Korean J. Chem. Eng., **19**(5), 773-779 (2002)

Oxygen Uptake Characteristics of Soil Inoculum Amended with Thiophene Derivatives

Chang Gyun Kim*† and Bohyun Chon

The Division of Environmental and Geosystem Engineering,
*Regional Research Center for Coastal Environments of Yellow Sea, Inha University,
253 Yong Hyun Dong, Nam Gu, Incheon 402-751, Korea
(*Received 5 November 2001 • accepted 5 June 2002*)

Abstract–This study was conducted to assess oxygen uptake characteristics of soil microcosm for biodegradation of sulfolane and thiolane mainly observed in a waste disposal site. The microcosm was obtained from the site and then spiked with nutrients and levels of contaminants (i.e. thiolane and sulfolane) in a closed vessel. The amount of oxygen consumed for sulfolane was peaked at 1450 μL for 200 mg/*l* and then decreased at greater than 500 mg/*l*. Sulfolane was completely degraded below 40 mg/*l*. It indicates that longer period of adaptation would be needed to completely degrade at greater than 500 mg/*l*. Apart from, the highest oxygen consumption was accomplished at 38.5 μL for 1 mg/*l* of thiolane, but it was dropped to a negligible level for 20 mg/*l*. That is, increasing thiolane concentration correspondingly decreased oxygen demand due to its inhibition against microcosm. Nevertheless, the unit amount of oxygen consumption for thiolane was well proportional to the cumulative oxygen uptake. It is concluded that sulfolane can be readily biodegradable in an aerobic condition, while degradation of thiolane was considerably inhibited against the given soil microorganisms even though its concentration was extremely lower by hundredths than that of sulfolane.

Key words: Thiolane, Sulfolane, Oxygen-uptake, Biodegradation

INTRODUCTION

The leachate from a waste disposal site has contaminated an unconfined alluvium overlying a weathered aquifer in Brisbane Australia. In the past 20 years, leaks through the interceptor trench have been detected in the site. The major organic contaminants identified are heterocyclic sulfur compounds (i.e. sulfolane and thiolane) as shown in Fig. 1. The historical information indicates that an ammonia plant has employed Sulfinol process adopting sulfolane to remove carbon dioxide in an air stream and subsequently waste sulfolane has been produced. Waste sulfolane sludge was eventually disposed into the site [Choi et al., 2000].

Biodegradation of thiolane has been poorly studied even though a few studies have been done on biodegradation of thiophene derivatives. Fedorak et al. [1988] reported that *n*-alkyl-substituted tetrahydrothiophenes can be biodegraded by bacterial and fungal cultures in 28 days. The bacteria used for their experiments were found to be gram-positive, aerobic, and non-motile rods. Grimalt et al. [1991] presented that thiolanes in crude oils are more easily decomposed than benzo[b]thiophenes. Alam et al. [1990] reported that *Escherichia coli* NAR30 can degrade thiophenes as a result of successive mutations of three novel genes (*thd*A, C, and D).

To our knowledge, not many works have focused on oxygen consumption behavior in conjunction with their potential biodegradation in soil cultures. This work thus aims to assess oxygen consumption characteristics since sulfolane and thiolane were oxidized by using an apparatus being modified from Anderson [1982]. In the long run, the oxygen consumption was related to a potential biodegradation on contaminants given.

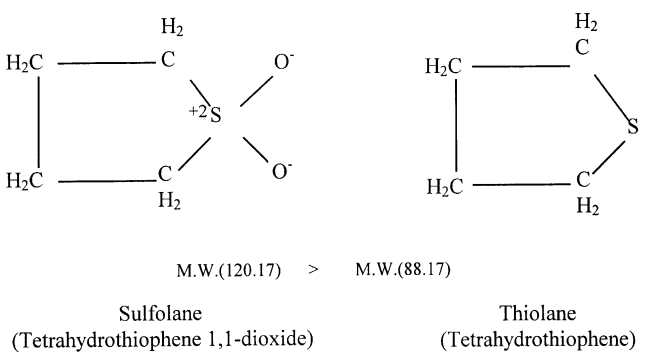

Fig. 1. Structural formula of sulfolane and thiolane used for oxygen-uptake characteristics on a soil microcosm.

MATERIALS AND METHODS

1. Soil and Groundwater Sampling

The soil samples were collected by using a Selby tube (45 cm L×4.8 cm I.D.) from unsaturated zone at 1 m deep below the ground surface in the site. Prior to sampling, a Selby tube was steam cleaned (Gerni 660 Turbo Laser, 2100 to 220 psi) and rinsed with 70% ethanol to prevent cross contamination. After a soil sample was obtained, both ends of the tube were sealed with parafilm. On the other hand, groundwater sample was obtained by using a sterilized stainless bailer (50 cm L×2 cm D) from a reference well that was 2 km from the study area. The bailer was previously sterilized by autoclaving at 121 °C and 15 psi for 15 min on the three consecutive days (Tomy, High Pressure Steam Sterilizer, ES-315). The groundwater samples were then collected in cleaned, sterile amber glass bottles (500 ml) with Teflon-lined stopper. Soil and groundwater samples were chilled on ice during transport and stored at 4 °C until they were analyzed or used in the studies.

†To whom correspondence should be addressed.
E-mail: cgk@inha.ac.kr

The soil types were characterized by XRD (Philips) while size distribution was characterized by Malvern MasterSizer/E. In addition, the soil samples were air dried for 5 days, then crushed with mortar and pestle followed by grinding with a ring mill. These samples were also analyzed for cationic exchange capacity and organic carbon content (LECO WR-112 Carbon Determinator).

For determining concentration of contaminants, 2 ml of groundwater sample was extracted with 2 ml of methylene chloride in a small glass vial by hand-shaking for 1 minute [Kim et al., 1999]. The separated aqueous layer was discarded. Any moisture retained in the methylene chloride extract was removed by dried Na_2SO_4. The samples were then analyzed by gas chromatography (GC). A Perkin Elmer AutoSystem gas chromatography equipped with a flame ionization detector (FID) was employed. A 30 m long (0.25 mm I.D.) and 0.25 μm thick DB5 (5% phenyl methyl polysiloxane) column was used for sulfolane analysis. High purity helium was used as the carrier gas at a flow rate of 1.7 ml/min. The injector and detector temperatures were 300 and 350 °C, respectively. FID response was monitored with a Perkin-Elmer LCI-100 integrator. Sulfolane concentrations were determined by comparing peak areas against calibration curves previously prepared.

The concentration of thiolane was determined by injecting the 25 μl of filtered sample into a Waters High Performance Liquid Chromatography (HPLC) which was equipped with UV spectrophotometer at 215 nm. A 150 mm long (3.9 mm I.D.) symmetry C_{18} column was used and $CH_3CN : H_2O$ (50 : 50) was introduced as an eluent at a flow rate of 1 ml/min. UV response was recorded with a Waters 740 emulator. Thiolane concentrations were determined by comparing peak areas against calibration curves prepared in advance.

Inductively Coupled Plasma Atomic Emission Spectrophotomer (ICPAES) was also used to characterize various inorganic compounds in groundwater which can prove the presence of any potential toxic compounds.

Table 1. Preparation of solution for inorganic salts, trace metals, and vitamins [prepared in 1 L of filtered (0.45 μm) groundwater]

	Chemicals	Quantity (g)
Inorganic salts	K_2HPO_4	3
	KH_2PO_4	3
	$(NH_4)SO_4$	6
	NaCl	6
	$MgSO_4 \cdot 7H_2O$	1.23
Trace metals	$FeCl_3 \cdot 6H_2O$	5
	$MgCl_2 \cdot 6H_2O$	1
	$MnCl_2 \cdot 6H_2O$	1
	$CaCl_2 \cdot 2H_2O$	1
	$CoCl_2 \cdot 6H_2O$	0.3
	$NiCl_2 \cdot 6H_2O$	0.2
	$CuSO_4 \cdot 5H_2O$	0.1
	$ZnSO_4 \cdot 7H_2O$	0.1
	H_3BO_3	0.1
	$Na_2MoO_4 \cdot 2H_2O$	0.1
	$AlCl_3 \cdot 6H_2O$	0.05
Vitamins	Biotin	0.002
	Folic acid	0.002
	Pyridoyine monohydrochloride	0.01
	Riboflavin	0.005
	Thiamine	0.005
	Nicotinic acid	0.005
	Pantothenic acid	0.005
	4-Aminbenzoic acid	0.005

2. Soil Culture Preparation

Microcosm mixtures were prepared by combining 200 g of soil with 100 ml of sterilized (0.45 μm) groundwater [Davis et al., 1994] in a sterile 1.225 L glass reactor. The mixtures of salts, metals and vitamins were prepared as present in Table 1 [Hobson, 1966; Romli, 1993]. 1 ml of mixed solution was then added into the reactor.

In order to obtain homogeneous mixed culture, the samples were then mingled by using a blender (Waring, USA) at a low speed for 10 min. Subsequently, samples were amended with resazurin (0.0002%) as a redox indicator. The cultures were then spiked with a stock solution of sulfolane to achieve a final concentration in the mixed medium ranging from 1 to 2,000 mg/*l*, while thiolane was adjusted in the range of 1 to 20 mg/*l* in the reactor. The range of concentrations was chosen to represent sulfolane and thiolane concentrations typically observed in the monitoring wells at the site. Finally, the test reactors were sealed with a PTFE-faced butyl rubber septum (4.4 cm I.D.).

On the other hand, three types of controls were prepared: 1) Positive control was prepared by adding 0.3 g of glucose into 200 g of non-sterilized soil medium with mixtures of nutrients to test viability of soil microorganisms. 2) Biologically inhibited controls were included in the study to monitor abiotic losses for the test constituents. That is, 200 g of soil was sterilized by autoclaving (121 °C, 15 psi) for 1 hr on the three consecutive days. The sterilized soils were then mixed with the filter-sterilized groundwater amended with 1,000 mg/*l* of mercuric chloride and mixtures of nutrients. 3) A blank control was also prepared to monitor natural degradation of sulfolane and thiolane. In other words, 200 g of non-sterilized soil containing nominal concentration of sulfolane and thiolane was tested since it was amended with salts, metals and vitamins. In addition, the absence of nutrients was also tested.

For proving aerobic experimental conditions, the gas present in the headspace of the control vessel (no-nutrient and not-sterilized) was obtained by using 10 ml Hamilton gas tight syringe through the sampling port and then analyzed by employing Perkin-Elmer Gas Chromatography equipped with TCD.

3. Apparatus

A reactor modified by Anderson [1982] was employed to determine oxygen consumption of soil inoculum by reading the manometric change. This is simply called a Warburg unit, which consists of 200 g of soil medium and a small quantity of alkali solution (10% w/v KOH) hanging in the headspace of the reactor as shown in Fig. 2.

Two legs of the manometer were constructed by using 10 ml of graduated pipette: a leg with 30 cm long (0.5 cm I.D.) PTFE tube connected through the stopper and the other opened to the atmosphere. Nominal volume of MilliQ water was introduced into manometers until the calibration mark opened to the air was reached.

As the test was being initiated, oxygen was consumed by the liv-

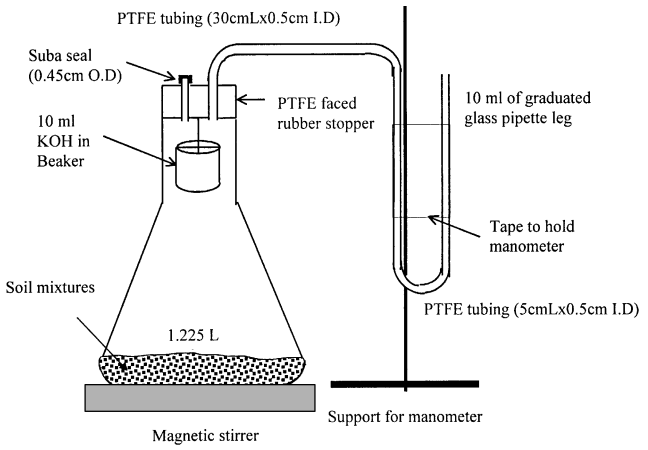

Fig. 2. Apparatus for manometric reading of oxygen consumption for various conditions on soil microcosm.

ing organisms during incubation at 20 °C while carbon dioxide was adsorbed into 10 ml of KOH (10% w/v). There was a subsequent change in height at manometer readings resulting from a decrease in the partial pressure of oxygen from the reactor. This reading was then corrected by atmospheric pressure changes of sterilized medium that occurred during incubation at 20 °C.

After a reading was taken every 24 hours, the pressure change was recalibrated by supplying purified air to the calibration mark being recovered.

4. Calculation of Oxygen Consumption

First, the manometer readings obtained from the test were corrected by the reading taken from atmospheric change as present in Eq. (1).

[Corrected reading for unit containing soil]
=[reading from soil unit]
−[reading from the sterilized control for atmospheric changes] (1)

The corrected reading taken as a unit of water pressure in mm H_2O was converted into volume of oxygen consumed by multiplying a "flask constant". Thereafter, the constant was subsequently determined by using Eq. (2) [Umbreit et al., 1964].

$$k = \frac{V_g \frac{273}{T} + V_f \alpha}{P_o} \quad (2)$$

where

k=flask constant (μl/mm H_2O)

V_g=volume of gas in flask and connecting tubes down to the reference mark of the fixed leg of the manometer (μl)

V_f=fluid volume in vessel (μl)

P_o=standard pressure (10,336 mm H_2O for distilled water)

α=gas (oxygen) solubility in reactive liquid (water) at 20 °C of incubation=0.03091

T=incubation temperature (K)

RESULTS AND DISCUSSION

1. Soil and Groundwater Characteristics

To remove the cobbles and rocks, the soil sample was sieved with 1 mm opening size screen. It was then characterized for soil types, organic carbon content and cationic exchange capacity as shown in Tables 2 and 3.

Table 2. Characterization of the soil sample used in the test

Parameters	Properties
Soil type	Silty clay sand
Clay (%)	11.02
Silt (%)	70.34
Very fine sand (%)	15.27
Fine sand (%)	3.34
Organic carbon (%)	0.001
pH	6.84
Cationic exchange capacity (meq/100 g)	8.40

Table 3. Characterization of groundwater used in the test

Compounds	Concentration (mg/*l*)	Analytical instruments
Thiolane	0	HPLC
Sulfolane	0	Gas chromatograph
S^{2-}	0.003	Spectrophotometer
SO_4	2.0	
DOC*	10.0	
Turbidity (FTU)	3.0	
pH	7.18	TPS 90-FLMV
TKN**	21.35	Standard methods
Ba	0.02	ICPAES*** (Model M+P)
Al	0.01	
Ca	3.72	
Fe	0.48	
K	2.84	
Mg	4.64	
Mn	0.02	
Na	124.0	
P	0.04	
S	9.0	
Si	23.5	
Sr	0.14	
As, B,Cd, Cr, Cu, Li, Mo, Se, Tl, V, Zn, Hg, Sc, Sn, Pb, Co, Ni, Sb, Bi, Be, Nb, Ag, Ti, Zr, U, La, Ce, Sm	Not detected	

*: Dissolved organic carbon; **: Total kjeldahl; ***: Inductively coupled plasma atomic emission spectrophotomer.

The soil type was classified as silty clay sand based upon a high silt content with lower amount of clay. The cationic exchange capacity was 8.4 meq/100 g due to low percentage of clay content (11.02%). The organic carbon content of the soil sample was determined to be approximately 0.001% by Leco combustion method [Kim et al.,

Table 4. Evaluation of flask constant

Parameters	Calculation	Value
k		88.60 μl/mm
V_g	flask (1225000 μl)+tube (30 L×0.5 cm ID: 5890 μl)-dry soil[200 g soil, moisture content: 20.07% (w/w), bulk density 1.616 g/cm^3: 98,923 μl]-beaker and string (4,000 μl)−water in soil sample (140,140 μl)−KOH (10,000 μl)=977827 μl	977827 μl
V_f	water in soil (140,140 μl)+KOH (10,000 μl)=150,140 μl	150,140 μl
P_o	10,336 mm for distilled water	10,336 mm
α	0.03091 for 20 °C	0.03091
T	20 °C	293 K

1999]. Sulfolane and thiolane were not detected from the groundwater samples taken from a reference well.

ICPAES was used for analysis of inorganic compounds in groundwater samples. The sample was scanned for 42 elements. Any toxic compounds were not observed in the analysis as shown in Table 3. The levels of compounds in the sample can be nevertheless used as nutrients for potential natural biodegradation of organic contaminants.

2. Calculation of Flask Constant

The sterilized control was used for measurement of atmospheric pressure changes that occurred during incubation and corrected for the reading in height taken for the soil samples tested. The corrected value is then converted into a volume by multiplying a "flask constant" using Eq. (2). It was consequently obtained at 88.60 μl/mm as shown in Table 4.

3. Leak and Blank Test

Prior to each experiment, the reactor connected into the manometer was slightly pressurized by compressed air to check any potential leak. There was no change observed in the manometer for 24 hours.

The presence of toxicants or a poor preparation of soil inoculum was verified as oxygen consumption for 0.3 mg of glucose was monitored. Fig. 3 shows that accumulated oxygen consumption was plotted against the incubation time while glucose was biologically degraded.

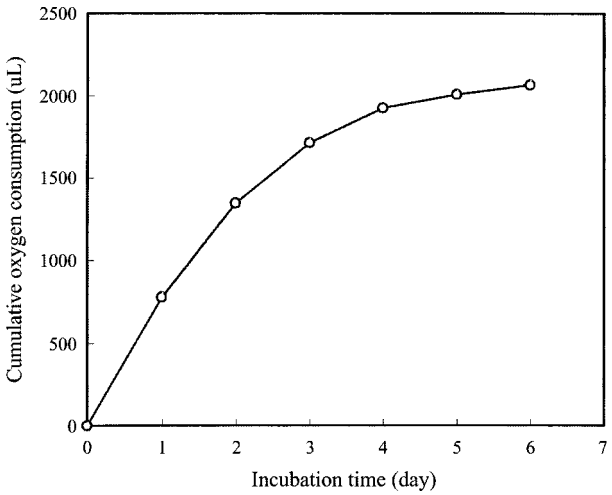

Fig. 3. Positive control of glucose (0.3 mg) on soil inocula observing cumulative oxygen consumption during test of period.

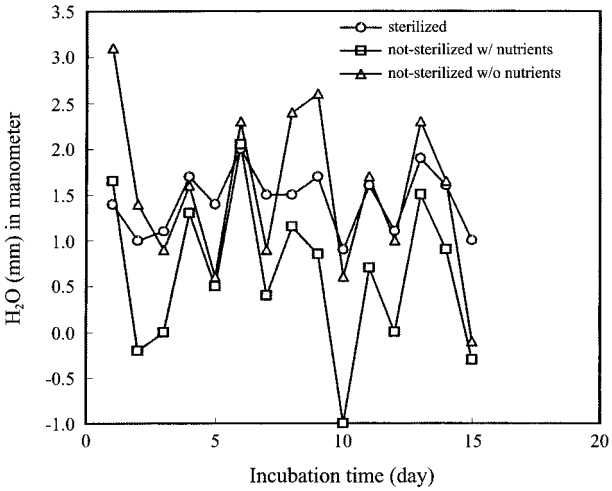

Fig. 4. Observed variation in the manometric readings of pressure change in mm H_2O for three controls.

The plot represented a typical curve for oxygen consumption made by microorganisms in a period. Oxygen was increasingly required at the beginning of incubation and then reached to the steady state.

Furthermore, three controls of a sterilized culture, nutrient amended and non-amended cultures were observed as shown in Fig. 4.

A manometric reading obtained from the sterilized control was positively observed ranging from 0.8 to 2.0 mm H_2O. Apart from that, two other controls, i.e., nutrients and non-nutrients soil inocula were variously influenced by atmospheric pressure change during the test period. Nevertheless, it appears that nutrient enriched control could use oxygen at the highest level among other controls, whereas non-nutrient control was similarly varied against atmospheric pressure change as for the sterilized control. It simply means that there may be not be capable of possible natural attenuation of organic contaminants. Exclusive characterization of head-space gas for three sets of controls revealed that negligible level of carbon dioxide was observed. Hydrogen and methane gas were not found. The experimental condition was thus clearly verified placed in aerobic conditions.

Finally, air volume in the reactor was checked to successfully accomplish aerobic biodegradation of sulfolane and thiolane, which was described in Eqs. (3) and (4), respectively.

$$C_4H_8SO_2+13/2O_2 \rightarrow 4CO_2+3H_2O+H_2SO_4 \quad (3)$$

Table 5. Theoretical oxygen demand for biodegradation of sulfolane and thiolane

Sulfolane		Thiolane	
Before incubation	After 15 days of incubation	Before incubation	After 15 days of incubation
912.21 mg/*l*	563.85 mg/*l*	13.15 mg/*l*	12.85 mg/*l*
126.63 ml O_2		0.17 ml O_2	

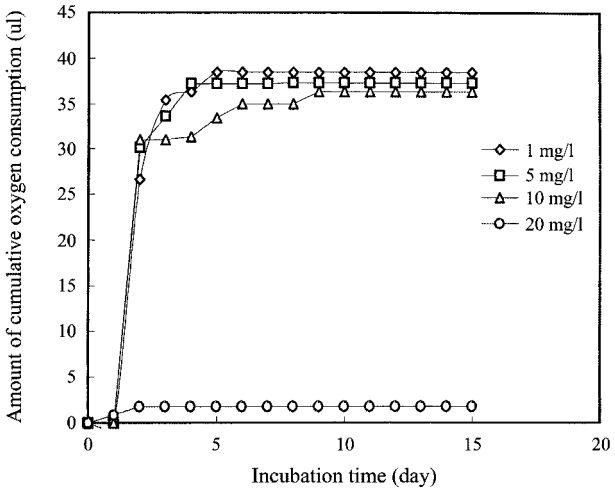

Fig. 5. Cumulative oxygen consumption for biodegradation of thiolane ranging from 1 to 20 mg/l in a lab prepared soil culture.

$$C_4H_8S+15/2O_2 \rightarrow 4CO_2+3H_2O+H_2SO_4 \quad (4)$$

Theoretical oxygen demand against the highest concentration of sulfolane and thiolane was compared to oxygen concentration in the reactor as described in Table 5. Oxygen volume in the reactor was calculated as 257.25 ml O_2, which was much higher than oxygen demands of sulfolane and thiolane showing 126.63 and 0.17 ml O_2.

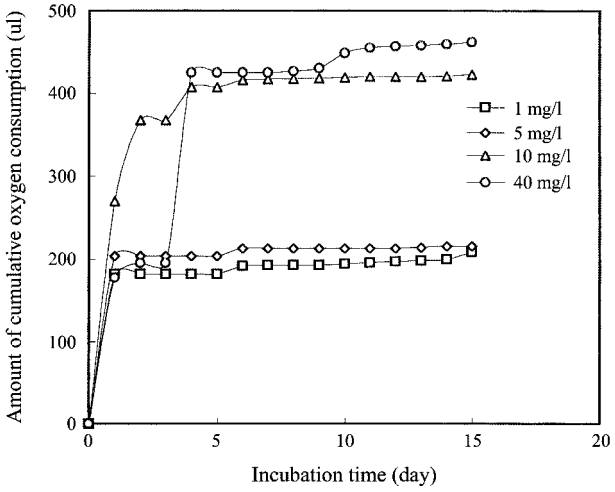

Fig. 6. Cumulative oxygen consumption for biodegradation of sulfolane ranging from 1 to 40 mg/l in a lab prepared soil culture.

4. Degradation Tests of Sulfolane and Thiolane

At first, degradation of thiolane was investigated for the range of concentrations as shown in Fig. 5.

The highest oxygen consumption was accomplished at 38.5 μL for 1 mg/*l* of thiolane and then the consumption dropped to negligible level for 20 mg/*l*. It indicates that soil mixtures of microorganisms may need an extended period to adapt to efficiently degrade thiolane even though relatively lower concentration of thiolane could be readily decomposed.

On the other hand, biodegradation of sulfolane was also investigated by measuring oxygen demand as shown in Figs. 6 and 7.

In lower concentrations ranging from 1.0 to 5.0 mg/*l*, oxygen was unvaryingly consumed up to 200 μL. By increasing sulfolane concentration to 10 mg/*l*, the oxygen consumption was correspond-

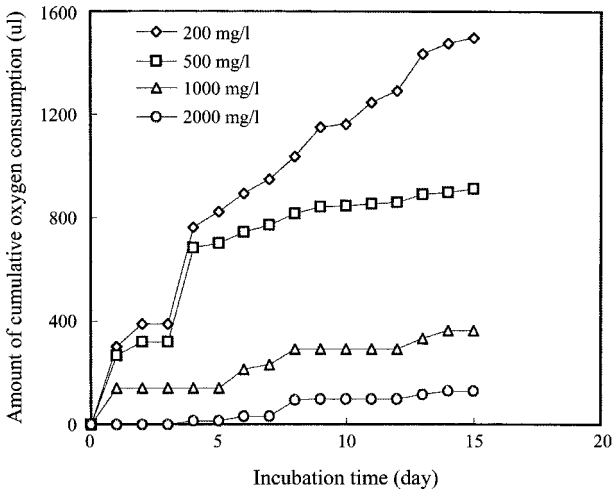

Fig. 7. Cumulative oxygen consumption for biodegradation of sulfolane ranging from 200 to 2,000 mg/l in a lab prepared soil culture.

Table 6. Residual concentrations of substrates after 15 days of incubation period

	Solution (200 ml)	Soil media	
		Before test being commenced	After 15 days
Thiolane (mg/*l*)	Sterilized	9.34	9.13
	1.0	0.68	0.59
	5.0	2.14	1.98
	10.0	4.58	4.49
	20.0	13.15	12.85
Sulfolane (mg/*l*)	Sterilized	10.30	10.34
	1.0	0.71	ND
	5.0	2.00	ND
	10.0	4.52	ND
	40.0	27.11	ND
	200.0	124.10	17.00
	500.0	148.40	78.10
	1000.0	415.42	220.30
	2000.0	912.21	563.85

ingly escalated twice and then peaked at 200 mg/*l* showing 1,500 μL of oxygen being consumed. However, increasing sulfolane concentration up to more than 500 mg/*l* unlikely decreased oxygen consumption in half, which was further proportionally decreased as the concentration of sulfolane was increased from 1,000 to 2,000 mg/*l*. It simply means that there existed a limiting concentration of sulfolane to be biologically degraded beyond 500 mg/*l*. In other words, it will take longer so that higher concentration of sulfolane above 500 mg/*l* can be biodegradably decomposed in a given soil microcosm. In the long run, the samples were analyzed to determine the residual concentrations of substrates as presented in Table 6. Table 6 shows clearly that sulfolane was significantly degraded in the concerned soil media while no critical degradation of thiolane was found in the given time period. Conversely, as shown in Fig. 8, the amount of oxygen consumed per unit mass of sulfolane degraded was not significantly reflected from cumulative oxygen consumption. Those were only marginally correlated.

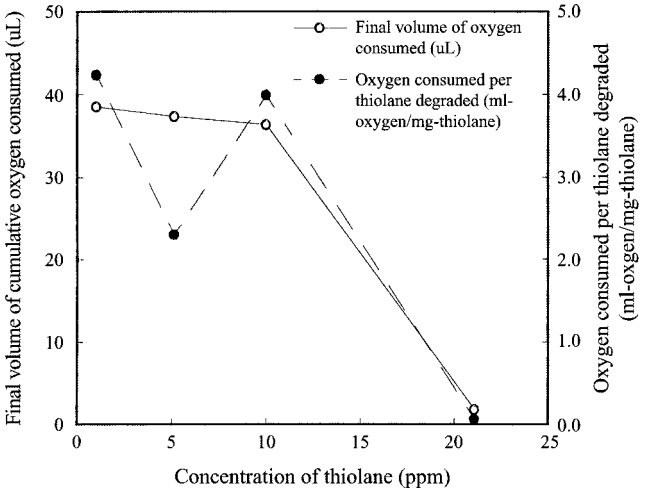

Fig. 8. Characteristics of oxygen consumption on various concentrations of thiolane incubated for 15 days in a lab prepared soil culture.

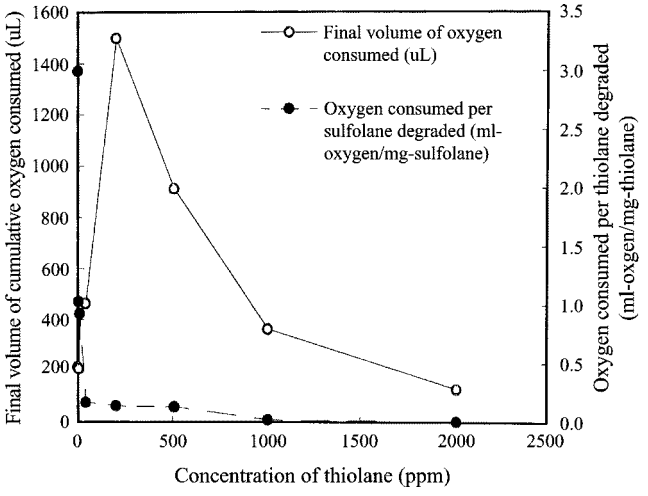

Fig. 9. Characteristics of oxygen consumption on various concentrations of sulfolane incubated for 15 days in a lab prepared soil culture.

On the other hand, as presented in Fig. 9, the unit amount of oxygen consumed for thiolane was well consistent with the cumulative oxygen uptake except for that obtained for 5 mg/l. It means that the degradation of thiolane can be agreeably estimated as conforming to oxygen demand. Therefore, oxygen consumption of thiolane can be likely used for both prediction of degradation and detection of possible limit of inhibition level, whereas the oxygen-uptake for sulfolane would be suitable for defining the potential range of biodegradable concentration applied for a given soil media.

CONCLUSION

Oxygen consumption of soil microcosms on contaminants was variously reflected from the type of contaminants and their concentration. Flask constant in this experiment was obtained as 88.60 μl/mm, which was used to calculate oxygen consumption for soil samples. Thiolane was insignificantly biodegradable at low concentration due to its intrinsic toxicity against microorganisms, while ranges of higher concentrations of sulfolane were readily degraded even though oxygen consumption observed from greater than 500 mg/*l* was starting to be decreased. It was supposed that it will take longer period of incubation so that certain level of sulfolane can be decomposed in a given soil culture. It is concluded that the oxygen consumption can be used for assessing potential degradation of contaminants (i.e., sulfolane and thiolane) and distinguishing critical limit of inhibition against the given microcosm.

ACKNOWLEDGEMENT

This work was partly supported by the Regional Research Center (RRC) program, the Ministry of Science and Technology (MOST) and the Korea Science and Engineering Foundation (KOSEF).

REFERENCES

Alam, K. Y., Worland, M. J. and Clark, D. P., "Analysis and Molecular Cloning of Genes Involved in Thiophene and Furan Oxidation by *E. coli*," *Applied Biochem. Biotech.*, **24/25**, 843 (1990).

Anderson, J. P. E., In: Page, A. L., Miller, R. H. and Keeney, D. R. (Eds.), "Soil Respiration, Methods of Soil Analysis, Chemical and Microbiological Properties," Agronomy No. 9, Part 2, 2nd Edition, 831 (1982).

Choi, Y. J., Kwon, T. I. and Yeo, Y. K., "Optimization of the Sulfolane Extraction Plant Based on Modeling and Simulation," *Korean J. Chem. Eng.*, **16**, 712 (2000).

Davis, J. W., Klier, N. J. and Carpenter, C. L., "Natural Biological Attenuation of Benzene in Ground Water Beneath a Manufacturing Facility," *Ground Water*, **32**, 215 (1994).

Fedorak, P. M., Payzant, J. D., Montgomery, D. S. and Westlake, D. W. S., "Microbial Degradation of *n*-alkyl Tetrahydrothiophenes Found in Petroleum," *Applied Environ. Microbiol.*, **54**, 1243 (1988).

Grimalt, J. O., Grifoll, M., Solanas, A. M. and Albaigés, J., "Microbial Degradation of Marine Evaporitic Crude Oils," *Geochimica et Cosmochimica Acta*, **55**, 1903 (1991).

Hobson, P. N., in: Norris, J. R. and Ribbons, D. W. (Eds.), "Rumen Bacteria, Methods in Microbiology," New York, Academic Press, Vol. 3B, 133 (1966).

Kim, C. G., Clarke, W. P. and Lockington, D., "Competitive Adsorption of Sulfolane and Thiolane on Clay Materials," *Korean J. Chem. Eng.*, **15**, 215 (1999).

Kim, C. G., Clarke, W. P. and Lockington, D., "Feasibility Test of Biological Degradation of Heterocyclic Sulfur Compounds in Aerobic State," *J. Environ. Sci. Health*, **A34**, 899 (1999).

Romli, M., "Modeling and Verification of a Two-stage High-rate Anaerobic Wastewater Treatment System," PhD thesis, Dept. of Chemical Engineering, University of Queensland, Australia (1993).

Umbreit, W. W., Burris, R. H. and Stauffer, J. F., "Manometric Techniques," Minneapolis, Burgess Publishing Co. (1964).