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Pilot scale study on PCB decomposition using a combination of photodechlorination and biodegradation

TOSHIO HAYAKAWA*, TAKASHI KYOTANI, TOMOYOSHI USHIOGI,
MINORU SHIMURA and KAZUhide KIMBARA

*Environmental Biotechnology Laboratory, Railway Technical Research Institute, 2-8-38 Hikari-cho,
Kokubunji, Tokyo 185-8540, Japan*

* TEL: 042-573-7316 FAX: 042-573-7349

* E-mail: hayakawa@rtri.or.jp

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Polychlorinated biphenyls (PCBs) are widespread environmental pollutants. Although microbiological PCB degradation has been studied for a few decades, it has not been applied for large-scale disposal of concentrated PCBs. We have already developed a method for the complete decomposition of PCBs using a combination of photodechlorination with biological degradation. Here we examine a pilot scale study on the degradation of commercial PCBs from a high voltage transformer. After optimizing the culture conditions, we could degrade 99.9975% and 99.99992% of 50 g of Kaneclor 1000 and 100 g of Kaneclor 500 within 9 days, respectively. Mass balance study showed that undesirable by-products such as polymerized PCBs were not produced. Dioxins including dibenzofuran and coplanar PCBs in the Kaneclor sample were also degraded completely by UV dechlorination and biodegradation. The concentration of PCBs in the final effluent was acceptable by the Japanese standard on wastewater, less than 3 µg/l. Based on the above results, we demonstrate that biological degradation combined with UV dechlorination is useful as a safe disposal method for PCBs.

Key words: Polychlorinated biphenyls, PCBs, Photodechlorination, Biodegradation

Introduction

Polychlorinated biphenyls (PCBs), a complex mixture produced by direct chlorination of the biphenyl nucleus, are chemically and thermally stable chemicals. It was used as an insulating oil in capacitors and transformers, hydraulic fluids, heat transfer fluids etc. Although the manufacture and use of PCBs have been banned since the 1970s in Japan, a large amount of PCBs still persist in the environment. Large quantities of PCBs also have been recovered and stored for future detoxification. Construction of PCB decomposition facilities which would have used thermal destruction processes have been planned several times, but were not accepted by the local community. The main reason for this objection was that the local communities were apprehensive that incomplete combustion of PCBs would result in the formation of dioxins. In 1992, the Japanese Ministry of Health and Welfare (JMHW) reported that 7% of stored transformers and capacitors containing PCBs could not be accounted for and most had probably disappeared. To avoid further pollution, JMHW authorized several methods utilizing chemical dechlorination and/or destruction of PCBs.

On the other hand, microorganisms that can degrade PCBs have been discovered all over the world^{1,5,13,15,23,26,27}. Many researches have referred to PCB degraders as potential candidates for the development of a safe decomposition process. Several reports, however, have also pointed out the limitations of biodegradation^{3,10,11}.

Recently, Shimura et al succeeded in complete degradation of a 100 mg/l solution of commercial PCB using biological degradation combined with photolysis by ultraviolet irradiation²⁵. To demonstrate the practical use of this combined method, a pilot scale study on the decomposition of commercial PCBs appears to be necessary. In this study, we examine a pilot scale degradation of PCBs derived from a high voltage transformer. The final effluent passed the Japanese Standards on PCB and dioxin concentration. To our knowledge, this is the first report on a practical decomposition process for commercial PCBs with PCB degrading bacteria.

2. Materials and Methods

2.1 PCBs

Kaneclor 1000 (KC1000, Kanegafuchi Chemical Com-

pany, Japan) equivalent to Aroclor T-1000 (Monsanto Industrial Chemicals Company, USA), which consists of 70% of Kaneclor 500 (KC500, Kanegafuchi Chemical Company, Japan) an equivalent to Aroclor 1254 (Monsanto Industrial Chemicals Company, USA) and 30% of trichlorobenzene, was used as the PCB sample. It was used as an insulating oil in the main transformer of electric trains. KC500, which was prepared from KC1000 by distillation, was used to determine the mass balance.

2.2 Component of the pilot plant

Figure 1 shows a schematic diagram of the PCB treatment system. There are two UV irradiation apparatuses each of which can contains 20 l of fluid. The first and second bioreactors have culturing capacities of 1000 l and 500 l, respectively. These bioreactors are equipped with two six-bladed impellers and an air sparger to maintain the aerobic microbial metabolism, respectively. All components can be sterilized by steam provided by a steamer.

PCBs were dissolved in isopropyl alcohol (IPA) and then irradiated in the UV irradiation apparatus. The UV treated mixture was subjected to biological degradation with two PCB degraders following IPA recovery by distillation *in vacuo*. Finally, effluent from the second bioreactor was stocked in a storage tank and released into the sewer under license of local government.

2.3 UV irradiation

UV Drinking Water Sterilization system, NFP-8 (Funakoshi, Japan) equipped with five low pressure mercury lamps (39 W) was used as the UV irradiation apparatus. Fifty grams of KC1000 (final concentration of 2500 mg/l) was dissolved in 15 l of IPA containing 5 l of water and 40 g of NaOH. This PCBs solution was irradiated with UV for about 50 hours at room temperature. For the mass balance determination, 50 g of KC500 was used as a sample.

2.4 Biological degradation

UV-treated mixture was concentrated and added to 500 l of *Comamonas testosteroni* strain TK102 culture in the first bioreactor²⁴. Five hundred liters of *C. testosteroni* TK102 culture was grown on biphenyl for 72 hours and then used for PCB degradation. Ten liters of fresh inoculum was seeded at the beginning of the subsequent experiment. The incubation was continued up to 96 hours at 30°C. The culture conditions are summarized in Table 1. A PCB degrader, *Rhodococcus opacus* strain TSP203¹⁷ was used for the second treatment to ensure complete PCB degradation. These bacteria were grown on synthetic minimal salt medium (W medium) supplemented with biphenyl¹³.

Five liters of concentrated PCBs mixture, 10 l of fresh inoculum, and biphenyl were added to the 500 l of *C.*

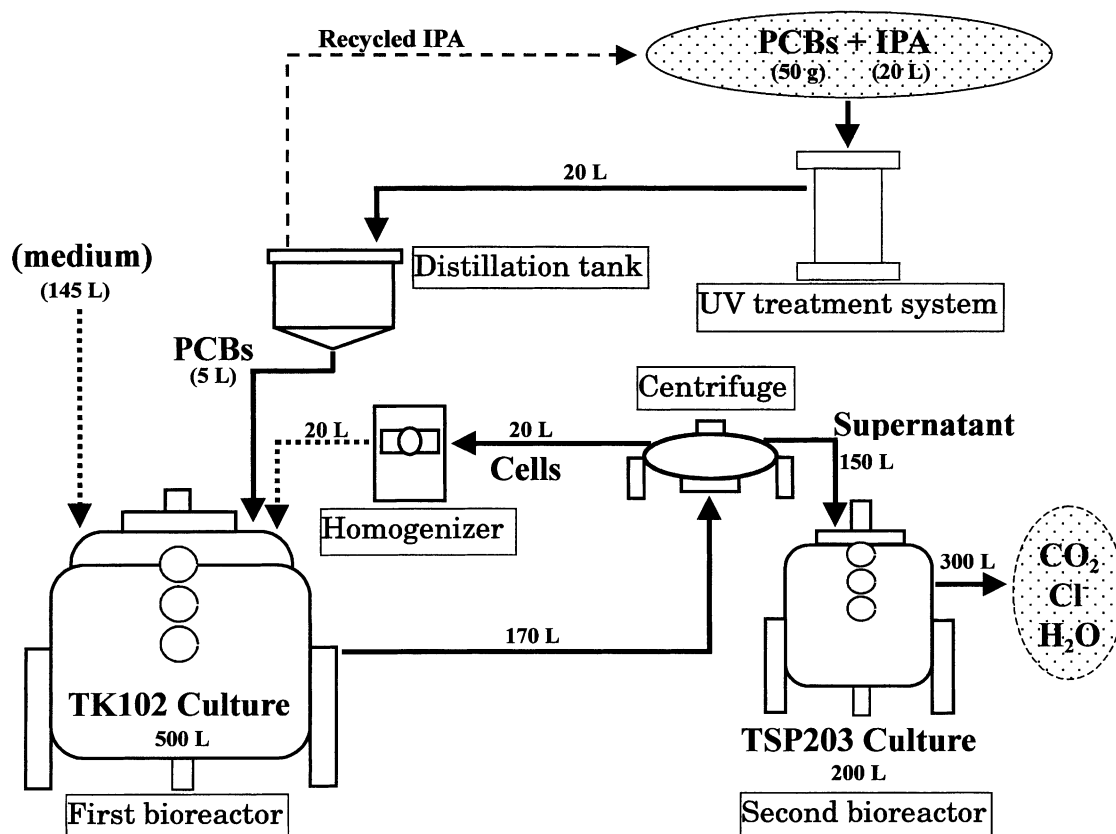


Fig. 1. Schematic diagram of the pilot plant.

The arrows show the basic process, while dotted arrows indicate additional process to maintain the continuous cultivation.

Table 1. Culture conditions used in pilot scale experiment.

	First bioreactor	Second bioreactor
Organism	<i>C. testosteroni</i> TK102	<i>R. opacus</i> TSP203
Culture volume (l)	500	350
Biphenyl (mg/l)	250~500	0
Fresh Inoculant (l)	10	200
Temperature (°C)	30	30
Aeration rate (l/min)	200	130
Agitation (rpm)	140	70

testosteroni TK102 culture. After 92 hours of incubation, 170 l of culture from the first bioreactor was transferred to a continuous-flow centrifugal separator (ADS250CS, Kokusan, Japan) at the rate of 700 ml/min. Twenty liter of the separated cells were disrupted by passing through a homogenizer (Lab100, Gaulin APV, Germany) at 100 MPa and put into the first bioreactor as nutrient. Subsequently, the next UV treated mixture was added to the first bioreactor with 145 l of fresh medium. This cycle was repeated upto 7 times. The 150 l of supernatant was put into the second bioreactor with 200 l of *R. opacus* TSP203 culture. After 96 hours treatment, culture was discharged into the sewer with permission of the local government following analysis of PCB concentration and other properties.

2.5 Analysis of PCBs, Total organic chlorine, and Cl⁻

10 ml of culture was taken from the bioreactors and PCBs were extracted with an equal volume of ethyl acetate. The organic layer was separated and evaporated under a stream of nitrogen. The remaining PCBs were dissolved in 1 ml of ethyl acetate and used for gas chromatography-mass spectrometry (GC-MS) analysis. A gas chromatograph (HP-5890II, Hewlett Packard Co., U.S.A.) equipped with a mass selective detector (HP-5971, Hewlett Packard Co., U.S.A.) and a HP-Ultra2 capillary column (50 m, 0.2 mm, 0.33 μ m, SE-54 equivalent, Hewlett Packard Co., U.S.A.) was used. MS Chemstation (HPG1034, Hewlett Packard Co., U.S.A.) was used for analysis of the GC-MS data. The Same samples were analyzed by Yakult Co. (Tokyo, Japan) by the Japanese standard methods (JIS K0093-1995) to confirm the PCBs concentration. Total organic chlorine (TOCl) and chloride ion (JIS K0102-98) were determined by Yakult Co.

2.6 Analysis of toxic and controlled substances

Dioxin, dibenzofuran, and co-planar PCBs in KC1000, UV treated samples, final effluent, and exhaust gas were analyzed by Japanese standard (JIS K0311-1999 and JIS K0312-1999). These measurements were taken by Japan Quality Assurance Organization (Tokyo, Japan). Mutagenicity of the treated samples were examined by Ames

test²⁾ which is a standard method to detect mutagen using bacteria. Biological oxygen demand (BOD) and suspended solid (SS) in final effluent were measured by oxygen electrode method (JIS K0102-1998) and gravimetric analysis (JIS K0102-1998), respectively.

2.7 Chemicals

All chemicals were obtained from Wako Pure Chemical Industries (Tokyo, Japan), except Kaneclor 1000.

3. Results

3.1 UV irradiation

The percent degradation of KC1000 was estimated to be 99.1% following UV treatment for 51 hours. The decrease in PCBs and accumulation of biphenyl during UV irradiation is shown in Fig. 2. The concentration of PCBs did not decrease in the initial 6 hours. After 6 hours, PCBs concentration decreased rapidly and there was an accumulation of biphenyl. Trace amounts of chlorinated compounds such as monochlorobenzofuran, dichloroethylmethylbenzene, and 1-chloro-4-(phenylethyl)-benzene were detected from the UV treated sample.

3.2 Biological degradation of KC1000

The initial PCB concentration in the first bioreactor in each experiment was below 500 μ g/l. The PCB concentration decreased to less than 30 μ g/l by biological degradation (Table 2). The remaining PCBs were subjected further degradation in the second bioreactor by *R. opacus* strain TSP203. The final concentration of PCBs was less than 3 μ g/l after the second biodegradation. In the experiment numbered 1-1, 1-4, 2-1, and 2-3, the PCB concentration was below the detection limit (0.5 μ g/l). Experiment 3 had been running for 7 weeks without any problems. In every experiment, the PCB concentration in the final effluent was below 3 μ g/l. The decomposition efficiency of KC1000 was estimated at 99.9975%.

3.3 Mass balance calculation

Fig. 3 shows the mass balance calculation of chlorine. 107.2 g of KC 500 contained 58.2 g of chlorine. It was estimated that 99.3% of chlorine in PCBs was converted to

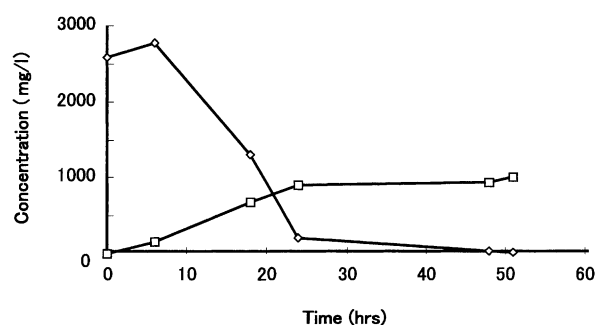


Fig. 2. The concentration of PCB (\diamond) and biphenyl (\square) during UV irradiation.

Table 2. Concentration of PCBs before and after biological treatment.

No.	Cycle	<i>C. testosteroni</i> TK102			<i>R. opacus</i> TSP203		
		Initial ($\mu\text{g/l}$)	Final ($\mu\text{g/l}$)	Time (h)	Initial ($\mu\text{g/l}$)	Final ($\mu\text{g/l}$)	Time (h)
1	1	256	10	88	4.3	ND ^{a)}	68.5
	2	291	4.7	65	2.0	0.8	69
	3	184	3.4	66	1.5	0.7	70
	4	149	2.5	66	1.1	ND ^{a)}	68
2	1	199	ND ^{a)}	92	ND ^{a)}	ND ^{a)}	89.5
	2	103	ND ^{a)}	92	ND ^{a)}	2.3	88
	3	105	0.5	92	ND ^{a)}	ND ^{a)}	93
3	1	182	0.5	90	ND ^{a)}	0.5	90
	2	440	16	92	6.9	1.7	113
	3	127	9.6	92	4.1	1.6	113
	4	132	30	92	12.9	2.8	94
	5	154	18	92	7.7	2.1	94
	6	160	15	92	6.5	1.5	93
	7	144	9.4	92	4.0	1.5	94

^{a)} Not detected

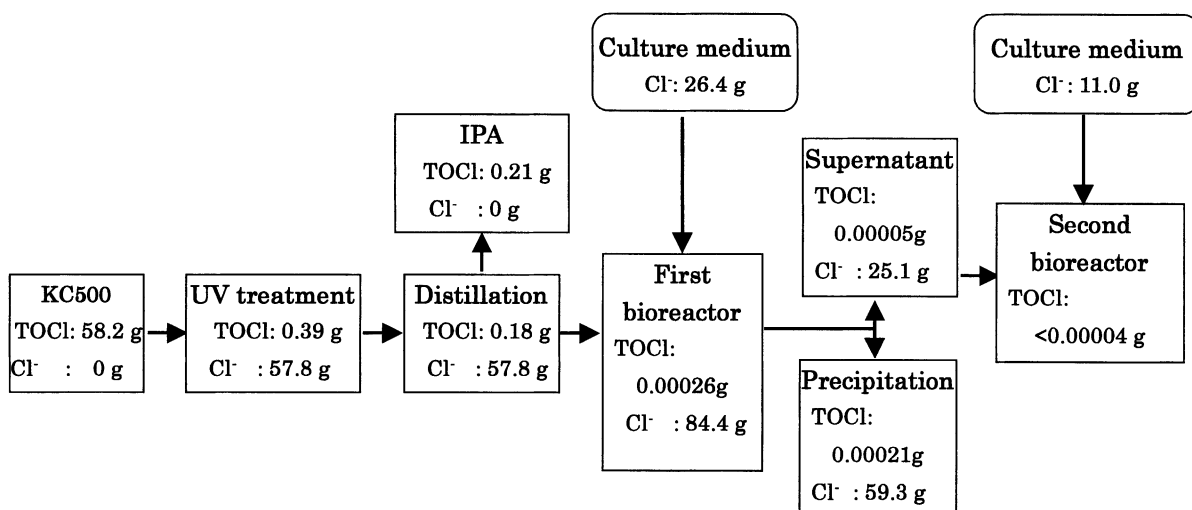


Fig. 3. Mass balance of total organic chlorine (TOCl) and chloride ion (Cl-) resulting from photodechlorination and biodegradation.

chloride ion. The total organic chloride in the final effluent was less than 0.00004 g. UV irradiation resulted in 99.64% dechlorination of KC500. The PCB concentration in the first bioreactor was 0.69 mg/l at the beginning of the biological treatment. The PCB concentration in the final effluent was less than 0.05 $\mu\text{g/l}$. This result shows that the decomposition efficiency of KC500 is more than 99.99992% (Fig. 4).

3.4 Properties of effluents

Dioxins were not detected in untreated solution of KC1000, UV treated mixture, final effluent from the second bioreactor and exhaust gas from the first bioreactor (Table 3). Pure KC1000 contained 4100 ng/g (150 ng-TEQ/g) of dibenzofurans. This concentration decreased to 10 ng/g (0 ng-TEQ/g) after the UV treatment and was never detected

in the final effluent from the second bioreactor and the exhaust gas from the first bioreactor. Pure KC1000 also contained 31000000 ng/g (7200 ng-TEQ/g) of co-planar PCBs. After UV treatment, 39 ng/g (0.04 ng-TEQ/g) of co-planar PCBs were detected. We could not detect co-planar PCBs in the final effluent of the second bioreactor. We also determined the concentration of chlorobenzenes, which is known as a precursor of dioxin. KC 1000 contained 400000 mg/l of total chlorobenzenes and 98.4% of them was determined to be trichlorobenzene. In the UV treated mixture, 78.7% of the total chlorobenzenes (290000 mg/l) was dichlorobenzene. The final effluent contained only 0.000048 mg/l of dichlorobenzene. The Ames test indicated there was no mutagen and concentration of BOD and SS was 63 mg/l and 43 mg/l, respectively. These result suggest that effluent from the pilot plant would not result

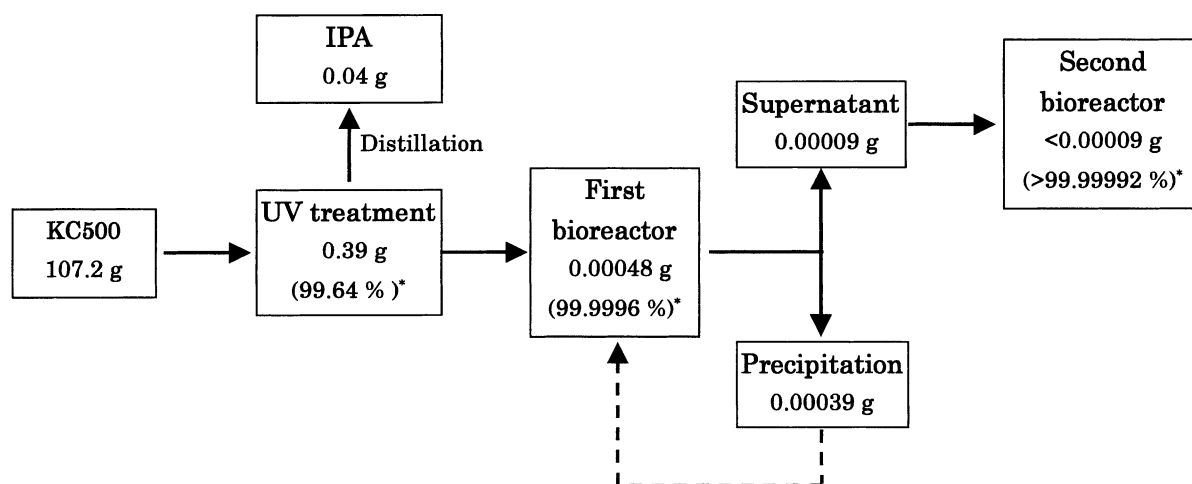


Fig. 4. Concentration of total PCB and degradation rates.
* Degradation rate

Table 3. Concentration of chlorinated organic compounds in the untreated and treated samples.

	KC1000	UV irradiation	Final effluent ^{a)}	Exhaust gas ^{b)}
Dioxins (ng/g)	ND ^{c)}	ND ^{c)}	ND ^{c)}	ND ^{c)}
Dibenzofuran (ng/g) (ng-TEQ/g)	41000 (150)	10 (0)	ND ^{c)}	ND ^{c)}
Co-planer PCBs (ng/g) (ng-TEQ/g)	3100000 (7200)	39 (0.04)	ND ^{c)}	ND ^{c)}
Chlorobenzenes (mg/l)	400000	290000	0.000048	—

^{a)} Effluent from the second bioreactor

^{b)} Exhaust gas from the first bioreactor

^{c)} not detected

in water pollution.

Discussion

It is well known that enzymatic oxidation by PCB degrading aerobic bacteria transform biphenyl and chlorobiphenyls to benzoic acid and chlorobenzoic acids, respectively. During microbial degradation, undesirable products, such as dioxin, would never be produced, because the catalytic activity of an enzyme is very specific. A commercial PCB mixture used in high voltage electrical equipment contains tetra-, penta-, and hexachlorinated biphenyls. The congener specificity of most PCB degraders, however, limits biodegradation to those congeners which have less than four chlorine substituents^{5,9)}. This congener specificity limits the practical utilization of microbes for the decomposition of commercial PCBs^{6,14)}. To overcome this problem, PCB congeners with more than four chlorine substituents must be eliminated prior to biodegradation.

Photodechlorination of PCBs was first reported by Safe and Hutzinger²¹⁾. They found that a hexachlorinated congener could be photolyzed in hexane or methanol by UV irradiation at 310 nm. Bunce et al have described solar

degradation of PCBs in the aquatic environment⁷⁾. Furthermore, various organic solvents such as hexane¹²⁾, cyclohexane²⁰⁾, iso-octane⁸⁾ and alcohol^{18,28)} have been used in PCB photolysis studies to enhance the rate of dechlorination. Although photolysis of PCBs appeared to be an effective and safe process, complete dechlorination is not achieved as there is competition for UV light between PCBs and the dechlorinated products²²⁾. In a previous paper²⁵⁾, we have demonstrated that photodechlorination was suitable as a pretreatment for complete degradation of PCBs by biodegradation.

In this study, we changed the solvent from methanol to alkaline IPA which provided a higher dechlorination yield¹⁹⁾. The concentration of total PCBs in the UV treated mixtures did not decrease in the initial 6 hours however this lag was transient (Fig. 2). GC-MS analysis indicated that the decrease in higher chlorinated PCBs resulted in the accumulation of lower chlorinated congeners, mainly monochlorinated congeners. The decrease in the concentration of total PCBs was accompanied by an increase in biphenyl (Fig. 2).

Trace amounts of several chlorinated organic compounds were found when KC1000 was exposed to UV irradiation. It is possible that some of these chlorinated

by-products were produced from PCBs by UV irradiation. Since most of these chlorinated by-products likely not detected from UV treated KC500, it is thought that the chlorinated by-products were derived from chlorobenzenes contained in the KC1000.

Biological degradation of PCBs have succeeded in laboratory-scale experiment. To evaluate, whether bacteria can be used for large-scale PCB decomposition, we examined the reproducibility of the results during degradation of PCBs on a pilot scale (Table 2). The final concentrations of PCBs in the effluent was always less than 3 $\mu\text{g/l}$, which is the Japanese standard of PCBs for effluent. In our experiments, the treatment process was run continuously 7 weeks. It appears that biological degradation is stable and can be applied for practical use.

The two PCB degraders used in this study have different characteristics. *C. testosteroni* TK102, used in the first bioreactor, degrades all trichlorobiphenyls and some tetrachlorobiphenyls in PCB concentrations upto 5000 mg/l. While *R. opacus* TSP203, used in the second bioreactor, can degrade all tetrachlorobiphenyls and some pentachlorobiphenyls in the presence of lower concentration (i.e. 25 mg/l) of PCBs. As a result of using a combination of these two PCB degraders, the rapid and safe decomposition of commercial PCBs can succeed.

Trace amounts of PCBs (below 100 $\mu\text{g/kg}$) remained in the bacterial cell pellet collected after the first biodegradation (data not shown). These PCBs appeared to be absorbed on the cell membrane and was recalcitrant to bacterial degradation probably because accessibility to degradation enzymes was reduced. To avoid discharge of the absorbed PCBs into the effluent, the bacterial cells were collected and reused as nutrient in subsequent cultures after disruption with a homogenizer.

Baxer et al have reported biochemical and photochemical degradation of PCBs in the environment⁹). These reports suggested that microbiological reactions convert PCBs to products which are susceptible to further degradation by photochemical process in environment. Based on their assumption, it would appear that higher chlorinated congeners that can not be degraded by microbes must accumulate in the environment. We on the other hand presume that aerobic bacteria attack the lower chlorinated PCBs which are supplied by dechlorination of highly chlorinated congeners.

The Waste Management Law of Japan requires that PCB detoxification should be done only by methods approved and authorized by the government. Several technologies for the decomposition of PCBs such as base-catalyzed decomposition, sodium dispersion method, supercritical water oxidation methods, etc. have been authorized. These chemical processes use chemicals under conditions of high temperature and pressure. Not only can photodechlorination and bacterial degradation be done at room temperature and atmospheric pressure, hazardous by-products are also not detected in the effluent. In con-

clusion, we have shown that this method is a safe method for PCB decomposition.

It is necessary to improve the degradation rate of this method for practical use. In this study, from mass balance studies we found KC500 is easier to degrade than KC1000. This difference in degradability could be because chlorobenzenes in KC1000 competed with PCBs for absorption of UV light. Therefore, the separation of chlorobenzenes by distillation from KC1000 prior to UV irradiation will increase efficiency of dechlorination. Any improvement in the ability of UV irradiation apparatus may also increase the degradation rate. Studies are under way to confirm these presumptions by the installation of distillation apparatus and enhanced UV irradiation system.

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