9
Organic carbon (%)	1.1	27.3
Total N (%)	0.1	3.4
NH_4^+-N (mg kg^{-1})	9.2	623
P_2O_5 (%)	ND	4.8
Bray P1 (mg kg^{-1})	19.1	ND
Total P (%)	0.002	2.14

- = Not detected

Table - 4: The residual concentration of toluene in soils after 48 hr of operation with the addition of various amounts of biosolids

	Treatments			
	MSBck	MSB_{5x}	MSB_{2x}	MSB_{1x}
Addition biosolids (g)	0	2	5	10
Residual concentration (µg g^{-1} - soil)	735	313	37.4	82.7
Toluene removal efficiency (%)	15	63	96	90
Initial removal rate, k (µg g^{-1}-soil · hr)	2.7	11.6	17.3	16.3

Table - 5: The residual concentration of naphthalene in soils after 60 hr of operation with the addition of various amounts of biosolids

	Treatments			
	MSBck	MSB_{5x}	MSB_{2x}	MSB_{1x}
Addition biosolids (g)	0	2	5	10
Residual concentration (µg g^{-1}-soil)	418	124	12.3	1.4
Naphthalene removal efficiency (%)	20	78	98	99
Initial removal rate, k (µg g^{-1}-soil · hr)	1.4	6.3	8.1	8.3

$B \times \beta_B \times (1-\alpha)$ and the above equations can be rewrite as the following two equations:

$$O_{u,sp} = [\{\beta_{S+C} \times (S + C) + (\alpha\, \beta_B\, B)\} \times (1 - Y_0)] \quad ....(5)$$

$$O_{u,ex} = \beta_{S+C} \times (S + C)] + [\beta_B \times B] \quad ....(6)$$

We use linear regression between the experimental $O_{u,ex}$ and amounts of different amounts of added biosolids (B) to get its slope (β_B) and intercept [$\beta_{S+C} \times$ (S+C)]. By using the linear regression between the experimental $O_{u,sp}$ and amounts of biosolids added (B) to get its slope [$\beta \times \beta_B \times (1 - Y_0)$] and intercept [$\beta_{S+C} \times (S+C) \times (1 - Y_0)$]. We can finally get the $\beta_{S+C} \times (S + C)$, β_B, and α value.

Results and Discussion

Basic characteristics of biosolids: Table 2 shows the concentration of different heavy metals in the tested biosolids [extracted by the toxicity characteristic leaching procedure (TCLP)]. These concentrations were lower than the TCLP limitations of hazardous wastes (EPA Taiwan, 2004; NIEA R201.13C). Khan and Scullion (2002) reported that the application of high concentration of heavy metal in the sludge had significant effect on decreasing the biomass of microorganisms after 3-7 weeks. Because of the lower concentration of heavy metal in the biosolids used in this study, and the tested the negative effect(the effect on decreasing the biomass of microorganisms) on the activity of microorganism were not observed.

Table 3 shows the contents of organic carbon and nutrients in the tested biosolids and soils. Biosolids has higher concentration of NH_3-N (623 mg kg^{-1}-soil) in relative to soil. The total P concentration in soil was only about 0.0002%. According to the analyzing result, the application of biosolids is able to increase the concentration of organic carbon and nutrients in the soils. The amount of microorganism in the biosolids (water content 70%) was about 4×10^8 CFU g^{-1}-biosolid, which revealed that considerable amounts of microorganisms were added in the tested soils with the application of biosolids.

Effect of biosolids on the biodegradation of toluene and naphthalene in soils: The initial concentrations of toluene and naphthalene were 867 and 500 $\mu g\ g^{-1}$-soil, respectively. After 48 and 60 hr of operation, the effect of biosolids on the biodegradation of the toluene and naphthalene is shown in Tables 4 and 5. The application of biosolids has enhanced the biodegradation rates of toluene and naphthalene the addition of difference amount of in the contaminated soils. For these contaminants, the biodegradation rate with the treatment of MSB_{5x} was about 4-fold higher than that without the addition of biosolids. These results were in accordance with the published studies that the presence of biosolids enhanced the removal efficiency and biodegradation rate of organic contaminants in soils. Similar to previous studies, biosolids have reinforcing effect on the biodegradation rates of organic contaminants (Margesin and Schinner, 2001; Duncan *et al.*, 2003; Sarkar *et al.*, 2005). In the treatments of higher ratios of biosolids (MSB_{5x} and MSB_{2x}), the toluene and naphthalene were in the removal efficiencies about 90-99%. Namkoong *et al.* (2002) reported that the effect of biosolids on microorganism included the supply of nutrients, trace elements, and degradable organic carbon (Rivera Espinoza and Dendooven, 2004; Ros *et al.*, 2003). The application of biosolids had different effects on the biodegradation of toluene and naphthalene. There was no lag period was observed on the toluene removal with the addition of biosolids, because toluene was relatively readily biodegradable. Regardless of the lower initial concentration of Toluene than Naphthalene, the biodegradation rates of Naphthalene were higher than Toluene in all the treatments.

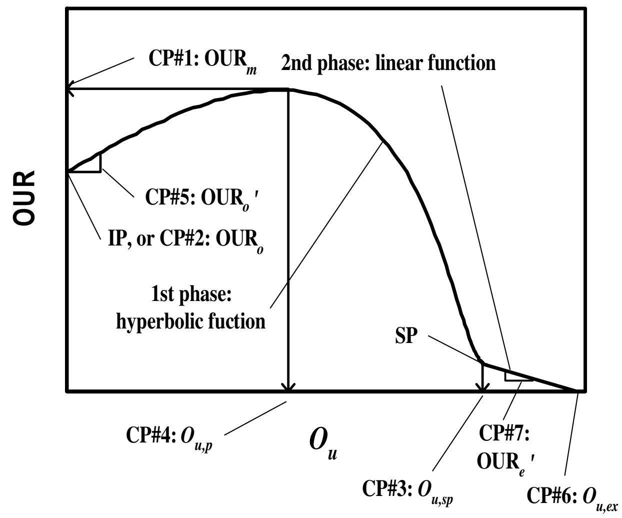

Fig. 1: Typical 2-phase respirogram of a respirometer (Wu *et al.*, 2004)

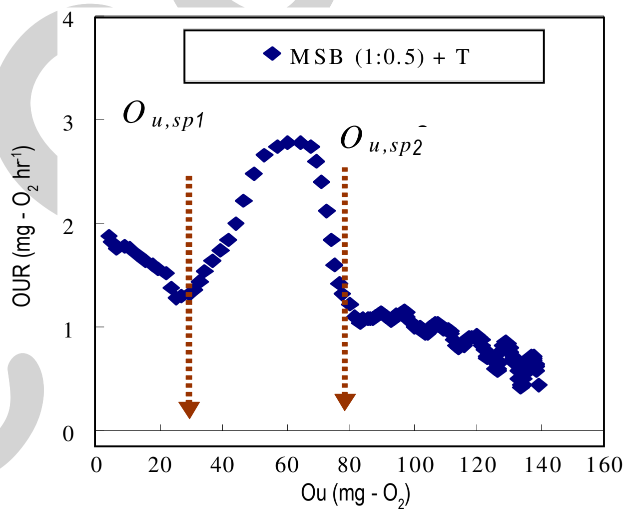

Fig. 2: The relationship between OUR and O_u of toluene-contaminated soil in the treatment of MSB (1:0.5)

For the toluene- and naphthalene-contaminated soils, the application of biosolids has higher removal efficiency in the treatment of MSB_{2x} in relative to others (Table 4 and 5). The removal efficiency and k value (zero-order reaction) of MSB_{2x} was 96% and 17.3 mg g^{-1} hr in the toluene-contaminated soil. Although MSB_{1x} has higher k value in relative to MSB_{2x} in naphthalene-contaminated soil, the difference of removal efficiency between them was only 1%. We thus

Table - 6: The value of $O_{u, sp}$ and $O_{u, ex}$ of toluene in soils after 48 hr of operation with the addition of various amounts of biosolids

Treatments	Added biosolid (mg)	Ratio (soil: biosolids; w/w)	$O_{u, sp1}$ (mg O_2)	$O_{u, sp2}$ (mg O_2)	$O_{u, ex}$ (mg O_2)
Soil + T[a]	0	0	0	23.0	37
MSB (1: 0.08) + T	1,200	0.08	0	41	75
MSB (1: 0.1) + T	1,500	0.1	0	35	91
MSB (1: 0.2) + T	3,000	0.2	8	57	110
MSB (1: 0.4) + T	6,000	0.4	22	58	144
MSB (1: 0.5) + T	7,500	0.5	26	80	194
MSB (1: 0.7) + T	10,500	0.7	23	85	200
MSB (1: 1) + T	15,000	1	27	80	343
MSB (1: 1.4) + T	21,000	1.4	32	82	460

[a] = Toluene-contaminated soil with a total concentration of 1,000 $\mu g\ g^{-1}$-soil

Table - 7: The value of $O_{u, sp}$ and $O_{u, ex}$ of naphthalene in soils after 60 hr of operation with the addition of various amounts of biosolids

Treatments	Added biosolids (mg)	Ratio (soil: biosolids; w/w)	$O_{u, sp1}$ (mg O_2)	$O_{u, sp2}$ (mg O_2)	$O_{u, ex}$ (mg O_2)
Soil + N[b]	0	0	0	3.8	6.3
MSB (1: 0.05) + N	250	0.05	1.5	13.6	35.1
MSB (1: 0.1) + N	500	0.1	3.2	16.5	38.5
MSB (1: 0.15) + N	750	0.15	4.6	24.7	63.3
MSB (1: 0.2) + N	1,000	0.2	5.0	26.6	74.2
MSB (1: 0.25) + N	1,250	0.25	5.5	29.3	80.0
MSB (1: 0.35) + N	1,750	0.35	10.0	38.9	117.0
MSB (1: 0.4) + N	2,000	0.4	8.4	39.6	146.4

[b] = Naphthalene-contaminated soil with a total concentration of 1,000 $\mu g\ g^{-1}$-soil

think that the treatment of MSB_{2x} has the best effect on the biodegradation of toluene and naphthalene in contaminated soils in relative to MSB_{5x} and MSB_{1x}.

Two phase respirogram of toluene and naphthalene biodegradation: The $O_{u, sp}$ and $O_{u, ex}$ in the different treatments of biosolids were calculated using the two-phase respirogram (OUR vs. O_u) and were shown in Table 6,7. The application of biosolids has higher $O_{u, ex}$ value in relative to that without biosolids. The respirogram (OUR vs. O_u) of soil without biosolids was not similar to the typical one with the addition of higher amounts of biosolids. There are three kinds of relationships between the OUR and O_u in the treatment of MSB (1:0.5) for toluene contaminated soils with the addition of biosolid (MSB_{2x}) (Fig. 2). The $O_{u, sp1}$ in Table 6, 7 was contributed by micro-organism themselves and the biodegradation of substrate in biosolids.

To calculate the α value by using $O_{u, sp}$ and $O_{u, ex}$: According to Fig. 2, biosolids was the major contribution for the oxygen uptake during the initial stage. In order to understand whether the oxygen consumption resulted from the consumption by the biodegradation of substrates in biosolids or by micro-organisms in soils, whether the contribution of oxygen is by substrate in biosolids, we have to calculate oxygen uptake percentage of substrate in biosolids (α value). By using the amounts of added biosolids, $O_{u, sp}$, and $O_{u, ex}$ in Tables 6, 7, the β_B value in the toluene- and naphthalene-contaminated soils was 0.02 and 0.06 (mg-BOD mg^{-1}-biosolid), respectively. The α value in the toluene- and naphthalene-contaminated soils was 0.2 and 0.1 (mg-BOD mg^{-1}-BOD), respectively. Because of the slower biodegradation rate of naphthalene compared with toluene, the microorganisms would continuously degrade the substrate in biosolids. Therefore, there was a higher β_B value in the naphthalene-contaminated soils. Because of the low á values of the two pollutants, we revealed that microorganisms contributed most of the oxygen uptake in this system. The β_B value was not a constant it may change with the properties. However, we need more experimental data to prove our assumption.

The experimental result indicated that the addition of biosolids enhanced the biodegradation rates and removal efficiencies of toluene and naphthalene in the contaminated soils showed that there was an enhanced effect of applying biosolids on the biodegradation of toluene (867 $\mu g\ g^{-1}$-soil) and naphthalene (500 $\mu g\ g^{-1}$-soil) in artificially contaminated soils. The biodegradation of organic materials in biosolids and the endogenous respiration of micro-organism of micro-organisms were the major contribution of oxygen uptake during the initial stage for soils amended with biosolids. Because of the slower biodegradation rate of naphthalene compared with toluene, the micro-organism would continuously degrade the organic materials in biosolids for soils contaminated by naphthalene. That would result in a higher β_B value in the naphthalene-contaminated soils. Because of the low α values of these two pollutants, we revealed that micro-organisms contributed most of the oxygen uptake in the biosolids added soil system. The β_B value is not a constant which changes with the kinds of pollutants and needed further study to prove.

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