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Removal of pentachlorophenol from wastewater by combined anaerobic–aerobic treatment

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Abstract

In this research, the feasibility of combined anaerobic–aerobic biotreatment of pentachlorophenol (PCP) was studied. PCP at 15 mg l^{-1} caused inhibition to both batch and continuous anaerobic systems. However, the recovery time was reduced significantly with higher biomass. With proper acclimation, an acetate-fed anaerobic column could degrade 93% of PCP with influent concentration of 15 mg l^{-1}. With an aerobic process (a conventional activated sludge system) alone, only 74% of 7.5 mg l^{-1} of PCP was removed. Using an activated sludge system as a polishing unit following the upflow anaerobic column up to 97% of 15 mg l^{-1} of PCP was removed.

Keywords: Acclimation; Acetate; Activated sludge; Aerobic; Anaerobic; Pentachlorophenol

1. Introduction and objectives

PCP is a common biocidal and a priority pollutant, widely used as a wood preservative at more than 500 commercial sites in the United States [4,5]. Along with other chlorophenols, PCP has been listed as a priority pollutant by the U.S. EPA (Environmental Protection Agency). It was also registered for use by the EPA as an insecticide, fungicide, herbicide and disinfectant. There is considerable interest in the toxicity and degradation of PCP because of its widespread distribution in contaminated soil, surface water, and groundwater [8,9]. PCP is a highly chlorinated organic compound which is acutely toxic to organisms at low concentrations but can be degraded

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microbially in anaerobic environments [18]. PCP has been detected in surface waters and sediments, rainwater, drinking water, aquatic organisms, soils and food, as well as human milk and urine. PCP releases to surface water occur through direct discharge and direct entry from numerous non-point sources, including treated wood [17].

PCP released into the environment may undergo transformation by natural chemical or biological processes. Biodegradation is a significant process under both aerobic and anaerobic conditions. Volatilization and photolysis do not appear to be important transport and transformation processes for PCP in soils [5]. PCP is stable to hydrolysis and oxidation, but the compound can be metabolized by microorganisms. Adsorption to soils and sediments is more likely to occur under acidic conditions than under neutral or basic conditions [17]. PCP can be degraded by bacteria under aerobic conditions [12,15,1,3]. Treatments of PCP-contaminated wastewater and groundwater have been performed successfully in pilot-scale and field studies by aerobic organisms such as *Flavobacterium* sp. [15,3,1] and *Rhodococcus* sp. [16]. Aerobic PCP biodegradation products include both oxidized residues and less chlorinated derivatives, which may be readily reduced back to PCP under anaerobic conditions [6]. PCP can also be mineralized to methane and CO_2 by anaerobic organisms [11].

Combined anaerobic–aerobic treatment is not new. Some industrial wastewaters such as meat packing and food processing wastes have been treated successfully in anaerobic–aerobic treatment systems [7]. When hazardous materials contain a mixture of hazardous organic compounds, it appears that a combined anaerobic–aerobic process will work better. With a mixture of volatile and non-volatile organics in the wastewater, an aerobic process alone cannot work because the volatile compounds will leave the system and will cause air pollution problems. The closed anaerobic system can not only confine the volatiles, but it can also have a better potential for biodegrading volatile compounds such as chlorinated hydrocarbons. For PCP, which is not very volatile, no information is currently available on such combined treatment. The anaerobic process involves 3 stages, i.e., hydrolysis and fermentation, acidogenesis, and methanogenesis [14]. The acetate utilizing methanogens are generally rate-limiting and are the most vulnerable in the presence of toxicants.

The objectives of this study were to investigate the fate and effects of PCP in batch and continuous anaerobic systems with acetate-utilizing methanogens and to determine the removal of PCP in anaerobic processes alone and in combined anaerobic–aerobic processes.

2. Materials and methods

2.1. Stock cultures

An acetate enrichment culture was used for both the anaerobic toxicity assay and the upflow anaerobic column study. The stock culture was developed by feeding nutrient and acetate as the sole carbon source to an anaerobically digested sludge collected from a municipal wastewater treatment plant. The constituents (in mg l^{-1}) of nutrient solution used for stock cultures and all experimental systems were: NH_4Cl, 1200; $MgCl_2$, 500;

KCl, 400; $Na_2S \cdot 9H_2O$, 300; $CaCl_2 \cdot 2H_2O$, 25; $(NH_4)_2HPO_4$, 80; $FeCl_2 \cdot 4H_2O$, 40; $CoCl_2 \cdot 6H_2O$, 2.5; KI, 2.5; $(NaPO_3)_6$, 10; $MnCl_2 \cdot 2H_2O$, 0.5; NH_4VO_3, 0.5; $ZnCl_2$, 0.5; $Na_2MoO_4 \cdot 2H_2O$, 0.5; H_3BO_3, 0.5; $NiCl_2 \cdot 6H_2O$, 0.5; Cysteine, 10; $NaHCO_3$, 10000. Volatile suspended solids (VSS) concentrations were maintained in the range 700–1000 mg l^{-1} by maintaining a 50 day solids retention time (SRT) in the stock cultures. Stock cultures were maintained in a fed-batch mode in 20 l carboys and were fed 1000–1200 mg COD l^{-1} (propionic acid) twice a week.

2.2. Anaerobic toxicity assay

Sixty-six serum bottles were used (including 9 controls) for the anaerobic toxicity assay (ATA) following the methods described by [13]. The bottles (150 ml) were filled with nitrogen and 45 ml of stock culture was anaerobically transferred to the serum bottles. Three ml of nutrient solution and 2 ml of yeast extract solution (50 g l^{-1}, Sigma Chemical, St. Louis, MO) were added to the serum bottles as supplemental nutrients. The serum bottles were incubated at 35 °C. To study the effect of biomass concentration, different VSS concentrations were used in the serum bottles (Table 1). Triplicate control bottles were run for each of the 3 VSS ranges (i.e., 900–950, 1400–1490, 1870–2050 mg l^{-1}). Reduction in daily total gas production from 50 μl addition of acetic acid

Table 1
Toxic effects and removal of PCP in acetate culture

VSS (mg l^{-1})	PCP spiking conc. (mg l^{-1})	Recovery [a] (days)	Removal [b] (%)	pH
950	1.0	6	78	7.2
1490	1.0	4	80	7.2
2000	1.0	4	83	7.4
910	1.5	7	79	7.3
1480	1.5	5	80	7.2
1870	1.5	4	81	7.5
900	2.0	F [c]	51	7.2
1490	2.0	4	75	7.3
2070	2.0	4	79	7.2
1430	3.0	5	51	7.4
2080	3.0	4	59	7.2
1400	5.0	F	25	7.2
2050	5.0	6	42	7.3
2010	10.0	F	39	7.3
2360	10.0	5	68	7.2
2040	15.0	F	22	7.4
2300	15.0	F	62	7.5
2400	20.0	F	69	7.5
3520	20.0	F	70	7.3

[a] When the daily gas production was within 10% of normal conditions (44 ml day^{-1}).
[b] At the end of the experiment.
[c] F = Failed.
Note: Triplicate control bottles were run for each of these VSS ranges.

(Kron Chemical, Dallas, TX) was used as an indicator of inhibition of the organisms. The gas volume was measured using a manometer filled with a 5% H_2SO_4 solution with sodium chloride, and colored with methyl red indicator for visibility of the liquid level. The bottles were allowed to stabilize to a time when daily gas production varied less than $\pm 10\%$ from the expected gas production for 7 consecutive days. Stoichiometrically (from Eq. (4)), 50 μl of pure acetic acid, at 35 °C, produces 22 ml CO_2 chemically (acid-bicarbonate reaction, Eq. (2)) and 22 ml of methane biologically (methanogenesis reaction, Eq. (3)).

$$CH_3COOH \rightarrow CH_3COO^- + H^+ \quad (1)$$

$$H^+ + HCO_3^- \rightarrow H_2O + CO_2 \quad (2)$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (3)$$

$$CH_3COOH \rightarrow CO_2 + CH_4 \quad (4)$$

Before the serum bottles were spiked with PCP, 3 ml samples of culture were drawn from each bottle for total suspended solids/volatile suspended solids (TSS/VSS) analyses. After stabilizing the bottles, the triplicate serum bottles were spiked with selected concentrations of PCP (Table 1). After the serum bottle study was completed, 2

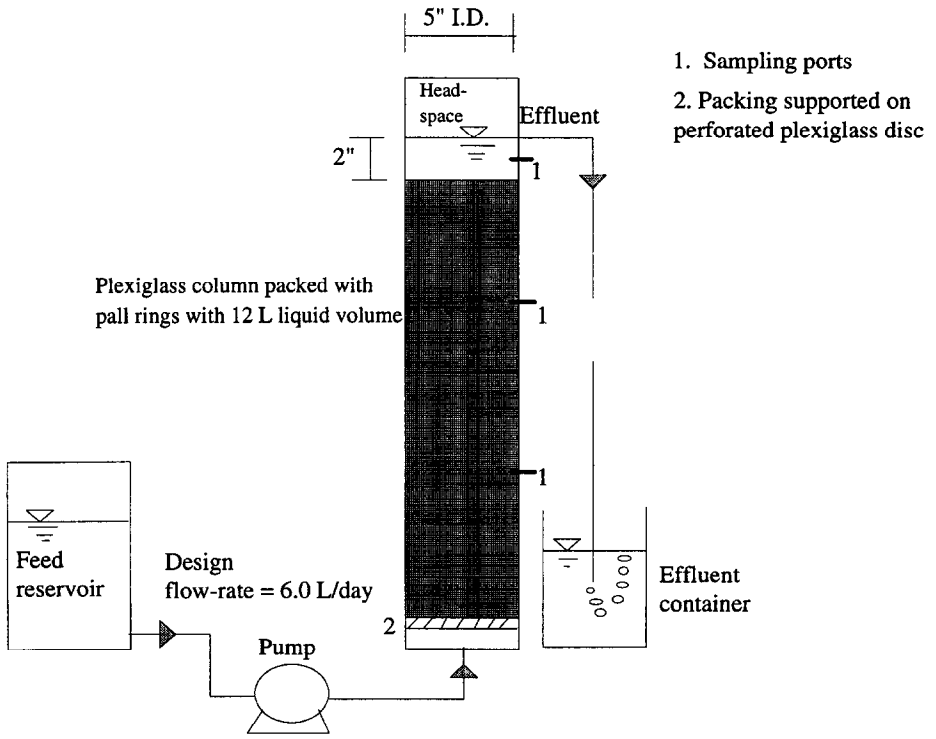

Fig. 1. Schematic diagram of the anaerobic upflow column.

Table 2
Operation schedule of the upflow anaerobic column

Day	Operation
1-37	Column start up, manual batch operation
38-72	Continuous operation
73	Steady state attained
74-91	5.0 mg/L of PCP was spiked
92-103	10.0 mg/L of PCP was spiked
104-128	PCP stopped
129-165	respiking with 7.5 mg/L of PCP
166-172	PCP stopped
173-218	Respiking with 7.5 mg/L of PCP
190-218	Collected effluent for activated sludge
219-263	15.0 mg/L of PCP was spiked
226-255	Collected effluent for activated sludge
264	End of study

10 ml samples of culture were taken from each bottle for TSS/VSS and pH analyses and another 2 ml samples for residual PCP concentration analyses with HPLC.

2.3. Upflow fixed film anaerobic column

A plexiglass column (12 l with 5″ I.D. and 4 ft in height) packed with polypropylene pall rings (Norton Process Services, Baton Rouge, LA) having 90% void space was used to study the fate and effects of PCP on methanogenesis at 35 °C. Fig. 1 shows the schematic view of the column. Acetate enrichment culture (4 l) was anaerobically transferred to the column, yeast extract (200 ml from 50 g l^{-1} stock) and nutrients (2 l) were added, and the column was maintained in a batch mode for 38 days by the manual operation for growing of organisms. Nutrients, acetic acid and yeast extract were added daily. The substrate (acetic acid) utilization rate was monitored daily. When the substrate utilization rate reached 2.0 g l^{-1} day^{-1}, the column was switched to continuous mode and was fed using a pump (Masterflex, model #L-07553-30, Cole-Parmer, Chicago, IL). Acetic acid solution (2000 mg l^{-1}) was pumped into the column from a 15 l plastic container at 6.0 l day^{-1} for a hydraulic retention time of 2 days. Four hundred and fifty ml of nutrient solution and 5 ml yeast extract were also pumped (using a similar pump) into the column once a day. On Day 74, when steady-state was reached, PCP spiking was started. Influent and effluent samples were collected daily for measuring PCP, pH, alkalinity, and volatile acids. Table 2 shows the major events during the 264 day operation.

2.4. Complete-mix aerobic system

This study was conducted with 2 completely mixed aerobic reactors. The plexiglass reactors were 5.5″ in height and 4.5″ in diameter. The volume of each reactor was 1.4 l.

The activated sludge was developed from a seed culture obtained from Jefferson Parish, LA, municipal wastewater treatment plant. Primary effluent (200 mg l^{-1} of COD) collected from the municipal wastewater treatment plant and glucose were used as the feed with a COD of 5000 mg L^{-1} at 200 ml day^{-1}. Two cassette pumps (Manostat, model#72510000, NY) were used to maintain the continuous feed. The hydraulic retention time was 7 days for both reactors.

The 2 reactors attained steady-state after 30 days; there were less than 5% variations in effluent concentrations of COD, total and volatile suspended solids. The effluent from the upflow anaerobic column and glucose (to keep the feed COD at the design level) were used as the influent to the first activated sludge reactor. This was performed between Days 190–218 and 226–255. The second activated sludge reactor was fed primary effluent and glucose and was spiked directly with 7.5 mg l^{-1} of PCP during the same period. Influent and effluent samples were collected daily for measuring COD, TSS/VSS, and PCP.

2.5. Analytical techniques

Acetate concentrations were measured with a gas chromatograph (Shimadzu, GC-14A). The analytical column was 3 mm I.D. × 5 mm O.D. × 1.7 m glass packed with 50 g of 60/80 mesh Carbopack C solid phase washed with Carbowax PEG 20 M/0.1% H_3PO_4 liquid phase (Supelco, Bellefonte, PA). The column temperature was 120 °C and the detector (FID) was maintained at 200 °C. Samples were acidified with formic acid to adjust the pH to less than 3. Total gas production from the serum bottles was measured with an acidified water-filled manometer. Methane was measured by gas chromatography using a Shimadzu GC-8A equipped with a 6′ × 1/8″ stainless-steel column packed with 60/80 Chromosorb 102 and a thermal conductivity detector. The column temperature was maintained at 60 °C and the detector was maintained at 170 °C. A Fisher Scientific Accumet 910 meter was used to measure pH. The chemical oxygen demand (COD) was measured using a Hach Kit (Model #45600) and a spectrophotometer (Spectronic Model #20D) at a wavelength of 620 nm. Biomass was measured gravimetrically as total suspended solids (TSS) and volatile suspended solids (VSS) by Methods 2540 D and 2540 E, respectively, from Standard Methods [2]. Sulfide was measured iodometrically by Method 4500 E from Standard Methods [2]. The total alkalinity (TA) and the bicarbonate alkalinity (BA) were measured by titrating with 0.2 N sulfuric acid to an end point of pH 3.7 and 5.7 following [10]. PCP concentrations were determined using a UV-equipped (254 nm) HPLC (Waters, Milford, MA). A reverse-phase Nova Pak C-18 column (Waters, Milford, MA) was used with 15% acetonitrile, 40% methanol, and 45% deionized water as eluent at a flow rate of 1.0 ml min^{-1}. The detection limit was 100 ppb. The eluent was filtered by using Millex-HV filter units fitted with 0.45 μm Durapore Membrane Filters (Waters, Milford, MA). The samples were prepared by extraction using a Prep Sep C-18 extraction column or Sep Pack C-18 cartridges. The cartridges were activated by passing 1 ml of methanol and 1 ml of deionized water. Two ml of filtered sample were then passed through the cartridge to sorb the organic matter which was then eluted by washing the cartridge with 2 ml of methanol.

3. Results and discussion

Fig. 2 shows the effects of 5.0 mg l^{-1} of PCP on acetate enrichment culture at different VSS concentrations (1400–1490 and 1870–2050 mg l^{-1}). Although a PCP concentration of 5.0 mg l^{-1} caused irreversible inhibition to the methanogens at 1400–1490 mg l^{-1} of VSS, the same PCP concentration caused only reversible inhibition to the methanogens at 1870–2050 mg l^{-1} of VSS. The results indicate a higher capacity to tolerate higher PCP concentrations at higher VSS. Fig. 2 also demonstrates the effects of PCP spiked with concentration of 2.0 mg l^{-1} on an acetate enrichment culture at 3 ranges of VSS concentrations (900–950, 1400–1490 and 1870–2050 mg l^{-1}). The results again showed that higher biomass or VSS can tolerate higher PCP toxicity.

Fig. 3 shows influent and effluent volatile acid concentrations in the column. The PCP concentration of 5.0 mg l^{-1} was first spiked to the column on Day 74 (Table 2). The effluent volatile acid was between 0 and 600 mg l^{-1} as CH_3COOH on the following 18 days. This demonstrates that the column had capacity to withstand 5.0 mg l^{-1} of PCP. On Day 92, the feed PCP concentration was increased to 10.0 mg l^{-1}. The effluent volatile acids were between 250 and 600 mg l^{-1} from Day 93 to Day 99. From Day 100, the effluent volatile acids increased sharply. Both PCP and acetate were stopped from Day 104 to Day 128 and only nutrients were added. On Day 129, when the acetic acid was about 300 mg l^{-1}, continuous spiking with 7.5 mg l^{-1} PCP was

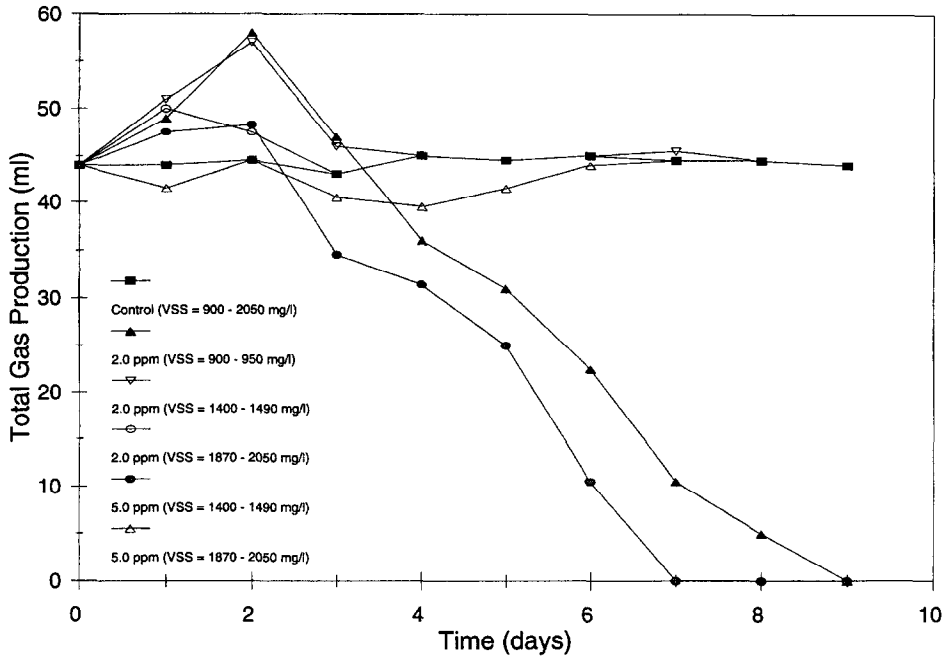

Fig. 2. Effects of PCP on total gas production from acetate.

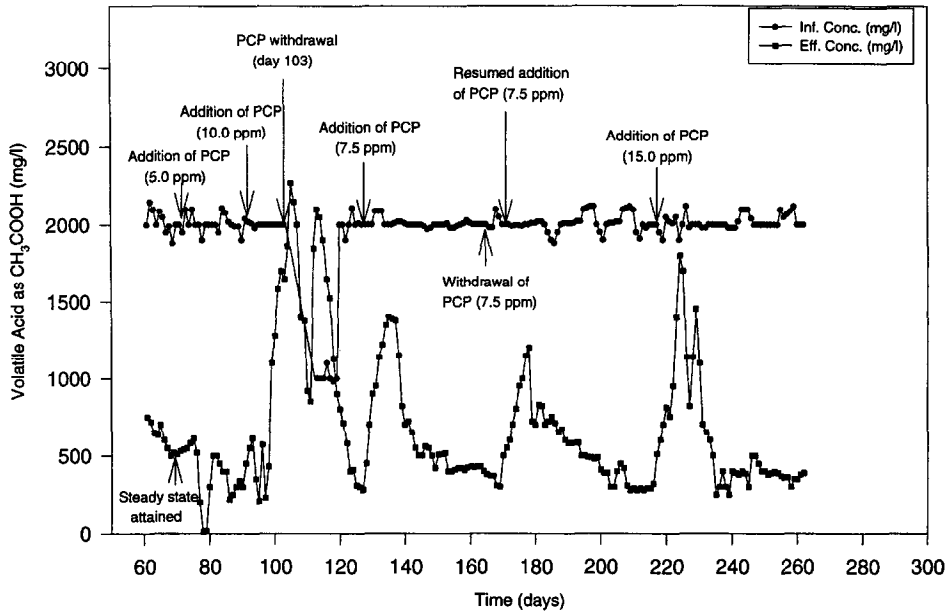

Fig. 3. Effect of PCP on upflow anaerobic column.

resumed. The column effluent volatile acids went up to 1420 mg l^{-1} on Day 136. From Day 137, the effluent volatile acid began to decrease very quickly. Between Days 166 and 172 no PCP was added. When respiked with 7.5 mg l^{-1} of PCP on Day 173, the results showed the effluent volatile acids went up to only 1200 mg l^{-1} indicating bacterial acclimation to PCP. From Day 219, a PCP concentration of 15.0 mg l^{-1} was spiked in the column. As expected, the effluent volatile acids increased and went up to 1850 mg l^{-1} on Day 225. From Day 226, the effluent volatile acids content went down sharply. This was further evidence of bacterial acclimation to PCP. On Day 232, the effluent volatile acids content went down to only 400 mg l^{-1}.

Fig. 4 shows the initial and residual PCP concentration in the column. The effluent PCP concentration increased to 2.0 mg l^{-1} on Day 80 and was less than 2.5 mg l^{-1} until Day 92 when the feed PCP was increased to 10.0 mg l^{-1}. The effluent residual PCP concentration increased sharply and reached 7.3 mg l^{-1} on Day 103 when PCP addition was stopped. With resumption of PCP addition on Day 129, the effluent residual PCP concentration went up to 2.1 mg l^{-1} by Day 136. However, from Day 137, the effluent residual PCP concentration began to decrease rapidly. When PCP was stopped on Day 166, the residual PCP went further down to less than 0.5 mg l^{-1}. After respiking with 7.5 mg l^{-1} PCP on Day 173, the effluent PCP increased to 1.8 mg l^{-1} on Day 180. Comparing the results of the first and the second spiking with 7.5 mg l^{-1} of PCP, it was seen that the effluent residual PCP concentration in the second case was much lower than the one in the first. This is clear evidence of bacterial acclimation to PCP toxicity. From Day 219, a PCP concentration of 15.0 mg l^{-1} was fed to the column. The effluent PCP concentration was only 1.0 mg l^{-1} on Day 250. The low

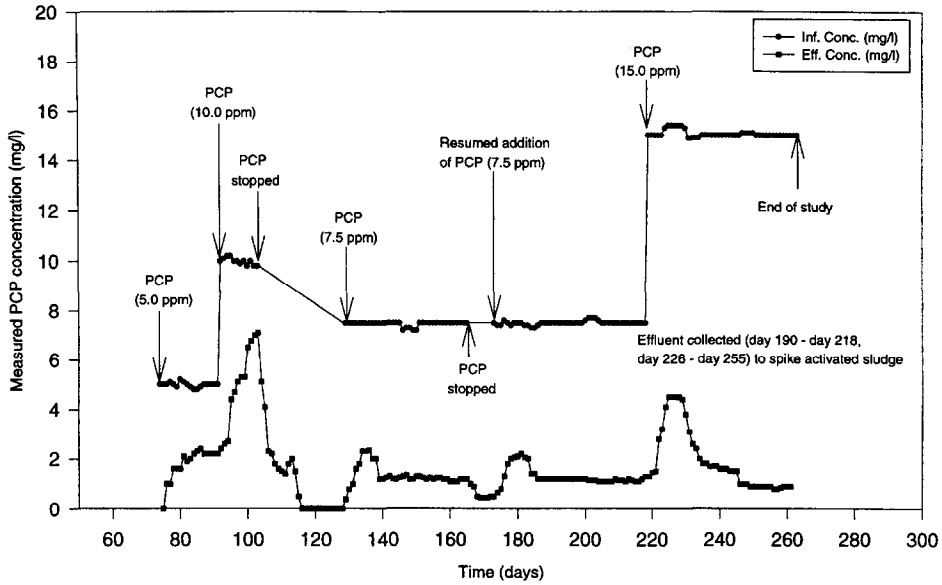

Fig. 4. PCP concentration in upflow anaerobic column.

effluent residual PCP concentration showed that the organisms had developed the capacity to remove PCP. The results also shows that the bacterial acclimation requires time and is facilitated by the gradual increase in PCP concentration. The overall removal

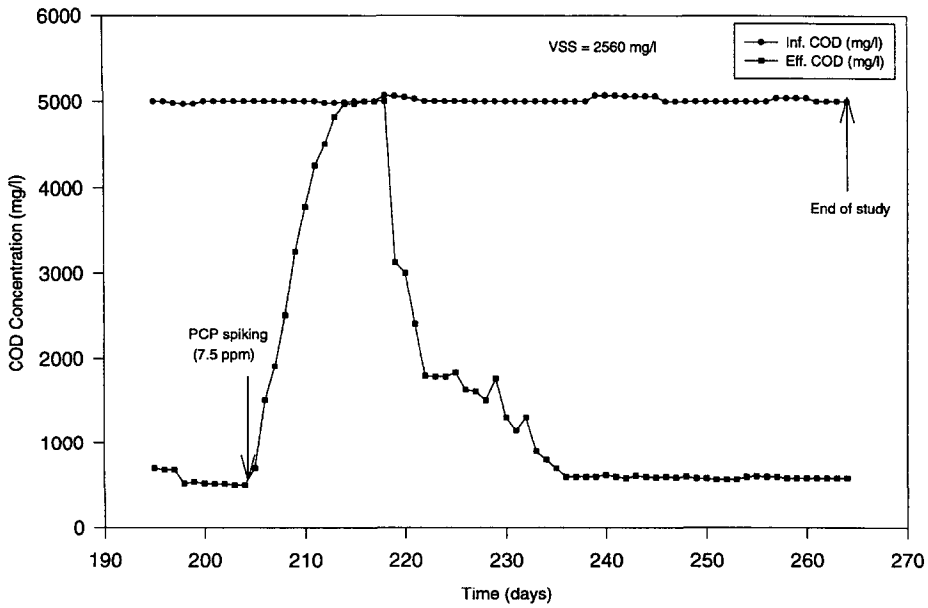

Fig. 5. Effects of PCP spiking with 7.5 ppm in aerobic reactor.

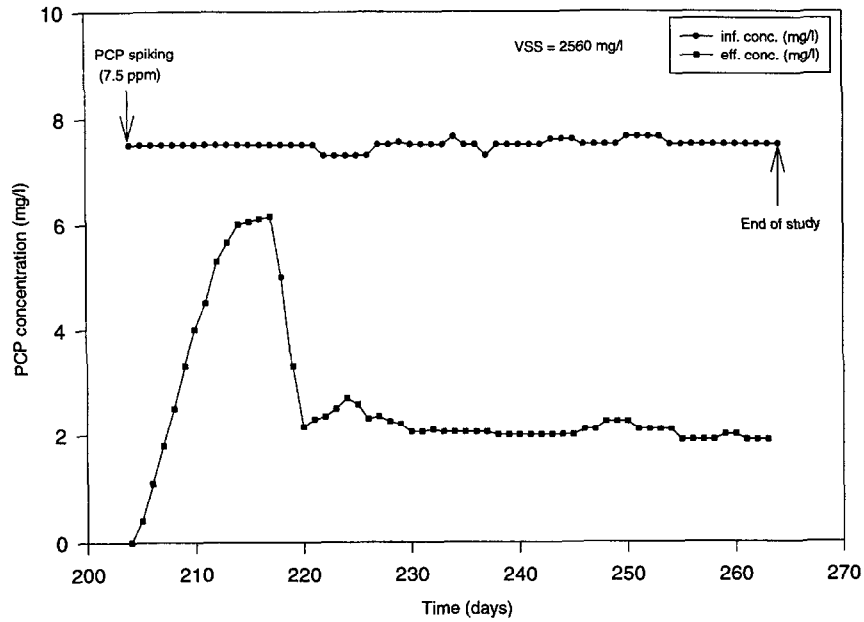

Fig. 6. Measured PCP concentration in acrobic reactor.

of PCP in the upflow anaerobic fixed-film column reached 93%. The effluent PCP concentration was less than 1.0 mg l^{-1} after acclimation (Fig. 4).

Fig. 5 shows the effects of PCP on an activated sludge system. A PCP concentration

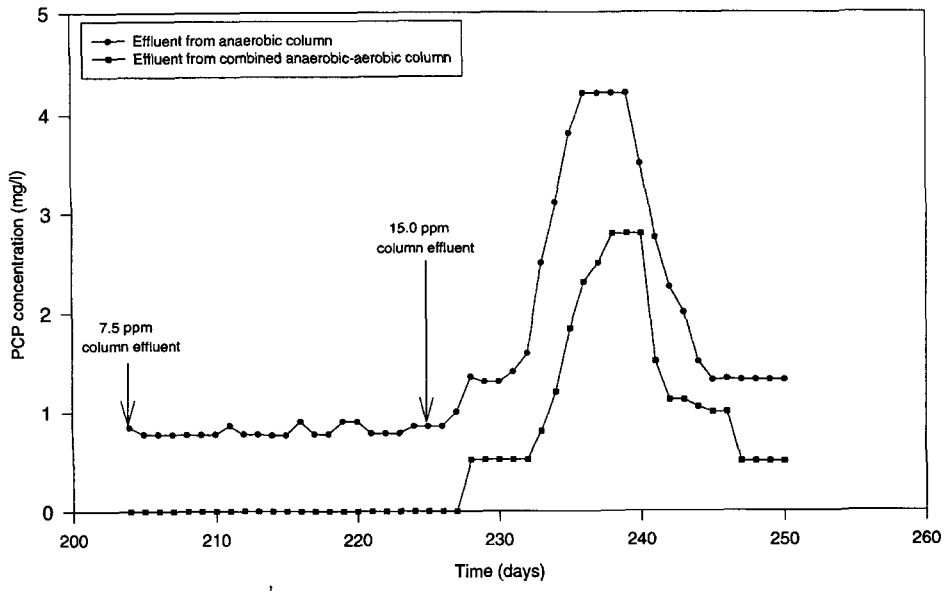

Fig. 7. Removal of PCP in the combined anaerobic–aerobic process column.

of 7.5 mg l^{-1} was directly spiked to the reactor on Day 204. The initial and residual COD concentrations were used for monitoring and controlling the system. At steady-state, the effluent residual COD concentration was 500 mg l^{-1}. On Day 213, the effluent residual COD concentration reached 5000 mg l^{-1} which was equal to the feed COD concentration. However, from Day 218, the effluent residual COD concentration began to decrease and gradually went down to about 500 mg l^{-1}. Fig. 6 shows the effluent PCP concentration went up to 6.5 mg l^{-1} on Day 213. From Day 214, the effluent PCP began to decrease and was 2.1 mg l^{-1} on Day 255. At the end of the study, the residual PCP concentration of 2.0 mg l^{-1} was reached and the total removal of PCP was only about 73%.

Fig. 7 shows the effluent PCP concentration from the upflow anaerobic column and the effluent PCP concentration from the combined anaerobic–aerobic system. The combined anaerobic–aerobic system showed greater removal (about 97%) of PCP than either the upflow anaerobic column (Fig. 4) or the aerobic reactor alone (Fig. 5). This showed the advantage of anaerobic treatment with an activated sludge system as a polishing step.

4. Conclusions

With proper acclimation, up to 15.0 mg l^{-1} of PCP does not cause significant toxicity in an upflow anaerobic column with an acetate enrichment culture. Up to 93% of 15.0 mg l^{-1} of PCP can be removed by the upflow anaerobic column with the acetate enrichment culture. Only 74% of 7.5 mg l^{-1} of PCP was removed by the aerobic system alone. Using the activated sludge system as a polishing unit following the upflow anaerobic column, 15.0 mg l^{-1} of PCP can be removed at 97% efficiency.

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