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Effects of Nutritional Sources on Degradation of Polychlorinated Biphenyls (PCBs) by *Pseudomonas* sp. P2

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The effects of nutritional sources on growth of *Pseudomonas* sp. P2 were investigated in medium containing biphenyl as a carbon source. To determine characterization of *Pseudomonas* sp. P2, the incubation time was determined to 100 h of the log phase in the growth curve. The optimal compositions for the growth of *Pseudomonas* sp. P2 degrading polychlorinated biphenyls (PCBs) were 1000 mg/L NH_4NO_3, 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, and 10 mg/L $FeSO_4 \cdot 7H_2O$. *Pseudomonas* sp. P2 showed the degradability of 59.3 %, 57.6%, 51.4%, and 48.7% at 500 mg/L, 1000 mg/L, 1500 mg/L, and 2000 mg/L of the PCBs within insulating oil after 100 h incubation under the optimum conditions, respectively.
Key words : Polychlorinated biphenyls, *Pseudomonas* sp., Characterization, Degradation, Insulating oil.

1. INTRODUCTION

Polychlorinated biphenyls (PCBs) represents many isomers with different numbers of chlorine substituted in biphenyl. While there are 209 different PCBs isomers (Bedard et al., 1987), the commercial PCBs are the mixture of these isomers and were used as insulating oil in the electrical transformers and capacitors (Tanabe, 1988; May et al., 1992).

PCBs has been concerned for several decades because of persistence in environment, accumulation in animal tissue, and potential carcinogen. PCBs was usually distributed as a mixture of PCBs isomers in environment (May et al., 1992). Although PCBs was recalcitrant, recent studies have shown that PCBs has been extensively dechlorinated in some environment. The biodegradability of PCBs is important to minimize its impact on biota (Furukawa, 1978).

Many works have been done to isolate strains degrading PCBs. Furukawa et al. (1979) demonstrated the metabolic fates of PCBs by *Alcaligenes* sp. Y42 and *Acinetobacter* sp. P6. Furukawa et al. (1983) also studied the biodegradability of PCBs, and its metabolic products by *Acinetobacter* sp. P6. Shields et al. (1985) also investigated the mineralization of PCBs by *Alcaligenes* sp. and *Acinetobacter* sp. Kim et al. (1986) paid attention to the degradation of PCBs by Alcaligenes aquamarinus. Kohler et al. (1988) studied the biodegradability of Aroclor 1254 by *Acinetobacter* sp. P6 and *Arthrobacter* sp. B1 B. Gibson et al. (1993) worked that *Pseudomonas* sp. LB400 was grown in medium containing biphenyl as a carbon source.

Some studies have also been conducted to understand the effects of environmental factors on microbial degradation of PCBs. Kim and Kim (1990) studied the effects of environmental factors on the degradation of Aroclor 1242 by *Pseudomonas* sp. Liu (1981) studied the PCBs biodegradation whose factors affecting degradation. Bedard et al. (1986) reported the characterization of microorganisms with regard to the ability to degrade PCBs.

If *Pseudomonas* sp. P2 is considered as strain biodegrading PCBs within insulating oil, it is essential that the optimum conditions for growth are determined. Investigation for nutritional sources could also provide useful information on the optimum conditions degradating PCBs within insulating oil by *Pseudomonas* sp. P2. Therefore, it was attempted to investigate the culture conditions of the *Pseudomonas* sp. P2 for further characterization.

2. Materials and Methods

2.1 Microorganism and Medium

The *Pseudomonas* sp. P2 isolated from the soil

on the Sinchun stream in Taegu was used (Choi, 1996). *Pseudomonas* sp. P2 was grown aerobically in 30 mL nutrient broth medium with 150 rpm shaking incubator for seed culture at 30℃ 12 h. The cells were then harvested by centrifugation, and washed throughly by deionized water.

The compositions of basal medium were 1000 mg/L $(NH_4)_2SO_4$, 1000 mg/L KH_2PO_4, 200 mg/L $MgSO_4 \cdot 7H_2O$, 100 mg/L NaCl, 10 mg/L $FeSO_4 \cdot 7H_2O$, and 20 mg/L $CaCl_2 \cdot 2H_2O$. Initial pH of medium was adjusted to 7.0 (Kim et al., 1986).

2.2 Effects of Nutritional Factors

The effects of 1000 mg/L of $(NH_2)_2CO$, $(NH_4)_2SO_4$, $NaNO_3$, NH_4Cl, NH_4NO_3, and KNO_3 as nitrogen sources were compared one another on the growth of *Pseudomonas* sp. P2. The optimum concentration of selected nitrogen was monitored between 250 mg/L and 2000 mg/L. The effects of 1000 mg/L of KH_2PO_4, K_2HPO_4, NaH_2PO_4, and H_3PO_4 as phosphorus sources were observed in the growth of *Pseudomonas* sp. P2. The optimum concentration of selected phosphorus was monitored between 250 mg/L and 2000 mg/L. Metal ions were selected to $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, NaCl, and $FeSO_4 \cdot 7H_2O$.

The 1000 mg/L biphenyl as a carbon source was added to 30 mL liquid medium. The initial optical density of *Pseudomonas* sp. P2 was adjusted to 0.25 at 660 nm. The *Pseudomonas* sp. P2 inoculated in liquid medium was incubated at 30℃ for 160 h with 150 rpm shaking incubator. The growth was monitored by measuring optical density at 660 nm with a UV/Visible spectrophotometer (Varian, Model CARY-1).

2.3 Degradation of PCBs

The insulating oil contained PCBs was obtained directly from transformer with Aroclor 1242 (Lee et al., 1979; Kim and Park, 1989). To check the influences of PCBs concentration, Pseudomonas sp. P2 was cultivated aerobically at 30℃ with 500 mg/L, 1000 mg/L, 1500 mg/L, and 2000 mg/L of PCBs within insulating oil. The initial cell mass was adjusted to 130 mg/L. The degradation of PCBs was obtained after 100 h of the log phase in the growth curve.

2.4 Analysis of PCBs

An analysis of PCBs in medium was carried out by gas chromatography in terms of perchlorination meaning that PCBs were converted to decachlorinated biphenyl (DCB). PCBs extraction from medium was accomplished with n-hexane by magnetic stirrer for 1 h. PCBs extracted from medium was perchlorinated to DCB by SbCl5 (Huckins et al., 1974; Trotter and Young, 1975; Kim and Moon, 1995).

The concentration of PCBs was analyzed by a Varian 3300 gas chromatography equipped with 63 Ni electron capture detector (ECD). The packing material of column (2 m long×0.6 cm i.d.) was packed with 3% OV-17 on 80/100 chrom. whp. The column temperature was 250℃ while the detector and inlet temperature were 260℃. The flow rates of N2 as carrier gas was 60 mL/min (Schutzmann et al., 1971; Armour, 1973; Moon et al., 1995).

3. Results and Discussion

3.1 Growth Curve

Fig. 1 showed the growth curve of Pseudomonas sp. P2 in medium containing 1000 mg/L biphenyl as a carbon source for 160 h incubation. After a lag phase for 20 h, Pseudomonas sp. P2 was increased rapidly until 100 h incubation. In the log phase,

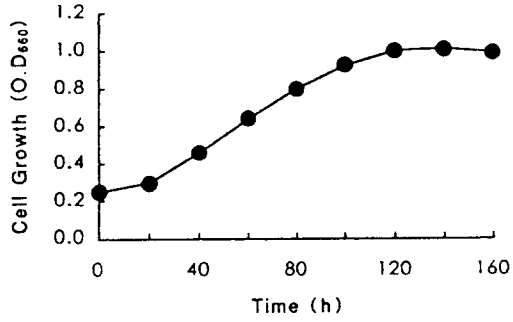

Fig. 1. Growth curve of *Pseudomonas* sp. P2 in medium containing 1000 mg/L biphenyl as a carbon source. Growth was observed by optical density at 660 nm by spectrophotometer.

Table 1. Effects of nitrogen sources on the growth of *Pseudomonas* sp. P2. Relative activity was calculated with O.D $_{660}$ after 100 h incubation

Nitrogen sources (1000 mg/L)	Relative activity (%)
NH_4NO_3	100.0
$(NH_2)_2CO$	82.1
$(NH_4)_2SO_4$	84.8
$NaNO_3$	85.4
NH_4Cl	85.9
KNO_3	80.4

the optical density of Pseudomonas sp. P2 was varied from 0.30 to 0.92. The maximum growth rate (μmax) was calculated 0.33 day^{-1} in the log phase. A maximum optical density 1.01 was obtained at 140 h, and a final optical density 0.99 was obtained at 160 h. For the study of optimum conditions, incubation time was determined at 100 h of the log phase in the growth curve.

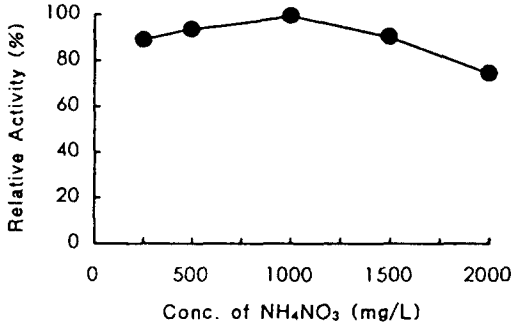

Fig. 2. Effects of NH_4NO_3 concentrations on the growth of *Pseudomonas* sp. P2. Relative activity was measured at O.D $_{660}$ after 100 h incubation.

3.2 Effects of Nitrogen Sources

The batch experiments were conducted to study the effects of nitrogen sources and nitrogen concentrations on the growth of Pseudomonas sp. P2. The effects of 1000 mg/L of NH_4NO_3, $(NH_2)_2CO$, $(NH_4)_2SO_4$, $NaNO_3$, NH_4Cl, and KNO_3 were shown in Table 1. The optimum nitrogen source was selected to be NH_4NO_3 shown maximum growth. Fig. 2 showed the effects of the concentrations of NH_4NO_3 as the nitrogen source. The optimal NH_4NO_3 concentration was decided on 1000 mg/L on the growth of *Pseudomonas* sp. P2.

Table 2. Effects of phosphorus sources on the growth of *Pseudomonas* sp. P2

Nitrogen sources (1000 mg/L)	Relative activity (%)
KH_2PO_4	100.0
K_2HPO_4	97.6
NaH_2PO_4	88.9
H_3PO_4	98.7

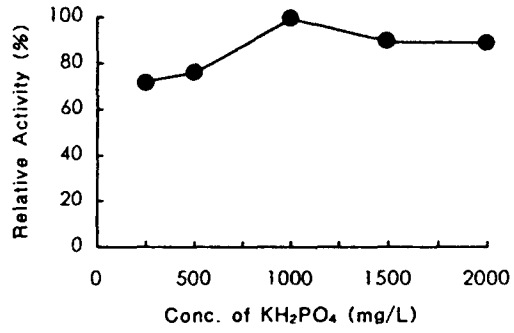

Fig. 3. Effects of KH_2PO_4 concentrations on the growth of *Pseudomonas* sp. P2.

3.3. Effects of Phosphorus Sources

The effects of phosphorus sources and phosphorus concentrations on the growth of *Pseudomonas* sp. P2 were examined in the batch experiments. The effects of phosphorus sources were examined in 1000 mg/L of KH_2PO_4, K_2HPO_4, NaH_2PO_4, and H_3PO_4. As shown in Table 2, the optimum phosphorus source was revealed to be KH_2PO_4. Therefore the optimal KH_2PO_4 concentration was investigated between 250 mg/L and 2000 mg/L. In Fig. 3, the optimal KH_2PO_4 concentration was confirmed to be 1000 mg/L on the growth of *Pseudomonas* sp. P2.

3.4. Effects of Metal Ions

To improve the growth of *Pseudomonas* sp. P2, the effects of metal ions were examined out as $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, NaCl, and $FeSO_4 \cdot 7H_2O$. Fig. 4 showed the effects of $MgSO_4 \cdot 7H_2O$. The relative activities of the growth at 0 mg/L and 250 mg/L of $MgSO_4 \cdot 7H_2O$ were 54.3% and 77.3%,

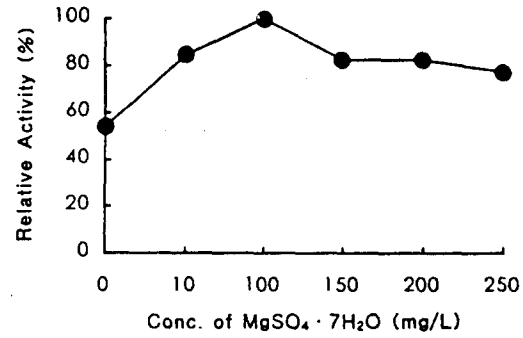

Fig. 4. Effects of $MgSO_4 \cdot 7H_2O$ concentrations on the growth of *Pseudomonas* sp. P2.

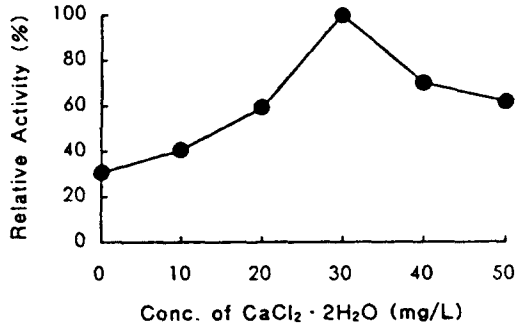

Fig. 5. Effects of $CaCl_2 \cdot 2H_2O$ concentrations on the growth of *Pseudomonas* sp. P2.

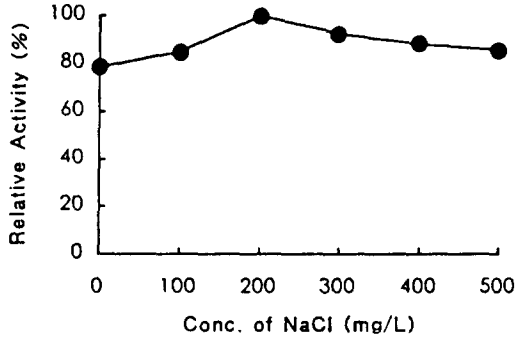

Fig. 6. Effects of NaCl concentrations on the growth of *Pseudomonas* sp. P2.

based on the criteria of 100 mg/L $MgSO_4 \cdot 7H_2O$, respectively. The relative activities of the growth in case of 0 mg/L and 50 mg/L of $CaCl_2 \cdot 2H_2O$ were 30.6% and 61.8%, based on the criteria of 30 mg/L $CaCl_2 \cdot 2H_2O$, respectively (Fig. 5). The optimum concentrations of $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$ were found to be at 100 mg/L and 30 mg/L, respectively.

The relative activities of the growth at 0 mg/L and 500 mg/L of NaCl were 78.6% and 85.7%, based on the criteria of 200 mg/L NaCl, respectively. The optimum concentration of NaCl was 200 mg/L (Fig. 6). The relative activities of the growth at 0 mg/L and 25 mg/L of $FeSO_4 \cdot 7H_2O$ were 37.5% and 82.5%, based on the criteria of 10 mg/L $FeSO_4 \cdot 7H_2O$, respectively. The optimum concentration of $FeSO_4 \cdot 7H_2O$ was 10 mg/L (Fig. 7).

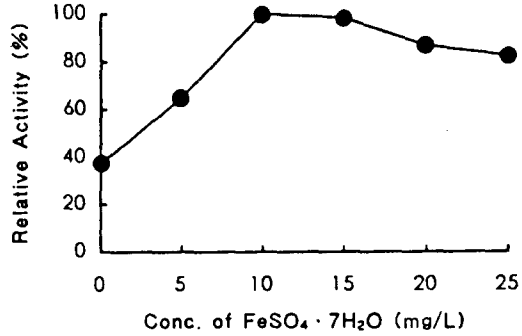

Fig. 7. Effects of $FeSO_4 \cdot 7H_2O$ concentrations on the growth of *Pseudomonas* sp. P2.

Table 3. Optimal compositions of medium to degrade polychlorinated biphenyls (PCBs) by *Pseudomonas* sp. P2

Components	Concentrations (mg/L)
PCB_s	1000
NH_4NO_3	1000
KH_2PO_4	1000
$MgSO_4 \cdot 7H_2O$	100
NaCl	200
$FeSO_4 \cdot 7H_2O$	10
$CaCl_2 \cdot 2H_2O$	30
Emulsifier[1)]	200
pH	7.0

1) Alkyl aryl ethoxylated phosphate as surfactant 101

3.5 Degradation of PCBs

To examine the effects of PCBs concentrations within insulating oil, *Pseudomonas* sp. P2 was cultivated in 500 mg/L, 1000 mg/L, 1500 mg/L, and 2000

mg/L of PCBs under the optimal medium compositions as indicated in Table 3.

To confirm degradability of PCBs by *Pseudomonas* sp. P2, the residual PCBs in medium was measured by gas chromatography after 100 h of the log phase in the growth curve. The chromatogram shown in Fig. 8 (A) was demonstrated by the standard decachlorinated biphenyl (DCB) with 6.5 min retention time. The DCB peaks of Fig. 8 (B) and (C) after perchlorination of samples were completely agreed with the peak of standard DCB. Comparing with Fig. 8 (B), the 57.6% degradation obtained at 1000 mg/L PCBs was confirmed to decrease peak height of DCB on Fig. 8 (C).

The degradation at 500 mg/L, 1000 mg/L, 1500 mg/L and 2000 mg/L of PCBs were 59.3%, 57.6%, 51.4% and 48.7%, respectively (Table 4). Choi (1996) reported that 1000 mg/L PCBs was degraded 2.0% at 40 h, 49.0% at 80 h, 60.0% at 120 h, and 60.0% at 160 h after incubation of *Pseudomonas* sp. P2. Based on Choi's report (1996), this study showed that the degradation rate of PCBs was not inhibited from 500 mg/L to 2000 mg/L of PCBs (Table 4). Similarly, Wong and Kaiser (1975) reported that inhibition of bacterial growth was not observed up to 1000 mg/L PCBs. Kim et al. (1986) reported that inhibition of PCBs degradation by *Alcaligenes aquamarinus* was occurred at 5000 mg/L PCBs.

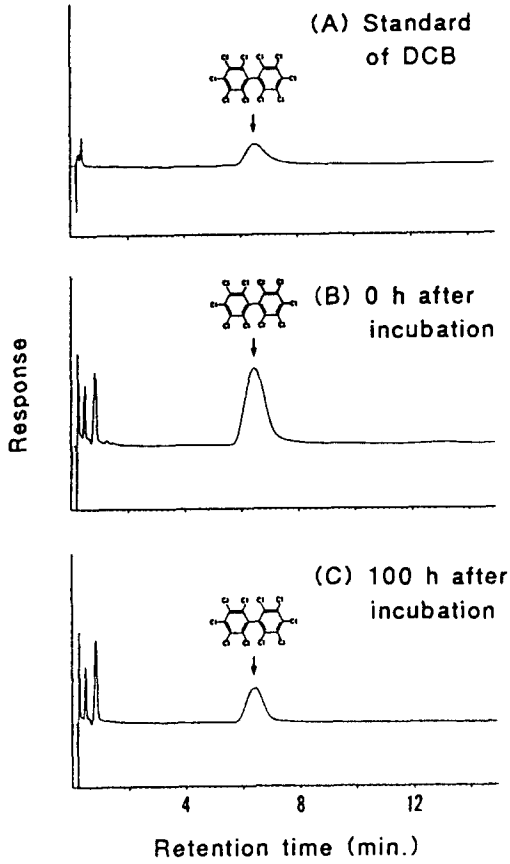

Fig. 8. GC-ECD chromatograms for analysis of polychlorinted biphenyls (PCBs). Standard decachlorinated biphenyl (DCB) in (A), perchlorinated medium after 0 h incubation in (B), and perchlorinated medium after 100 h incubation in (C).

Table 4. Degradation of polychlorinated biphenyls (PCBs) by *Pseudomonas* sp. P2 in medium containing PCBs as a carbon source after 100 h incubation

Concentrations (mg/L)	Degradation of PCBs (%)
500	59.3
1000	57.6
1500	51.4
2000	48.7

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Pseudomonas sp. P2에 의한 Polychlorinated Biphenyls (PCBs) 分解에 대한 營養原의 影響

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(1996년 1월 26일 접수)

유일한 炭素原으로서 Biphenyl을 包含하고 있는 液體培地에서 *Pseudomonas* sp. P2의 生育에 미치는 營養原의 效果에 관해 硏究하였다. *Pseudomonas* sp. P2의 特性을 調査하기 위한, 培養時間은 菌의 生育曲線에서 對數成長期 末인 100時間으로 하였다. PCBs를 分解하는 *Pseudomonas* sp. P2의 生育을 위한 最適 組成은 1000 mg/L NH_4NO_3, 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 $FeSO_4 \cdot 7H_2O$였다. *Pseudomonas* sp. P2에 의한 100時間 培養後 絶緣油 內에 있는 PCBs의 分解率은 500 mg/L에서 59.3%, 1000 mg/L에서 57.6%, 1500 mg/L에서 51.4%, 2000 mg/L에서는 48.7%였다.