한국환경위생학회지, 제22권 제3호 (1996)
Kor. J. Env. Hlth. Soc., Vol. 22, No. 3, pp 1~7 (1996)

Biodegradation of Polychlorinated Biphenyls (PCBs) within Insulating Oil by *Pseudomonas* sp. P2

Jung-Ho Kim, Sang-Ki Choi and Young-Ho Kim*

Dept. of Environmental Science, Kyungsan University, Kyungsan, 712-240, Korea
**Dept. of Food Science and Technology, Yeungnam University, Kyungsan, 712-749, Korea*

Pseudomonas sp. P2에 의한 絶緣油 內의 Polychlorinated Biphenyls (PCBs)의 分解

金政鎬 · 崔尙基 · 金榮鎬*

慶山大學校 環境科學科, *嶺南大學校 食品加工學科

국문초록

Polychlorinated Biphenyls(PCBs)의 生物學的 處理가 試圖되고 있으며, PCBs를 分解할 수 있는 微生物을 利用할 수 있다. 따라서 本 硏究에서는 폐기된 絶緣油의 生物學的 處理를 위하여 PCBs를 分解하는 菌을 分離하였으며, 分離된 균을 이용하여 絶緣油 內의 Polychlorinated Biphenyls(PCBs) 分解를 回分式 實驗에서 硏究하였다. 大邱의 新川으로부터 유일한 炭素原으로 Biphenyl을 包含하고 있는 固體培地에서 PCBs를 分解할 수 있는 *Pseudomonas* sp. P2 菌株를 分離하였다. PCBs의 용해도를 높이기 위해 사용된 유화제 alkyl aryl ethoxylated phosphate가 200 mg/L에서는 *Pseudomonas* sp. P2 균주의 성장에 영향을 미치지 않았다. 1000 mg/L의 Biphenyl과 PCBs에 *Pseudomonas* sp. P2 를 접종하여 160 時間 培養후에 Biphenyl과 PCBs의 分解가 各各 97.5%, 58.0%였다. Biphenyl 1000 mg/L에서 最大成長率 (μ_{max})은 0.34 day^{-1}, 合成係數(Y)는 0.59였다. PCBs 1000 mg/L에서의 最大成長率 (μ_{max})와 合成係數(Y)는 各各 0.29 day^{-1}, 0.26 였다. 따라서 鹽素가 結合되지 않은 Biphenyl는 鹽素가 結合된 PCBs 보다 分解가 빠르게 進行되었다. 또한 *Pseudomonas* sp. P2는 Biphenyl과 PCBs의 分解로 부터 誘導된 黃色의 分解代謝産物을 生成하였다. 본 연구에서는 *Pseudomonas* sp. P2 菌株가 絶緣油 內의 PCBs를 分解할 수 있다는 것을 確認하였다.

Keywords : Polychlorinated Biphenyls, Insulating Oil, *Pseudomonas* sp., Biodegradation.

I. Introduction

Polychlorinated biphenyls (PCBs) consists of a biphenyl accompanied with a number of chlorine. PCBs has 209 possible isomers with differing numbers and positions of the chlorines.[1] The commercial PCBs was used as insulating oil to the electrical transformers and capacitors. PCBs do not break down easily in the natural environment.[2] As a result, high concentrations of PCBs have been accumulated at significant levels in ecosystem.[3]

PCBs was first synthesized in 1881, and has been produced commercially since 1929.[4] The cumulative world production of PCBs is estimated to be 1.2×10^6 ton. It can be estimated that some amount of 780×10^3 ton of PCBs are still in use in older electrical equipment and other products, and some other amount of PCBs are deposited in landfills, dumped, and stored in the world.[4] It is estimated that Korea utilizes and stores about 205 ton of insulating oil containing PCBs.[5]

The studies on biodegradation of PCBs are essential to apply the treatment of PCBs within insulating oil. Kimbara *et al.*[6] described the isolation of *Pseudomonas* sp. degrading PCBs. Reichardt *et al.*[7] demonstrated the comparative biodegradation of biphenyl and chlorobiphenyls by microorganism. Wong and Kaiser[8] studied the biodegradability of biphenyl and PCBs by

Achromobacter sp. and *Pseudomonas* sp. Furukawa *et al.*[9] examined the relationships between chlorine substitution and biodegradability of PCBs by *Alcaligenes* sp. and *Acinetobacter* sp. The resistance of highly chlorinated PCBs to the degradation by bacteria poses a formidable problem to clean up the environment polluted with PCBs.[6]

Many studies have been done to isolate strains degrading PCBs,[5-9] but a few reports are available for the degradation of PCBs within insulating oil by microorganisms. To investigate the treatment of PCBs within insulating oil, this research was focused on the biodegradation of PCBs within insulating oil. We have attempted to measure the microbial parameters that describe the rates of PCBs substrate utilization in the batch system.

II. Materials and Methods

1. Chemicals

Biphenyl was purchased from Aldrich Chemical Co. Decachlorinated biphenyl (DCB) was purchased from DR. Ehrenstofer Co. (German). The alkyl aryl ethoxylated phosphate as surfactant 101 was used as an emulsifier. The insulating oil containing PCBs was obtained directly from transformer with Aroclor 1242.[10,11]

2. Microorganisms and Medium

The *Pseudomonas* sp. P2 was isolated from the soil on the Sinchun stream in Taegu. *Pseudomonas* sp. P2 was grown aerobically at 30℃ for 12 h in 30 mL nutrient broth medium with 150 rpm shaking incubator for seed culture. The cells were then harvested by centrifugation and washed throughly by deionized water.

The culture was maintained on the following basal medium with 1000 mg/L NH4NO3, 1000 mg/L KH_2PO_4, 100 mg/L $MgSO_4 \cdot 7H_2O$, 30 mg/L $CaCl_2 \cdot 2H_2O$, 200 mg/L NaCl, 10 mg/L $FeSO_4 \cdot 7H_2O$, pH 7.0, and deionized water 1L.[12]

3. Batch Experiments

The 1000 mg/L of biphenyl or PCBs as a carbon source were added to 30 mL liquid medium. The initial cell mass was adjusted to 160 mg/L at 1000 mg/L biphenyl and 130 mg/L at 1000 mg/L PCBs. *Pseudomonas* sp. P2 was grown aerobically at 30℃ for 160 h with 150 rpm shaking incubator. The determination of cell mass was carried out by filtrating with filter membrane (0.22 μm of pore size). After filtration, cell was dried at 90℃ for 2 h, and determined the cell mass (mg/L).

4. Analysis of Biphenyl and PCBs

An analysis of biphenyl and PCBs in medium was carried out by gas chromatography with perchlorination meaning that PCBs were converted into decachlorinated biphenyl (DCB).[13,14] The extraction of PCBs in 30 mL medium was accomplished by mixing the medium with 100 mL hexane by a magnetic stirrer for 1 h. The mixture was quantitatively transferred to a 250 mL separate funnel, the medium was discarded, and the hexane layer was dried over anhydrous Na_2SO_4. Add 0.5 mL $SbCl_5$, and maintain at 165℃ for 16 h. Add 5 mL of 30% HCl to minimize precipitation of oxychloride. Add 10 mL hexane, and shake thoroughly for 3 min. Percolate the hexane layer with 2 g of anhydrous Na_2SO_4, and ready for analysis by gas chromatography.[15]

PCBs was analyzed by the Varian 3300 gas chromatography equipped with ^{63}Ni electron capture detector. Gas chromatography parameters were shown in Table 1.

Table 1. Gas chromatography parameters for the analysis of decachlorinated biphenyl (DCB) converted from biphenyl and polychlorinated biphenyls (PCBs)

Parameters	Conditions
Instrument	Varian 3300
Detector	Electron capture detector
Column	2 m×0.6 cm, stainless
Packing material	3% OV-17, chrom. whp 80/100 mesh
Temperature	Injection 260℃, Column 250℃, Detector 260℃
Carrier gas	N_2 60 mL/min
Recorder	Chromate V 2.2

III. Results and Discussion

1. Isolation

Using the enrichment method, we obtained the colonies which grew in the presence of biphenyl as the sole carbon source from soil at the Sinchun stream in Taegu. Ten types of colonies with apparently different growth rates were appeared on basal medium agar plate. Among the 10 strain, strain P2 was showed the highest growth rate (Table 2).

To confirm the relation between the growth rate and the exhaustion of biphenyl, residual of biphenyl in medium was determined by the scanning of absorption spectrum from 200 to 300 nm after 120 hr of growth (Fig. 1). Fig. 1 (A) was showed a absorption peak of standard biphenyl at 240~300 nm. To confirm the degradation of biphenyl was characterized by a decrease in absorbance at 270~285 nm. Residual biphenyl in medium by strain P2 was showed a lower absorbance rather than that by control (Fig. 1 (B)). The biodegradability of biphenyl by strain P2 was confirmed. Therefore, strain P 2 was selected for degradation of biphenyl and PCBs. The isolated strain P2 should be identified as a strain of *Pseudomonas* sp. by Bergey's manual of systematic bacteriology.[16,17]

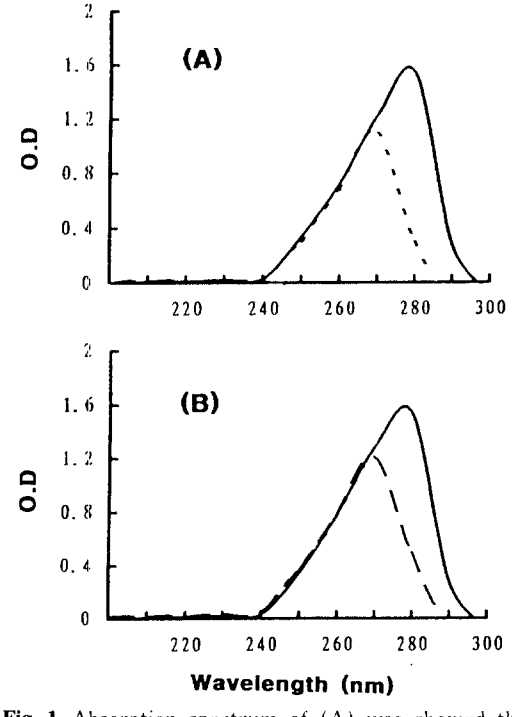

Fig. 1. Absorption spectrum of (A) was showed the standard biphenyl with 500 mg/L (——) and 50 mg/L (- - -) in hexane. Absorption spectrum of (B) was indicated the spectrum of residual biphenyl extracted from the medium of control (——) and strain P2 (- - -) after 120 h incubation.

2. Effects of Emulsifier

PCBs is hydrophobic and slightly soluble in water. The water solubilities of Arochlor 1242 and PCBs of insulating oil at room temperature were reported to 0.703 mg/L, and 0.698 mg/L, respectively.[10] The insulating oil was contained with Arochlor 1242.[11] The 1000 mg/L PCBs within insulating oil as a carbon source in medium needs to be required. Therefore, the emulsifier was added to the medium in order to increase 0.703 mg/L of water solubility of PCBs to 1000 mg/L PCBs in the medium. Liu[18] used the sodium ligninsulfonate as emulsifier to increase the solubility of Aroclor 1221. Bedard[19] also used Triton X-100 as emulsifier to increase the solubility of PCBs. The emulsifier used this experiment was 200 mg/L alkyl aryl ethoxylated phosphate as surfactant 101.

Table 3 showed the effects of alkyl aryl

Table 2. Comparison on the growth of isolated strains in medium containing 1000 mg/L biphenyl as a carbon source for 60 h incubation at 30°C

Strains	Cell growth (OD_{660})[1]	Strains	Cell growth (OD_{660})
P1	0.52	P6	0.32
P2	0.64	P7	0.30
P3	0.49	P8	0.35
P4	0.37	P9	0.36
P5	0.34	P10	0.37

1) The initial optical density of cells was adjusted to 0.25 at 660 nm.

ethoxylated phosphate from 100 mg/L to 50000 mg/L in medium consisting of glucose as a carbon source. The growth was not inhibited by a alkyl aryl ethoxylated phosphate from 100 mg/L to 1000 mg/L. Alkyl aryl ethoxylated phosphate was found to be the inhibition of the growth from 3000 mg/L to 50000 mg/L. The 200 mg/L alkyl alryl ethoxylated phosphate used in medium has no inhibition on the growth of *Pseudomonas* sp. P2.

3. Batch Experiments with Biphenyl

The relationships between growth of *Pseudomonas* sp. P2 and residual biphenyl during a batch culture were shown in Fig. 2. The gas chromatogram degrading biphenyl were shown in Fig. 3. The degradation of biphenyl by *Pseudomonas* sp. P2 was confirmed with the peak height of decreased DCB after 80 h incubation and 110 h incubation (Fig. 3 (B) and (C)). After a lag period of about 20 h, the growth of *Pseudomonas* sp. P2 and degradation of biphenyl were commenced, indicating that the growth of *Pseudomonas* sp. P2 was parallel to the degradation process of biphenyl.

The cell mass was increased from an initial value of 160 mg/L to 610 mg/L for 160 h incubation. Biphenyl was rapidly degraded

Table 3. Effects of alkyl aryl ethoxylated phosphate as an emulsifier on the growth of *Pseudomonas* sp P2. Cultivation was performed with medium using glucose as a carbon source. Relative activity was calculated with $O.D_{660}$ after 24 h incubation

Conc. of Emulsifier (mg/L)	Relative activity(%)	Conc. of Emulsifier (mg/L)	Relative activity(%)
0	100.0	3000	73.4
100	103.2	5000	58.9
200	103.4	7000	35.5
500	103.8	10000	19.2
700	102.2	30000	18.3
1000	102.5	50000	17.2

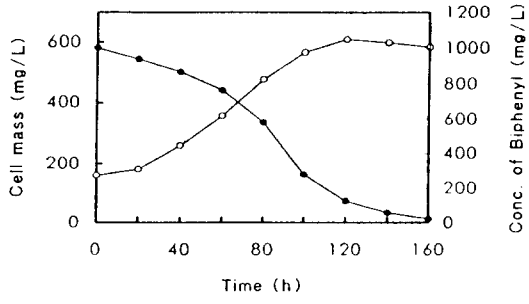

Fig. 2. Time course of growth (○——○) and degradation of biphenyl (●——●) in medium with 1000 mg/L biphenyl as a carbon source by *Pseudomonas* sp. P2.

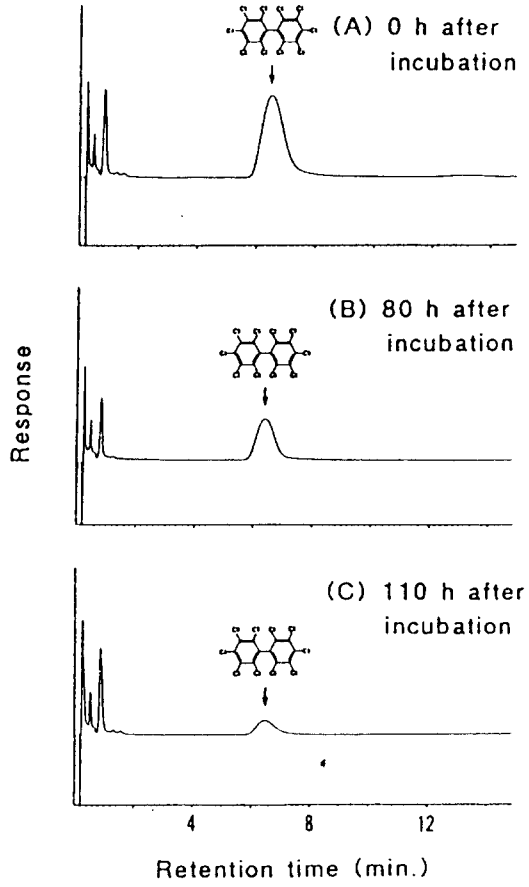

Fig. 3. GC-ECD chromatogram for the analysis of biphenyl in a media containing 1000 mg/L biphenyl during the culture of *Pseudomonas* sp. P2. Medium with 1000 mg/L biphenyl after 0 h incubation in (A), 80 h incubation in (B), and 110 h incubation in (C) were extracted with hexane and perchlorinated with SbCl5 to DCB. Then DCB was analyzed by GC.

between 20 h and 120 h of incubation. The biphenyl of substrate was decreased from 1000 mg/L to 130 mg/L at 120 h cultivation with 593 mg/L cell mass. After 160 h incubation, 97.5% of the initial amount of biphenyl was degraded. Degradation of biphenyl and growth of *Pseudomonas* sp. P2 were retarded after 120 h incubation (Fig. 2).

4. Batch Experiments with PCBs

The relationships between growth of *Pseudomonas* sp. P2 and residual PCBs during a batch culture were shown in Fig. 4. A lag period in the growth curve of *Pseudomonas* sp. P2 for PCBs degradation was 40 h. Kohler *et al.*[20] also reported the degradation of PCBs by *Acinetobactor* sp. P6 and *Arthrobacter* sp. B1B after a lag period of 40 h. A log period of the growth of *Pseudomonas* sp. P2 was reached to 100 h (Fig. 4).

The residual PCBs in medium was measured by gas chromatography after derivatizating with DCB. The chromatogram shown in Fig. 5 demonstrated that PCBs was converted into the fully chlorinated DCB. The degradation of PCBs by *Pseudomonas* sp. P2 was followed by analyzing PCBs at different time intervals (Fig. 4). After 80 h incubation, 49.0% of 1000 mg/L PCBs was utilized. After 100 h incubation, PCBs was varied from 1000 mg/L to 430 mg/L.

The yellow-colored intermediates were observed from biphenyl and PCBs by *Pseudomonas* sp. P2. The yellow compounds were gradually disappeared during further cultivation. Furukawa *et al.*[9] was reported that the yellow intermediate was a meta cleavage products. Those yellow compounds might be a meta cleavage intermediates (2-hydroxy-6-oxo-(pentachlorophenyl)hexa-3,4,5,-trichloro-2,4,-dienoic acid) on degradation of PCBs as des-

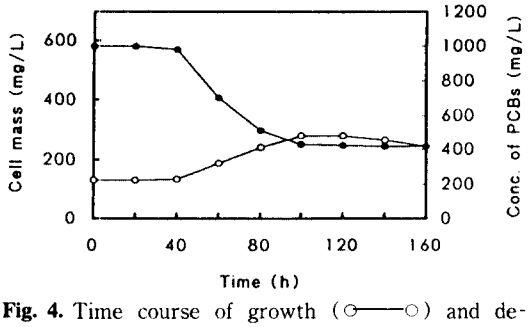

Fig. 4. Time course of growth (○——○) and degradation of polychlorinated biphenyls (PCBs) (●——●) in medium with 1000 mg/L PCBs as a carbon source by *Pseudomonas* sp. P2.

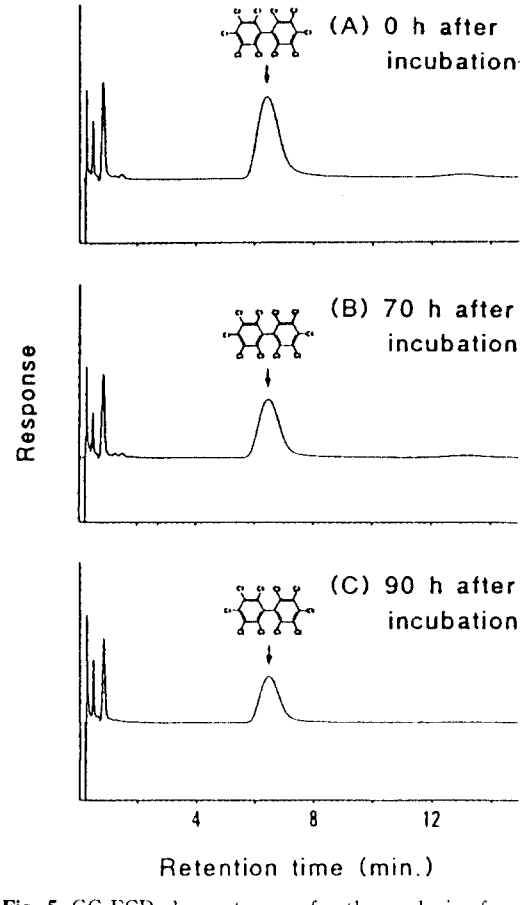

Fig. 5. GC-ECD chromatogram for the analysis of polychlorinated biphenyls (PCBs) in a media containing 1000 mg/L polychlorinated biphenyls (PCBs) during the culture of *Pseudomonas* sp. P2. Medium with 1000 mg/L polychlorinated biphenyls (PCBs) after 0 h incubation in (A), 70 h incubation in (B), and 90 h incubation in (C) were extracted with hexane and perchlorinated with SbCl5 to DCB. Then DCB was analyzed by GC.

cribed previously.[21]

5. Growth Kinetics

The kinetic coefficients on the growth of *Pseudomonas* sp. P2 were calculated from substrate utilization obtained in batch experiment (Fig. 2 and Fig. 4). The equations for the calculation of the specific growth rate (μ) and the yield coefficient (Y) during the log phase of the growth of *Pseudomonas* sp. P2 were as follows: 22)

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

$$Y = -\frac{X_2 - X_1}{S_2 - S_1}$$

where, X is cell mass (mg/L), and

S is concentration of substrate (mg/L).

In Table 4, the maximum growth rate (μ_{max}) and yield coefficient (Y) on μ_{max} of *Pseudomonas* sp. P2 for biphenyl and PCBs were shown. The maximum growth rate (μ_{max}) and yield coefficient (Y) of *Pseudomonas* sp. P2 on 1000 mg/L biphenyl were 0.34 day-1 and 0.59, respectively. The measured values of μ_{max} and Y for 1000 mg/L PCBs were 0.29 day^{-1} and 0.26, respectively.

Furukawa *et al.*[9] studied the relationships between chlorine substitution of PCBs and biodegradability of PCBs by two species of *Alcaligenes* and *Acinetobacter*. Focht and Brunner[23] studied the kinetics of metabolism with biphenyl and PCBs by *Acinetobacter* strain P6. Wong and Kaiser[8] demonstrated that the ability of the bacteria to degrade PCBs was decreased with percent increase of chlorination. They showed that the chlorination of the biphenyl retarded the bacterial degradation. Table 4 showed that the microbial degradation have related to the degree of chlorination. This result indicated that the unchlorinated biphenyl was much more rapidly disappeared than highly chlorinated biphenyls.

Table 4. Kinetics from the growth of *Pseudomonas* sp. P2 in the medium containing biphenyl or polychlorinated biphenyls (PCBs) as a carbon source

Substrate	Growth rate	Yield coefficient
	μ_{max} (day^{-1})	Y[1]
Biphenyl	0.34	0.59
PCBs	0.29	0.26

1) Yield coefficient (Y) was calculated on μ_{max}.

IV. Summary

The biodegradation of polychlorinated biphenyls (PCBs) within insulating oil was investigated in the batch experiments with *Pseudomonas* sp. P2. The growth inhibition of *Pseudomonas* sp. P2 was not shown in case of the 200 mg/L alkyl aryl ethoxylated phosphate of emulsifier. After 160 h incubation, the degradation of biphenyl and PCBs was 97.5% and 58.0%, respectively. The maximum growth rate (μ_{max}) and yield coefficient (Y) on 1000 mg/L biphenyl were 0.34 day^{-1} and 0.59, respectively. The values of maximum growth rate (μ_{max}) and yield coefficient (Y) on 1000 mg/L PCBs were 0.29 day^{-1} and 0.26, respectively. Biphenyl was disappeared much more rapidly than PCBs in batch culture. These results indicated that PCBs within insulating oil was apparently utilized as a carbon sources by *Pseudomonas* sp. P2.

V. Acknowledgment

This research was supported by a grant from the Korea Science and Engineering Foundation (No, 951-0606-035-2) in 1995~1996.

References

1) Bedard, D.L., Wagner, R.E., Brennan, M.J., Haberl, M.L. and Brown Jr., J.F. : Extensive degradation of Aroclors and environmentally transformed polychlorinated biphenyls by *Alcaligenes eutrophus* H850. *Appl. Environ. Microbiol.*, **53**(5), 1094-1102, 1987.

2) Harrad, S.J., Sewart, A.P., Alcock, R., Boum-

phrey, R., Burnett, V., Duarte-Davidson, R., Halsall, C., Sanders, G., Waterhouse, K., Wild, S.R. and Jones, K.C. : Polychlorinated biphenyls (PCBs) in the British environment : sinks, sources and temporal trends, *Environ. Pollut.*, **85**, 131-146, 1994.

3) Liu, D. : Biodegradation of Aroclor 1221 type PCBs in sewage wastewater, *Bull. Environ. Contam. Toxicol.*, **27**, 695-703, 1981.

4) Tanabe, S. : PCB problems in the future : Foresight from current knowledge." *Environ. pollut.*, **50**, 5-28, 1988.

5) Chosun-ilbo, Oct. 23. Seoul., pp.35, 1995.

6) Kimbara, K., Hashimoto, T., Fukuda, M., Koana, T., Takagi, M., Oishi, M. and Yano, K., : Isolation and characterization of a mixed culture that degrades polychlorinated biphenyls, *Agric. Biol. Chem.*, **52**(11), 2885-2891, 1988.

7) Reichardt, P.B., Chadwick, B.L., Cole, M.A., Robertson, B.R. and Button, D.K. : Kinetic study of the biodegradation of biphenyl and its monochlorinated analogues by a mixed marine microbial community, *Environ. Sci. Technol.*, **15**(1), 75-79, 1981.

8) Wong, P.T.S. and Kaiser, K.L.E. : Bacterial degradation of polychlorinated biphenylsII-Rate studies, *Bull. Environ. Contamin. Toxicol.*, **13**(2), 249-255, 1975.

9) Furukawa, K., Tonomura, K. and Kamibayashi, A. : Effect of chlorine substitution on the biodegradability of polychlorinated biphenyls, *Appl. Environ. Microbiol.*, **35**(2), 223-227, 1978.

10) Lee, M.C., Chian, E.S.K. and Griffin, R.A. : Solubility of polychlorinated biphenyls and capacitor fluid in water, *Water Research*, **13**, 1249-1258, 1979.

11) Kim, G.-J. and Park, J.-J. : A study on PCB analysis of insulating oil in waste condenser, *Kor. J. Environ. Toxicol.*, **4**, 11-17, 1989.

12) Choi, S.-K. : Biodegradation of polychlorinated biphenyls (PCBs) by *Pseudomonas* sp. P2, Master Thesis, Kyungsan Univ., Kyungsan, pp.1-17, 1996.

13) Armour, J.A. : Quantitative perchlorination of polychlorinated biphenyls as a method for confirmatory residue measurement and identification, *J. Assoc. Off. Anal. Chem.*, **56**, 987-993, 1973.

14) Moon, C.-H., Choi, S.-K. and Kim, J.-H. : Determination of polychlorinated biphenyls in the soil by perchlorination, *J. Korean Environ. Sci.*, **4**(3), 249-258, 1995.

15) Kim, J.-H. and Moon, C.-H. : Residual polychlorinated biphenyls (PCBs) in the sediment of the Kumho river, *Korean J. Environ. Agric.*, **14**(3), 272-281, 1995.

16) Krieg, N.R., and J.G. Holt. : Section 4, Gram negative aerobic rods and cocci. p. 140-219. *In* Bergey's manual of systematic bacteriology. Vol. 1. Williams & Wilkins, Baltimore, 1984.

17) Kim, J.-H., Choi, S.-K., Moon, C.-H., Park, M.-K., Kim, Y.-H., Suh, S.-K., Woo, C.-J. and Park, H.-D. : Isolation and characterization of *Pseudomonas* sp. P2 degrading polychlorinated biphenyls (PCBs), *J. Micro. Biotec.*, **6**, 167-172, 1996.

18) Liu, D. : Biodegradation of Aroclor 1221 type PCBs in sewage wastewater, *Bull. Environ. Contam. Toxicol.*, **27**, 695-703, 1981.

19) Bedard, D.L., Unterman R., Bopp L.H., Brennan M.J., Haberl M.L. and Johnson C. : Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls, *Appl. Environ. Microbiol.*, **51**(4), 761-768, 1986.

20) Kohler, H.-P.E., Kohler-Staub, D. and Focht, D. D. : Cometabolism of polychlorinated biphenyls : Enhanced transformation of Aroclor 1254 by growing bacterial cells, *Appl. Environ. Microbiol.*, **54**(8), 1940-1945, 1988.

21) Chaudhry, G.R. and Chapalamadugu, S. : Biodegradation of halogenated organic compounds, *Microbiol. Rev.* **55**(1), 59-79, 1991.

22) Shuler, M.L. and Kargi, F. : Bioprocess engineering bisic concepts, Prentice-Hall, New Jersey, pp.148-198, 1992.

23) Focht, D.D. and Brunner, W. : Kinetics of biphenyl and polychlorinated biphenyl metabolism in soil, *Appl. Environ. Microbiol.*, **50**(4), 1058-1063, 1985.