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Factors affecting the biodegradation of PCP by *Pseudomonas mendocina* NSYSU

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Abstract

A pentachlorophenol (PCP) degrading bacterium was isolated from PCP-contaminated soils and identified as *Pseudomonas mendocina* NSYSU (*P. mendocina* NSYSU). The main objectives of this study were to (1) clarify the factors affecting the ability and efficiency of PCP biodegradation by *P. mendocina* NSYSU, and (2) optimize the use of this bacterium in bioremediation of PCP. Microcosm experiments were conducted to fulfill the objectives. In batch cultures, *P. mendocina* NSYSU used PCP as its sole source of carbon and energy and was capable of completely degrading this compound. This was confirmed by the stoichiometric release of chloride ion. Moreover, *P. mendocina* NSYSU was able to mineralize a high concentration of PCP (150 mg/L). Results from the oxygen concentration experiment reveal that the growth of *P. mendocina* NSYSU was inhibited under low oxygen and anaerobic conditions. Results indicate that the optimal growth conditions for *P. mendocina* NSYSU include the following: slightly acidic ($6 < pH < 7$), aerobic, and relatively moderate ambient temperature ($20\,^{\circ}C < \text{temperature} < 30\,^{\circ}C$) conditions. Addition of extra carbon sources (sodium acetate and glucose) could not enhance the PCP biodegradation. No PCP byproducts were detected after eight days of incubation in this study. This suggests that *P. mendocina* NSYSU is able to effectively biodegrade PCP and its biodegradation byproducts without the accumulation of inhibitory toxic compounds. Results from this study could be used to assist the optimization of its use in bioremediation of PCP.

Keywords: *Pseudomonas mendocina* NSYSU; Groundwater contamination; PCP; Microcosm; Biodegradation

1. Introduction

Chlorophenols have been used as biocides or as starting components for pesticide production [1–3]. Among them, pentachlorophenol (PCP) and its sodium salt have been widely used as wood preservative owing to their toxic effect on bacteria, mold, algae, and fungi. The US Environmental Protection Agency (EPA) has listed PCP as a priority pollutant because of its proven carcinogenicity and toxicity, as well as for the large number of known PCP-contaminated sites worldwide [2,4].

The biodegradation of PCP has been studied in both aerobic and anaerobic systems. A number of researchers have studied anaerobic PCP degradation in aquatic, sludge, and soil environment [1,2,5–8]. Reductive dechlorination has been suggested as the primary PCP biodegradation mechanism [6,9–11]. Under anaerobic conditions, chlorine can be removed from the aromatic ring by reductive dechlorination resulting in partially or fully dechlorinated product which is then more susceptible to either aerobic or anaerobic attack [10]. The aromatic ring is thus totally dechlorinated prior to ring cleavage. Further degradation results in the production of methane and carbon dioxide [10]. Under aerobic

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conditions, the biodegradation pathways of PCP are more diverse than under anaerobic conditions. Ring cleavage can occur either before or after removal of the chlorine substituents, giving rise to a whole array of intermediates of varying toxicity [10,12]. Isolation of PCP-degrading microorganisms from the soils, sediments, or sludges has been reported [10–15]. Application of the enriched PCP-degrading microorganisms would significantly enhance the PCP removal [10,12,15].

Previous reports of PCP degradation by *Pseudomonas* spp. are rare. *Pseudomonas cepacia* could only degrade PCP in the presence of supplemental carbon sources [16]. Radehaus and Schmidt [13] described that PCP could be mineralization by *Pseudomonas* sp. strain RA2. *Pseudomonas* sp. strain SR3 has been shown to be able to bioremediate soil and water contaminated with PCP [5]. Tomasi et al. [17] demonstrated that *P. cepacia* AC1100 metabolized PCP to the corresponding chlorohydroquinone. *Pseudomonas* sp. Bu34 was suggested to be able to biodegrade the highly PCP-contaminated soils, water, and wood products [18]. Thakur et al. [7] showed that *Pseudomonas* sp. strain IST103 was capable of utilizing PCP as sole carbon and energy source. Results from previous studies indicate that groundwater samples collected around a formal PCP manufacturing plant located in southern Taiwan were contaminated by PCP [19,20]. Site soils consist of grayish sandy clay loam, brown sandy clay loam with brown and grayish-brown mottles, and light gray sandy loam with yellowish-brown mottles. The site soils are moderately acidic and well drained on low lands. The surficial aquifer sediments are a relatively uniform sand to silty sand. Depth to the seasonal high water table is approximately 1.2 m. According to the results from hydrogeologic tests, the average hydraulic conductivity of the host geologic material is 3×10^{-3} cm/s, and the groundwater slop is approximately 0.1%. In our preliminary study, a PCP degrading bacterium was isolated from PCP-contaminated aquifer sediments and identified as *P. mendocina* NSYSU (*P. mendocina* NSYSU) [20]. The main objectives of this study were to (1) clarify the factors affecting the ability and efficiency of PCP biodegradation by *P. mendocina* NSYSU, and (2) optimize the use of this bacterium in bioremediation of PCP. This paper describes the effects of growth conditions of *P. mendocina* NSYSU and PCP utilization by this bacterium.

2. Materials and methods

2.1. Method of isolation

A PCP-degrading bacterium was isolated from the aquifer sediments collected from the studied PCP site. Sediments (1 g) were added to 100 mL of an inorganic salts solution (defined broth) containing 10 mg of PCP/L [11,13]. The defined broth contained the following components at the specified concentrations (units are in mg/L of water): KH_2PO_4, 800; Na_2HPO_4, 800; $Mg_2SO_4{\cdot}7H_2O$, 200; $CaCl_2{\cdot}2H_2O$, 10; NH_4Cl, 500; plus 1 mL of trace metal solution which include $FeSO_4{\cdot}7H_2O$, 5; $ZnSO_4{\cdot}7H_2O$, 4; $MnSO_4{\cdot}4H_2O$, 0.2; $NiCl{\cdot}6H_2O$, 0.1; H_3BO_3, 0.15; $CoCl_2{\cdot}6H_2O$, 0.5; $ZnCl_2$, 0.25; and EDTA, 2.5. After 14 days of incubation at 30 °C (160 rpm) in a Gyrotory Shaker, 1 mL of the enrichment culture was transferred to 100 mL of defined medium containing 20 mg of PCP/L. This medium was incubated at 30 °C (160 rpm) for 14 days. The above procedures were repeated until the concentration of PCP in the medium reached 40 mg/L. After the incubation processes, serial dilutions were plated on the defined medium containing inorganic salts, 40 mg of PCP/L, and 15 g of defined agar (Noble class, Difco)/L. Each separate colonies appearing on the defined agar after 2 weeks of incubation at 30 °C was restreaked on fresh agar, and then before they were transferred back to liquid medium. By transferring serial subcultures to liquid medium with PCP concentrations of 40 mg/L, PCP degraders were enriched.

2.1.1. PCP biodegradation experiments

Microcosm biodegradation experiments were conducted to evaluate the ability of the isolated PCP-degrader (*P. mendocina* NSYSU) on PCP biodegradation. In this experiment, PCP was used as the sole carbon source. Each 160 mL of serum bottle (microcosm) contained 1 mL of bacterial culture (OD = 0.2), 100 mL of inorganic salts solution (defined medium), and PCP, and sealed with Teflon-lined rubber septa. The initial PCP concentrations in the serum bottles included 20, 40, 80, 100, 150, and 320 mg/L. The headspace of each bottle was filled with air to provide sufficient oxygen for aerobic PCP biodegradation. The varied PCP concentrations used in this experiment were to evaluate if the bacterium could mineralize higher PCP concentrations. Serum bottles were incubated at 30 °C in a Gyrotory shaker (160 rpm) in the dark. The defined medium contained the following components at the specified concentrations (units are in mg/L of water): KH_2PO_4, 800; Na_2HPO_4, 3200; $MgSO_4{\cdot}7H_2O$, 200; $CaCl_2$, 10; NH_4Cl, 500; plus 1 mL of trace elements which include (units are in mg/L of water) $FeSO_4{\cdot}7H_2O$, 500; $ZnSO_4{\cdot}7H_2O$, 4000; $MnCl_2{\cdot}4H_2O$, 200; $CoCl_2{\cdot}6H_2O$, 500; $NiCl{\cdot}6H_2O$, 100; H_3BO_3, 150; EDTA, 2500. The final pH value was 7.2 in each bottle. The medium solution was autoclaved before use. Control bottles contained 250 mg/L of $HgCl_2$ and 500 mg/L of NaN_3. The inocula used for the control bottles were autoclaved before use. PCP concentration measurement was performed during the incubation period. Duplicate microcosms were sacrificed at each time point and analyzed for PCP concentrations. Five millilitres of solution was taken from each bottle for PCP analysis by puncturing the Teflon-lined rubber septa with a glass syringe. Organic compound analyses were performed in accordance with US EPA Method 604, using a HP 6890 Gas Chromatograph (GC). Spectrophotometry method [21] was also used occasionally to verify the PCP concentrations determined by the GC method.

2.2. Effects of other carbon sources and environmental factors on PCP biodegradation

Effects of other carbon sources (glucose and sodium acetate) and environmental conditions [pH, temperature, dissolved oxygen (DO)] on PCP biodegradation by *P. mendocina* NSYSU were evaluated in this study. In the carbon addition experiment, glucose and sodium acetate were applied as the primary substrates and added in serum bottles. Each 160 mL of serum bottle contained 1 mL of bacterial culture (OD = 0.2), 100 mL of inorganic salts solution, PCP, and glucose (or sodium acetate). The initial PCP concentrations in the serum bottles prepared in this test were ranged from 24 to 36 mg/L. The initial glucose (or sodium acetate) concentrations in the serum bottles prepared in this test were approximately 500 mg/L.

In the pH control experiment, phosphoric acid (H_3PO_4, 0.2 M) and sodium hydroxide (NaOH, 0.5 M) were used to adjust pH values in the solution. Six different pH values (pH 5, 6, 6.5, 7.5, 8, 9, and 10) were used to evaluate the effects of pH values on PCP biodegradation. In the temperature control experiment, four different incubation temperatures (10, 20, 30, and 40 °C) were used to evaluate the effects of temperature on PCP biodegradation. In the oxygen control experiment, a 2-L respirator (Mituwa KMJ-2B) was used to control the DO values (7.6, 3.8, 0.3, and 0.01 mg/L) and temperature (30 °C) in the solution to evaluate the effects of temperature on PCP biodegradation. The construction, incubation conditions, and analyses for live and control bottles were described in the above section.

2.3. Determination of PCP biodegradation byproducts and chloride ion

Biodegradation of PCP was also determined by measuring the possible degradation byproducts and chloride ion using GC–mass spectrometer (MS) and a chloride ion electrode connected to an Orion pH/ISE meter, respectively. The GC–MS was operated with the computer system MS ChemStation (Hewlett Packard, USA) following the procedures described in Susarla et al. [22]. In this experiment, microcosms were constructed by inoculating resting cells of *P. mendocina* NSYSU in each 160 mL of serum bottle, which contained 1 mL of inocula (OD = 0.5), 100 mL of inorganic salts solution, and PCP. The initial PCP concentrations in the serum bottles were 40 mg/L. The construction, incubation conditions, and analyses for live and control bottles were described in the above section.

3. Results and discussion

3.1. PCP biodegradation experiments

One bacterium, which could tolerate higher concentrations of PCP, was isolated from the tested soils. The PCP degrader is a gram-negative, oxidase- and catalase-positive, aerobic rod-shaped bacterium, and is motile in soft agar. The PCP-degrading bacterium is identified as *P. mendocina* NSYSU [20]. The utilization of PCP as a carbon source for growth in a liquid mineral medium by this bacterium under aerobic conditions was conducted. Fig. 1 shows the PCP consumed by *P. mendocina* NSYSU using PCP as the sole carbon source (primary substrate). Results reveal that PCP in microcosms with concentrations in approximately 20, 40, 80 and 100 mg/L, respectively, was rapidly dechlorinated after a 6-day lag period. No PCP removal was detected in all control microcosms (data not shown). Approximately 99% of the PCP was removed within 12 days of incubation in these four groups of microcosms. However, a longer lag period (10 days) was observed for the microcosms with relatively high PCP concentration (150 mg/L), although a complete PCP removal was also observed on day 18 in this group of microcosms. No PCP removal was detected in microcosms with PCP concentrations of 320 mg/L after 20 days of incubation. This might be due to the following causes: (1) limitation of biomass; (2) limitation of oxygen; and (3) toxicity effect of the higher PCP concentration on the growth of bacteria activity. Results from this study indicate that *P. mendocina* NSYSU could be applied to the future remedial application at PCP-contaminated sites.

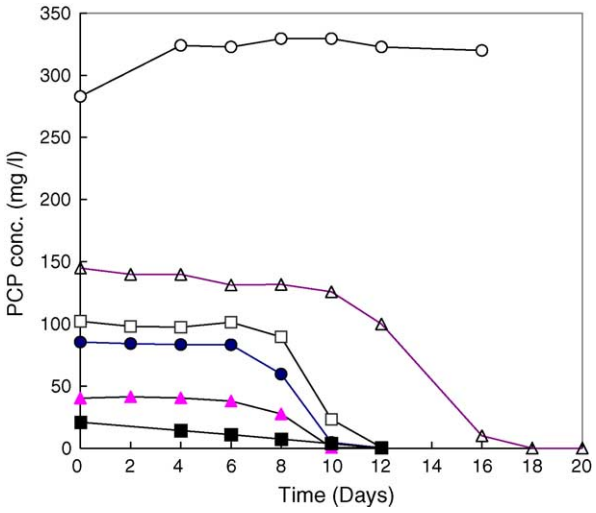

Fig. 1. Degradation of PCP by *P. mendocina* NSYSU. Cells were incubated in chemically defined medium containing various concentration of PCP. PCP initial concentration. (■) 20 mg/L; (▲) 40 mg/L; (●) 80 mg/L; (□) 100 mg/L; (△) 150 mg/L; (◯) 320 mg/L.

3.2. Effects of other carbon sources and environmental factors on PCP biodegradation

Fig. 2 shows the effects of carbon source (glucose or sodium acetate) addition on PCP biodegradation by *P. mendocina* NSYSU. Glucose and sodium acetate were used as the primary substrates in this experiment. Complete PCP removal was observed on day 10 in microcosms with extra carbon

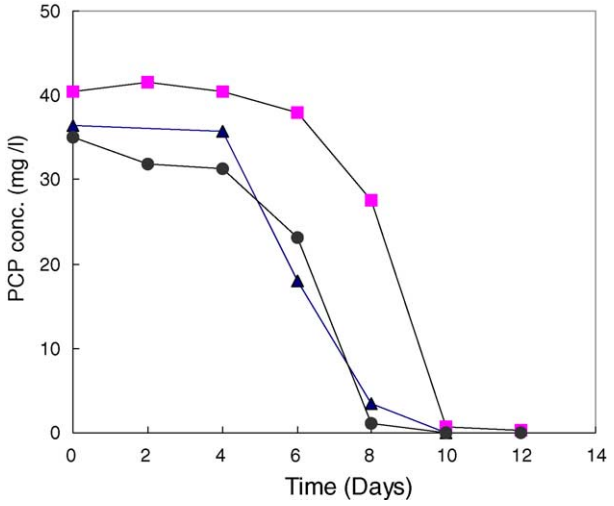

Fig. 2. Effect of various additional carbon sources on the degradation of PCP by *P. mendocina* NSYSU. (■) PCP (40 mg/L); (▲) PCP + sodium acetate; (●) PCP (40 mg/L) + glucose (500 mg/L).

sources (glucose or sodium acetate). Although a shorter lag period (4 days) was observed for these two groups of microcosms, results reveal that the addition of glucose or sodium acetate could not significantly enhance the biodegradation of PCP. These results might be attributable to the fact that *P. mendocina* NSYSU was isolated from PCP-contaminated soils and was not receiving inputs of glucose (or acetate) from natural sources. Thus, the preferential removal of more biodegradable carbon sources (glucose and acetate) by *P. mendocina* NSYSU was not observed in this experiment. The other possible cause is that the cometabolism is not the dominant biodegradation mechanism of PCP by this bacterium. Thus, the addition of other carbon source could not affect the PCP degradation rate.

Fig. 3 shows the effects of pH values on PCP biodegradation by *P. mendocina* NSYSU. Results indicate that pH 6 was the optimal value for *P. mendocina* NSYSU to biodegrade PCP. Complete PCP removal was observed on day 6 without any lag period in microcosms with the pH value of 6. Complete PCP removal was also observed in microcosms with pH 6.5, and no lag period was observed. However, a slightly longer incubation time (8 days) was required in this group of microcosm (pH 6.5). Results also show that longer lag periods were observed in microcosms with pH higher than 7.5. A 10-day lag was observed in microcosms with a pH value of 9. Biodegradation of PCP was inhibited in relatively acidic (pH 5) and alkaline (pH 10) conditions. No PCP removal was detected within the 18-day incubation period in microcosms with pH values of 5 or 10.

Fig. 4 shows the effects of incubation temperature on PCP biodegradation by *P. mendocina* NSYSU. Results indicate that 30 °C was the optimal incubation temperature for *P. mendocina* NSYSU to biodegrade PCP. Complete PCP removal was observed on day 12 with a 4-day lag period in microcosms incubated at 30 °C. A 10-day lag period for

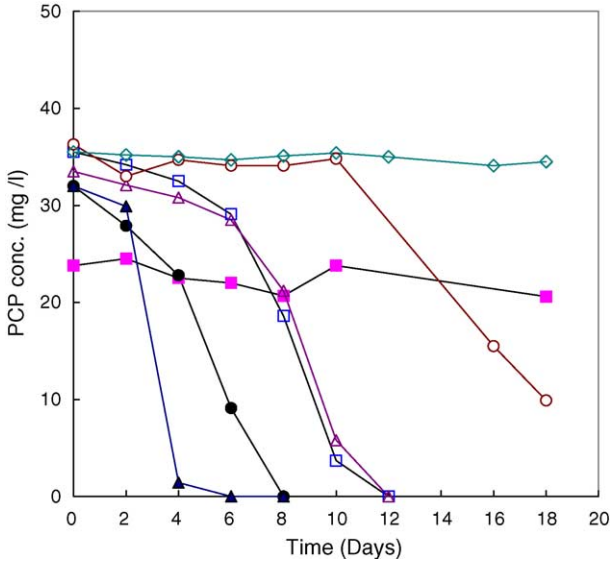

Fig. 3. Effect of pH on the degradation of PCP by *P. mendocina* NSYSU. Cells were incubated in chemically defined medium containing PCP at different pH. (■) pH 5; (▲) pH 6; (●) pH 6.5; (□) pH 7.5; (△) pH 8; (○) pH 9; (◊) pH 10.

PCP biodegradation was detected in microcosms incubated at 20 °C. No PCP removal was observed in microcosms incubated at 10 and 40 °C.

Fig. 5 shows the effects of DO on PCP biodegradation by *P. mendocina* NSYSU. Results indicate that more effective PCP removal rates can be obtained under aerobic conditions (DO = 7.5 and 3.8). Lower PCP degradation rate was obtained when DO dropped to 0.3 mg/L. No PCP removal was observed within the 16-day incubation period under the extremely low DO conditions (DO = 0.01 mg/L). Results indicate that the isolated PCP-degrader, *P. mendocina* NSYSU, is an aerobic bacterium, which could perform effec-

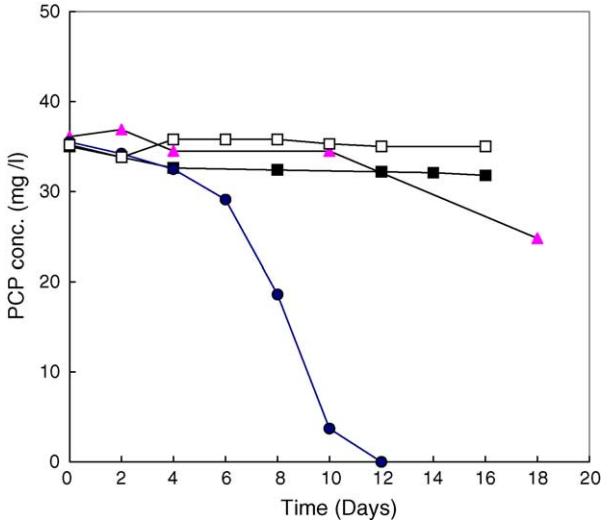

Fig. 4. Effect of temperature on the degradation of PCP by *P. mendocina* NSYSU. Cells were incubated in medium containing PCP at different temperature. (■) 10 °C; (▲) 20 °C; (●) 30 °C; (□) 40 °C.

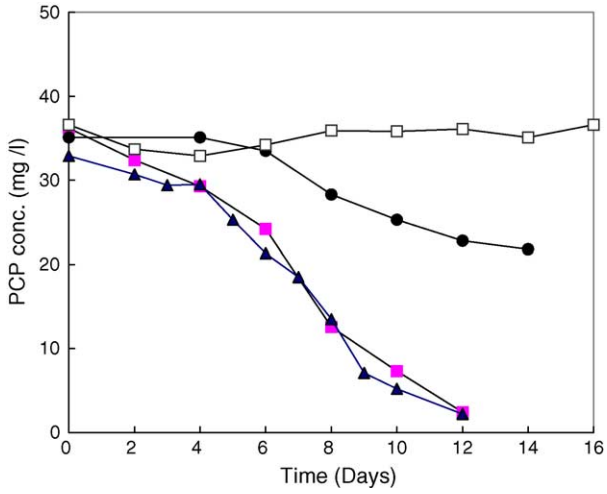

Fig. 5. Effect of dissolved oxygen on the degradation of PCP by *P. mendocina* NSYSU. Cells were incubated in chemically defined medium containing PCP under different oxygen concentration. Dissolved oxygen concentration: (■) 7.6 mg/L; (▲) 3.8 mg/L; (●) 0.3 mg/L; (□) 0.01 mg/L.

tive PCP biodegradation ability under aerobic conditions. The PCP degradation ability would be significantly inhibited under low oxygen conditions.

3.3. Determination of PCP biodegradation byproducts and chloride ion

This experiment was conducted to further confirm the degradation of PCP by measuring the possible degradation byproducts and produced chloride ion. Fig. 6 presents the disappearance of 40 mg of PCP/L and the release of 25.4 mg of chloride/L during the PCP biodegradation process. Compared to the results from previous PCP biodegradation experiments which bacterial culture with lower density (OD = 0.2), more effective PCP removal rate was observed in this experiment. This might be due to the effect that relatively higher bacterial culture density was used (OD = 0.5) in this experiment. Results show that PCP concentration dropped from 40 mg/L to below detection limit (1 μg/L) within 8 days of incubation. Thus, all PCP was degraded within 8 days without any lag period. Because the molecular weight of PCP consists of 66.7% chloride, the decrease in 40 mg/L of PCP concentration on day 8 in this experiment would correspond to the release of 26.68 mg of chloride/L. Results show that approximately 27.8 mg of chloride/L was detected at the end of the experiment on day 8 (Fig. 6). This reveals that the measured chloride concentration had a close match with the theoretical value. In the control microcosms, approximately 3.4 mg/L of chloride ion concentration was detected throughout the experiments with no decrease in PCP concentrations. This indicates that the depletion of PCP corresponded with the dechlorination reactions and release of chloride ions in the microcosms.

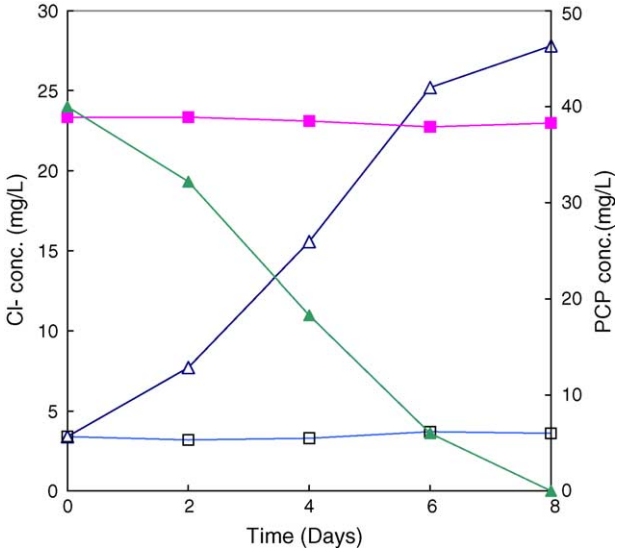

Fig. 6. Degradation of PCP by *P. mendocina* NSYSU resting cells (OD_{600} = 0.5, 8.9×10^8 cells/mL). (▲) PCP concentration (with resting cells); (△) chloride ion concentration (with resting cells); (■) PCP concentration of sterile control (no cells); (□) chloride ion concentration of sterile control (no cells).

GC–MS was applied to determine the degradation byproducts of PCP in this microcosm experiments on days 6 and 8. However, only low levels of 2,4,6-trichlorophenol (6 μg/L), (2,4-dichlorophenol (15 μg/L), 4-chlorophenol (13 μg/L), and 2-chlorophenol (28 μg/L) were detected on day 6. Moreover, no byproducts were detected on the last sampling day (day 8) (including live and control bottles). This suggests that *P. mendocina* NSYSU is capable of biodegrading PCP and its degradation byproducts effectively without the production of inhibitory toxic compounds.

4. Conclusions

A PCP-degrading bacterium was isolated from PCP-contaminated soils and identified as *P. mendocina* NSYSU. Batch experiments were conducted to evaluate the ability of the isolated PCP-degrader on PCP biodegradation and determine the optimal environmental conditions on PCP biodegradation. Conclusions of this study include the following:

(1) The isolated PCP-degrader, *P. mendocina* NSYSU, could use PCP as its sole carbon and energy sources and was capable of completely degrading this compound. This is confirmed by the stoichiometric release of chloride. Thus, substitute or other primary substrates are not required in the practical application.
(2) Higher PCP concentrations would cause the decrease in bacterial activity and increase in the lag period during the PCP degradation process. PCP concentrations of less than 150 mg/L were degraded effectively by *P. mendocina* NSYSU. Thus, the PCP loading into the remediation system inoculated with the PCP-degrader should be controlled within an acceptable range during the practical remedial application.

(3) Addition of extra carbon sources (sodium acetate and glucose) could not significantly enhance the PCP biodegradation. This might be due to the effect that *P. mendocina* NSYSU had been acclimated under the high PCP environment for a long period of time.
(4) The optimal growth temperature and pH values for *P. mendocina* NSYSU were 30 °C and pH 6, respectively. Results from the oxygen concentration experiment reveal that the growth of *P. mendocina* NSYSU was inhibited under low oxygen and anaerobic conditions. To obtain a more efficient PCP removal rate, the remedial system could be operated under slightly acidic ($6 < pH < 7$), aerobic (DO > 3 mg/L), and relatively moderate temperature conditions (20 °C < temperature < 30 °C).
(5) Results show that maintaining a high bacterial population and activity would significantly enhance the effectiveness of the remedial system on PCP removal.
(6) No biodegradation byproducts were detected after eight days of incubation in the byproduct experiment. This suggests that *P. mendocina* NSYSU is able to biodegrade PCP and its degradation byproducts effectively.

This paper describes the effects of growth conditions of *P. mendocina* NSYSU and PCP utilization by this bacterium. Results have clarified the factors affecting the ability and efficiency of PCP biodegradation by *P. mendocina* NSYSU. This could assist the optimization of its use in bioremediation of PCP. Future applications of this bacterium in practical remedial tasks include the following:

(1) If *P. mendocina* NSYSU exists at a PCP-contaminated site, natural bioremediation could be considered as a remedial option for this site if environmental and growth conditions for this bacterium are appropriate.
(2) If environmental conditions are not appropriate for the growth of this bacterium, enhanced bioremediation could be applied at the PCP site to increase the biodegradation rate.

Some bioremediation systems (e.g., biobarrier system, bioreactor) inoculated with the PCP-degrader, *P. mendocina* NSYSU, could be used in situ or on-site for field application.

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References

[1] K.A. McAllister, H. Lee, J.T. Trevors, Microbial degradation of pentachlorophenol, Biodegradation 7 (1996) 1–40.
[2] C.D. Litchfield, M. Rao, Pentachlorophenol biodegradation: laboratory and field studies, in: G.A. Lewandowski, L.J. DeFilippi (Eds.), Biological Treatment of Hazardous Wastes, Wiley, New York, 1998, pp. 271–283.
[3] J. Chi, G.L. Huang, A multimedia fugacity river model of pentachlorophenol in south drainage canal, China, J. Environ. Sci. Health A37 (2002) 113–125.
[4] Federal Register, National primary and secondary drinking water regulations, Fed. Reg. 54, 1989.
[5] S.M. Rensnick, P.J. Chapman, Physiological properties and substrate specificity of a pentachlorophenol-degrading *Pseudomonas* species, Biodegradation 5 (1994) 47–54.
[6] B. Tartakovsky, M.F. Manuel, D. Beaumier, C.W. Greer, S.R. Guiot, Enhanced selection of an anaerobic pentachlorophenol-degrading consortium, Biotechnol. Bioeng. 73 (2001) 476–483.
[7] I.S. Thakur, P.K. Verma, K.C. Upadhaya, Involvement of plasmid in degradation of pentachlorophenol by *Pseudomonas* sp. from a chemostat, Biochem. Biophys. Res. Commun. 286 (2001) 109–113.
[8] R.M.M. Abed, N.M.D. Safi, J. Koster, D. de Beer, Y. El-Nahhal, J. Rullkotter, F. Garcia-Pichel, Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of petroleum compounds, Appl. Environ. Microbiol. 68 (2002) 1674–1683.
[9] Y.T. Wang, S. Muthukrishnan, Z. Wang, Reductive dechlorination of chlorophenols in methanogenic cultures, J. Environ. Eng. 124 (3) (1998) 231–238.
[10] A. Vallecillo, P.A. Garcia-Encina, M. Pena, Anaerobic biodegradability and toxicity of chlorophenols, Water Sci. Technol. 40 (1999) 161–168.
[11] B. Tartakovsky, M.J. Levesque, R. Dumortier, R. Beaudet, S.R. Guiot, Biodegradation of pentachlorophenol in a continuous anaerobic reactor augmented with *Desulfitobacterium frappieri* PCP-1, Appl. Environ. Microbiol. 65 (1999) 4357–4362.
[12] G.V.B. Reddy, M.H. Gold, Degradation of pentachlorophenol by *Phanerochaete chrysosporium*: intermediates and reactions involved, Microbiol. 146 (2000) 405–413.
[13] P.M. Radehaus, S.K. Schmidt, Characterization of a novel *Pseudomonas* sp. that mineralize high concentrations of pentachlorophenol, Appl. Environ. Microbiol. 58 (1992) 2879–2885.
[14] S.K. Banerji, R.K. Bajpai, Cometabolism of pentachlorophenol by microbial species, J. Hazar. Mater 39 (1994) 19–31.
[15] P. Jin, S.K. Bhattacharya, Toxicity and biodegradation of chlorophenols in anaerobic propionate enrichment culture, Water Environ. Res. 69 (1997) 938–947.
[16] J.S. Karns, J.J. Killbane, S. Duttagupta, A.M. Chakrabarty, Metabolism of halophenols by 2,3,5-trichlorophenoxyacetic acid-degrading *Pseudomonas cepecia*, Appl. Environ. Microbiol. 46 (1983) 176–1181.
[17] I. Tomasi, I. Artaud, Y. Bertheau, D. Mansuy, Metabolism of polychlorinated phenols by *Pseudomonas cepacia* AC1100: determination of the first two steps and specific inhibitory effect of methimazole, J. Bacteriol. 177 (1995) 307–311.
[18] S.G. Lee, B.D. Yoon, Y.H. Park, H.M. Oh, Isolation of a novel pentachlorophenol-degrading bacterium, *Pseudomonas* sp. Bu34, J. Appl. Microbiol. 85 (1998) 1–8.
[19] China Petrochemical Development Corp. (CPC), Tainan An-Shun Plant Investigation Report, Taipei, Taiwan, 1998.
[20] C.T. Chai, Characterization of PCP-degrading bacteria isolated from a PCP-contaminated site, Master thesis, National Sun Yat-Sen University, Kaohsiung, Taiwan, 2002.
[21] R.U. Edgehill, R.K. Finn, Microbial treatment of soil to remove pentachlorophenol, Appl. Environ. Microbiol. 45 (1983) 1122–1125.
[22] S. Susarla, S. Masunaga, Y. Yonezawa, Reductive dechlorination pathways of chloroorganics under anaerobic conditions, Water Sci. Technol. 34 (1996) 489–494.