

ASSESSMENT OF THE ABILITY OF SLUDGE TO DEGRADE PCP UNDER ANAEROBIC CONDITIONS

R. M. L. Bolaños, M. H. R. Z. Damianovic, M. Zaiat and E. Foresti*

Laboratório de Processos Biológicos, Departamento de Hidráulica e Saneamento,
Escola de Engenharia de São Carlos (EESC), Fax: +(55) (16) 3373-9550,
Universidade de São Paulo (USP), Av. Trabalhador São-Carlense 400,
CEP 13560-590, São Carlos - SP, Brazil.
E-mail: eforesti@sc.usp.br

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Abstract - The capacity of sludge from different sources to degrade pentachlorophenol (PCP) was evaluated. Three 2.5 liter reactors (R₁, R₂, and R₃) were inoculated with different anaerobic sludges, semi continuously fed and maintained in orbital motion at 30±1°C. R₁ was inoculated with aerobic sludge and river sediment collected downstream from a pulp and paper plant. R₂ received sludge from an anaerobic reactor treating effluents from a paper recycling plant and R₃ received anaerobic sludge from a biodigester treating industrial and domestic effluents. The sludges were first acclimatized to a culture medium generally recommended for organochloride anaerobic degradation studies. The reactors were then subjected to increasing concentrations of PCP from 0.05 to 10.0 mg.l⁻¹. PCP degradation and metabolite formation were monitored using gas chromatography, and the effects of PCP on the anaerobic process were verified by monitoring pH, volatile fatty acids, alkalinity, total suspended solids, and chemical oxygen demand. It was found that PCP did not affect reactor performance. All the sludges displayed the best PCP degradation capacity at a concentration of 0.2 mg.l⁻¹, producing fewer chlorinated metabolites than when higher PCP concentrations were applied. R₁ consistently produced fewer chlorinated metabolites, confirming the hypothesis that pre exposure to chlorinated compounds improves the sludge's capacity to degrade PCP.

Keywords: Anaerobic process; Wastewater; Biodegradation; Organochlorine; PCP (pentachlorophenol); Reductive dechlorination.

INTRODUCTION

Among other factors, ecological devastation has been attributed to the presence of resistant synthetic organic compounds in the environment and their bioaccumulation or transformation into toxic products. Some of these compounds are particularly important, such as the organochloride compounds released into the environment in increasing amounts by their application in a variety of activities.

The attention of researchers of health and the environment has focused heavily on this problem because of the wide diversity of organochloride

compounds that not only have biocidal properties, but are also toxic and mutative for bioterrestrial and aquatic organisms. These compounds are recalcitrant, directly impacting the environment and waste treatment systems (Field et al., 1995).

Chlorophenols are usually highly toxic and, owing to poor microbial degradation, accumulate in environments such as wastewater, sewage, groundwater, leachate, freshwater, and marine sediments and in hot waste streams from the pulp and paper industry (Larsen et al., 1991). Chlorophenols are used industrially primarily as insecticides, herbicides, and fungicides and as

*To whom correspondence should be addressed

preservatives for wood, glue, paint, vegetable fibers, and leather. These compounds have been found in soils around wood-preserving plants, in groundwater leaching from contaminated soils, and in surface runoff or direct industrial waste discharge (Flora et al., 1994).

Pentachlorophenol (PCP) is one of the most extensively studied organochlorines because it is highly insoluble in water, extremely toxic, and generally resistant to attack by microorganisms. Its relative resistance to biological degradation, which is the reason for its use as a preservative, creates a pollution problem when this compound enters the environment (Galil & Novac, 1995). When exposed to these types of compounds, however, microorganisms such as bacteria and fungi can develop the ability to use them as a source of carbon, causing their own biodegradation (Hagglblom et al., 1994). Biological processes can be used to degrade these residues because these processes convert wastes into inoffensive and completely mineralized products (Field et al., 1995) or into less resistant or toxic compounds.

When organochlorides are biodegraded in anaerobic systems, biodegradation occurs mainly as a result of the first ring being broken or the halogen being changed by hydroxyl radicals (Pereira, 1993). However, some highly chlorated organic compounds appear to be resistant to the anaerobic degradation process (Mohn & Tiedje, 1992). Under anaerobic conditions, chlorines can be removed from the aromatic ring by reductive dechlorination, resulting in partially or fully dehalogenated products, which are then more susceptible to either aerobic or anaerobic attack (Krumme & Boyd, 1988).

In the past few years, several studies have shown that halogenated phenols are reductively dehalogenated in sewage sludge, aquatic sediments, and soils. Some of these studies indicate that reductive dehalogenation reactions may thrive in methanogenic environments (Madsen & Aamand, 1991). One of the difficulties involved in biodegrading organochlorine compounds is their toxicity to microorganismic cultures, which hinders the degradation process in ways that are not yet fully understood (Mohn & Tiedje, 1992).

This paper discusses the results of a comparative study of the capacity of sludges from different sources to transform PCP under anaerobic conditions. These results should support the choice of sludge for inoculating anaerobic reactors.

MATERIALS AND METHODS

The experimental apparatus was set up to conduct studies in semi continuous anaerobic reactors installed in a temperature-controlled chamber kept at $30 \pm 1^\circ\text{C}$. The three reactors (R_1 , R_2 , and R_3) consisted

of 2500 ml amber-colored flasks with rubber stoppers, with 2450 ml of useful volume (liquid medium plus sludge). Two glass tubes were inserted through the stoppers: one was connected to a gas meter to release biogas and the other was used to siphon off liquid to drain the reactor and for weekly feeding by means of a peristaltic pump (Watson-Marlow type 202 model M1). The reactors were kept in continuous orbital motion at 45 rpm in order to maintain effective contact between sludge and substrate.

Three different inocula were used in the experiments. R_1 was inoculated with sludge taken from an aerobic reactor treating pulp and paper processing wastewater mixed with anaerobic river sediment, collected downstream from the effluent discharge of the processing plant. Therefore, this sediment had been preexposed to chlorinated organic compounds from the plant's effluents. R_2 was inoculated with granulated sludge from an up-flow anaerobic sludge (UASB) reactor treating paper recycling wastewater. This sludge was chosen because it contained sulfate-reducing bacteria, purportedly participants in the process of degradation of haloaromatics. R_3 received sludge from the biodigester of a municipal wastewater treatment plant. This sludge was used due to the diversity of effluents treated in the plant (domestic sewage and several types of industrial waste). A control reactor (R_C) was also run without the addition of PCP to compare the influence of PCP on the performance of the substrate degradation process. The control reactor was inoculated with a mixture of the sludges used in reactors R_1 , R_2 , and R_3 .

Initially, the reactors were filled with 1200 ml of culture medium, 850 ml of a saline solution (0.85% NaCl) and 400 ml of sludge with a concentration of volatile suspended solids of approximately 20 mg.l^{-1} , so the initial VSS concentration in each reactor was around 4 g.l^{-1} . The reactors were operated semi continuously with 1200 ml of liquid medium drained off from each reactor weekly. After each drainage, the same volume of fresh substrate was added to maintain the total volume of the reactor.

The culture medium used in this study was prepared according to Angelidaki et al. (1990) and modified with a vitamin solution described by Madsen and Aamand (1991) and a reductive solution of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (292 mg.l^{-1}). Glucose (331 mg.l^{-1}), sodium acetate (419 mg.l^{-1}), and sodium formate (1650 mg.l^{-1}) were used as carbon sources. This solution containing carbon sources resulted in a total COD of 1000 mg.l^{-1} .

The reactors used for testing PCP degradation were operated for 308 days, during which the pH, temperature, chemical oxygen demand (COD), total

volatile acids (TVA), alkalinity, and volatile suspended solids (VSS) were monitored according to the Standard Methods for Examination of Water and Wastewater (1998).

The biogas composition was determined by chromatography, using a Gow-Mac gas chromatograph (series 150) equipped with a thermal conductivity detector and a Porapak-Q column (2 meters in length with an internal diameter of 1/4").

The specific rate of substrate conversion to methane was calculated from the volume of methane produced, considering the theoretical value of $0.43 \text{ l CH}_4 \cdot \text{g}^{-1}$ of organic matter as COD at 30°C . Methane production was quantified by displacement of alkaline solution, aiming at the removal of carbon dioxide.

PCP degradation was evaluated by detection of intermediate degradation compounds, since the reductive dehalogenation of PCP is known to occur through the formation of metabolites (tetrachlorophenol - TeCP, trichlorophenol - TCP, dichlorophenol - DCP, chlorophenol - CP). These compounds were detected in the samples using an HP 5890 series II gas chromatograph equipped with electron capture and a split/splitless injection system and five DB-5 capillary columns (30 m long with an internal diameter of 0.25 mm). A hydrogen flow of $1 \text{ ml} \cdot \text{min}^{-1}$ was used as the drag gas. Hexane solutions of highly purified standards (Supelco) were used to determine the retention time of each compound in the column.

The samples from the reactors under study were extracted in HPLC-grade hexane using the methodology described by Cass et al. (2000).

The microorganisms in each reactor were examined one month after their inoculation, two months after beginning the PCP additions, and at the end of the experiment. The microorganisms were examined under phase contrast and fluorescence microscopy at 1000x using an Olympus model BH2 binocular microscope. Prior to the microscopic observations, the samples were fixed on plates containing 2% agar.

Six months after inoculation, PCP and the

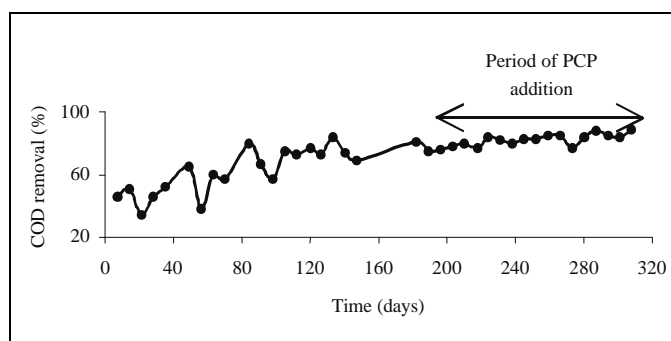
carbon-source-containing substrate were added weekly to the reactors by drainage/feeding operations. The PCP concentration in the feed was increased progressively from 0.05 to $10.0 \text{ mg} \cdot \text{l}^{-1}$.

RESULTS AND DISCUSSION

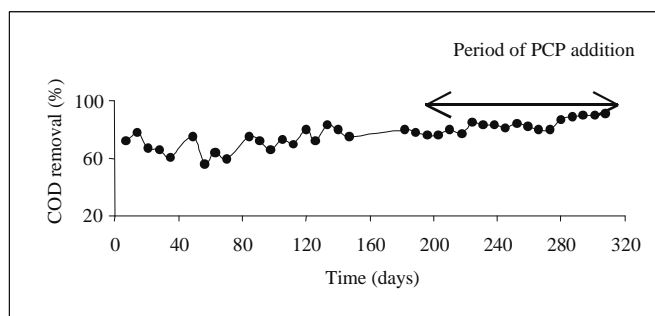
The experiment was divided into three stages, the first corresponding to the acclimatization period (day 0 to day 120), the second comprising a long period of reactor stabilization (day 121 to day 182) without the addition of PCP, and the third consisting of the period with addition of PCP (day 183 to day 308). In the third stage, the PCP concentration was increased from 0.05 to $2.0 \text{ mg} \cdot \text{l}^{-1}$ throughout 12 weeks (2 weeks at $0.05 \text{ mg} \cdot \text{l}^{-1}$, 2 weeks at $0.10 \text{ mg} \cdot \text{l}^{-1}$, 4 weeks at $0.20 \text{ mg} \cdot \text{l}^{-1}$, 2 weeks at $0.50 \text{ mg} \cdot \text{l}^{-1}$, 1 week at $1.0 \text{ mg} \cdot \text{l}^{-1}$, and 1 week at $1.5 \text{ mg} \cdot \text{l}^{-1}$).

The effluent pH varied similarly even after the addition of PCP, with values ranging from 7.9 to 8.5 (mean value of 8.2 ± 0.2) throughout the experiment in all the reactors assayed. Effluent bicarbonate alkalinity ($2082 \pm 91 \text{ mg CaCO}_3 \cdot \text{l}^{-1}$) was consistently higher than the influent values ($1389 \pm 35 \text{ mg CaCO}_3 \cdot \text{l}^{-1}$) throughout the experiments in all the reactors, even after the addition of PCP. It is well known that the generation of bicarbonate under anaerobic conditions is a very reliable indicator of process stability.

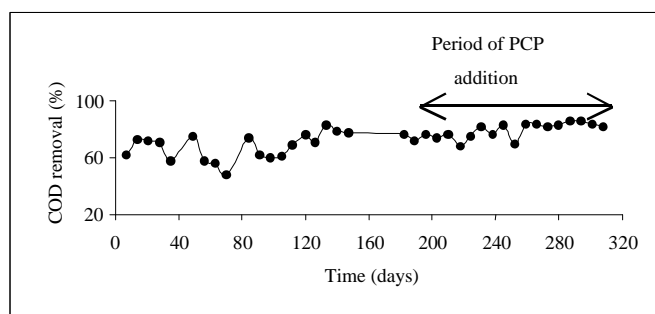
The average COD removal efficiencies of the four reactors were similar during the second phase of operation, before the addition of PCP (R_1 : $76 \pm 5\%$; R_2 : $77 \pm 5\%$; R_3 : $74 \pm 7\%$; R_C : $77 \pm 4\%$). COD removal efficiency was lower for R_1 ($56 \pm 14\%$) at the beginning of operation (first 105 days) than for R_2 ($68 \pm 7\%$) and R_3 ($64 \pm 8\%$), probably due to the characteristics of the inoculum sludge, which consisted of a mixture of aerobic and anaerobic sludge. However, although it took longer to stabilize, the performance of R_1 was similar to that of the other reactors throughout the experimental period (Figure 1).



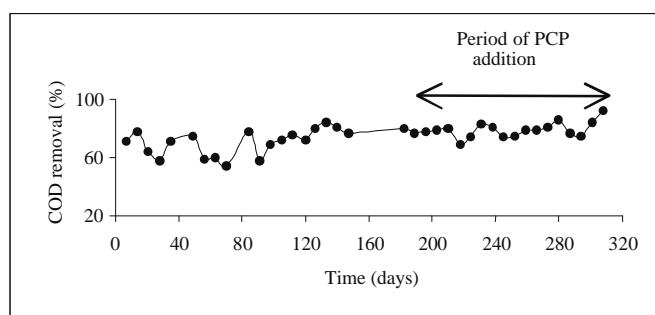
(a)



(b)



(c)



(d)

Figure 1: Temporal variation in COD removal efficiency. (a) R_1 : Paper and pulp plant sludge; (b) R_2 : Paper recycling sludge; (c) R_3 : Sewage treatment plant sludge; (d) R_C : Control reactor

During the third operational period (with addition of PCP), the removal of organic matter (as COD) was $82 \pm 4\%$, $83 \pm 5\%$, $79 \pm 6\%$, and $81 \pm 5\%$ for reactors R_1 , R_2 , R_3 , and R_C , respectively. The TVA concentration for the four reactors was 160 ± 20 mg $\text{HAc} \cdot \text{l}^{-1}$ before addition of PCP and 120 ± 13 mg $\text{HAc} \cdot \text{l}^{-1}$ when PCP was added, corroborating the statement that reactor performance was not affected by the PCP.

In Table 1 the rates of substrate conversion into methane during the third operational phase (when PCP was added) are listed for PCP concentrations ranging from 2.0 to 10.0 mg $\cdot \text{l}^{-1}$, while in Table 2 the ratios of the conversion rates in reactors fed with PCP to that obtained in the control reactor (without the addition of PCP) are shown. The initial concentration of biomass in the reactor was used to

calculate the conversion rate, since this concentration remained practically constant throughout the experiments. Cell synthesis was probably similar to the loss of biomass during each reactor discharge.

Immediately following the addition of a concentration of 2.0 mg $\cdot \text{l}^{-1}$ of PCP, the substrate conversion rates observed in R_1 and R_3 were 32% lower than that observed in the control reactor. In R_2 , the decrease was about 20%.

With PCP concentrations of 3.0 to 8.0 mg $\cdot \text{l}^{-1}$, the conversion rates in R_1 were consistently higher than those observed in the control reactor. The maximum rate obtained with a PCP concentration of 8.0 mg $\cdot \text{l}^{-1}$ was 68% above the value recorded for the control. At PCP concentrations of 10.0 mg $\cdot \text{l}^{-1}$, however, the conversion rate in R_1 was only 40% of that observed in the control reactor and the residual PCP concentration

was 6.56 mg.l⁻¹ (Table 3), indicating accumulation of this compound and possible process inhibition.

The conversion rates in R₂ were similar to those observed in the control for PCP concentrations up to 6.0 mg.l⁻¹. For PCP concentrations of 8.0 and 10.0 mg.l⁻¹, the reaction rates were, respectively, 62% and 48% higher than that observed in the control reactor, but the residual PCP concentration in the reactor was

2.1 mg.l⁻¹ (Table 3).

The substrate conversion rates in R₃ were negatively effected by the addition of PCP, with rates remaining 19% to 45% lower than those observed in the control reactor. When initial PCP concentrations of 6 and 10 mg.l⁻¹ were applied, the residual PCP concentration was lower than 1 mg.l⁻¹ and only traces of TeCP were detected (Table 3).

Table 1: Rates of substrate conversion into methane for each reactor

| PCP (mg.l ⁻¹) | Rate of substrate conversion into methane (gCH ₄ - COD.gSSV ⁻¹ .day ⁻¹) | | | |
|---------------------------|---|----------------|----------------|----------------|
| | R ₁ | R ₂ | R ₃ | R _c |
| 2.0 | 0.017 | 0.020 | 0.017 | 0.025 |
| 3.0 | 0.060 | 0.054 | 0.023 | 0.043 |
| 4.0 | 0.025 | 0.019 | 0.018 | 0.023 |
| 6.0 | 0.045 | 0.030 | 0.025 | 0.031 |
| 8.0 | 0.057 | 0.055 | 0.024 | 0.034 |
| 10.0 | 0.017 | 0.062 | 0.023 | 0.042 |

Table 2: Ratios of the rates of substrate conversion in the reactors to the rate observed in the control reactor (without the addition of PCP)

| PCP (mg.l ⁻¹) | R ₁ /R _c | R ₂ /R _c | R ₃ /R _c |
|---------------------------|--------------------------------|--------------------------------|--------------------------------|
| 2.0 | 0.68 | 0.80 | 0.68 |
| 3.0 | 1.40 | 1.25 | 0.53 |
| 4.0 | 1.09 | 0.82 | 0.78 |
| 6.0 | 1.45 | 0.97 | 0.81 |
| 8.0 | 1.68 | 1.62 | 0.71 |
| 10.0 | 0.40 | 1.48 | 0.55 |

Table 3: Residual concentrations of chlorophenols in the reactors after 1 week of operation

| PCP added | R ₁ | | | | R ₂ | | | | R ₃ | | | |
|-----------|----------------|------|------|------|----------------|------|-----|------|----------------|------|-----|-----|
| | PCP | TeCP | TCP | DCP | PCP | TeCP | TCP | DCP | PCP | TeCP | TCP | DCP |
| 0.2 | t. | 0.5 | 0.34 | 1.57 | t | 0.26 | * | 0.33 | t | t | * | 2.8 |
| 0.5 | t. | 0.71 | * | * | t | t | t | * | t | t | t | * |
| 1.5 | t. | t. | t | * | t | * | * | * | 0.56 | 0.16 | t | * |
| 6.0 | 0.95 | 0.40 | t | * | 2.1 | * | * | * | 0.31 | t | * | * |
| 10.0 | 6.56 | 0.10 | * | * | 2.1 | * | * | * | 0.76 | * | * | * |

t - trace, * - undetected

These results indicate that the inoculum played an important role in the anaerobic treatment of the PCP-containing substrate. The sludge taken from an aerobic reactor treating pulp and paper processing wastewater mixed with anaerobic river sediment (R₁) had the best conversion rates, while the anaerobic sludge taken from a biodigester in a municipal wastewater treatment plant (R₃) was negatively affected by adding PCP, showing lower reaction rates than the control. The granulated sludge taken from an up-flow anaerobic sludge (UASB) reactor treating wastewater from a paper recycling plant provided the best conversion rates for the highest PCP concentration assayed.

The chlorinated metabolites (TeCP, TCP, DCP, CP) in the reactor supernatant were also analyzed for the purpose of evaluating the overall PCP degradation process. Intermediate compounds were not detected in the chromatographic analyses of the first samples containing low PCP concentrations (0.05 to 0.1 mg.l⁻¹), but they appeared in the chromatograms of samples taken after higher PCP concentrations were applied (Table 3).

PCP, TeCP, TCP, and DCP were detected in R₁ when 0.2 mg.l⁻¹ of PCP was applied. TeCP and TCP metabolites were detected for PCP concentrations of up to 6.0 mg.l⁻¹, while only TeCP was detected at an influent PCP concentration of 10.0 mg.l⁻¹.

After the addition of 0.2 mg.l⁻¹ of PCP, TeCP and DCP were detected in the supernatant of R₂, indicating the dehalogenation of the original compound.

PCP accumulation was observed in R₃ for initial concentrations of 3.0 mg.l⁻¹ or more. TeCP, TCP, and DCP were detected in the supernatant when PCP concentrations of up to 1.5 mg.l⁻¹ were applied. For initial PCP concentrations of 1.5 to 6.0 mg.l⁻¹, only TeCP was detected and no intermediate metabolites with a PCP concentration of 10.0 mg.l⁻¹ were found (Table 3).

The R₁ sludge showed a greater capacity to degrade PCP into less chlorinated metabolites, even at the higher PCP concentrations assayed in this study, reinforcing the hypothesis that preexposure of the inoculum to the compound improves its degradative capacity. However, as mentioned previously, the production of methane in R₁ was found to decrease when the reactors were subjected to 10.0 mg.l⁻¹ of PCP, clearly indicating a reduction in methanogenic activity under this condition.

Duff et al. (1995) observed the inhibition of methanogenesis at PCP concentrations of 2.0 to 3.0 mg.l⁻¹ in batch tests. Using batch reactors inoculated with water and sediment from several lakes and incubated at a temperature of 25°C, Hale et al. (1990) found that the dehalogenation of 2,3-, 2,4- and 2,6-DCP was faster in sediments adapted to organochlorine compounds than in unadapted sediments. Mikesell and Boyd (1986) observed that sludges from an anaerobic bioreactor, adapted to degrade 2-, 3-, 4-CP and used in combination, degraded PCP completely.

The structures resembling actinomycetes observed in sediments adapted to organochlorine compounds require further study to elucidate the role of these microorganisms in the PCP degradation process. Studies have shown that this group of Gram-positive bacteria can transform and degrade xenobiotic compounds. Haggblom et al. (1994) identified an actinomycete, denominated *Rhodococcus chlorophenicus*, that mineralizes PCP.

Methanogenic consortia (*Methanosaeta*-sp, *Methanosarcina*-sp, and *Methanobacterium*-sp) were seen in the sludges under study, indicating the importance of anaerobic bacteria in the degradation of highly chlorinated compounds and confirming the findings of Mohn and Tiedje (1992), Wu et al. (1993), and Krumme and Boyd (1998).

CONCLUSIONS

The results obtained in this work indicate that the inoculum played an important role in the anaerobic

treatment of the PCP-containing substrate.

The reactors inoculated with sludge taken from an aerobic reactor treating pulp and paper processing wastewater mixed with anaerobic river sediment (R₁) provided PCP degradation into less-chlorinated metabolites, even at the higher PCP concentrations assayed in this study. This performance likely resulted from the preexposure of the inoculum sludge to organochlorines. The conversion rates in these reactors were consistently higher than that observed in the control reactor for PCP concentrations up to 8 mg.l⁻¹, but the reactor was affected when subjected to a PCP concentration of 10.0 mg.l⁻¹.

The specific level of substrate conversion into methane in the reactors appeared to be unaffected by addition of PCP up to concentrations of 6.0 mg.l⁻¹ in the reactor inoculated with granulated sludge from an up-flow anaerobic sludge reactor treating paper recycling wastewater (R₂). At a PCP concentration of 10.0 mg.l⁻¹, the performance of this reactor was clearly better than that of the others, showing the best performance according to all the available evaluation parameters under this condition.

The reactor that received sludge from a bioreactor of a municipal wastewater treatment plant (R₃) had the worst performance, with rates of conversion to methane that were lower than those observed in all the reactors, including the control reactor.

The reactors showed the best PCP degradation capacity at a concentration of 0.2 mg.l⁻¹ of PCP, producing fewer chlorinated metabolites and consuming the original compound. A microscopic examination of the sludge revealed methanogenic archaea, indicating that, at the concentrations assayed, PCP was not toxic to these microorganisms.

The *Methanospirillum*-sp-like cells and actinomycetes in PCP-degrading sludges deserve special attention in future research in order to elucidate the importance and extent of their participation in the processes of biodegradation of organochlorine compounds.

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