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Performance of Anaerobic Granules for Degradation of Pentachlorophenol

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Anaerobic granules degrading pentachlorophenol (PCP) with specific PCP removal activity up to 14.6 mg/g of volatile suspended solids per day were developed in a laboratory-scale anaerobic upflow sludge blanket reactor at 28°C, by using a mixture of acetate, propionate, butyrate, and methanol as the carbon source. The reactor was able to treat synthetic wastewater containing 40 to 60 mg of PCP per liter at a volumetric loading rate of up to 90 mg/liter of reactor volume per day, with a hydraulic retention time of 10.8 to 15 h. PCP removal of more than 99% was achieved. Results of adsorption of PCP by granular biomass indicated that the PCP removal by the granules was due to biodegradation rather than adsorption. A radiotracer assay demonstrated that the PCP-degrading granules mineralized [^{14}C]PCP to $^{14}CH_4$ and $^{14}CO_2$. Toxicity test results indicated that syntrophic propionate degraders and acetate-utilizing methanogens were more sensitive to PCP than syntrophic butyrate degraders. The PCP-degrading granules also exhibited a higher tolerance to the inhibition caused by PCP for methane production and degradation of acetate, propionate, and butyrate, compared with anaerobic granules unadapted to PCP.

Pentachlorophenol (PCP) is one of the biocides that was widely used in the United States, mainly for the preservation of wood and wood products. Along with other chlorophenols, PCP has been listed as a priority pollutant by the U.S. Environmental Protection Agency (16). Under aerobic conditions, PCP can be degraded by bacteria (2, 8, 29, 34) and fungi (23, 33). Aerobic organisms such as *Flavobacterium* spp. (8, 34, 37) and *Rhodococcus* spp. (2, 12, 35) have been successfully used in pilot-scale and field studies for the treatment of PCP-contaminated wastewater and groundwater. PCP can also be completely mineralized to methane and CO_2 by anaerobic microorganisms (27). Reductive dechlorination of PCP occurs prior to complete mineralization in digested sludges and soils (3, 24, 26–29, 31, 41). Combined systems of an anaerobic fluidized bed plus trickling-filter or aerated lagoons were used to treat chlorophenolic waste from the paper pulp bleaching process (12, 35). Chlorophenols were removed from the wastewater by 50 to 60%, and mineralization of added PCP to CO_2 was observed by use of radiotracer assay. However, only the overall system removal of chlorophenols was reported, and the role of anaerobic fluidized bed in dechlorination was not clear. PCP removal or dechlorination was reported in semi-continuous-flow, stirred-sewage sludge digestors (11), in a bioreactor which was partially packed with glass beads to treat a mixture of *meta*-, *ortho*-, and *para*-chlorophenols (19), in an anaerobic fixed-film reactor (13), and in upflow anaerobic sludge blanket (UASB) reactors with anaerobic granular sludge (14, 28, 42). In some cases, the dechlorination of PCP was not carried out completely, resulting in the appearance of lesser chlorinated phenols (28, 42). The volumetric PCP loading rates of the anaerobic reactor systems mentioned above were ca. 2.2 mg of PCP per liter of reactor volume per day or less.

We have developed methanogenic PCP-degrading granules on a synthetic wastewater containing PCP, acetate, propionate, butyrate, and methanol in a laboratory-scale UASB reactor at 28°C (5, 6). The maximum PCP removal rate of the granules was as high as 14.6 mg/g of volatile suspended solids (VSS) per day, and a stable volumetric PCP removal rate of 90 mg of PCP per liter per day was achieved. The purpose of this article was to examine the feasibility of the development of methanogenic granules with high dechlorinating activity and to investigate the performance of the granules in treating wastewaters containing high PCP concentrations in a laboratory-scale UASB reactor system.

MATERIALS AND METHODS

Chemicals and gases. All chemicals (analytical grade) except PCP were obtained from Sigma Chemical Co. (St. Louis, Mo.); PCP was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). Nitrogen gas and gas mixtures of N_2-CO_2 (95:5 and 70:30) were obtained from Linde Division, Union Carbide Corp. (Warren, Mich.), and passed over heated (370°C) copper filings to remove traces of O_2.

Analytical methods. Methane, methanol, and volatile fatty acids (VFA) were determined by using gas chromatography as described elsewhere (43, 44). PCP in solution was determined with high-performance liquid chromatography (HPLC). Samples (1.0 ml) were mixed with 0.5 ml of acetonitrile on a vortex mixer, centrifuged for 5 min at 5,500 × *g* with an Eppendorf 5415 centrifuge (Brinkmann Instruments, Inc., Westbury, N.Y.), and filtered through 0.45-μm-pore-size syringe filters (Acrodisc LC13; Gelman Sciences Co., Ann Arbor, Mich.). A Waters HPLC system, consisting of the model 501 pump, the Lambda-Max model 481 UV detector, and the model 740 data module, was used. Samples were injected by using a Rheodyne 7010 injector fitted with a 50-μl loop. Separation was accomplished with a Waters Radial-Pak C-18 column. The mobile phase consisted of acetonitrile and 5% aqueous acetic acid (8:2, vol/vol). The flow rate of

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the mobile phase was 1.2 ml/min. PCP was measured at $A_{300.5}$. The biomass was estimated by determining suspended solids (SS) or VSS as described in reference 1. The specific gravity of granules was determined as described in reference 1. The size distribution of granules was measured by photographic methods (39).

Toxicity test. Serum vials (158 ml in volume; Wheaton Scientific, Millville, N.J.) and pressure tubes (27 ml; Bellco Glass, Inc., Vineland, N.J.) with butyl rubber stoppers (Bellco Biotechnology, Vineland, N.J.) were used to test the toxicity of PCP to the granules. Medium and stock solutions were prepared anaerobically under an N_2 gas atmosphere, as described previously (15). The basal medium for the toxicity assay contained the following components (per liter): NaCl, 0.5 g; NH_4Cl, 0.5 g; $NaHCO_3$, 9 g; K_2HPO_4, 1.45 g; KH_2PO_4, 0.75 g; $MgCl_2 \cdot 6H_2O$, 0.20 g; $CaCl_2 \cdot 2H_2O$, 0.10 g; resazurin, 0.001 g; and trace element solution, 10 ml (15, 17). The tubes or vials were pressurized with a gas mixture of N_2-CO_2 (70%:30%) to 1 atm (1 atm = 101.29 kPa) of gauge pressure prior to the test. An $Na_2S \cdot 9H_2O$ solution (2.5%, wt/vol) was used as the reductant (0.04 ml/10 ml of medium). The final pH was 7.2 to 7.3.

A two-step test was used for the determination of toxicity. A methane production test, similar to the method of Owen et al. (32), was used to estimate the overall inhibition of methane production. A fatty acid degradation test was conducted to identify the effect of PCP on individual microbial trophic groups.

Methane production tests were performed with 27-ml pressure tubes containing 10 ml of medium. The initial concentrations in the test medium were approximately 5 mM acetate, 5 mM propionate, and 5 mM butyrate. The tubes were prewarmed in a 35°C shaking water bath and then inoculated with 0.3 ml of granules (which contained 50 g of SS per liter) by using a 1-ml glass syringe with an 18-gauge needle. Prior to addition to the tubes, the granules were homogeneously disrupted by passing them through a syringe with a 23-gauge needle. One hour after inoculation, gas samples were taken to obtain starting values. Methane production was subsequently monitored every 12 h for 5 days. The final biomass concentration was assayed at the conclusion of the test.

A fatty acid degradation test was done in 158-ml serum vials containing 50 ml of the medium. VFA were added to the basal medium to achieve starting concentrations of 4 to 8 mM acetate, 8 mM propionate, and 8 mM butyrate. Granules (0.5 ml per sample) directly withdrawn from the reactor were used as the inocula in the serum vials. After incubation in a shaking water bath (35°C), liquid samples and gas samples were taken every 4 to 8 h during the initial 2 days and every 8 to 24 h thereafter until the methane production stopped. The biomass concentration was determined at the conclusion of the test and used for specific activity calculations. The increase of biomass during the test was insignificant and, thus, ignored. The specific degradation rates (dS/dt, in millimoles per gram of SS per day) can be easily calculated. For propionate, the calculation is:

$$dS/dt_p = (S_{p1} - S_{p2}) \cdot V/[(t_2 - t_1) \cdot X] \quad (1)$$

where V is the volume of medium (in liters), S_{p1} and S_{p2} are propionate concentrations (in millimoles per liter) in the medium at times t_1 and t_2, respectively, and X is the biomass (in grams of SS). The specific butyrate degradation rate (dS/dt_b) is:

$$dS/dt_b = (S_{b1} - S_{b2} + S_{ib1} - S_{ib2}) \cdot V/[(t_2 - t_1) \cdot X] \quad (2)$$

where S_{b1}, S_{b2}, S_{ib1}, and S_{ib2} are the butyrate (b) and isobutyrate (ib) concentrations (in millimoles per liter) at times t_1 and t_2, respectively. This equation takes into account the reversible isomerization between butyrate and isobutyrate that occurs (40, 46). In addition to the isomerization, valerate and 2-methylbutyrate were formed from butyrate and propionate during the fatty acid degradation (43). These VFA were, however, produced at very low levels and not considered in the calculation of the rates for propionate and butyrate. The degradation rate of acetate (dS/dt_a) is calculated as follows:

$$dS/dt_a = (S_{a1} - S_{a2}) \cdot V/[(t_2 - t_1) \cdot X] + dS/dt_p + 2 \cdot dS/dt_b \quad (3)$$

where S_{a1} and S_{a2} are the acetate concentrations (in millimoles per liter). In equation 3, the acetate sources consist of acetate in the medium and acetate produced during syntrophic propionate and butyrate conversions as follows (7, 25, 36):

$$\text{Propionate}^- + 3H_2O \rightarrow \text{acetate}^- + HCO_3^- + 3H_2 + H^+ \quad (4)$$

$$\text{Butyrate}^- + 2H_2O \rightarrow 2\ \text{acetate}^- + 2H_2 + H^+ \quad (5)$$

Sources of granules and inoculum. VFA-degrading granules developed on a VFA mixture consisting of acetate, propionate, and butyrate (39) were used as the core biocatalyst for the development of PCP-degrading granules and a radiotracer assay. Anaerobic mixed-culture enrichments developed from the samples collected from contaminated sites and sludges and enriched with PCP were also used as inocula (4, 5). Brewery waste-degrading granules obtained from a full-scale UASB reactor treating brewery wastewater (G. Heileman Brewery Co., LaCrosse, Wis.) were used as a control for the radiotracer assay.

Adsorption assay. The adsorption of PCP to granule biomass was tested in anaerobic pressure tubes. Each tube contained 10 ml of the basal medium which was supplemented with potassium phosphate (20 mM), $NaHCO_3$ (20 mM), sodium acetate (5 mM), sodium propionate (2.5 mM), and sodium butyrate (2.5 mM). A sodium PCP stock solution was added anaerobically to the tubes to achieve initial PCP concentrations of 2.9, 5.2, 9.4, 13.5, and 20.2 mg/liter, respectively. These tubes were divided into three groups. In the tubes of the first group, the medium was reduced by sodium sulfide (0.5 mM) and then inoculated with 0.5 ml of the anaerobic granules by using a syringe with a 23-gauge needle. The medium of the second group was also reduced by sodium sulfide. The medium in the tubes of the third group was not reduced. The tubes of both the second and the third groups were not inoculated with granules. Liquid samples were withdrawn from each tube of the second and the third groups to determine the initial PCP concentration. All of the tubes were incubated in a shaking water bath (30°C) at 100 strokes per min for 14 h to obtain equilibrium of adsorption. Afterwards, the tubes were opened. The PCP concentrations in the tubes of the second and the third groups were determined to check if any loss of PCP occurred during the incubation period. For the first group, the liquid was separated from the biomass by centrifugation and then used for the determination of PCP concentrations. The biomass pellet of each tube was resuspended in 1.4 ml of the

TABLE 1. Basal composition of media used for laboratory-scale UASB reactor tests

Medium	Methanol (mM)	Acetic acid (mM)	Propionic acid (mM)	Butyric acid (mM)	COD[a] (g/liter)	KH_2PO_4 (mM)	Na_2SO_4 (mM)	$FeSO_4$ (mM)	NH_4Cl (mM)	NaOH (mM)	NaCl (g/liter)	$MgCl_2$ (g/liter)	$CaCl_2$ (g/liter)	Resazurin (g/liter)	Trace element[b] (ml/liter)
A	33	50	25	25	10	3	0.1	0.05	6.6	50	0.5	0.2	0.1	0.001	0.5
B	16.5	25	12.5	12.5	5	3	0.1	0.05	6.6	25	0.5	0.2	0.1	0.001	0.5
C	12.5	5.0	7.5	12.5	2.6	3	0.1	0.05	1.32	14	0.5	0.2	0.1	0.0005	0.25
D	10	5.0	2.5	2.5	1.48	3	0.1	0.05	1.32	8	0.25	0.2	0.1	0.0005	0.2
E	8	4	2	2	1.2	3	0.1	0.05	1.32	8	0.2	0.2	0.1	0.0005	0.2

[a] COD was calculated on the basis of stoichiometry of full oxidation by oxygen to CO_2. The ratios (in grams of COD per moles) are 48, 64, 112, and 160 for methanol, acetate, propionate, and butyrate, respectively. The COD contributed by PCP was not incorporated.
[b] Trace element solution (15).

basal medium and incubated at 30°C for an additional 6 h to release the sorbed PCP. After the second incubation, the liquid was separated by centrifugation and used for the determination of PCP. Finally, the wet weight of biomass in each tube was determined. Experimental results indicated that the PCP concentration in the tubes from the second and third groups did not change after incubation.

The adsorption and desorption patterns were characterized by the Freundlich isotherm:

$$X/M = KC_e^{1/n} \quad (6)$$

where X is the mass of PCP sorbed on biomass at equilibrium (in milligrams of PCP), M is the biomass used (in grams of SS), C_e is the PCP concentration in liquid phase at equilibrium (in milligrams of PCP per liter), K is a Freundlich constant (in milligrams per gram), and $1/n$ is a Freundlich constant (nondimensional). The constants K and $1/n$ were determined by using nonlinear least-squares analysis.

Reactor feed composition. The media composition for the reactor is shown in Table 1. The concentrations of chemicals added to the medium were changed during the experimental period. The carbon sources used were methanol, acetic acid, propionic acid, and butyric acid. The PCP concentration varied depending on the experimental requirement. Medium A was used for the start-up of the reactor. Media B, C, D, and E were used to test the operational performance of the reactor.

Laboratory-scale UASB reactor. A water-jacketed glass column reactor (2.1-cm diameter, 28-cm length) was used. The reactor volume was 100 ml, and the settler volume was 225 ml. The total reactor system volume was 325 ml. All parts were either of glass (column, settler), stainless steel, or butyl rubber. A schematic diagram of the reactor system is illustrated in Fig. 1. The reactor was started with medium A, which contained 50 mM acetate, 25 mM propionate, and 25 mM butyrate (representing 10 g of chemical oxygen demand [COD] per liter), and inoculated with VFA-degrading granules (19 ml) and a PCP-degrading enrichment (12 ml). The reactor was operated at 28°C with continuous feed supplied by a minipulse peristaltic pump (Gilson Medical Electronics, Middleton, Wis.). The recycle of treated effluent from the top settler to the reactor inlet (recycle flow, 1 liter/h) was accomplished by using another peristaltic pump. Gas samples for methane and hydrogen analysis were withdrawn from the gas sampling port (Fig. 1). Liquid samples for VFA analysis were withdrawn from the sampling ports on the reactor. Gas production was measured by liquid displacement of an HCl solution (0.1 N) in an inverted graduated cylinder. The specific growth rate of the granule bed was estimated on the basis of the increase in granule bed volume:

$$G = (V_t - V_0)/[0.5 \cdot (V_t + V_0) \cdot t] \quad (7)$$

where G is the specific granule bed growth rate (in milliliters per milliliter per day), V_0 is the initial volume of the granule bed (in milliliters), and V_t is the volume of the granule bed (in milliliters) after t days of operation.

Radiotracer assay. Radiotracer experiments were performed in anaerobic serum vials (16 ml in volume) containing 3 ml of the basal medium (pH 7.0) and a VFA mixture (3.0 mM acetate, 1.5 mM propionate, and 1.5 mM butyrate). The headspace of the vials was pressurized with an N_2-CO_2 (70:30) gas mixture. A mixture of PCP and [^{14}C]PCP solution was used to achieve an initial PCP concentration of 1.5 mg/liter and a radioactivity of 2.25×10^5 dpm per vial. Granules were disrupted in the basal medium, which was reduced by 0.4 mM of sodium sulfide, and then used as the inoculum (2 ml per vial). Four replicates were used. After inoculation, the vials were incubated at 30°C.

The mineralization of PCP was determined by monitoring

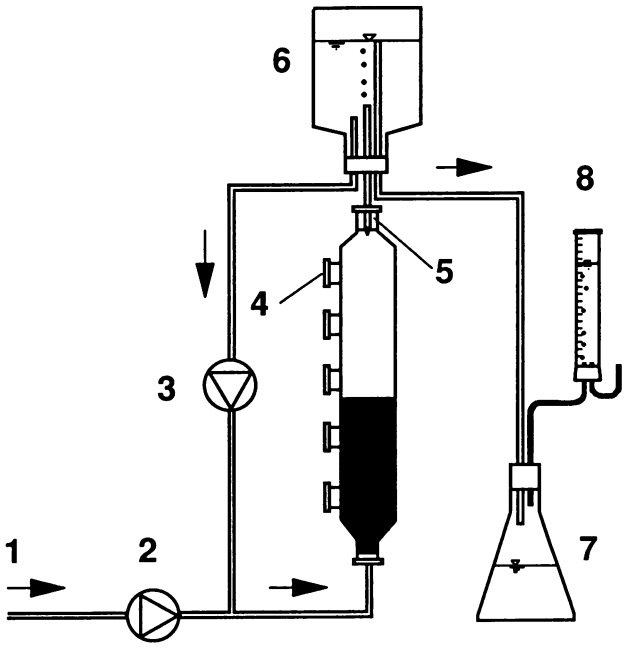

FIG. 1. Schematic diagram of laboratory-scale UASB reactor system. The lower darkened portion of the reactor represents granules. 1, Medium reservoir; 2, feed pump; 3, recycle pump; 4, sampling port for PCP and VFA; 5, sampling port for gas analysis; 6, settler; 7, effluent collecting flask; 8, gas volume measurement.

TABLE 2. Effect of PCP on methane production from a VFA mixture by the VFA- and PCP-degrading granules

Granules[a]	Relative methane production[b] at PCP concn of:			
	2.5[c]	5.0	10	20
VFA degrading	0.43	0.13	0.038	0.016
PCP degrading	0.93	0.27	0.21	0.12

[a] The amount of the granules inoculated was approximately 1.0 g of SS per liter.
[b] Relative methane production was calculated on the basis of the average methane produced after 5 days in control tubes being equal to 1.0.
[c] PCP concentrations expressed in milligrams per liter.

TABLE 3. Effect of PCP on the VFA degradation rates by the VFA- and PCP-degrading granules

Granules	PCP concn (mg/liter)	Relative activity[a]		
		Acetate	Propionate	Butyrate
VFA degrading	0	1.0	1.0	1.0
	1.0	0.69	0.087	0.50
	2.5	0	0	0.33
PCP degrading	0	1.0	1.0	1.0
	1.0	0.73	0.42	0.72
	2.5	0.42	0.064	0.36

[a] Relative activity was calculated on the basis of the maximum specific fatty acid degradation rates determined in controls (no addition of PCP). Before adaptation to PCP, the degradation rates were 35.2, 5.6, and 24.3 mmol/g of SS per day for acetate, propionate, and butyrate, respectively. After the adaptation, the rates were 33.1, 5.9, 17 mmol/g of SS per day for acetate, propionate, and butyrate, respectively.

the production of $^{14}CH_4$ and $^{14}CO_2$. $^{14}CH_4$ and $^{14}CO_2$ were quantified by the method of Nelson and Zeikus (30) by using a gas proportional counter (model 894; Packard Instruments, Downers Grove, Ill.) coupled with a series 580 TCD gas chromatography (GOW-MAC Instrument Co., Bridgewater, N.J.) equipped with a thermal conductivity detector at a column temperature of 100°C. Helium was used as the carrier gas (flow rate, 50 ml/min). The test was continued until the $^{14}CH_4$ and $^{14}CO_2$ concentrations in the headspace stopped increasing. The aqueous fraction in the vials was then separated by centrifugation at 10,000 × *g* for 10 min. The radioactivity of the aqueous fraction was determined by using a liquid scintillation counter (model LS 3801; Beckman Instruments Inc., Fullerton, Calif.) with external stand

RESULTS

Toxicity of PCP to VFA degradation by granules. The VFA-degrading granules were tested prior to using them as an inoculum to develop PCP-degrading granules. The PCP-degrading granules were tested for toxicity after receiving PCP-containing feed for over 200 days. The inhibition levels of the PCP to methane production by VFA- and PCP-degrading granules were determined by using the methane production test. The initial concentrations of PCP used were 2.5, 5.0, 10, and 20 mg/liter. The methane produced in 5 days was used to estimate activity and inhibition (Table 2). The methane production in each test tube was normalized as relative methane production by using 1.0 as the average methane production in control tubes. On the basis of these results, the inhibition of PCP increased as the PCP concentration increased. The PCP-degrading granules that had been acclimated to the presence of PCP had a much higher tolerance to PCP. A PCP concentration of 2.5 mg/liter was significantly inhibitory to VFA-degrading granules but not to the PCP-degrading granules.

Fatty acid degradation tests provide insight into the inhibition of PCP to syntrophic-propionate degraders, syntrophic butyrate degraders, and acetate-utilizing methanogens, the major VFA-degrading trophic groups observed in anaerobic granules. PCP concentrations of 1.0 and 2.5 mg/liter were utilized. The effects of PCP on the initial degradation rates of acetate, propionate, and butyrate by the VFA- and PCP-degrading granules are summarized in Table 3. Methane production by VFA-degrading granules was inhibited at 1.0 mg or more of PCP per liter (Fig. 2A), while methane production by PCP-degrading granules was not inhibited at 1.0 mg/liter and only slightly inhibited at 2.5 mg/liter (Fig. 2E). The inhibition of degradation of the three fatty acids differed significantly. The propionate degraders were the most sensitive to the addition of PCP in both types of granules, and acetate-utilizing methanogens were the next most sensitive (Fig. 2 and Table 3). At 1.0 mg of PCP per liter, propionate degradation by VFA-degrading granules was inhibited immediately and almost completely, whereas propionate degradation by PCP-degrading granules was initially slightly inhibited but recovered over time. The addition of 1.0 mg of PCP per liter partially inhibited acetate degradation rates for both VFA- and PCP-degrading granules (Table 3). In general, PCP-degrading granules exhibited a higher tolerance to PCP inhibition than VFA-degrading granules.

Development of PCP-degrading granules in UASB reactor. Operational results during the first 200 days are illustrated in Fig. 3. The COD loading rate was initially low (Fig. 4A) and then increased to 15 g/liter/day by day 25. Afterwards, the loading rate was maintained between 8 and 15 g/liter/day because the objective of this study was to obtain a high PCP removal rate rather than a high COD loading rate.

The concentration of PCP in the influent, the PCP loading rate, and the PCP concentration inside the reactor as well as PCP removal are summarized in Table 4. Initially, the feed did not contain PCP. After 5 days, PCP at 0.5 m/liter was supplied in the feed. The PCP loading rate was increased gradually to a higher level (36 mg/day per reactor). The PCP concentration in the influent was increased to 60 mg/liter (Fig. 3B and Table 4). During this period, PCP removal was greater than 98%. Higher effluent PCP concentrations (0.5 to 1.0 mg/liter) were observed when the influent PCP concentration was increased from 30 to 60 mg/liter. The effluent PCP concentration then decreased. At the end of this operational period, the PCP concentration in the reactor was observed to be 0.1 to 0.2 mg/liter.

Methanol was detected in the effluent only during the initial 3 days after start-up. Acetate (1 to 5 mM), propionate (1 to 4 mM), and butyrate (0.05 to 0.5 mM) were observed throughout the operational period (Fig. 3C). During the first 50 days of the operation, the propionate degraders in the reactor appeared to be sensitive to any increase in the influent PCP concentration and the PCP loading rate. After this time, increases in PCP loading rate did not appear to affect propionate degradation. This is consistent with the results from toxicity assays performed with the PCP-degrading granules.

After start-up, the granule bed in the reactor grew continuously (Fig. 3D). Initially, a part of inoculated enrichment (floc-like sludge) was washed from the column reactor into the settler. This sludge was recycled back to the reactor once

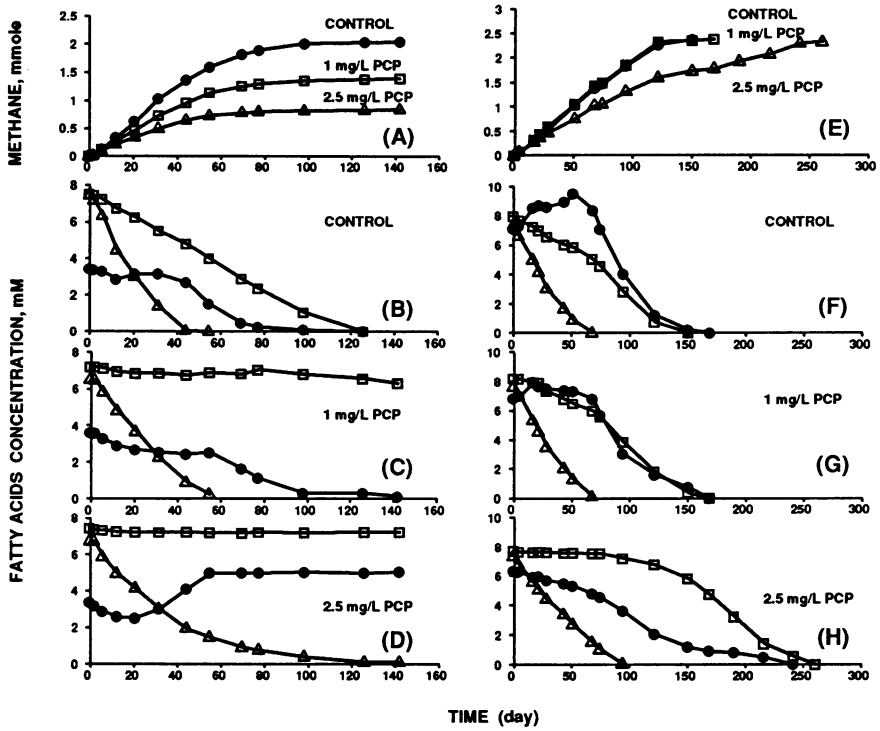

FIG. 2. The effect of PCP concentration on VFA degradation by VFA-degrading granules (A, B, C, and D) and PCP-degrading granules (E, F, G, and H). (A and E) methane production; (B and F) VFA degradation in the absence of PCP; (C and G) VFA degradation in the presence of 1 mg of PCP per liter; (D and H) VFA degradation in the presence of 2.5 mg of PCP per liter. Symbols in B to D and F to H: ●, acetate; □, propionate; △, butyrate.

per day for 10 days. A rapid specific growth rate of granule bed (0.046 ml/ml/day) was observed during the first 20 days. Subsequently, the volume of the granule bed stabilized at 60 to 70 ml (Fig. 3D). Excess granules were continuously washed from the granule bed to the settler. The granules and flocs that settled in the settler were removed periodically from the reactor system. The PCP-degrading capability of the granules was identified by comparison of the adsorption

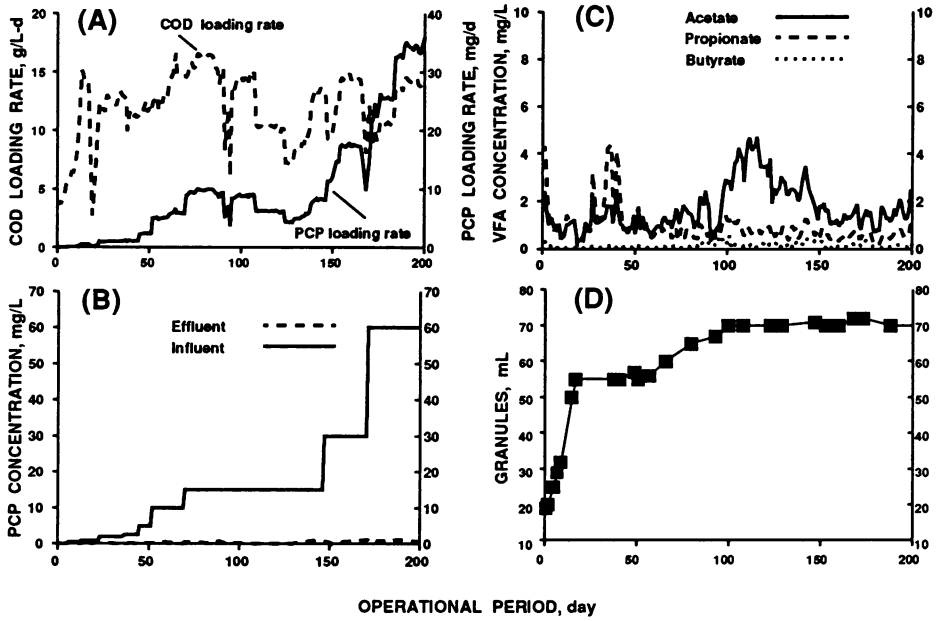

FIG. 3. Operational results during development and performance of PCP-degrading granules. (A) Volumetric COD loading rate and PCP loading rate; (B) PCP concentrations in the influent and effluent; (C) VFA concentration in the reactor; (D) granule bed volume in the reactor.

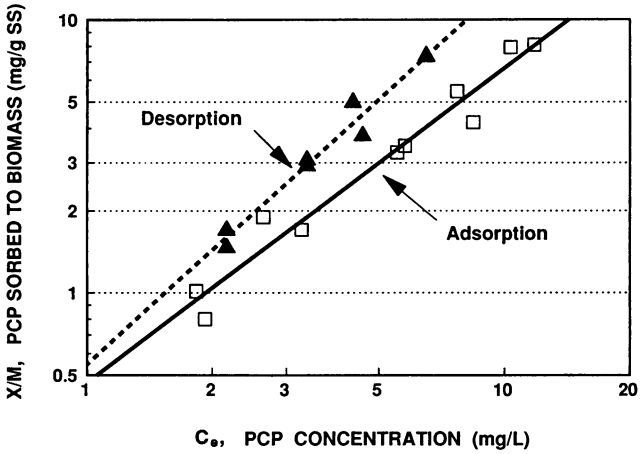

FIG. 4. Freundlich isotherm plots and best-fit lines for adsorption and desorption of PCP by VFA-degrading granules. Symbols: □, adsorption isotherm; ▲, desorption isotherm.

capacities of VFA-degrading granules and the specific PCP removal rates in the reactor and by the radiotracer assay.

Adsorption of PCP to granules. PCP is a weak organic acid (pK_a = 4.75). The adsorption of PCP in water-solid system is influenced by pH, organic solvent, and ionic strength (20). The sorption of chlorophenols including PCP onto anaerobic granules could be described by using the Freundlich isotherm (18). The test conditions used in this study were similar to those in the reactor (pH 7.0 and 30°C). A precise adsorption or desorption isotherm of PCP could not be obtained by using PCP-degrading granules since dechlorination was carried out rapidly by these granules. Therefore, VFA-degrading granules were used because these granules did not show any capability to degrade PCP (as shown below) and had microbial populations similar to those of the PCP-degrading granules. The Freundlich adsorption isotherm and desorption isotherm of PCP for the VFA-degrading granules are presented in Fig. 4. Significant adsorption and desorption of PCP were observed. The Freundlich constants K and $1/n$ were 1.15 and 0.470 for the adsorption (with a regression coefficient [r^2 = 0.961), and 1.38 and 0.548 for the desorption (r^2 = 0.970), respectively. The desorption isotherm was different from the adsorption isotherm. This indicates that a part of PCP was irreversibly sorbed to the biomass. This difference is not likely due to conversion of PCP to other compounds because no other chlorinated phenols were detected. Assuming that the adsorption and desorption of PCP for PCP-degrading granules was the same as those for VFA-degrading granules and that the PCP concentration in the reactor was 0.5 mg/liter, only 0.83 mg of PCP could be removed by 1 g (SS) of granules due to adsorption. At an equilibrium concentration of 0.5 mg/liter, the granules in the reactor (approximately 60 ml) only could sorb about 2.5 mg of PCP. The biodegradation of PCP had likely begun by day 22 because by that time about 5.6 mg of PCP had been fed to the reactor, on the basis of the amount of PCP removed. At the end of the operational period (day 171 to day 205), the PCP removal rate (5.5 to 9.2 mg/g of VSS per day) was much higher than the adsorption capacity at respective PCP concentrations observed in the reactor.

TABLE 4. PCP concentrations in the reactor and PCP removal after start-up[a]

Days	Influent PCP (mg/liter)	PCP loading (mg/day/reactor)	PCP in reactor (mg/liter)	PCP removal (%)
1–5	0	0	0	0
5–12	0.5	0.11–0.20	ND[b] (0.5)	>99
13–22	1.0	0.4–0.6	ND	>99
23–37	2.0	0.95–1.1	0.2–0.5	75–90
38–44	2.5	1.1–1.2	ND (0.2)	>99
45–51	5.0	2.2–2.4	0.2–0.5	96
52–69	10	4.9–5.9	0.1–0.2	98–99
70–147	15	5.0–8.9	0.1–0.8	99–95
148–170	30	11.3–17.8	0.5–1.0	98–97
171–205	60	21.8–36.5	0.1–1.0	98–99

[a] During the operational period, the reactor was fed medium A.
[b] ND, not detected (<0.1 mg/liter).

Radiotracer assay results. At day 144, the mineralization of PCP was tested by using PCP-degrading granules in serum vials with [^{14}C]PCP as the tracer. One day after the addition of [^{14}C]PCP to granules incubated at 30°C, production of $^{14}CH_4$ and $^{14}CO_2$ was observed. The $^{14}CH_4$ and $^{14}CO_2$ production reached the maximum levels within 1 week. After 44 days of incubation, the vials were opened to estimate the [^{14}C]carbon balance. The results from four replicates indicated that 52% ± 2% of total [^{14}C]carbon added as [^{14}C]PCP was recovered as $^{14}CH_4$ gas, and 18% ± 2% was recovered as $^{14}CO_2$ gas. The aqueous fraction contained 21% ± 3% [^{14}C] carbon, which was composed of $H^{14}CO_3^-$ and other soluble components. The remaining [^{14}C]carbon was likely associated with the biomass. The anaerobic degradation pathway of PCP was proposed via phenol as an intermediate (3, 27, 47). On the basis of calculations of the theoretical yield for methane and CO_2 by using the equation of Symons and Buswell (38), the percentages of total carbon in phenol converted to methane and CO_2 are 58 and 42%, respectively. Thus, our radiotracer assay results demonstrated that PCP was almost completely mineralized to CH_4 and CO_2 by the microorganisms in the granules.

As controls, VFA-degrading granules and brewery-waste-degrading granules were also tested. After 44 days of incubation with PCP, neither $^{14}CH_4$ nor $^{14}CO_2$ was observed in the serum vials inoculated with either of these granules. This indicated that these granules did not possess detectable PCP-mineralizing capability.

Characteristics of PCP-degrading granules. The granules developed on PCP-containing feed were yellowish brown to grey in color, indicating a low content of ferrous sulfide. The diameter of the granules ranged from 0.1 to more than 5 mm. On the basis of size analysis, most granules were less than 2.0 mm (Fig. 5A). However, the large granules (>2.0 mm) contributed a great part of the granule bed volume (Fig. 5B). The specific gravity of the granules was determined to be 1.02. The biomass density of the granule bed in the reactor was approximately 50 g of SS per liter of granules. The ratio between VSS and SS was determined to be 0.88. Microscopic examination revealed that the PCP-degrading granules contained *Methanothrix*-like rods as prevalent species. Some *Methanothrix*-like bacteria grew as long filaments, and others grew as rods in chains of three to five cells. *Methanosarcina* sp. and *Methanobacterium* sp. were also observed as prevalent methanogenic species in the granules.

Performance of the PCP-degrading granules. Besides medium A, the operational performance of the PCP-degrading granules was tested by using media B, C, D, and E with decreasing COD concentrations of 5, 2.6, 1.48 and 1.2 g/liter, respectively (Table 1). Each medium was fed to the reactor

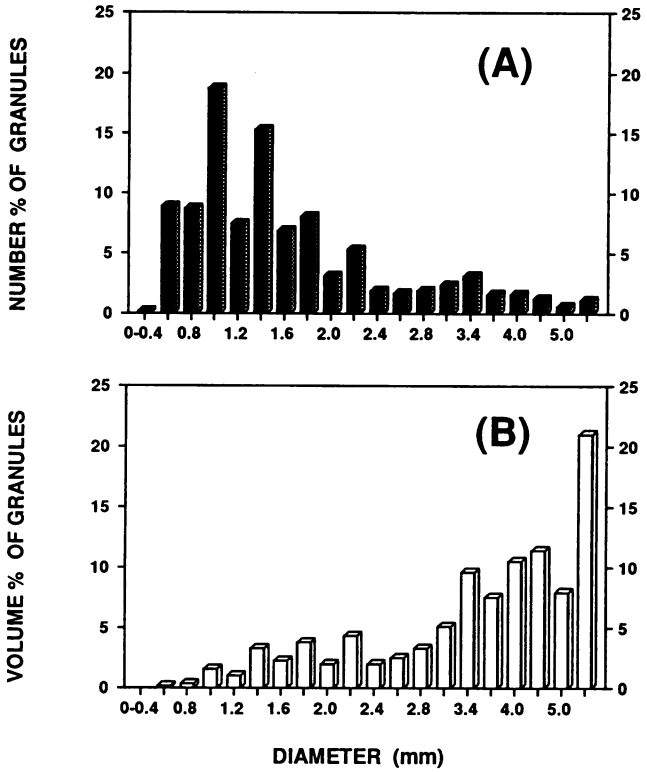

FIG. 5. Size distribution of PCP-degrading granules. The total number of the granules used for the determination was 639. (A) Diameter of the granules versus the number of the granules; (B) diameter of the granules versus the volume of the granules.

for at least 1 month. A portion of the granules in the reactor was periodically removed to monitor the specific growth rate of the granule bed. During the operation, the feed rates were adjusted to obtain a low VFA concentration in the reactor, i.e., the concentrations of acetate, propionate, and butyrate were less than 1.0, 0.5, and 0.05 mM, respectively. The effluent methanol concentration was below detection limits (<0.05 mM). The pH in the reactor was maintained between 6.9 and 7.3. The hydrogen partial pressure in the biogas was low (<20 ppm). The methane content in the biogas was 70 to 72%. The operational results for PCP and COD removal are summarized in Table 5. In all cases, the PCP concentration in the effluent was near or below the detection limit in this study (<0.1 mg/liter) except when the influent PCP concentration (medium A) was 60 mg/liter. Chlorophenols in the effluent other than PCP were not detectable (<0.1 mg/liter) during the entire operational period. Decreases in the influent VFA and methanol concentrations (and, therefore, COD concentration) as well as the COD loading rates did not influence PCP removal activity. A lower specific growth rate of the granule bed was observed when lower specific COD loading rates were applied. The specific PCP removal rates achieved in stable operation were approximately 13.6 to 14.6 mg of PCP per g of VSS per day at a hydraulic retention time of 10.8 h.

The COD removal rates of the granules under these operational conditions were observed to be 0.5 to 1.5 g of COD per g of VSS per day. The maximum specific COD removal rates were observed to be approximately 2.2 g of COD per g of VSS per day on the basis of methane production (0.35 liter of CH_4 per g of COD removed at standard conditions) when the reactor was perturbed with higher concentrations of VFA to achieve 10 mM acetate, 5 mM propionate, and 5 mM butyrate in the reactor.

TABLE 6. Maximum PCP degradation rate test[a]

Day	Influent PCP (mg/liter)	Effluent PCP (mg/liter)	Flow rate (ml/h)	PCP removal (mg/day)	PCP removal rate (mg/g of VSS/day)
1	40	1.0	20.5	19.2	16.6
2	40	4.3	24.8	21.2	18.3
3	40	6.6	28.1	22.5	19.4
4	40	13.1	32.7	21.1	18.2

[a] The reactor was fed with medium E and contained 26 ml of the PCP-degrading granules (1.16 g of VSS).

The maximum PCP degradation rate. The maximum PCP degradation rate was tested in the reactor containing 26 ml of granules by increasing the influent rate until 13 mg of PCP per liter of effluent was observed (Table 6). The maximum specific PCP removal rate was observed to be 18 mg/g of VSS per day. However, at this high level of PCP, acetate-utilizing methanogens and VFA degraders were almost irreversibly inhibited. The methanogenic performance did not recover until after 2 weeks. During the inhibition, 3,4,5-trichlorophenol (3,4,5-TCP), 2,4,6-TCP, 3,5-dichlorophenol (3,5-DCP), and 2,4-DCP were detected (at greater than 0.2 mg/liter) in the reactor effluent.

DISCUSSION

During the last decade, studies on the development of anaerobic granules to obtain high COD removal performance have been targeted. Most published protocols for the development of granules suggest that toxic compounds or inhibi-

TABLE 5. Typical operational performance of PCP-degrading granules fed with different media at 28°C

Medium	HRT[a]	Granules (ml)	Influent PCP (mg/liter)	Effluent PCP (mg/liter)	PCP removal rate			Influent COD (mg/liter)	COD removal (%)	COD loading rate		Granule bed growth (ml/ml/day)
					mg/g of VSS/day	mg/liter of granules/day	mg/liter · day[b]			g/g of VSS/day	g/liter/day	
A	15	70	60	<0.5	9.2	400	97	10	98–99	1.5	16.2	0.032
B	15	50	30	<0.1	5.8	288	48	5	>95	1.06	8.1	0.022
C	15	48	30	<0.1	6.0	300	48	2.6	>90	0.57	4.2	0.012
D	10.8	42	40	<0.1	13.6	680	88	1.48	>85	0.51	3.3	0.011
E	10.8	39	40	<0.1	14.6	730	88	1.2	>80	0.46	2.7	ND[c]

[a] HRT, hydraulic retention time.
[b] Calculated on the basis of total reactor volume.
[c] ND, not determined.

tors have a negative effect on the development of granules (21, 22, 45). The present study demonstrates that stable anaerobic granules can be developed in the presence of toxic xenobiotics such as PCP with VFAs and methanol as cosubstrates.

The volumetric PCP removal rate (88 to 97 mg/liter/day, with >99% removal) obtained in a laboratory-scale UASB reactor was much higher than that obtained in a lab-scale digester (0.1 mg/liter/day, with >99% removal) (11), an anaerobic partially packed bed reactor (0.51 mg/liter/day, with 35% removal) (19), a laboratory anaerobic fixed-film reactor with glucose and phenol as substrate (approximately 0.7 mg/liter/day, with 98% removal) (13), and a lab-scale UASB reactor with phenol and glucose as substrate (2.2 mg/liter/day, with 99% removal) (14). The volumetric PCP removal rate in the laboratory system was limited by the reactor configuration because a high volume of settler (approximately 70% of the total volume of the reactor system) was used in this study. A higher volumetric PCP removal rate could be obtained if the PCP-degrading granules were applied in a pilot- or full-scale UASB system with approximately 30 to 40% of the total volume for the sludge settler. Compared with an aerobic fixed-film system, which can remove approximately 99% of the PCP with a PCP loading rate of 360 mg/liter/day (37), an anaerobic system with PCP-degrading granules may still be an alternative for treating PCP-contaminated wastewaters, especially those with high COD contents, since an anaerobic system can save the energy used for aeration.

PCP is inhibitory to methanogenesis (11, 12, 41, 43). Methane production by unacclimatized digested sludge was inhibited at 0.2 to 0.4 mg of PCP per liter (11). In this study, PCP concentrations of 1.0 to 2.5 mg/liter were inhibitory to methanogens and acetogens in VFA-degrading granules. Cosubstrates must be supplied since PCP cannot be used as the sole carbon source to develop anaerobic granules. According to the suggested biodegradation pathways of PCP and other chlorophenols that have been identified as intermediates during PCP dechlorination, phenol is the key intermediate (3, 10, 27, 47). It is known that anaerobic phenol degradation yields adipic, caproic, acetic, succinic, and propionic acids (9). Caproate can be degraded by syntrophic butyrate degraders (25, 36). Succinate can be converted to propionate. Phenol can also be degraded via benzoate to acetate by dechlorinating enrichments from freshwater sediments (10, 47). Therefore, in this study, acetate, propionate, butyrate, and methanol were used as the carbon sources for supporting the growth of anaerobic organisms involved in PCP dechlorination and phenol mineralization.

The metabolic pathways of reductive dechlorination of PCP were identified by several individual batch experiments and yielded a complex pathway based on the intermediate chlorophenols observed (3, 13, 14, 27, 31). The dechlorination of PCP could take place at any position, i.e., *ortho*, *meta*, or *para*. The concentration of intermediate chlorophenols was too low to be detected (<0.1 mg/liter) during normal reactor operation. 2,4,6-TCP, 3,4,5-TCP, 2,4-DCP, and 3,5-DCP were observed only when PCP degradation, methanogenesis, and VFA degradation were inhibited by the presence of a high concentration of PCP. This indicates that the reductive dechlorination of PCP was initiated by PCP-degrading granules in both the *meta* as well as the *ortho* position. Toxicity assays demonstrated that the intermediate 3,4,5-TCP at a concentration of 1.0 mg/liter was more toxic than PCP, whereas the same concentrations of 2,4,6-TCP and 2,4-DCP did not affect the VFA-degrading activity (43). This strongly suggests that dechlorination at the *meta* position may be the main pathway of PCP dechlorination by these anaerobic granules (3, 6).

The extent of inhibition by PCP of acetate-utilizing methanogens, syntrophic propionate degraders, and syntrophic butyrate degraders influences the complete mineralization of PCP. For successful development of PCP-degrading granules, the initial determination of the inhibition level of PCP to different trophic groups was important. On the basis of the toxicity assay, syntrophic propionate degraders and acetate-utilizing methanogens were more sensitive to PCP than syntrophic butyrate degraders (Fig. 2). The PCP concentration in the reactor was maintained lower than 1.0 mg/liter for the development of PCP-degrading granules, a safe level based on the results of the toxicity assays. The recycle of effluent to dilute the influent PCP concentration is perhaps required when the influent PCP concentration is higher than 1.0 mg/liter.

For wastewater containing PCP with relatively high organic matter content (2 to 10 g of COD per liter), both PCP and COD can be effectively removed by the PCP-degrading granules (Table 5). The lowest COD loading rate used was approximately 0.46 g of COD per g of VSS per day or 2.7 g of COD per liter per day for the reactor system. At these loading rates, dechlorination and degradation of PCP were achieved. Effluent PCP concentrations were below the detection limit (<0.1 mg/liter). On the basis of the results obtained, it would be possible to treat wastewater containing PCP with a COD concentration of 1.2 g/liter or more. However, to treat wastewater or groundwater which contain low levels of organic carbon source, further studies may be needed to determine how low a COD loading rate is necessary to maintain dechlorination activity.

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