Biolog. J. Armenia, *Special issue: Cyclodextrins*, 2001

INNOVATIVE APPROACH FOR REMOVAL AND BIODEGRADATION OF CONTAMINATED COMPOUNDS IN SOIL BY CYCLODEXTRINS

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Hydroxypropyl-β-cyclodextrin (HP-β-CD) was used for the remediation of contaminated soil. Very highly efficient removal was obtained by using the kneading method. A simple calculation model for the equilibrium amount of dissolution of biphenyl was proposed on the basis of the equilibrium adsorption assumption. The decomposition of the biphenyl by the activated sludge depended on both the type of CD and the degree of inclusion of biphenyl with CD. The direct decomposition of biphenyl in the kneaded soil slurry was applied to the bioremediation of the contaminated soil treatment by activated sludge.

Использован гидроксипропил – β – циклодекстрин для обработки и оздоровления загрязненных почв. Достигнут очень высокий результат с применением метода смешивания. Предложена простая расчетная модель для равновесия количества растворения бифенила на основе уравнения адсорбции. Разложение бифенила активированным илом зависит как от типа ЦД, так и от степени включения бифенила в ЦД. Использовано прямое разложение бифенила в размешанной почвенной жиже с помощью обработки активированным илом для биообработки и оздоровления почв.

Օգտագործվել է հիդրօքսիպրոպիլ- β – ցիկլոդեքստրինը (ՀՊ- β – ՑԴ) աղտոտված հողերի մշակման և առողջացման համար: Ստացվել է շատ բարձր արդյունք խառնման մեթոդի կիրառմամբ: Ադսորբցիոն հավասարության հիման վրա առաջարկվել է պարզ հաշվարկային մոդել բիֆենիլի լուծելիության քանակական հավասարության համար: Ակտիվացված տիղմի բիֆենիլի քայքայումը միջոցով կախված է ինչպես ՑԴ տիպից, այնպես էլ ՑԴ-ի հետ բիֆենիլի ներառման աստիճանից: Հողերի կենսաբուժման և մշակման համար կիրառվել է հողային խառնված զանգվածում բիֆենիլի ուղղակի քայքայում ակտիվացված տիղմով մշակմամբ:

Introduction

Contamination of soil by PCB and dioxin has been one of the serious pollution problems. The compounds are poorly soluble and toxic substances and have a high affinity with soil, so that the effective removal of the compounds from the soil is an important technical problem [1]. Various methods have been applied for the remediation of contaminated soil [2, 3]. The remediation process is very complex, but commonly divided into two steps; the removal of strongly adhered compounds from soil, and the decomposition of the removed compounds.

Cyclodextrins (CDs) are cyclic oligosaccharides from the enzymatic treatment of starch. The compounds have a relatively apolar cavity in which hydrophobic organic compounds can be stably included. Cyclodextrins can help to solubilize poorly soluble

substances by formation of inclusion complexes. Molnár *et al.* [4] reported that randomly methylated β-cyclodextrin is very effective for treating polluted soils. Fenyvesi [5] has reviewed extensively the application of CDs for the decontamination of soil and the ground water, and suggested that a great advantage of applying CDs is their biodegradability by the soil microflora. Wang and Brusseau [6], and Fenyvesi *et al.* [7] used hydroxypropyl-β-cyclodextrin (HP-β-CD) for the solubilization of low-polar organic compounds, and transportation of these substances adhered on the soil surface. Fava *et al.* [8] developed soil reactors to study the intrinsic *ex-situ* bioremediation of chronically PCB-contaminated soil, indicating that both HP-β-CD and γ-CD significantly enhanced the biological degradation of the soil contaminated compounds. Furuta *et al.* [9] kneaded biphenyl polluted soil with HP-β-CD and found that the removal and biodegradation of biphenyl is markedly enhanced by kneading soil with HP-β-CD.

The aim of this study was the development of a process for the removal of the contaminated compounds from soil by including the compounds selectively in the cavity of cyclodextrin. In this work, to obtain the fundamental knowledge for the remediation of soil by means of cyclodextrin, biphenyl was used as a model contaminating compound. We carried out the fundamental studies such as the enhancement of the solubility of biphenyl by cyclodextrin, the desorption of biphenyl from the soil by kneading the mixture of the contaminated soil and cyclodextrins using a twin-screw kneader, and the biodegradation of the included biphenyl in an aerobic system by activated sludge. The effect of kneading and the concentration of HP-β-CD on the rate of desorption of biphenyl from the soil was investigated by measuring the dissolution rate of biphenyl into the solution. On the basis of the solubility and the adsorption isotherm of the biphenyl on the soil, the amount of the removal of biphenyl was estimated and compared with the experimental values.

Experimental Methods

Materials. Biphenyl was obtained from Wako Chemicals (Tokyo, Japan). Hydroxypropyl-β-cyclodextrin (HP-β-CD), methyl-β-CD, Maltosyl-β-CD, and β-CD were purchased from Ensuiko Sugar Refining Co. (Tokyo, Japan). β-LCD was an oligomer of about five β-CDs purchased from Katayama Chemical Co. (Osaka). Other chemicals were of reagent grade. The model soil ("Andisol") was gathered in the campus of Tottori University. The content of N, C, and H in the Andisol was 0.226, 5.61, and 1.16 %, respectively.

Measurement of solubility of biphenyl in various CD solutions. Various concentrations (0.2 – 2 mmol/L) of CD solutions of 100 mL were prepared in 300 mL Erlenmeyer flasks with a screw cap. Ten mg of biphenyl was added to the CD solution, and incubated at 30 ℃ for 72 hours in a rotary shaker. Then, 5 mL of the aqueous solution was sampled in a test tube and centrifuged at 3,000 rpm for 10 min. Three mL of the supernatant was put a mixture of chloroform and water (1:4 v/v), and the included biphenyl was extracted into the chloroform phase by heating and mixing at 80 ℃ for 15 min. The concentration of biphenyl in chloroform was determined by gas chromatography. The analytical conditions were: glass column (2 m × 2 mm I.D.) packed with PEG-20M on 80/100 mesh Chromosorb W, column temperature 190 ℃, injection temperature 200 ℃, and nitrogen as the carrier gas.

Adsorption isotherm of biphenyl with Andisol. Four grams of Andisol was mixed with aqueous biphenyl solution of various concentrations in a 100 mL Erlenmeyer flask, followed by incubation at 30 ℃ for 72 hours. Six mL of the solution was centrifuged at 3,000 rpm. Four mL of the supernatant was sampled and biphenyl was determined by the same procedure as described above. The adsorbed quantity of biphenyl on the Andisol was calculated from the mass balance of biphenyl.

Preparation of the model contaminated soil. Forty grams of the vacuum-dried Andisol was mixed with 4 mg of biphenyl and 16 mL of water. The mixture was put into a twin screw kneader [10]

and kneaded at 30 °C for 30 min to obtain a model contaminated soil, in which 100 μg of biphenyl was adsorbed on a unit gram of dry soil (called as the contaminated soil of 100 ppm).

Inclusion and removal of the adsorbed biphenyl from the contaminated soil by kneading. Forty grams of the model contaminated soil was mixed with HP-β-CD at concentrations of 5 to 50 molar-folds to the initial biphenyl content in the soil. Then, the mixture was kneaded in the twin screw kneader for 30 min at a water content of 40 %, including the adsorbed biphenyl into HP-β-CD. Five grams of the kneaded sample was mixed with 10 mL water in a 100 mL Erlenmeyer flask with a screw cap, and shaking at 30 °C and 72 rpm in a rotary shaker. At prescribed time intervals, 7 mL of water was sampled and the concentration of biphenyl was determined by the same procedure as described above.

Aerobic treatment of the included biphenyl solution. In order to investigate the effect of the extent of inclusion on the biodegradation of biphenyl, two types of solution were prepared. One was a fully inclusion complex solution of 100 ppm biphenyl with 50 molar-folds of HP-β-CD by mixing at 30 °C for 72 hours. In the other case solutions of the two compounds were simply mixed at the same mixing ratio. Twenty-five mL of each solution was mixed with the same volume of concentrated aerobic sludge, which was a gift from Kurita Kogyo Co. Ltd. (Tokyo), and incubated at 30 °C. The concentration of biphenyl in the mixture was measured at prescribed intervals by gas chromatography as described above. For practical application of biodegradation by the activated sludge method, contaminated soil kneaded with HP-β-CD was directly incubated with the activated sludge. A model contaminated Andisol of 300 ppm (300 μg of biphenyl adsorbed on a unit gram of dry soil) was kneaded with HP-β-CD of 50 molar-fold, followed by mixing and incubating with the aerobic sludge of 50 mL at 30 °C and 85 rpm. At prescribed intervals, 1 mL of the slurry was sampled, and the total concentration of biphenyl in the slurry was measured by a similar procedure described above.

Results and Discussion

Enhancement of solubility of biphenyl in various cyclodextrin solutions. The solubility of biphenyl (mmol/L) in various CD solutions against the concentration of CD in the solution (mmol/L) is illustrated in Figure 1. β-CD, HP-β-CD, and methyl-β-CD markedly increased the solubility of biphenyl, while for Maltosyl-β-CD the solubility was slightly lower than with the other three CDs. The solubility changed linearly with an increase in the concentration of CD. However, above 1000 mg/L of the CD, the solubility in β-CD began to decrease because of the crystallization and the sedimentation of the inclusion complex. Assuming that a one-to-one complex was formed, the association constant K_c of biphenyl for each CD can be calculated from the slope s of the solubility line as [10]:

$$K_c = \frac{s}{b(1-s)} \tag{1}$$

where b is the interception of the line with y-axis in mg/L. Table 1 shows the association constant K_c of biphenyl for the various types of CD. Though β-CD has the highest value of K_c, it could not be used because of the sudden decrease of solubility above the CD concentration of 1000 mg/L. HP-β-CD and methyl-β-CD have also a higher value of K_c, indicating that these CDs have the potential ability of removing biphenyl from the contaminated soil. Therefore, we concluded that HP-β-CD was the most suitable among all the CDs.

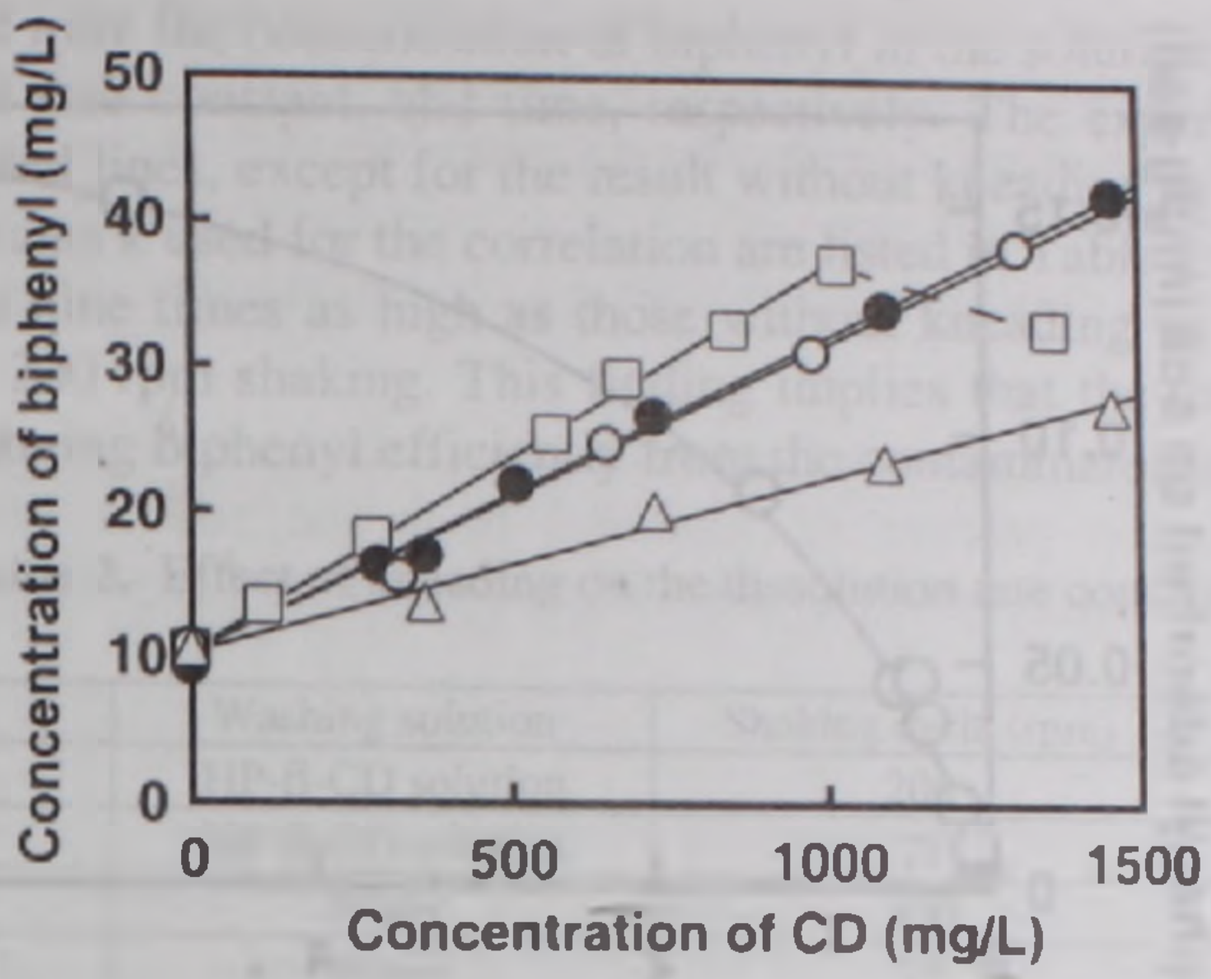

Figure 1. Solubility of biphenyl in various kinds of CD solutions. ●: HP-β-CD, ○: Methyl-β-CD, □: β-CD, ▽: Maltosyl-β-CD. Temperature was 30 °C. Solid lines are the linear correlation lines. Association constants of biphenyl to CD can be calculated by the slope and the y-interception of the line.

Table 1. The association constant of biphenyl with various CDs

	HP-β-CD	Methyl-β-CD	β-CD	Maltosyl-β-CD
K_c (L/mg)	0.00257	0.00247	0.00305	0.00142

Adsorption isotherm of biphenyl with Andisol. Figure 2 shows the adsorption isotherm of biphenyl with Andisol at 30°C. The amount of adsorbed biphenyl was markedly dependent on the content of organic compounds in the soil. The experimental data could be correlated well with the following Langmuir equation:

$$q = \frac{0.21C}{1 + 2.1C} \tag{2}$$

where q represents the amount of adsorbed of biphenyl (mg/g-dry soil) and C the concentration of biphenyl in the solution (mg/L) at equilibrium conditions.

Effective removal of biphenyl from the contaminated soil by kneading. The effectiveness of the kneading on the removal of biphenyl from the contaminated soil was investigated by measuring the dissolving rate of the included biphenyl from the soil into water. Figure 3 shows the dissolving time-course of biphenyl from the soil kneaded with HP-β-CD and the soil without kneading. A HP-β-CD solution of equal concentration (50 molar-fold to the initial biphenyl concentration in soil) was used as a dissolving medium in case of the soil without kneading. For the soil kneaded with HP-β-CD, biphenyl released and dissolved markedly faster, particularly at the lower shaking rate of 72 rpm. This implies that during kneading biphenyl in the soil was effectively included in HP-β-CD and removed from the soil. However, the equilibrium concentration of dissolution showed the same value regardless of the kneading, though the equilibrium concentration for the soil without

kneading could not be reached at 72 rpm shaking. It was also found that kneading for 60 min was enough to reach the equilibrium concentration at both 72 and 200 rpm shaking.

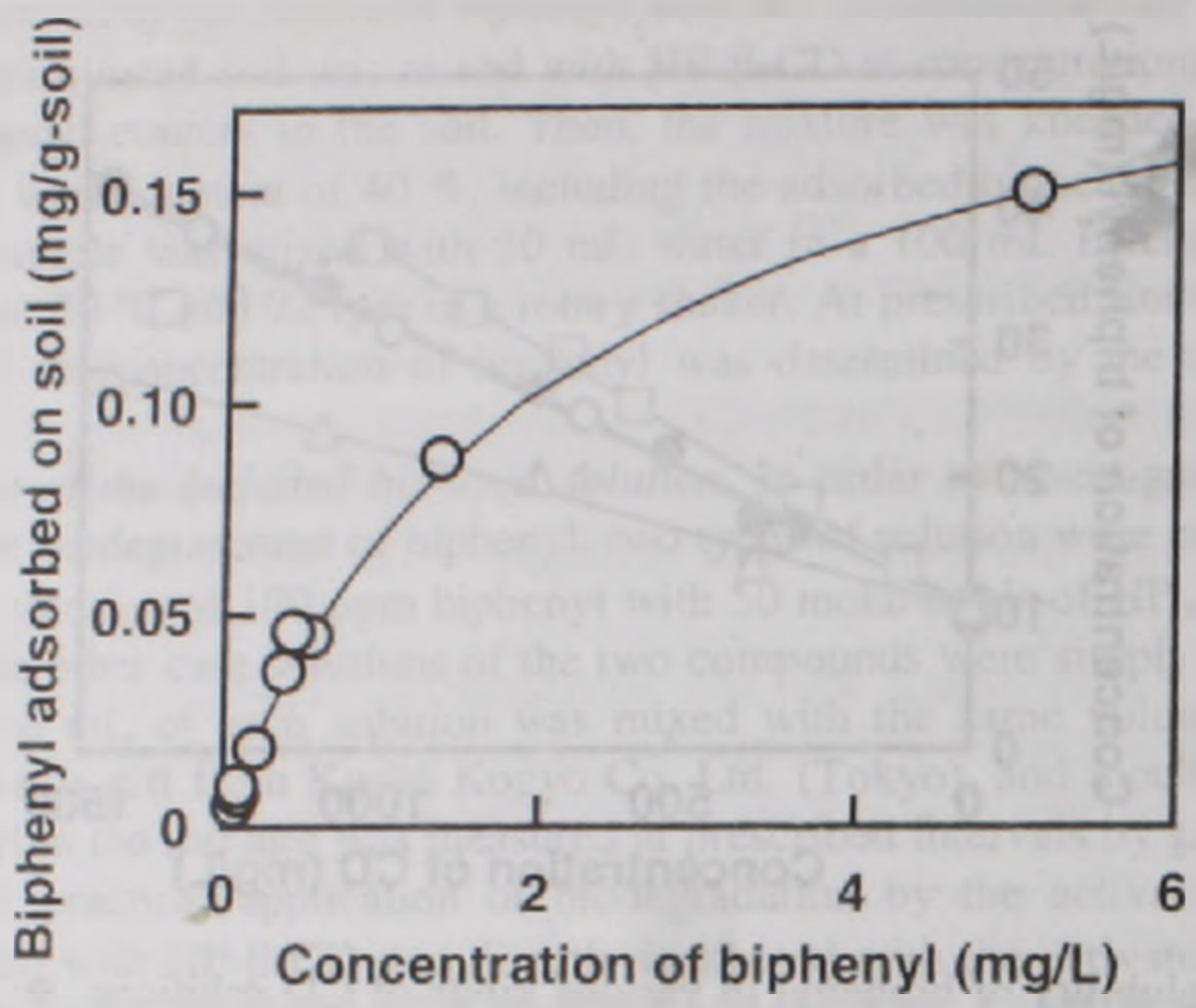

Figure 2. Adsorption isotherms of biphenyl with Andisol. ○: Equilibrium amount of biphenyl adsorbed on Andisol. Solid line is the correlation curve by equation (2).

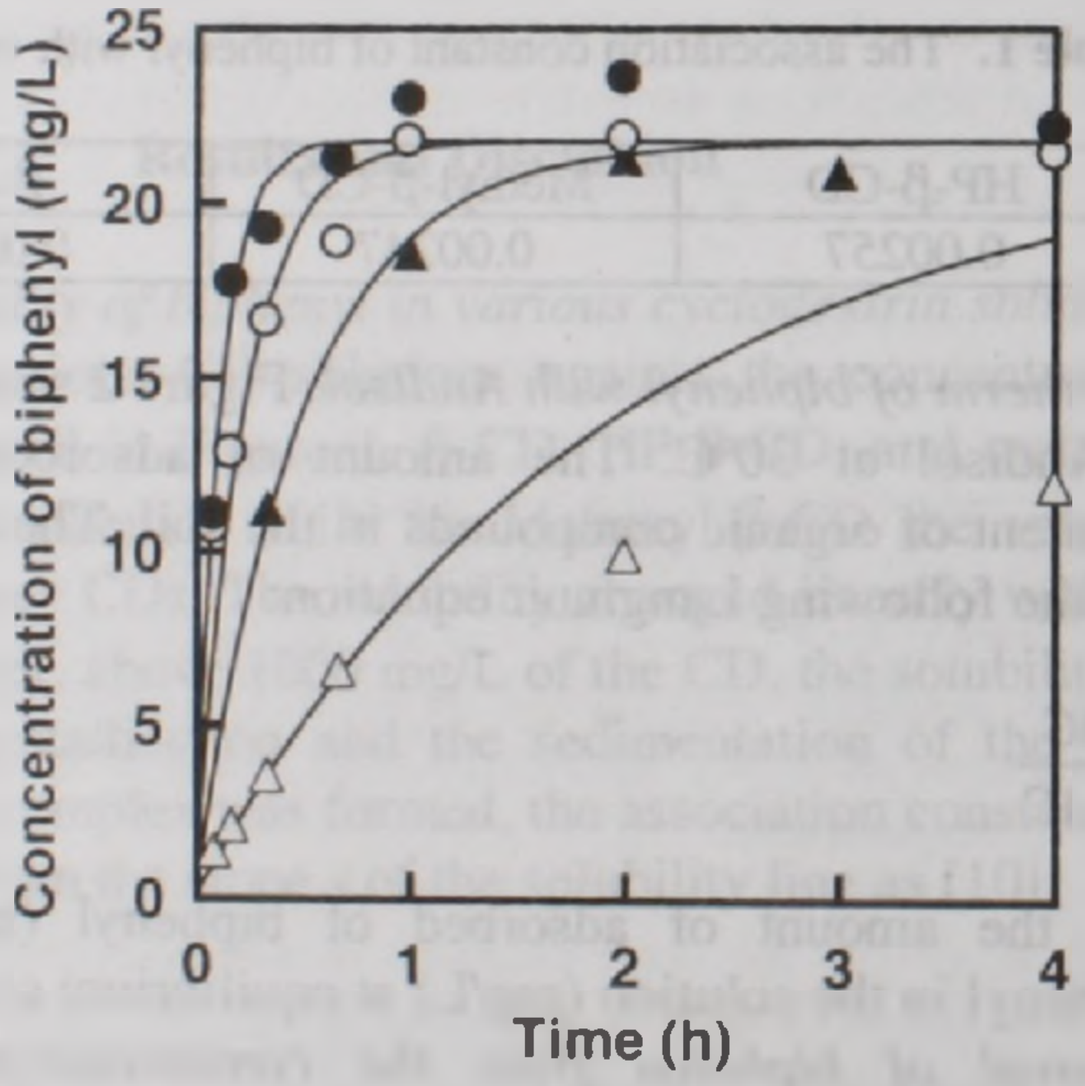

Figure 3. Effect of kneading on the rate of removal of biphenyl. ○, ●: Kneading with HP-β CD. △,▲: Without kneading. Closed symbols represent shaking at 200 rpm, and open symbols at 72 rpm. The solid lines are the correlation lines by equation (3). Initial biphenyl concentration in soil was 100 μg/g-dry soil. The molar ratio of the added HP-β-CD to the initial amount of biphenyl was 50. The water content during kneading was 40 % on dry basis.

The solid lines in Figure 3 represent the correlation curves by the following equation of the step response:

$$C = C_{max}(1 - e^{kt}) \tag{3}$$

where C, C_{max}, k, and t are the concentration of biphenyl in the solution, the maximum value of C, the dissolution rate constant, and time, respectively. The experimental values fitted well with the calculated lines, except for the result without kneading at 72 rpm shaking. The dissolution rate constants k used for the correlation are listed in Table 2. The values of k with kneading were about nine times as high as those without kneading at 72 rpm shaking, and 4.5 times as high at 200 rpm shaking. This finding implies that the use of a kneader has a high potential in removing biphenyl efficiently from the contaminated soil.

Table 2. Effect of kneading on the dissolution rate constant k

	Washing solution	Shaking cycle (rpm)	k (1/s)
Kneaded soil	HP-β-CD solution	200	2.43×10^{-3}
Kneaded soil	HP-β-CD solution	72	1.25×10^{-3}
Natural soil	Water	200	0.57×10^{-3}
Natural soil	Water	72	0.14×10^{-3}

The rate of removal of biphenyl from the soil under various concentrations of HP-β-CD. The rate of removal of biphenyl from soil was also investigated by changing the amount of HP-β-CD in kneading. As shown in Figure 4, both the dissolution rate and the maximum concentration of the included biphenyl increased considerably as the concentration of HP-β-CD increased. The solid lines in Figure 4 represent the correlation curves according to the equation (3) for $k = 1.25 \times 10^{-3}$ (1/s). The experimental values fitted well with the calculated lines using a unique value of k, indicating that the amount of HP-β-CD added during kneading did not affect the dissolution rate k but the maximum concentration of dissolution C_{max}.

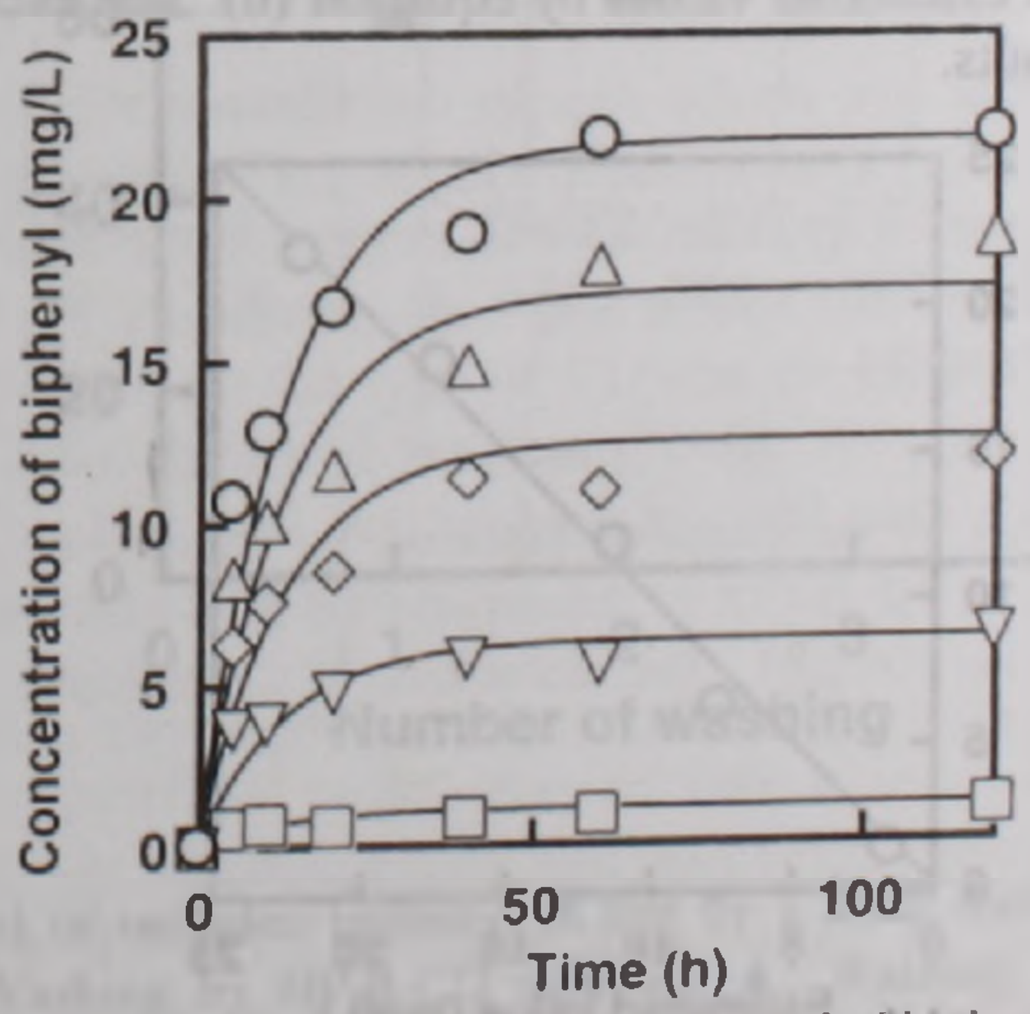

Figure 4. Effect of HP-β-CD concentration on the removal of biphenyl. The initial biphenyl concentration in soil, the added amount of HP-β-CD, and the water content during kneading are the same as in Figure 3. The solid lines are the correlation curves by equation (3). The dissolved rate constant k was the same value of 1.25×10^{-3} (1/s) for all the values of HP-β-CD added. The kneading conditions were the same as in Figure 3.

Estimation of the maximum concentration of biphenyl in solution. The maximum concentration of biphenyl in solution was estimated with the following assumptions:

i) In aqueous solution, cyclodextrin C_{CD}, biphenyl C_B and the inclusion complex C_{BCD} are equilibrated with each other, as given by equation (4);

$$C_B + C_{CD} \xleftrightarrow{K_c} C_{BCD} \tag{4}$$

where K_c is the association constant of equation (1).

ii) The dissolution process of biphenyl from the soil is assumed to be in an equilibrium. Therefore, equation (2) should hold between the adsorbed biphenyl and the free biphenyl in the solution.

Under these assumptions, the mass balance equation of biphenyl could be obtained as given by equation (5):

$$mq_0 = (K_c C_B C_{CD} + C_B)V + m\frac{k_1 C_B}{1 + k_2 C_B} \tag{5}$$

where m, q_0, V, k_1, and k_2 are the mass of the dry soil, the biphenyl adsorbed initially on the soil, the liquid volume, and the Langmuir constants in equation (2), respectively. Substituting equations (1) and (2) into equation (5), one could obtain a quadratic equation of C_B, which could be solved to give (6):

$$C_B = \frac{-\{(K_c C_{CD} + 1)Vk_2 + mk_1 - mq_0\} + \sqrt{\{(K_c C_{CD} + 1)Vk_2 + mk_1 - mq_0\}^2 - 4(K_c C_{CD} + 1)Vk_2 mq_0}}{2(K_c C_{CD} + 1)V} \tag{6}$$

Figure 5 shows the comparison between the equilibrium concentration of biphenyl C_B at 60 min shaking and the calculated values by equation (6). The estimated values fitted well with the experimental results.

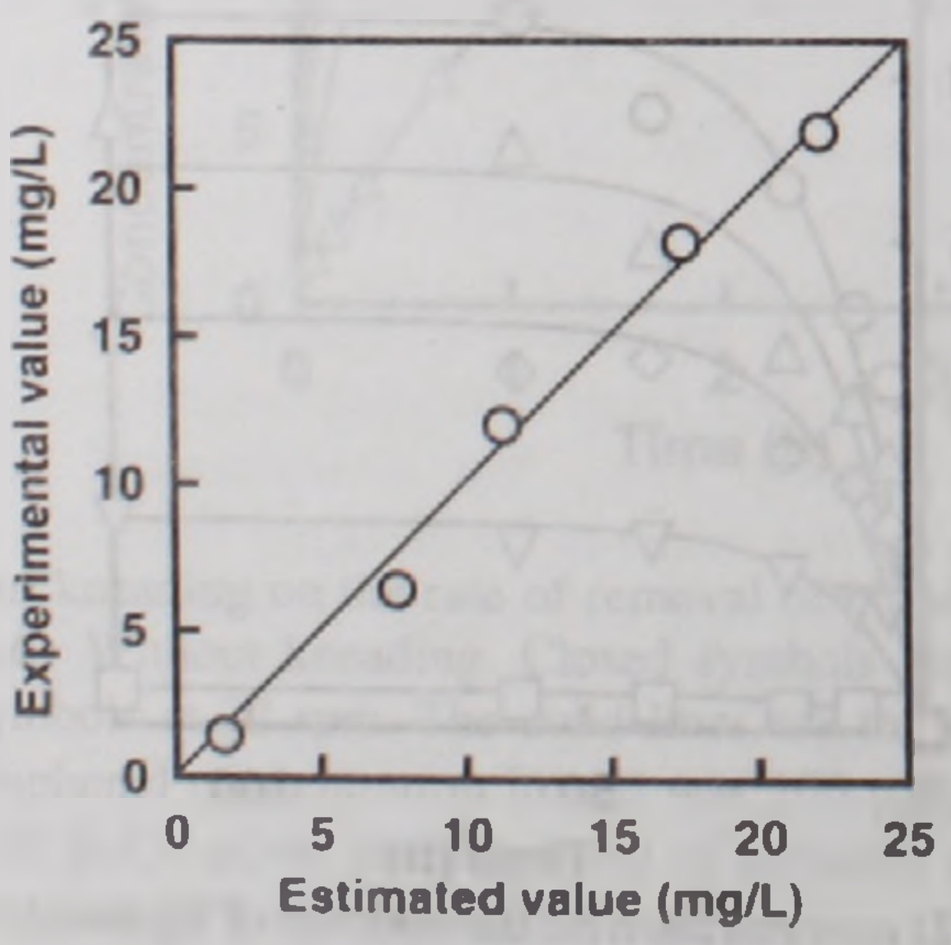

Figure 5. Estimation of the maximum concentration of biphenyl in solution. ○: 60 min shaking. The kneading conditions were the same as in Figure 3.

Washing out of the included biphenyl in the soil by a batch multi-stage washing operation. Since the included biphenyl by kneading exists in the soil, one needs the washing operation for several times to decrease the biphenyl concentration below an adequate level. The kneaded soil with HP-β-CD was washed three times by using distilled water or HP-β-CD solution with shaking at 200 rpm for 60 min. The concentration of biphenyl in the solution was measured after each washing step. At the same time, the biphenyl concentration at the i-th washing step C_{Bi} was estimated by a modified equation (6) as:

$$C_B = \frac{-\{(K_c C_{CD}+1)Vk_2 + nk_1 - mq_{i-1} - \alpha_{i-1}\} + \sqrt{\{(K_c C_{CD}+1)Vk_2 + nk_1 - mq_{i-1} - \alpha_{i-1}\}^2 - 4(K_c C_{CD}+1)Vk_2(mq_{i-1} - \alpha_{i-1})}}{2(K_c C_{CD}+1)V} \quad (7)$$

where q_i is the biphenyl adsorbed on the soil after the i-th washing and α_i ($\alpha_0 = 0$) is the amount of biphenyl remaining in the solution after the i-th washing in the void of the soil layer. Figure 6 shows the total removal of biphenyl against the number of washings. After washing 3 times, biphenyl was totally removed from the soil when HP-β-CD solution was used as the washing solution. On the other-hand, the total removal efficiency remained unchanged when water was used. In Figure 6, the removal by shaking for 5 min is also shown. The total removal efficiency increased markedly between the first and the second washing, though the removal was not perfect after washing 3 times. The solid and dotted lines in Figure 6 are the estimates of the removal by equation (7), indicating satisfactory results obtained by a simple calculation.

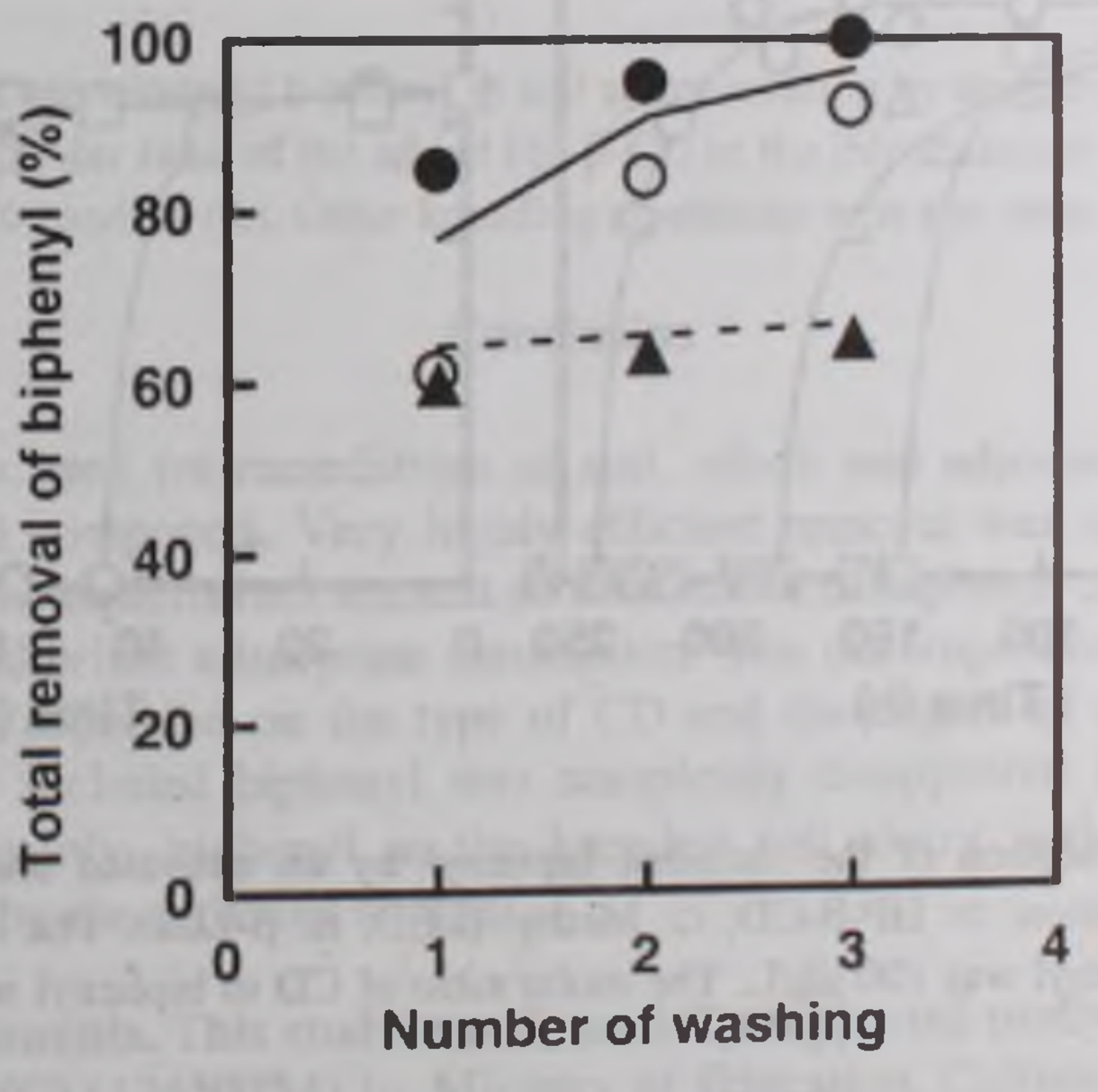

Figure 6. Removal of included biphenyl in soil by a batch multi-stage washing operation. ○, ●: Washing by HP-β-CD solution. ▲: Washing by water. Closed symbols represent shaking for 60 min, and open symbols for 5 min. The solid and dotted line are the estimated removal curves by equation (7). The kneading conditions were the same as in Figure 3.

Biodegradation of biphenyl solution by an activated sludge under aerobic conditions. Two types of biphenyl solution were incubated with an activated sludge under aerobic conditions. One was a perfectly included biphenyl solution with CD (called as "included solution"). In the other case, biphenyl and CD were simply mixed in the activated sludge solution just before the incubation (called as "simply mixing solution"). Three kinds of CDs such as HP-β-CD, methyl-β-CD, and β-LCD were used for the inclusion of biphenyl. The time-courses of biphenyl in both solutions are illustrated in Figure 7, (a) for the simply mixing solution and (b) for included solution. For the simply mixing solution, sudden decomposition of biphenyl was observed after 100 to 200 hours following inoculation as shown in Figure 7. Biphenyl mixed with methyl-β-CD could be decomposed fastest, and totally degraded in 150 hours. The rate of decomposition using HP-β-CD was the slowest and 240 hours were needed for nearly complete degradation. On the other hand, biphenyl in the included solution could be degraded surprisingly fast for all kinds of CDs. Contrary to the simply mixing case, HP-β-CD was the most suitable one, and biphenyl in the included solution completely disappeared in 45 hours, about five times faster than in the simply mixing case. For the other CDs, the deradation time decreased less. These findings imply that the degree of inclusion of biphenyl with CD was very important for the effective biodegradation by activated sludge.

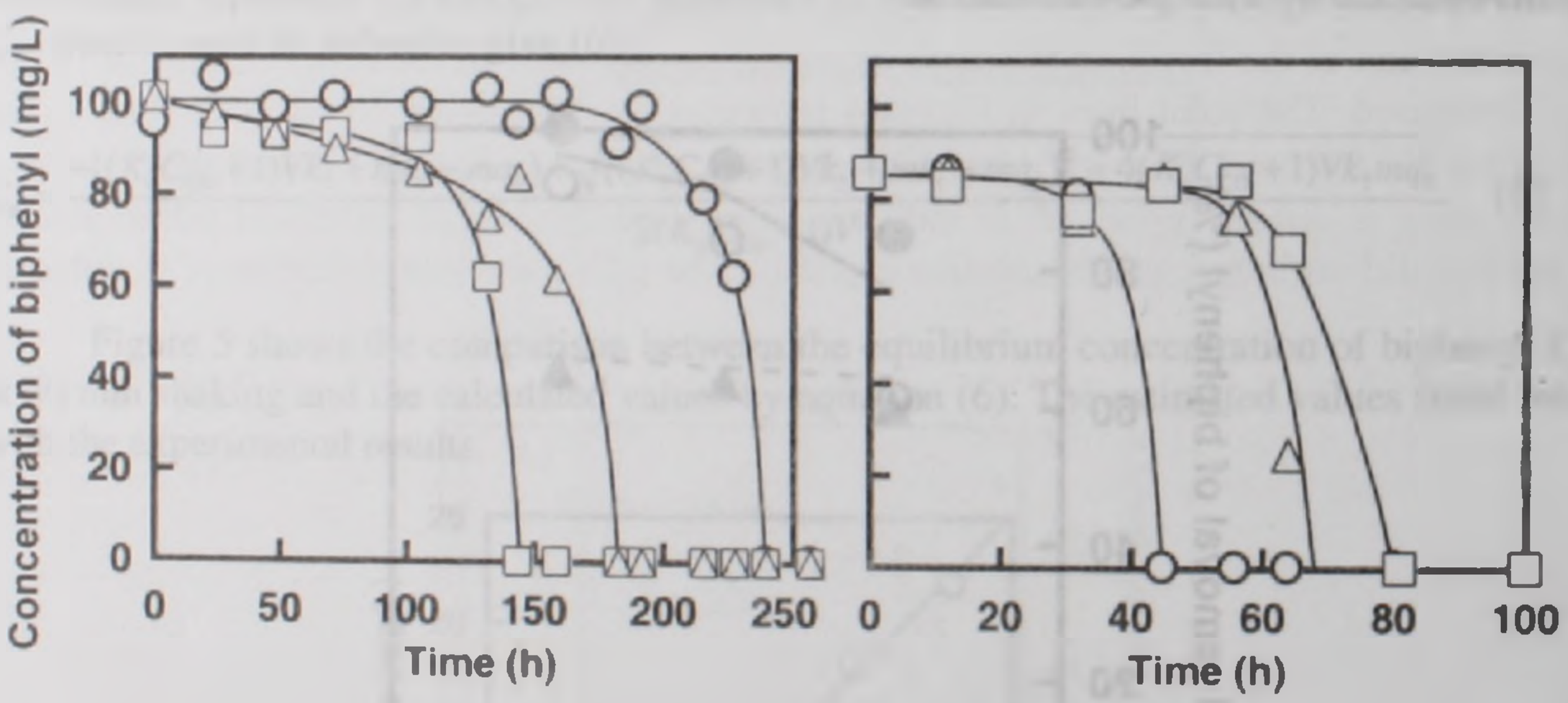

Figure 7. Degradation of the included biphenyl by an activated sludge under an aerobic condition. ○: HP-β-CD, □: Methyl-β-CD, Δ: β-LCD. The initial concentration of biphenyl was 100 μg/L. The molar ratio of CD to biphenyl was 50. Incubated at 30 °C.

Biodecomposition of biphenyl in soil slurry solution by an activated sludge. As mentioned above, biphenyl in the kneaded soil with HP-β-CD should be washed several times for complete removal from the soil, resulting in a large amount of the waste water. To simplify the treatment process and reduce the waste water, biphenyl in the kneaded soil with HP-β-CD was directly treated by the activated sludge. Figure 8 illustrates the degradation time-course of biphenyl in the kneaded soil with the addition of HP-β-CD of 10 and 50 molar-folds to the initial biphenyl content. The degradation rate of biphenyl was proportional to the added amount of HP-β-CD. Biphenyl concentration decreased gradually from the

beginning of the incubation, and no sudden decrease of the concentration could be observed as can be seen in Figure 7(b). However, the time needed for the complete disappearance was nearly the same in both cases. This implies that biphenyl in the kneaded soil would be perfectly included during kneading with HP-β-CD, and that the direct treatment of the kneaded soil by the activate sludge would be practical for the bioremediation of the contaminated soil.

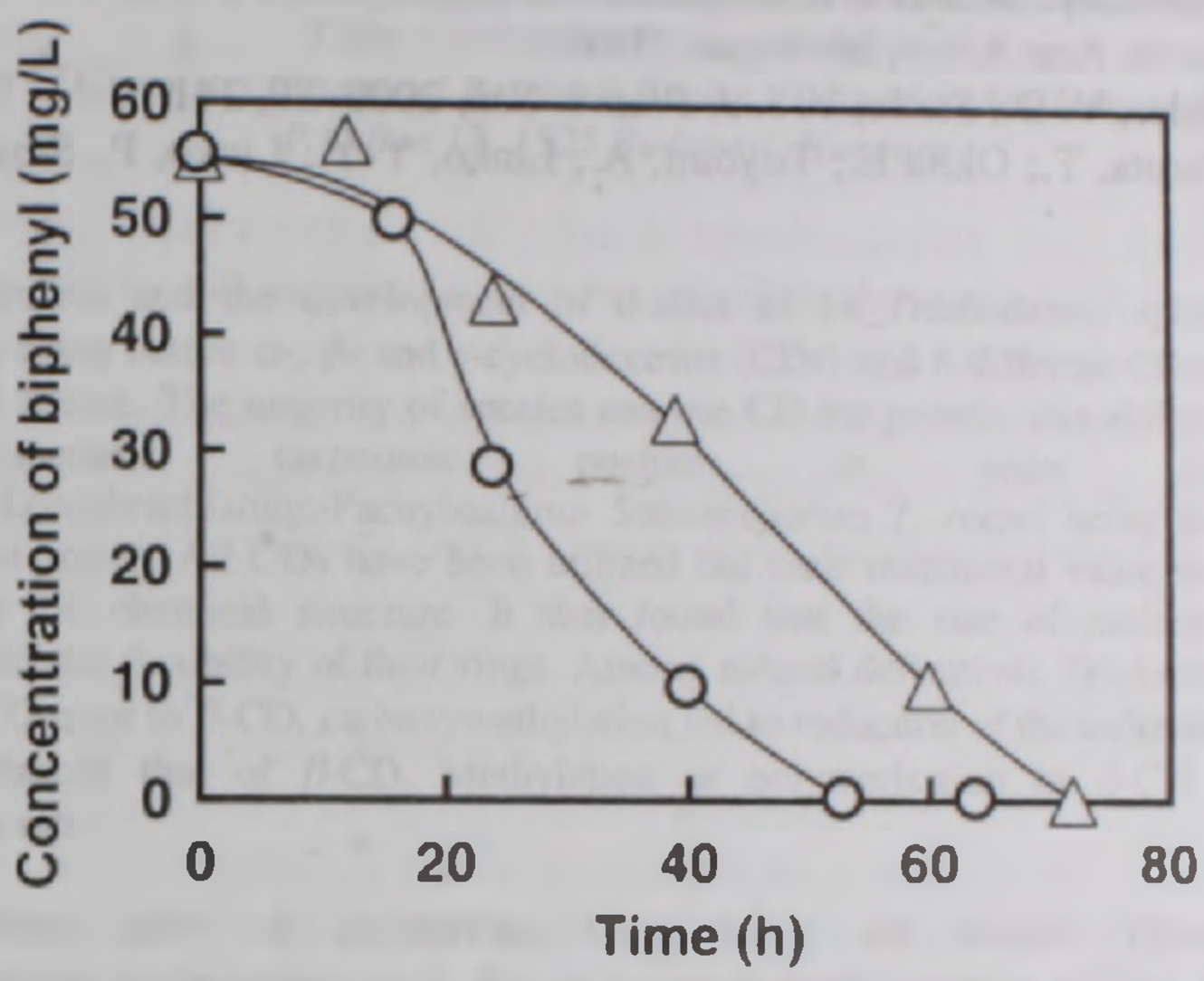

Figure 8. Decomposition of biphenyl in soil slurry solution by an activated sludge. The molar ratio of the added HP-β-CD to the initial amount of biphenyl in soil was 50 (○) and 10 (Δ). Other kneading conditions were the same in Figure 3.

Conclusins

HP-β-CD was used for remediation of soil, which was adsorbed with biphenyl as a model contaminated compound. Very highly efficient removal was obtained by using the kneading method. The equilibrium amount of dissolution of biphenyl could be calculated on the basis of the equilibrium adsorption assumption. The decomposition of the biphenyl by the activated sludge depended on the type of CD and the degree of inclusion of biphenyl with CD. The fully included biphenyl was completely disappeared in 40 to 80 hours of incubation. Consequently, biphenyl in the kneaded soil slurry with HP-β-CD could be successfully treated by the activated sludge.

Acknowledgements. This study was financially supported partly by a grant-in aid for Scientific Research (C) (12650754) by Ministry of Education, Culture, Sports, Science and Technology, and partly by Research Institute of Innovative Technology for the Earth (RITE).

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