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Pentachlorophenol contaminated groundwater bioremediation using immobilized *Sphingomonas* cells inoculation in the bioreactor system

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Abstract

Pentachlorophenol (PCP) has been used as a wood preservative for more than 100 years. The extensive use of PCP has widely contaminated soil and groundwater. PCP is toxic to living organisms. The main objective of this research was to inoculate the pure PCP-degrading bacterium strain *Sphingomonas chlorophenolica* PCP-1, isolated from PCP-contaminated soils, into PCP-contaminated groundwater for remediation purposes. The factors that influenced the bioremediation were explored with batch experiments using the inoculated immobilized and suspended cells as inoculation. A biological treatment system inoculated with immobilized cells was set up to estimate the microbial capability to degrade PCP. The results indicated that the suspended and immobilized cells could be inoculated into PCP-contaminated groundwater without adding other supplementary nitrogen and phosphate sources in batch conditions. Moreover, PCP decomposition was accompanied with released Cl^- and decreasing pH value. The optimum HRT in the bioreactor system was 12.6 h. PCP removal in the bioreactor remained stable and PCP removal efficiency was higher than 92% at this phase. Furthermore, PCP concentration in the biotreatment system effluent remained undetectable. It is possible to bioremediate PCP-contaminated groundwater using immobilized *S. chlorophenolica* PCP-1 cells in a bioreactor system. The proposed biological treatment system could be maintained for at least for 2 months.

Keywords: Biodegradation; Pentachlorophenol (PCP); Immobilized cells; *Sphingomonas chlorophenolica* PCP-1; Sodium-alginate

1. Introduction

The development of human industrial and agricultural activities has lead to the synthesis of new organic compounds known as xenobiotics [13]. Chlorophenolic compounds belong to them. Chlorophenols have been widely used as bactericides, insecticides, herbicides, fungicides, wood preservatives and dye intermediates [9]. These compounds have caused severe environmental problems because of their extensive world wide application. Among the chlorophenols, pentachlorophenol (PCP) is a priority pollutant widely used as a general biocide in commercial wood treatment [8]. PCP is toxic to all forms of life because it is an oxidative phosphorylation inhibitor. Moreover, PCP is recalcitrant to degradation because of its stable aromatic ring system and high chlorine content. It thus persists in the environment [14].

Bioremediation is an option that offers the possibility for destroying or rendering harmless various contaminants using natural biological activity. As such, bioremediation uses relatively low-cost, low-technology techniques that generally have high public acceptance and can often be carried out on site [20]. Generally, the rate and extent of biodegradation are greater in a bioreactor system than in situ or in solid-phase systems because a contained environment is more manageable and hence more controllable and predictable. In addition, microorganism immobilization is effective for many applications in closed, controlled bioreactor systems. The encapsulation procedure is simple, applicable to a range of microorganisms without detrimental effects and a variety of carriers allow choices for various applications [5]. A good approach to solve serious PCP pollution problems in Taiwan is to use indigenous strains isolated from contaminated sites. Our research group isolated a PCP-degrading bacterium in Taiwan, identified as *Sphingomonas chlorophenolica* PCP-1 [22]. Initial studies have shown that this strain has the potential of degrading high PCP concentrations. In this research, strain *S. chlorophenolica*

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PCP-1 was immobilized with alginate and used to remediate PCP-contaminated groundwater by setting up a bioreactor system. Factors that influenced the inoculation were explored first using batch experiments. Then the biological treatment system was set up to estimate the microbial capability to degrade PCP.

2. Materials and methods

2.1. Organism, media and culture conditions

The *S. chlorophenolica* PCP-1 used in all tests was obtained from PCP-contaminated soils in Taiwan [22]. This strain was isolated from an acclimated mixed culture, purified and maintained on R2A agar [16]. Some experimental procedures were performed in media containing the inorganic culture media mentioned by Yang et al. [22].

2.2. Synthesis and physiochemical characteristics of artificial PCP-contaminated groundwater

Because of the geographical and statutory limitations, it was difficult to obtain the original PCP-contaminated groundwater to perform the laboratory research. Therefore, the PCP-contaminated groundwater used in this research was artificial. The artificial PCP-contaminated groundwater was comprised of natural groundwater and PCP, and PCP concentration range was between 120 and 160 mg l^{-1}. Groundwater used in this research was from the aquifer below the authors' institution. PCP was purchased from Sigma (St. Louis, MO, USA), and its purity was higher than 99%. To evaluate whether the natural groundwater was suitable for application, the groundwater parameters were determined such as chemical oxygen demand (COD), ammonia (NH_3-N), chloride (Cl^-), phosphorus (PO_4-P), hardness, pH and iron (Fe).

2.3. Procedure of cell immobilization

The cell immobilization procedure was performed according to Wang et al. [21]. About 4% (w/v) sodium-alginate and 4% (w/v) $CaCl_2$ were prepared and sterilized separately. Different bacterial cell concentrations maintained in inorganic culture media (OD_{600} value between 0.1 and 0.4 units) were mixed with sodium-alginate solution. The volumetric ratio of cell suspension and sodium-alginate solutions was 1:10, respectively. After adequate mixing, the cell–alginate mixture was dropped into a 4% $CaCl_2$ aqueous solution using a peristaltic pump (MP-1000, EYELA, Japan) and sterile silicone tubing fitted to the end of a standard 200-μl yellow tip to form beads. In order to shape and solidify the immobilized beads, the solution was kept steady for at least 5 h. The beads were then washed twice with sterilized water and collected by decantation. After preparing the immobilized cells with various cell concentrations, their ability to degrade PCP was determined using batch reactor experiments.

2.4. Batch reactor experiments

2.4.1. PCP removal with suspended bacterial culture

To achieve PCP removal in the artificial PCP-contaminated groundwater, an experiment was conducted using a series of 125-ml batch reactors. Each reactor contained 40-ml of the natural groundwater with a pure bacteria culture. The initial cell concentration was between 0.1 and 0.4 OD_{600} units. 150 mg l^{-1} PCP was added and the Erlenmeyers flasks were stoppered with cotton. The reactors were shaken at 120 rpm in the dark at 30 °C to observe PCP removal and the chloride release.

2.4.2. PCP removal with the immobilized bacterial culture

To investigate whether the bacterial cells were damaged during the immobilization process, PCP removal using the immobilized cells at various cell concentrations mentioned in Section 2.3 was studied. The protocol was the same as the experiment using suspended bacterial cultures as the inocula, but the different immobilized culture and the inorganic culture media replaced the suspension culture and the natural groundwater, respectively. Moreover, the volumetric ratio of the immobilized cells and the experimental liquid medium was 1:4.

2.5. The semi-continuous batch reactor experiment

PCP degradation efficiency and stability of immobilized cells in the artificial PCP-contaminated groundwater was also measured in semi-continuous batch reactors. The immobilized cells used here were recovered and collected from the batch experiment of immobilized cells so that the stability of immobilized cells degrading PCP could also be realized. Five 1-l flasks were prepared, each with 500 ml of natural groundwater and the appropriate amount of the immobilized beads with or without bacterial cells, and PCP concentration was adjusted to 150 mg l^{-1}. The reactors were then sampled periodically to observe PCP removal and chloride release under aerobic conditions. When PCP was completely consumed, treated groundwater in the flasks was replaced with fresh PCP-contaminated groundwater.

2.6. Setting up the bioreactor treatment system

The reactor and process in this research are illustrated in Fig. 1. There were three parts in the total system, the influent tank, bioreactor and the settling tank. The influent tank volume was 20 l. It was exposed directly in the atmosphere. The total bioreactor volume was 4 l. The working volume was 2.5 l. This reactor was used with an agitation system, pH, oxidation–reduction potential (ORP) and dissolved oxygen (DO) meter (Inpro 3030, Mettler Toledo, Switzerland; P14805-SC-DPAS K8S, Mettler Toledo, Switzerland; Inpro 6900, Mettler Toledo, Switzerland). The agitation system was equipped with an air pump with two gas outlets, pipelines and aeration vents put at the bottom of the reactor by gravity. When air pump pumped air into groundwater, bubbles released from aeration vents increased not only the fluidity of the immobilized cells but also oxygen concentration in the groundwater. The reac-

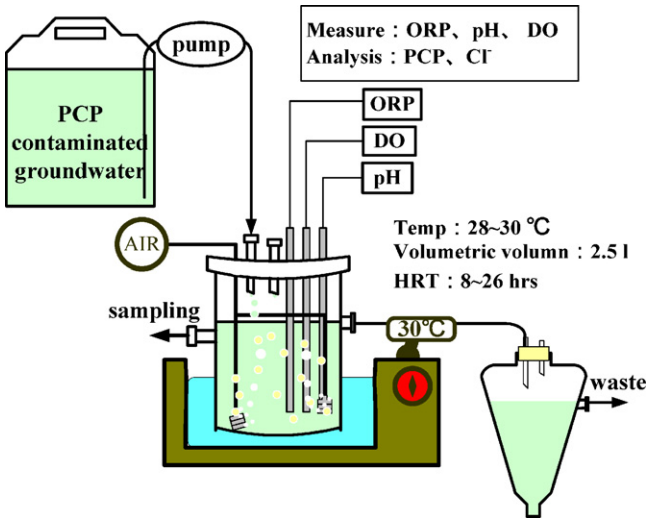

Fig. 1. Schematic diagram of the experimental reactor and process.

tor temperature was maintained at 28–30 °C with a water bath. The artificial PCP-contaminated groundwater stored in the influent tank was pumped into the reactor with a peristaltic pump (MP-1000, EYELA, Japan). The settling tank with an overflow was added after the bioreactor, and the treated groundwater in the settling tank was removed using an overflow process.

2.7. The continuous reactor experiment

For continuous studies, the immobilized cell activity was tested for about 70 days in a 4-l reactor. The appropriate amount of immobilized cells (ca. 500 ml) was placed into the reactor with 2 l inorganic medium and 150 mg l^{-1} PCP. To induce the PCP degrading activity, no influent and effluent were used in this phase. After induction, the artificial PCP-contaminated groundwater was allowed to flow into the reactor. The bioreactor HRT was adjusted between 8 and 26 h to obtain the optimum operating conditions. Daily reactor system monitoring included PCP and chloride concentrations in the influent, reactor and effluent. Furthermore, pH, ORP and DO values were also measured daily.

2.8. Analytical methods

The cell suspensions were clarified by centrifugation at 8000 rpm for 3 min. The PCP concentration in the supernatant was analyzed using high performance liquid chromatography (HPLC, Hitachi L7100 system, Tokyo, Japan) with a UV detector. The analysis condition was the same as the one used in the study of Yang et al. [22], and the PCP detection limit was 0.5 mg l^{-1}. The pH and OD_{600} were measured using a pH meter (PHM82, standard pH meter, Radiometer, Copenhagen, Denmark) and Spectrophotometer (Beckman Du® 530, CA, USA) at 600 nm, respectively. To transfer OD value to more compared value, cell biomass was determined from a standard curve relating OD to dry weight. An OD value of 0.7 represented 0.5 mg (dry weight) per ml for *S. chlorophenolica* PCP-1 cells harvested in the exponential phase of growth. This relationship was linear up to at least an OD value of 0.7. COD was determined using the closed reflux colorimetric method according to the method 508C in the Standard Methods for the Examination of Water and Wastewater [2]. The ammonia concentration was measured using the indophenol blue method [4]. Hardness was estimated using the titrimetric method [4]. Ion chromatography (IC, Dionex 100, Sunnyvale, CA, USA) was used to analyze anion Cl^-. The Fe and phosphate concentrations were determined using the phenanthroline [3] and the ascorbic acid methods [4], respectively.

Table 1
The physiochemical characteristics of the natural groundwater

Item	Data
pH	7.28–7.58
COD	19–26 mg l^{-1}
PO_4-P	0.25–0.56 mg l^{-1}
NH_3-N	Not detectable
Iron	0.02 mg l^{-1}
Hardness	180 mg l^{-1}
Total heterotrophs[a]	7.7×10^2 CFU ml^{-1}

[a] The medium for total hetertrophs was R2A agar medium.

3. Results and discussion

3.1. The physiochemical characteristics of the artificial PCP-contaminated groundwater

Because it was difficult to obtain the original PCP-contaminated groundwater to perform the laboratory research, the PCP-contaminated groundwater used in this research was artificial. The natural groundwater was from the aquifer below the authors' institution and its physiochemical characteristics are listed in Table 1.

3.2. PCP removal by free cells in the artificial PCP-contaminated groundwater

The removal of 160 mg l^{-1} PCP using various amounts of strain *S. chlorophenolica* PCP-1 grown in suspended form in the artificial PCP-contaminated groundwater is shown in Fig. 2. The results indicated that 160 mg l^{-1} PCP was completely degraded within 25 h under different bacterial biomass (dry weight) in the groundwater. Using a higher bacterial concentration in the reactor shortened the PCP degradation periods. Moreover, ca. 110 mg l^{-1} chloride was released by each bacterial concentration within the same period of time, and the amount of chloride ions released fit well with the theoretical values expected from the PCP mineralization. From the results mentioned above, PCP could be degraded in the groundwater without adding supplementary nitrogen and phosphate sources. From Table 1, nitrogen source in the groundwater seemed to be absent because of undetectable NH_4-N (lower than detection limit 0.01 mg l^{-1}). However, when chloride concentration in the groundwater was measured using IC, NO_3^- ion signal was also detected without quantitatively determining (data not shown). In addition, PCP degradation accompanied slight OD value increasing while biomass concentration was 0.14 g l^{-1} (dry weight) (data not

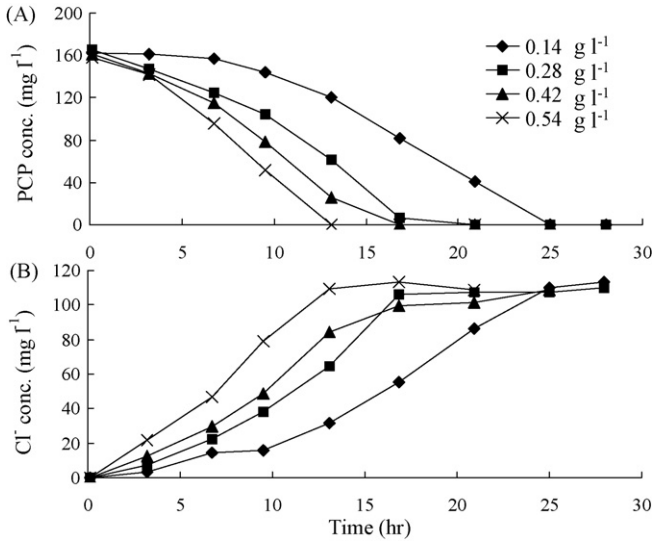

Fig. 2. 160 mg l^{-1} PCP degradation using the suspended cultures in the artificial PCP-contaminated groundwater in the batch reactors.

shown). Therefore, NO_3^- concentration in the groundwater should be enough for supporting PCP degradation and bacterial growth although its concentration was not quantitatively determined. Many *Sphingomonas* spp. can be isolated from oligotrophic environments like the ocean, distilled water, as well as from soil and/or water, and clinical environments [7]. Thus, this might also be the reason that this strain could grow well and maintain its ability to degrade PCP in the artificial PCP-contaminated groundwater.

3.3. PCP removal by immobilized cells in the inorganic culture medium

Fig. 3 shows that 120 mg l^{-1} PCP was degraded using immobilized cells containing various amount of bacterial biomass (dry weight) within alginate in the inorganic medium. The result

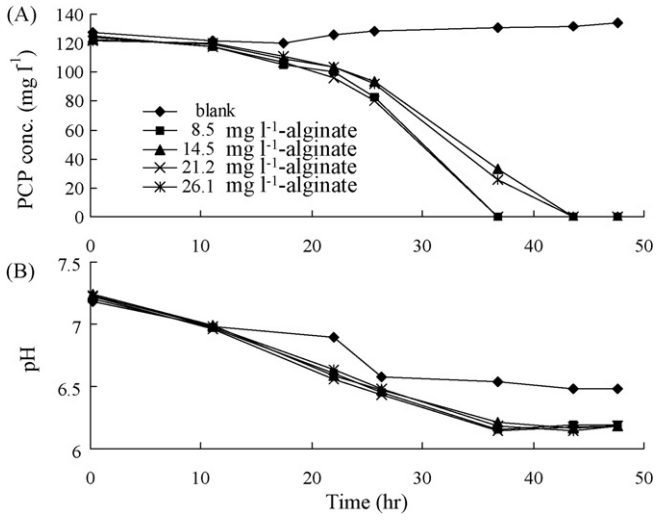

Fig. 3. 120 mg l^{-1} PCP degradation using immobilized cells inoculating various amounts of bacterial cells in the inorganic medium.

revealed that 8.5 and 21.2 mg (cell dry weight) per liter alginate immobilized cells both could remove 120 mg l^{-1} PCP within 36.8 h, and 120 mg l^{-1} PCP was degraded completely using 14.5 and 26.1 mg l^{-1} (cell dry weight) alginate-immobilized cell within 43.6 h. Furthermore, PCP degradation was accompanied with a decrease in the pH caused by dechlorination. Cassidy et al. immobilized strain *Pseudomonas* sp. UG30 with κ-carrageenan. The κ-carrageenan-encapsulated UG30 cells mineralized 100 μg ml^{-1} PCP within 2 days [5]. Comparing both results, PCP degrading rate of the immobilized *Sphingomonas* cells was better than the immobilized *Pseudomonas* cells. Besides, there was no significant PCP concentration variation during alginate bead inoculating, and this was the same as the result mentioned by Hu et al. [11]. Thus, the physical adsorption of PCP was negligible here. However, there was no trend between the immobilized cell amount and the PCP degradation period. The possible reason might be that cell activation in the immobilized beads was limited. The lag phase of each immobilized cell was 10 h. In our previous study using suspended cells as inoculums, there was also a significant lag phase at the initial period. This indicated that cell encapsulation in alginate microbeads did not reduce the lag phase. Stormo and Crawford hypothesized that immobilization might induce shorter lag phase due to protection from PCP toxicity [19]. However, this phenomenon was not observed here. Furthermore, alginate has been more widely used and studied than κ-carrageenan due to the mild encapsulation process conditions [18]. Hu et al. immobilized *Flavobacterium* cells with calcium alginate and polyurethane (PUF), respectively. Their result revealed that immobilization in calcium alginate beads was mild while more cells were inactivated or killed during immobilization in PUF [11]. The lack of loss in PCP degrading activity in this study also confirmed that the immobilization in calcium alginate beads was without deleterious effect.

3.4. PCP removal by the semi-continuous batch reactors

To confirm PCP degradation stability of the immobilized cells in the artificial PCP-contaminated groundwater, PCP removal using the recovered immobilized cells from batch experiment was investigated under semi-continuous conditions. The semi-continuous batch reactor results are shown in Fig. 4. While the PCP-contaminated groundwater replaced inorganic medium, 150 mg l^{-1} PCP was degraded entirely within 14.2 h by each recovered immobilized cells except alginate beads (blank). Strain *S. chlorophenolica* cells could degrade higher PCP concentration without lag phase after preinducing with PCP, and the time needed to degrade the PCP by un-induced cells was two times longer than the time needed to degrade PCP using induced cells [23]. The immobilized cells used in this experiment were recovered from batch experiment so their PCP degrading activities was already induced. Thus, PCP removal efficiency here was much better than that mentioned in Section 3.3. Furthermore, PCP degradation accompanied a decrease in pH because of production of HCl. After refreshing the PCP-contaminated groundwater, 150 mg l^{-1} PCP was also removed within 12.7 h, and the pH decreased from 8.5 to 7.5. The pH range in this

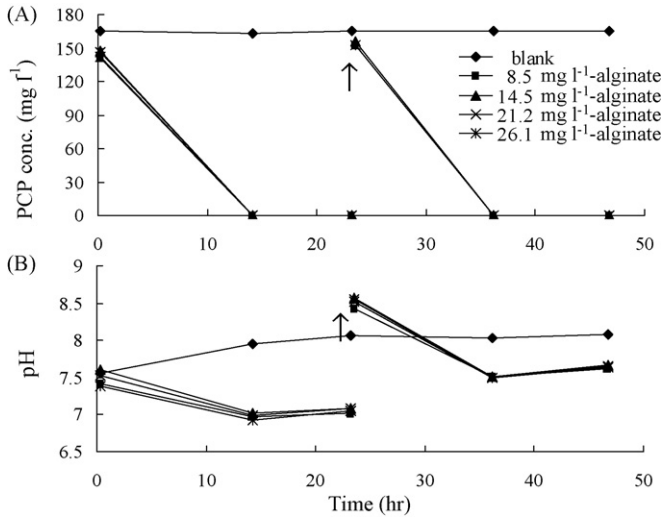

Fig. 4. 150 mg l^{-1} PCP degradation in the semi-continuous batch reactors. The arrow means fresh PCP-contaminated groundwater replacement.

experiment was between 7 and 8.5. In our previous study, the optimum pH for PCP removal by *S. chlorophenolica* PCP-1 was between 6.9 and 7.6. When the initial pH was higher than 8.3, PCP removal rate became lower [22]. In this experiment, PCP removal rate was not influenced significantly by the pH variation. This might be due to the protection from immobilization, and the alginated carrier provided the cells a buffered area against the environmental variation. Adlercreutz et al. immobilized *Gluconobacter oxydans* cells with alginate. Their result indicated that immobilized cells maintained a constant oxidative activity with variations in pH and temperature during glycerol oxidation into dihydroxyacetone, while decreases were observed with free cells [1]. According to the immobilized cells results mentioned above, 8.5 mg l^{-1} (cell dry weight) alginate-immobilized cells was chosen for the next continuous bioreactor experiment.

3.5. PCP removal using the bioreactor treatment system

Running the continuous-culture bioreactor using free cells led to serious wash out. This originated mainly from the low carbon source concentration in the groundwater and the bacterial growth characteristics. The ability of strain *S. chlorophenolica* PCP-1 to compete with other microorganisms was weak so the selected medium for its isolation should not be too nutrient rich [22]. Thus, even adding supplementary glucose as a carbon source to enhance the growth of *S. chlorophenolica* PCP-1 did not help to enhance PCP removal. Rutgers et al. investigated growth yield coefficients of *Sphingomonas* sp. strain P5 on various chlorophenols in chemostat culture. The observed growth yield coefficients on chlorophenols were rather low as compared to growth on non-aromatic compounds. They further inferred that low growth yield may contribute to the recalcitrance of chlorinated aromatic compounds under natural conditions and hamper the competitive behavior of the PCP-degrading population since less energy is available for growth. This may also prevent the population reaching a size sufficient to achieve significant biodegradation rates [17]. Depending on our previous

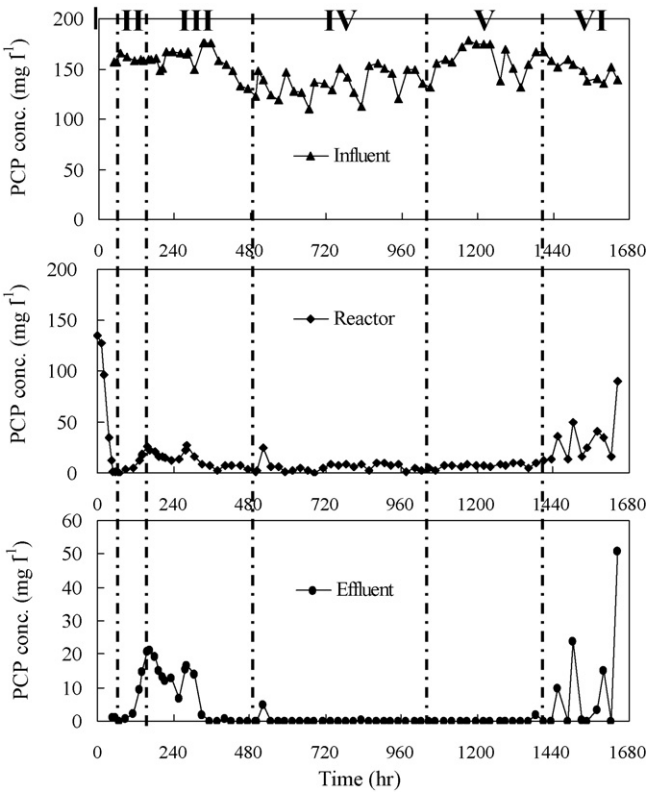

Fig. 5. 150 mg l^{-1} PCP removal using the continuous-culture bioreactor. Phase I: preincubation; phase II: HRT was 25.7 h; phase III: HRT was 20.9 h; phase IV: HRT was 16.4 h; phase V: HRT was 12.6 h; phase VI: HRT was 8 h.

studies and experiences, it was difficult to isolate pure PCP-degrading bacterium from PCP-contaminated site even though the contamination history was quite long. However, if strain *Sphingomonas* cells were immobilized with alginate, the competition problem will be solved and there will be enough PCP degraders staying in the bioreactor to enhance the total degradation efficiency. Thus, the well-maintained PCP degradation and the optimum operated condition of the biotreatment system should be explored after initial research.

Fig. 5 shows the continuous-culture bioreactor result. The goal of phase I was to preincubate the immobilized cells in the bioreactor. About 140 mg l^{-1} of PCP was removed within 44 h. After the pre-incubation phase, the PCP-contaminated groundwater became the influent, and the HRT was 25.7 h (phase II). PCP removal rate could not be well maintained because of the gradual increase in the effluent PCP concentration. According to our previous result, although the time for PCP removal at low PCP concentration was shorter than that at high PCP concentration, PCP removal rate at low concentration was much lower than the removal rate at the high PCP concentration [22]. Thus, to solve this problem, the HRT was shortened to be 20.9 h (phase III). The result indicated that PCP removal rate was not stable at the beginning of phase III, but became quite stable in the later phase III period. At steady state, PCP removal efficiency in the bioreactor was 95.7% at 353 h. PCP removal efficiency remained constant before entering phase IV. The HRT in phase IV was 16.4 h, and PCP removal efficiency was nearly 100% because the PCP concentration in the effluent remained undetectable (below

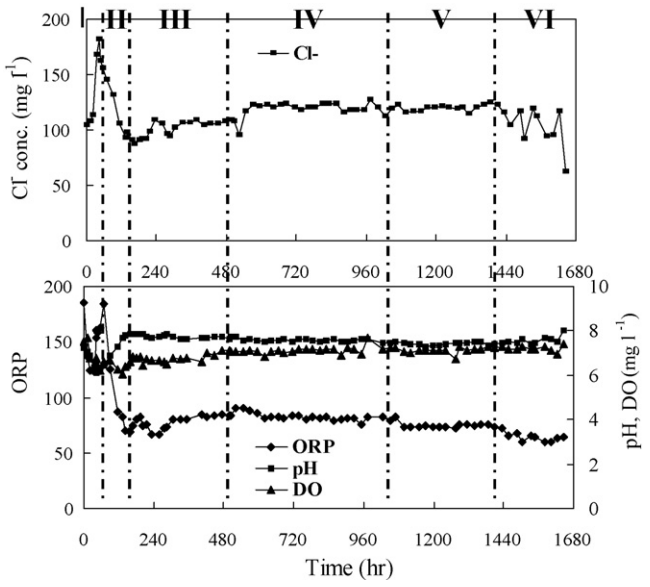

Fig. 6. The profiles of pH, ORP, DO and chloride concentration in the continuous-culture bioreactor. Roman numerals meant different HRT phases.

PCP detection limit 0.5 mg l^{-1}). To realize the limitation of the HRT, the HRTs were further shortened to 12.6 and 8 h, respectively. When the HRT was 12.6 h, PCP removal efficiency in the bioreactor was well maintained. The PCP concentration in the bioreactor was always below 10 mg l^{-1}. Furthermore, the PCP level in the effluent remained undetectable. However, the biotreatment system deteriorated when the HRT was adjusted to 8 h. The loading rate at this HRT (ca. 18.75 mg l^{-1} h^{-1}) might be too high and the immobilized cells could no longer remove the PCP. The PCP concentrations in the bioreactor and effluent tank both had a significant variation. This variable condition was not restored even though the HRT was extended to 12.6 h or even longer (data not shown).

The profiles of pH, ORP, DO and Cl^- concentration in the bioreactor are shown in Fig. 6. Chloride concentration became higher and higher during pre-incubation phase (phase I) because PCP was degraded and there were no influent and effluent. Then, the groundwater as influent was pumped into the bioreactor, and chloride concentration was gradually diluted during phase II even if PCP removal efficiency was not stable. After shortening HRT to 20.9 h, chloride concentration was lower than 100 mg l^{-1} at the beginning but became higher than 100 mg l^{-1} after middle of this phase. During phases IV and V, the average chloride concentration was 120.2 mg l^{-1}, except for the beginning of phase IV. The variation in chloride concentration reflected the variation in the PCP concentration in each phase in the bioreactor comparing Figs. 5 and 6. Chloride variation trends also answered to the PCP variation trends in influent and effluent (data not shown). Besides, ORP value range in bioreactor was between 60 and 90 and pH value range was between 7.3 and 8.03 after phase II. DO value was between 6 and 7.50 after pumping groundwater into the bioreactor.

The optimum HRT should be 12.6 h. During the continuous experiment, the size of the beads of the immobilized cells compared to the condition at the beginning became smaller. However, the function of the immobilized cells was well maintained. This result might originate from the hardness of the groundwater. Most commonly, a cell suspension in a sodium-alginate solution is dropped into a solution of $CaCl_2$. The Ca^{2+} ions cross-link with the alginic acid molecules into a firm polymeric matrix [19]. When the alginate beads make contact with a calcium sequestrant such as phosphate, they will weaken or dissolve [10]. Calcium alginate beads swell and brake easily during operation in stirred reactors and fixed-bed columns [11]. In this study, the phosphate concentration was very low, but the water hardness was relatively high. The groundwater hardness helped to preserve the shape and structure of the immobilized cells during the continuous experiment. Moreover, the native nitrogen concentration (NO_3-N) in the groundwater should be also enough for bioreactor long period operation.

Generally, low microbial growth, poor flocculation and instability problems (containing xenobiotics) can make an activated sludge process very difficult to apply to groundwater treatment. Wastewater contaminated with xenobiotics is usually treated in a fixed film reactor on a plastic support [6]. King applied a fixed film bioreactor to treat a lagoon contaminated with 36 mg l^{-1} PCP, 37 mg l^{-1} polynuclear aromatics (PNAs) and a total COD of 6700 mg l^{-1}. The reason they chose a biological treatment method was that equipment could rapidly be brought to the site and remediation could be completed within several months [12]. The immobilized cells were applied to degrade PCP in this study. The advantages are increase retention of the active biomass in the reactor and better oxygen transfer in the fixed film. The system operated longer if the HRT was maintained constant. O'Relly and Crawford monitored the activity of immobilized *Flavobacterium* cells exposed to 10 mg l^{-1} and 50 mg l^{-1} PCP for a long period. In the 10 mg l^{-1} PCP system, the PCP degradation rate over the first 15 days was 0.43 mg/g-foam day. This rate decreased to 0.06 mg g^{-1} foam day^{-1} for the period between day 136 and day 150. However, no degradation activity was detected in the 50 mg l^{-1} system after 22 days [15]. In our system, the PCP degrading activity could be maintained for at least two months at 150 mg l^{-1} PCP without other supplementary phosphate and nitrogen sources. From these results, it could be concluded that this method has the potential to bioremediate PCP-contaminated groundwater with immobilized *S. chlorophenolica* PCP-1 cells, and the biological treatment system could be maintained for a long period.

4. Conclusions

The purpose of this research was to immobilize strain *S. chlorophenolica* PCP-1 with alginate to investigate the possibility to bioremediate pentachlorophenol contaminated groundwater under batch and continuous-bioreactor conditions. The results can be summarized as follows:

(1) In the batch experiments, the suspended and immobilized cells could be inoculated into the PCP-contaminated groundwater without adding supplementary nitrogen and phosphate sources. When PCP was degraded using sus-

pended cells there was a quantitative increase of chloride in the groundwater.

(2) 120 mg l^{-1} PCP was removed by immobilized cells containing various amounts of bacterial cells in the inorganic medium. Moreover, there was no significant PCP removal when inoculating alginate beads without cells. This result demonstrated that the physical adsorption of PCP was negligible.

(3) The semi-continuous batch reactor result revealed that when the PCP-contaminated groundwater replaced the inorganic medium, 150 mg l^{-1} PCP was completely removed within 14.2 h by each reactor inoculating immobilized cells. After feeding with fresh PCP-contaminated groundwater, 150 mg l^{-1} PCP was also removed within 12.7 h.

(4) The continuous-culture bioreactor result using immobilized cells as inoculum indicated that when the HRT was 25.7 h, PCP removal rate could not maintain well because of the increasing of the PCP concentration in the effluent. After shortening the HRT to be 20.9 h, PCP removal rate was not stable at the beginning, but became quite stable in the later period. At the steady state, PCP removal efficiency in the bioreactor was 95.7% at 353 h and the removal efficiency for PCP maintained constant before entering the next phase. The HRT in the next phase was 16.4 h, and PCP removal efficiency was nearly 100% because the PCP concentration in the effluent was below the limit of detection.

(5) From the above results the proposed biological wastewater treatment system has good potential to bioremediate PCP-contaminated groundwater with immobilized cells. This biological treatment system could be maintained for a long period.

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