The Journal of Microbiology, September 2003, p.189-195 Vol. 41, No. 3

Influence of Transition-Metal Cofactors on the Reductive Dechlorination of Polychlorinated Biphenyls (PCBs)

O-Seob Kwon*, Young-Jin Kim§, Kyung-Je Cho, Jin Ae Lee, Young Eui Kim, In Young Hwang, and Jae Hyun Kwon

School of Environmental Science and Engineering, and Nakdong River Environmental Research Center, Inje University, Gimhae 621-749, Korea

(Received July 4, 2003 / Accepted September 2, 2003)

To enhance the reductive dechlorination of polychlorinated biphenyls (PCBs) under anaerobic conditions, we examined the adjunctive effects of cobalt (Co) and nickel (Ni), which are the central metals of transition-metal cofactors of coenzyme F_{430} and vitamin B_{12}, respectively, on the dechlorination of Aroclor 1248. After 32 weeks of incubation, the average numbers of chlorines per biphenyl in culture vials supplemented with 0.2, 0.5, and 1.0 mM of Co reduced from 3.88 to 3.39, 2.92, and 3.28, respectively. However, the numbers of chlorine after supplementing with Ni decreased from 3.88 to 3.43, regardless of the Ni concentrations. The observed congener distribution patterns of all vials with different conditions were similar to the pattern produced by the dechlorination process of H' after 21 weeks of incubation, and these patterns were unchanged up to week 32, except for vials supplemented with 0.5 and 1.0 mM of Co. In vials containing 0.5 mM of Co, *meta*-rich congeners, such as 25/25-, 24/25-, and 25/23-chlorobiphenyls (CBPs), which were found as accumulated products of dechlorination in other conditions, were further dechlorinated, and 25/2-, 24/2-, and 2/2-CBPs were concomitantly increased after 32 weeks of incubation. In this case, the congener distribution was similar to the dechlorination pattern of process M. From these results, we suggested that the enrichment of cultures with Co might stimulate the growth of specific populations of *meta*-dechlorinators, and that populations might promote a change in the dechlorination process from H' to M, which is known to be less effective on the dechlorination of the more highly chlorinated congeners of PCBs.

***Key words*:** transition metal, reductive dechlorination, Aroclor 1248, dechlorination pattern

The microbial dechlorination of polychlorinated biphenyls (PCBs) has been observed in many river, lake, and estuarine sediments (see references in Bedard and Quensen, 1995). Although this process is the only biological process known to degrade highly chlorinated PCB congeners, a small number of studies have reported the isolation of anaerobic microorganisms capable of dechlorinating PCBs (May *et al*., 1992; Cutter *et al*., 2001), though the mechanism of this reductive dechlorination is still not fully known. Many have investigated the effects of environmental parameters on the reductive dechlorination of PCBs, and the enhancement of the microbial dechlorination of PCBs by adding various carbon and energy sources and surfactants to sediments (see references in Bedard and Quensen, 1995), however, their addition has generally failed to enhance the dechlorination of PCBs. Recently, some investigators found that the addition of halogenated aromatic compounds can enhance the dechlorination of PCBs by stimulating the growths of dechlorinators (Bedard *et al*., 1996; Deweerd and Bedard, 1999; Cho *et al*., 2002).

Anaerobic bacteria are rich in the metallo-organic cofactors and methanogens are especially rich in corrinoids (Dangel *et al*., 1987). Vitamin B_{12} is representative of these corrinoids, and is found in various microorganisms. Coenzyme F_{430} is a nickel(II) porphinoid, which is uniquely present in all methanogens, and functions as the prosthetic group of methyl coenzyme reductase that mediates the final step in methanogenesis. It has been suggested that cobalt-containing cobalamins and coenzyme F_{430} are involved in the catalysis of the reductive dehalogenation of various chlorinated hydrocarbons by anaerobic bacteria (Gantzer and Wackett, 1991; Smith and Woods, 1994). Ye *et al*. (1995) suggested that methanogens may be responsible for the dechlorination of PCBs under culture conditions selected for methanogenic bacteria. The finding of May *et al*. (1992) was also consistent with Ye's hypothesis that methanogens are responsible for dechlorination. Dechlorination of PCBs was most advanced in methanogenic conditions, however, methane production was not essential for dechlorination, because

§ Present address: Chung-Eui Middle School, Euijeongbu 480-090, Korea
* To whom correspondence should be addressed.
(Tel) 82-55-320-3249; (Fax) 82-55-334-7092
(E-mail) envkos@inje.ac.kr

the addition of 2-bromoethane sulfonate, an inhibitor of methane production, did not affect dechlorination (Rhee *et al*., 1993a)

In this study, we examined the influence of two transition metals, nickel and cobalt, upon the reductive dechlorination of PCBs, using Aroclor 1248 as a substrate for a dechlorinating anaerobic microbial consortium.

Materials and Methods

Analysis of Ni and Co in culture sediments

The concentrations of Ni and Co in Owasco Lake sediments used in this study were analyzed by the method of Tessier *et al.* (1979). 10 ml of sodium acetate solution (0.5 M, pH 8.0) was added to 200 mg of air-dried sediment in a 50 centrifuge tube, mixed for 1 h, and centrifuged for 10 min at 3,000 rpm. The supernatants obtained were used for the analysis of the exchangeable fractions of metals. To the remaining sediments, 10 ml of sodium acetate solution (1.0 M, pH 5.0) were added and mixed for 5 h. The slurries were centrifuged for 10 min at 3,000 rpm, and the supernatants were analyzed for carbonate or easily reducible fractions. After these extraction steps, 10 ml of hydroxylamine hydrochloric acid solution (0.04 M) was added to the sediments, which were then heated for 12 h at 90°C, and centrifuged. The supernatants were analyzed for reducible fractions. After the treatment of previous steps, 5 ml of hydrogen peroxide (33%, pH 2.0) and 5 ml of nitric acid (0.02 M) were added to the sediments and mixed at 80°C until bubbles no longer appeared from the solution. 5 ml of ammonium acetate solution (3.2 M) was added, mixed for 1 h, and centrifuged. The supernatants obtained were analyzed for their organic fractions. After the previous extractions, the sediments were added to the 50 ml serum vials. 5 ml of acid mixture (nitric acid : fluoric acid : perchloric acid=4 : 4 : 1, v/v) was added and heated at 200°C to dryness. After cooling, 10 ml of 1% nitric acid solution was added and centrifuged, and the supernatants were analyzed for the lattice fractions of Ni and Co. The supernatants extracted by each step were analyzed using an atomic adsorption spectrophotometer (Perkin-Elmer 3100, USA).

Preparation of PCBs-dechlorinating cultures and the experimental set-up

PCB-free, air-dried, sieved sediments from Owasco Lake, NY, USA, were spiked with Aroclor 1248 (AccuStandard, USA) in hexane to yield a total PCB concentration of 300 μg/g on a sediment dry-weight basis. After the hexane have been evaporated, the PCB-spiked sediments were made into slurries containing 10% sediment (w/v on a dry-weight basis) with reduced synthetic minimal medium (Balch *et al.*, 1979) in an anaerobic chamber (Coy Laboratory Products, USA) with an $N_2/CO_2/H_2$ atmosphere (85:5:10). The minimal medium contained the redox indicator resazurine at a final concentration of 0.0001%. To ensure the homogeneous distribution of PCBs, the sediment slurry was stirred overnight with a magnetic stirrer. Batch incubations were prepared by dispensing 50 ml of the sediment slurry into serum vials (120 ml) and sealing them with a Teflon-lined stopper and aluminum crimp seals in an anaerobic chamber. The vials were autoclaved and, except for the control, inoculated with 2 ml of supernatant of the PCBs-acclimatized sediment slurry, prepared as described in a previous study (Kwon *et al.*, 2001). All culture vials were set up in duplicate.

To investigate the influence of Ni and Co on the dechlorination pattern of PCBs, solutions of Co ($CoCl_2 \cdot 6H_2O$) and Ni ($NiCl_2 \cdot 6H_2O$) (Fluka, Swiss) were added to vials containing Aroclor 1248 at a final concentration of 0.2, 0.5, and 1.0 mM using a syringe filter (0.22 μm, 25 mm; Corning, USA) in an anaerobic chamber. All vials were incubated at room temperature and 2 ml portions of the sediment slurry were removed at predetermined time intervals for PCB analysis in an anaerobic chamber. Samples were collected using a Pasteur pipette while slurries were continuously mixed on a magnetic stirrer.

PCB extraction and analysis

The collected samples were extracted with acetone and hexane by ultrasonication, treated with tetrabutylammonium hydrogen sulfate and sodium sulfite to remove elemental sulfur, and cleaned up on a 4% deactivated Florisil column (Rhee *et al.*, 1993a). PCB analysis was performed using a gas chromatograph (Hewlett-Packard 5890II) equipped with a ^{63}Ni electron capture detector, a HP Ultra II fused silica capillary column (25 m × 0.2 mm, 0.11 μm thickness), a HP 7673 autosampler, and a HP 3396 integrator. The gas chromatography conditions used have been described elsewhere (Rhee *et al*., 1993a). PCBs were quantitated on a HP Ultra II column using a calibration standard containing equal amounts of Aroclors 1221, 1016, 1254, and 1260 (0.2 μg/ml of each in hexane) (Rhee *et al*., 1993a). All of the chromatographic data were collected and processed on a microcomputer by using a HP 3365 Series II ChemStation chromatography data system. The mole percentage of PCB congeners and the average number of chlorines per biphenyl were calculated based on the concentration of each congener. Coeluting congeners were assumed to be present in equal proportions for the calculations.

Results and Discussion

To determine the amounts of Ni and Co required to enhance the dechlorination of PCBs without inhibiting microbial growth and activity, we analyzed the concentrations of these transition-metals in sediments. The total concentrations of Ni and Co, analyzed by the method of

Table 1. Concentrations of the various forms of nickel and cobalt in the sediments used in this study (μM)

species	1	2	3	4	5	sum
Ni	37.3 ± 3.0	3.0 ± 0.0	219.4 ± 9.0	59.7 ± 6.0	371.6 ± 10.4	691.0 ± 13.4
Co	11.3 ± 1.6	25.8 ± 4.8	82.3 ± 4.8	12.9 ± 4.8	67.7 ± 8.1	200.0 ± 9.7

1. exchangeable fraction including absorbed and cation exchangeable metals.
2. easily reducible fraction including carbonate and weak oxides.
3. reducible fractions mainly Fe-Mn oxides.
4. organic fraction associated with organic matters of sulfur species.
5. lattice fraction included in mineral lattices.

Tessier *et al.* (1979), were 691 and 200 μM, respectively (Table 1); however, they were present primarily in the forms of Fe-Mn oxides or mineral lattices, which have limited bioavailabilities. Among the various forms of metals, the bioavailable species of metals are exchangeable, easily reducible, or organic fractions and organisms can use bioavailable species more easily than the other ones (Forstner, 1989). The sediments used in this study contained 0.1 and 0.05 mM of bioavailable forms of Ni and Co, respectively. IC_{50} (inhibitory concentration 50) of Ni and Co to *Streptomyces coelicolor* were 0.05 and 5.8 mM, respectively (Abbas and Edwards, 1990). Maximum tolerant concentration of *Alcaligenes eutrophus* CH34 to Ni and Co was 2.0 and 10 mM, respectively (Mergeay *et al.*, 1985), and *A. eutrophus* A5 was found to be able to degrade Aroclor 1242 at a Ni concentration of 1.0 mM (Springael *et al.*, 1993). From these results, we decided to add 0.2, 0.5, and 1.0 mM of Ni and Co to culture vials, and then investigated the influence of these metals on the dechlorination characteristics of Aroclor 1248.

The time course of the dechlorination, expressed as total chlorine atom (Cls) numbers per biphenyl, showed that the Aroclor 1248-spiked sediment slurries were dechlorinated after a 9-week period (Fig. 1). Up to 21 weeks of incubation, Cls numbers per biphenyl were not significantly different among vials supplemented with various concentrations of Co, and ranged from 3.42 to 3.49 or overall from 9.6% to 11.4% reduction from the original Aroclor 1248 (Fig. 1A). In the vial containing 0.5 mM of Co, however, dechlorination continued until 32 weeks of incubation, and the Cls number per biphenyl was reduced to 2.89 equivalent to an overall reduction of 25.1%. The dechlorination rate under this condition was 4.45×10^{-8} mol-Cl/g-sediment/week, which was twice than those of the vials supplemented with 0 or 0.2 mM of Co. Dechlorination also continued in the vial containing 1.0 mM of Co, but the reaction rate was less than that of 0.5 mM of Co. Unlike Co, Ni did not enhance the dechlorination of Aroclor 1248 (Fig. 1B). After 21 weeks of incubation, total Cls per biphenyl in vials supplemented with 0, 0.2, 0.5, or 1.0 mM of Ni ranged from 3.46 to 3.50, and from 3.42 to 3.44 or an overall reduction of 11.1% as compared with the original Aroclor 1248 after 32 weeks. The average rate of dechlorination in the vials containing the Ni supplement was 2.26×10^{-8} mol-Cl/g-sediment/week, and

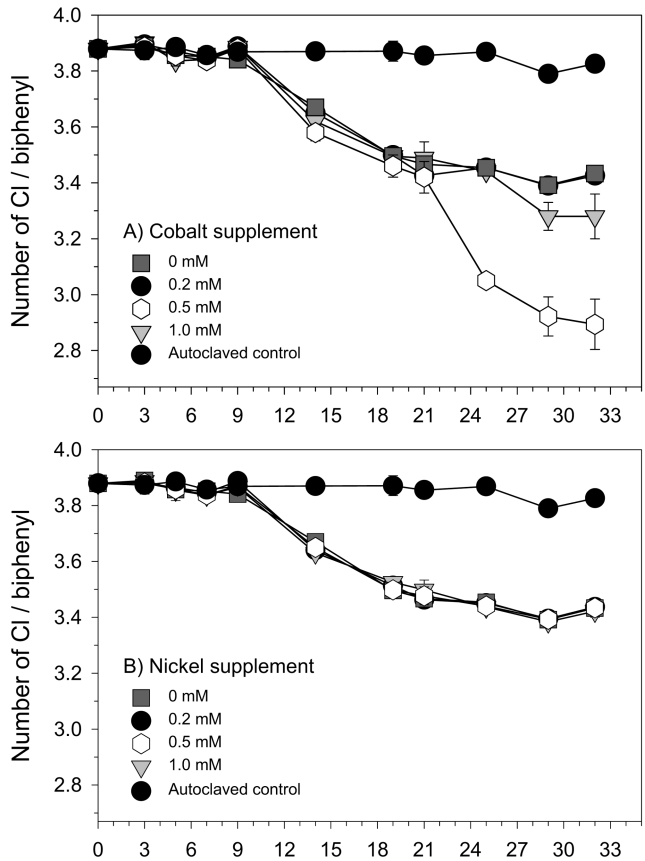

Fig. 1. Changes of total chlorine per biphenyl of Aroclor 1248 with the supplementation of transition metals. Data represent the means of duplicate cultures.

that with no addition of Ni was 2.22×10^{-8} mol-Cl/g-sediment/week.

The optimization of environmental parameters, such as PCB concentration, moisture content, and some haloaromatic compounds, increased the population of PCB-dechlorinators and enhanced the dechlorination rates (Kim and Rhee, 1999; Rhee *et al.*, 2001; Cho *et al.*, 2002). The omission of trace metals resulted in a slight negative impact on the rate and the extent of Aroclor 1242 dechlorination by Hudson River microorganisms (Abramowicz *et al.*, 1993). The sediments used in this study contained only 0.05 mM of bioavailable Co (Table 1) and the synthetic mineral medium had about 0.005 mM of dissolvable Co (Balch *et al.*, 1979). Although we did not know the iden-

tities of the dechlorinating microorganisms present and not determine the influence of Co on the sediment microbial populations in this study, the reduced level of dechlorination occurred in the vial supplemented with 1.0 mM of Co than in the vial with 0.5 mM of Co (Fig. 1) was probably due to the toxic effect of the Co upon the sediment microorganisms. The growth of *A. eutrophus* JMP134 and *Pseudomonas putida* PRS2015, which are able to degrade 2,4-D and 3-chlorobenzoate, respectively, was found to be inhibited at a Co concentration of 0.1 mM (Chatterjee *et al*., 1981; Don *et al*., 1985), although other bacteria were found to be able to tolerate a Co concentration of more than 1.0 mM (Mergeay *et al*., 1985; Abbas and Edwards, 1990).

Analysis of the PCB homolog distribution over incubation time also showed that the extents and patterns of dechlorination were different according to the amounts of cobalt supplemented (Fig. 2). After 9 weeks of incubation, all vials showed a similar decrease in the amounts of penta- and tetra-chlorobiphenyls (CBPs) and a dramatic increase in tri-CBPs. Clearest differences were observed in terms of the accumulation of di-CBPs and the diminution of tetra-CBPs after 21 weeks of incubation; in the vial supplemented with 1.0 mM of Co, di-CBPs increased and tetra-CBPs decreased, while in vials with 0.2 and 1.0 mM of Co relatively little changed. In vials supplemented with 0.2, 0.5, and 0.5 mM of Ni, no difference in the PCB homolog distribution was observed (data not shown). Mono-CBPs were not detected under any condition.

The mole percentages of Aroclor 1248 congeners after 32 weeks of incubation are presented in Fig. 3, which compares the dechlorinating patterns in the presence of different metal levels. After 32 weeks of incubation, the chromatographic pattern obtained from an Aroclor 1248-spiked sediment slurry without Co or Ni addition showed that decreases involved 234/245- + 245/34-, 34/34- + 236/34-, 234/24-, 245/24-, 245/25-, 24/34-, 25/34-, and 2,4,5/4-CBPs with concomitant increases in 24/25-, 25/25-, 25/4- + 24/4-, 24/3-, 25/3-, 236- + 26/3-, and 2/3-CBPs (Fig. 3B). The chromatographic pattern obtained from culture vials supplemented with 0.5 mM of Ni (Fig. 3C) was very similar to the that obtained with no addition, which demonstrated that Ni has no effect (Fig. 1) on the pattern of dechlorination. The observed congener distribution patterns, obtained from vials without Co or Ni addition and with 0.5 mM of Ni, were similar to the pattern produced by the dechlorination process known as H' (Bedard and Quensen, 1995), in which only *meta*- and *para*-Cls adjacent to others are removed, but not isolated chlorines. In the vial supplemented with 0.5 mM of Co, 6 congeners of

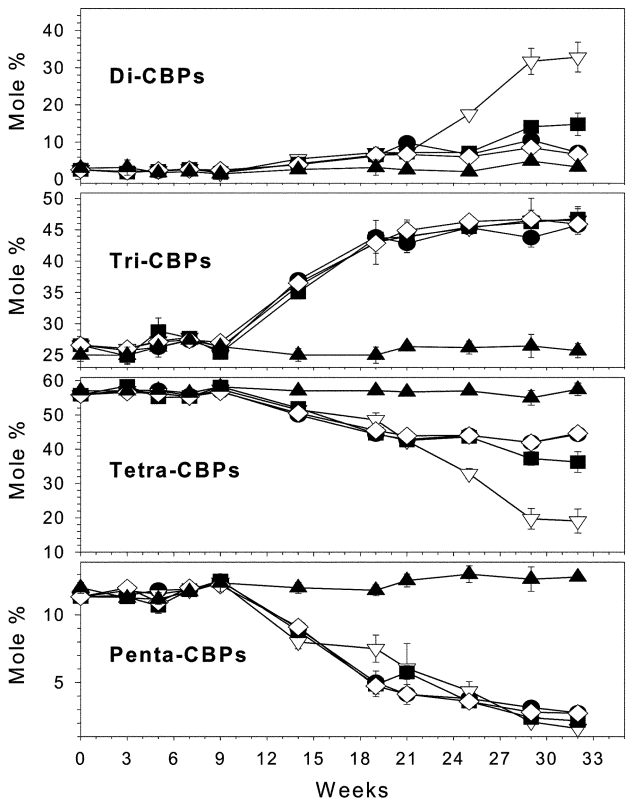

Fig. 2. Changes in the homolog distribution of Aroclor 1248 with the supplementation of cobalt over a 32-week incubation period. Data represent the means of duplicate cultures. (◇, 0 mM Co; ●, 0.2 mM Co; ▽, 0.5 mM Co; ■, 1.0 mM Co; ▲, Autoclaved control)

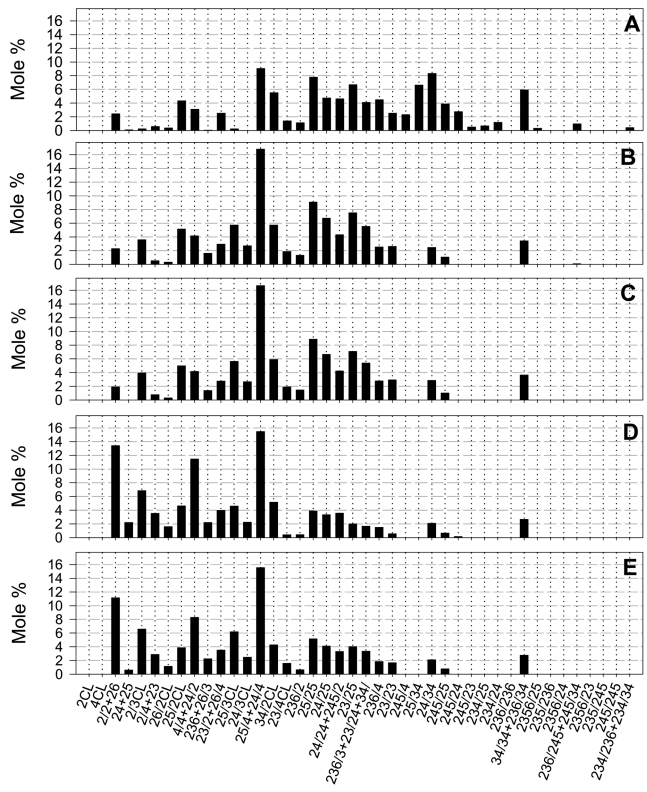

Fig. 3. Mole percentages of Aroclor 1248 congeners in sediments slurries supplemented with cobalt or nickel after 32 weeks of incubation. (A, Autoclaved control; B, No addition; C, 0.5 mM Ni; D, 0.5 mM Co; E, 0.5 mM Co and 0.5 mM Ni)

tetra-CBPs (236/4-, 236/3- + 23/24-, 23/25-, 24/24- + 245/2-, 24/25-, and 25/25-CBPs) were additionally decreased with concomitant increases in tri- and di-CBPs such as 4/4- + 24/2-, 26/2-, 2/4- + 23-, 2/3-, 24- + 25-, and 2/2- + 26-CBPs (Fig. 3D). In particular, major *meta*-rich congeners, such as 25/25-, 24/25-, and 23/25-CBPs, which were found as accumulated products of the dechlorination when Co or Ni were not added or in the presence of 0.5 mM of Ni (Fig. 3B and 3C), were further dechlorinated, and products, such as 25/2-, 24/2-, and 2/2-CBPs, were concomitantly increased. The congener distribution pattern of supplementation with 0.5 mM of Co was similar to that of the dechlorination process known as M, in which flanked and unflanked *meta*-Cls are removed and this process is less effective on the more highly chlorinated PCBs (Bedard and Quensen, 1995). Supplementation with 0.5 mM of Co and 0.5 mM of Ni showed the same reduction in tetra-CBPs and accumulation of tri- and di-CBPs (Fig. 3E); however, the extent of these changes was less than observed for 0.5 mM of Co only.

Dechlorination of PCBs occurred mainly via the removal of *meta*- and *para*-Cls. After an incubation time of 21 weeks, dechlorination of Aroclor 1248 occurred to similar extents regardless of added metal levels, though *para*-dechlorination was somewhat faster than *meta*-dechlorination (Fig. 4). The inoculating sediments used in our previous study showed the same preferential removal of *para*-Cls in Aroclor 1248, especially in slurry condition (Kwon *et al.*, 2001). After 32 weeks, however, dechlorination occurred predominantly from the *meta*-position in vials supplemented with 0.5 or 1.0 mM of Co (Fig. 4). In vials containing 0.5 mM of Co, *meta*-Cls were reduced by

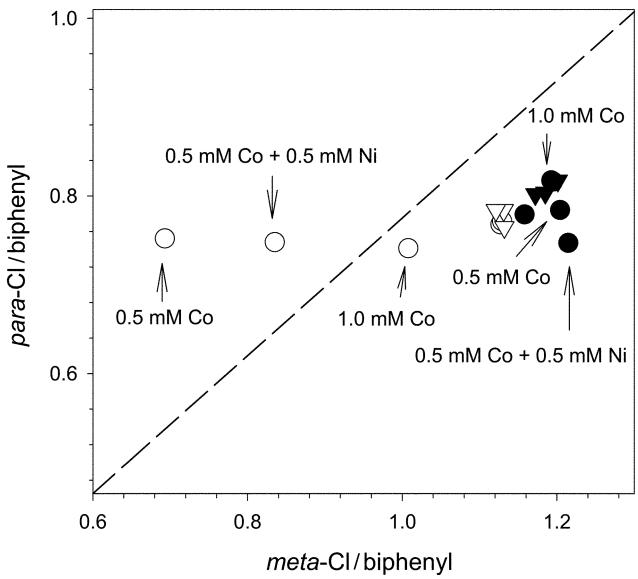

Fig. 4. Average number of *meta*-Cl versus *para*-Cl in samples supplemented with cobalt (circle) or nickel (triangle) after 21 (black color) and 32 (white color) weeks of incubation. The dashed line represents the proportional removal of *meta*- and *para*-Cl from Aroclor 1248.

42.0% (from 1.19 to 0.69) and *para*-Cls by only 8.5% (from 0.82 to 0.75) after 21 and 32 weeks of incubation. *Meta*-dechlorination also occurred during this period in vials containing 1.0 mM of Co and in those containing 0.5 mM of Co and 0.5 mM of Ni, but the extent of *meta*-dechlorination was greater in the presence of 0.5 mM of Co. In vials containing the other supplements, *meta*- and *para*-Cls were hardly reduced during this period.

Although little is known about the environmental factors required for the dechlorination of PCBs, some investigators have identified factors that enhance or inhibit dechlorination. In a biphenyl enrichment experiment using Aroclor 1254 (Rhee *et al.*, 1993b), dechlorination rates were found to be no different in biphenyl-enriched and non-enriched cultures for up to 13 months of incubation. After this period, however, one of the principal dechlorinated products of Aroclor 1254, 24/4-CBP, was dechlorinated to 2/4-CBP in a non-enriched vial, whereas biphenyl- enrichment selectively inhibited the *para*-dechlorination of 24/4-CBP for up to 24 months. Bedard *et al.*, (1996) found that addition of 25/34-CBP to slurries of Aroclor 1260 stimulated selective *para*-dechlorination and suggested that 25/34-CBP enriched a population of PCB-dechlorinating microorganisms that could use it as an electron accepter. The enrichment of sediment microorganisms with some haloaromatic compounds (HACs) enhanced only the *meta*-dechlorination of Aroclor 1248 and other HACs increased both *meta*- and *para*-dechlorination (Cho *et al.*, 2002). Some investigators have also shown that inhibitors, temperature, PCB concentration, and other factors favor different dechlorinating microorganisms (Bedard and Quensen, 1995). The results of these studies clearly demonstrate that there are many dechlorinating microorganisms and that they have different abilities.

Supplementation of 0.5 mM of Co to the culture vial enhanced the *meta*-dechlorination of Aroclor 1248 (Fig. 4) and the dechlorination pattern changed from a process H' type to a process M type (Fig. 3). According to these results and the results of other investigator's, we propose that the enrichment of cultures with Co may stimulate the growth of a specific population of *meta*-dechlorinators after a long incubation time, and that subsequently *meta*-rich dechlorinated products of Aroclor 1248, such as 25/25-, 25/23-, and 25/24-CBPs, are further dechlorinated to 2/2-, 25/2-, and 24/2-CBPs. Cobalt acts as a cofactor of vitamin B_{12}, which can catalyze the reductive dechlorination of chlorophenols at the *para* and *meta* positions (Gantzer and Wackett, 1991; Smith and Woods, 1994). Methanogens are rich in corrinoids such as vitamin B_{12}, and believed to be one of the physiological groups capable of PCB dechlorination (Ye *et al.*, 1995). When methanogens were inhibited with 2-bromoethanesulfonate in sediments containing Aroclor 1248, the extent of dechlorination was reduced due to the absence of the further dechlorination of some *meta*-rich products of the initial

dechlorination (Kim and Rhee, 1999). Two populations involved in the further dechlorination of the *meta*-rich congeners of Aroclor 1248 differed by two orders of magnitude (Cho *et al*., 2000). We enriched methanogens in serum vials containing short chain fatty acids and yeast extract, and the existence of methanogens was confirmed by methane analysis (Kwon *et al*., 2001). This enriched culture of methanogens was supplemented to the newly prepared culture vials containing Aroclor 1248 and Co was added at a concentration of 0.5 mM to investigate their combined effect on the dechlorination of Aroclor 1248. The extent and the pattern of dechlorination, however, was similar to that of 0.5 mM of Co alone (data not shown). Since we did not add PCBs to the methanogen enrichment medium, the enriched cultures might not have had the activity of PCBs dechlorination and, thus, the addition of methanogens had no effect on the dechlorination of Aroclor 1248. Acclimation is very important in the biodegradation of many recalcitrant compounds (Alexander, 1994).

The more highly chlorinated PCB congeners were described as being the most carcinogenic (Safe, 1993). Among the 209 congeners of PCBs, 11 congeners substituted with chlorine in both *para*-positions, in at least two *meta*-positions, and/or the mono-*ortho*-position are classified as coplanar PCBs. These congeners are commonly referred to as dioxin-like and bind with great affinity to the aryl hydrocarbon receptor (Safe, 1993). The present results show that supplementation with adequate amounts of cobalt promotes the *meta*-dechlorination of PCBs, and reflects enrichment of microorganisms with *meta*-dechlorinating ability. Therefore, if we can selectively enrich appropriate dechlorinating microorganisms, it may be possible to degrade recalcitrant congeners, such as coplanar PCBs.

Acknowledgments

This work was supported by the Inje Research and Scholarship Foundation in 2001.

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