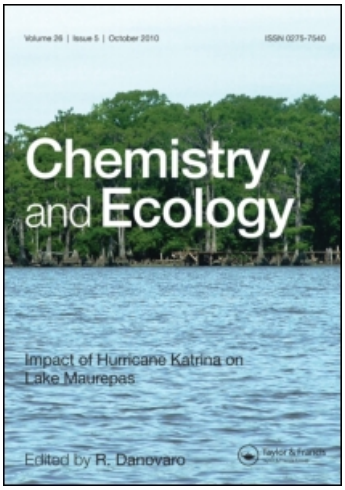

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Anaerobic Degradation of Chlorophenols By 2,4-Dichlorophenol-Adapted Microbial Communities at Different Concentrations

Bea-Ven Chang[a]; Mon-Bor Liou[a]; Shaw-Ying Yuan[b]; Tzu-Ming Pan[c]
[a] Department of Microbiology, Soochow University, Taipei, Taiwan, R.O.C. [b] National Institute of Environmental Analysis, Environmental Protection Administration, Taipei, Taiwan, R.O.C. [c] National Institute of Preventive Medicine, Taipei, Taiwan, R.O.C.

To cite this Article Chang, Bea-Ven , Liou, Mon-Bor , Yuan, Shaw-Ying and Pan, Tzu-Ming(1995) 'Anaerobic Degradation of Chlorophenols By 2,4-Dichlorophenol-Adapted Microbial Communities at Different Concentrations', Chemistry and Ecology, 10: 1, 115 — 125
To link to this Article: DOI: 10.1080/02757549508035335
URL: http://dx.doi.org/10.1080/02757549508035335

Chemistry and Ecology, 1995, Vol. 10, pp. 115–125
Reprints available directly from the publisher
Photocopying permitted by license only

ANAEROBIC DEGRADATION OF CHLOROPHENOLS BY 2,4-DICHLOROPHENOL-ADAPTED MICROBIAL COMMUNITIES AT DIFFERENT CONCENTRATIONS

BEA-VEN CHANG,[1]* MON-BOR LIOU,[1] SHAW-YING YUAN[2] and TZU-MING PAN[3]

[1]*Department of Microbiology, Soochow University, Taipei, Taiwan, R.O.C.;* [2]*National Institute of Environmental Analysis, Environmental Protection Administration, Taipei, Taiwan, R.O.C.;* [3]*National Institute of Preventive Medicine, Taipei, Taiwan, R.O.C.*

(Received 4 January 1994; in final form 17 January 1994)

In this study, we investigated the potential for reductive dechlorination of 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), and pentachlorophenol (PCP) by municipal sewage sludge adapted to 2,4-DCP at different concentrations. 2,4-DCP was completely dechlorinated within 4 weeks. After 18 weeks' incubation, 2,4,6-TCP was also completely dechlorinated and the residue of PCP was 0, 44.46, 51.96% at 0.5, 5, and 50 $\mu g\ ml^{-1}$, respectively. The 2,4-DCP adapted communities initially removed the ortho-chlorine from PCP of 5.0, 50 $\mu g\ ml^{-1}$, following an ortho > para > meta order of chlorine removal. Intermediate products were 3,4,5-TCP, 3,5-DCP, 3-CP (3-chlorophenol), phenol, benzoate and hexanoic acids, whereas PCP (0.5 $\mu g\ ml^{-1}$) indicated a preference for meta-chlorine removal. The intermediate product of 2,4,6-TCP at three concentrations were 2,4-DCP, phenol, benzoate and hexanoic acid. These products were identified by GC-MASS spectrometry. The effects of supplements, including sodium citrate (0.08 mM), sodium pyruvate (0.18 mM), sodium sulphate (0.14 mM) had a direct stimulatory effect on the dechlorination of 2,4,6-TCP and PCP after treatment for 4 weeks, but dechlorination was inhibited after 8 weeks.

KEY WORDS: Anaerobic degradation, chlorophenols, adaptation, sewage sludges

INTRODUCTION

Chlorophenols are a group of primary-pollutant xenobiotic compounds (Keith and Telliard, 1979) widely used in many industries for the production of biocides (Guthrie *et al.*, 1984). These compounds are generally very toxic (Yokoyama *et al.*, 1988) and owing to poor microbial degradation, accumulate in environments such as water, sewage, ground water, leachate, freshwater and marine sediments (Yuan *et al.*, 1991).

Several options are currently available for the bioremediation of chlorophenols under both aerobic (Valo and Salkinoja-Salonen, 1986) and anaerobic (Mikesell and Boyd, 1988) conditions. A number of bacteria that biodegraded chlorophenol under aerobic conditions has been isolated (Stanlake and Finn, 1982; Saber and Crawford, 1985; Topp *et al.*, 1988; Haggblom *et al.*, 1988). More recent work has focused on the anaerobic degradation of chlorophenols added to sewage sludge. Mikesell and

*Address correspondence to: Dr. Bea-Ven Chang, Department of Microbiology, Soochow University, Taipei, Taiwan, R.O.C. Tel:886-8819471 ext 6859, 6880 Fax:886-2-8831193

Boyd (1985) observed reductive dechlorination in several anaerobic sludges. They (Mikesell and Boyd, 1986) also observed that PCP degradation in anaerobic sludge could be enhanced when the microorganisms in the sludges were first adapted to dechlorinate a mixture of monochlorophenols. Bryant *et al.* (1991) reported that 2,4-DCP-adapted communities initially removed the ortho-chlorine from PCP, whereas the 3, 4-DCP-adapted communities initially removed the para-chlorine from PCP.

Several factors, such as temperature (Larsen *et al.*, 1991), pH (Holliger *et al.*, 1992), redox potential (Katner, 1991), sulphate and organic carbon supplements (Kohring, 1989; Kuhn, 1990) can affect the anaerobic degradation and fate of xenobiotic compounds. The effect of various concentrations of chlorophenol on the rate of dechlorination is not well known.

In this study, we investigated the dechlorinative potential and metabolites of 2,4,6-TCP and PCP by municipal sewage sludge adapted to 2,4-DCP at various concentrations. Furthermore, the influence of sodium citrate, sodium pyruvate and sodium sulphate supplements on dechlorination of chlorophenol were reported.

MATERIALS AND METHODS

Chemicals

PCP, 2,4,6-TCP, 2,4-DCP were obtained from Supelco Co., USA. All other chemicals were reagent or HPLC-grade as necessary.

Media

Mineral salts: medium A contained, in grams per litre, the following: NH_4Cl 2.7 g, $MgCl_2.6H_2O$ 0.1 g, $CaCl_2.2H_2O$ 0.1 g, $FeCl_3.4H_2O$ 0.02 g, KH_2PO_4 0.35 g, K_2HPO_4 0.27 g, yeast extract 5 g, resazurine 0. 001 g, Na-thioglycollate 0.5 g. The medium was first adjusted to pH 7.0 and then sterilized at 121°C for 20 min.

Adaptation of Sludge

Sludges with microbial communities were collected from primary anaerobic digestors of Di-Hua Municipal Sewage Treatment Plant of Taipei City. The adaptation processes consisted of adding 2,4-DCP (20 $\mu g\ ml^{-1}$) to the sampled sludge at 24 day intervals on four occasions, and the sludge incubated at 30°C in an anaerobic chamber (Forma Scientific, Model 1025 S/N) during each interval. The final prepared sludge was used as 'adapted sludge' for further studies.

2,4,6-TCP and PCP Dechlorination Studies

The fate of 2,4,6-TCP and PCP was examined in adapted sludge; all experiments were carried out in 125 ml serum bottles with 45 ml of medium, and 5 ml of adapted sludge with microbial communities. Bottles were capped with butyl rubber stoppers and wrapped in aluminium foil to prevent photolysis of chlorinated phenols. The experiments were initiated by adding 2,4,6-TCP and PCP to replicate active and control samples to yield final concentrations of 0.5, 5, and 50 $\mu g\ ml^{-1}$. All processes were performed in an anaerobic chamber. All bottles were incubated at 30°C in the dark without shaking. Aqueous samples were taken from all treatment bottles

periodically and residual chlorinated phenols and metabolite concentrations were measured.

Dechlorination with Addition of the Sulphate, Acetate and Pyruvate

The influence of electron donors on degdradation of 2,4,6-TCP and PCP (50 μg ml^{-1}) was investigated with sodium sulphate (0.08 mM), sodium acetate (0.18 mM) and sodium pyruvate (0.14 mM). All the processes were subject to the conditions mentioned above.

Analysis of Phenolic Compounds

GC methods

Samples of halogenated phenols (0.5 μgml^{-1}) were acidified with HCl to pH 2, extracted with dichloroethane, and acetylated by adding 0.5 ml of acetic anhydride and extracted with 2 ml of hexane. The extracts were analyzed on a HP 5890 gas chromatograph equipped with electron capture detector, ultra-fused silica capillary column. The injection temperature was 240°C. The detection temperature was 300°C. The column temperature was held at 100°C for 5 min and increased to 235°C at 5 min. Nitrogen was used as both the carrier and make-up gas at a rate of 0.8 and 65 ml min^{-1}, respectively. Samples of halogenated phenols (5 and 50 μg ml^{-1}) were also acidified to pH < 2 with HCl and extracted twice with dichloroethane, analyzed with flame ionization detectors and a SP-1240 column. Nitrogen (30 ml min^{-1}), air (30 ml min^{-1}), hydrogen (20 ml min^{-1}) were used as the carrier gas. Injection temperature was 220°C. The detector temperature was 270°C, the column temperature was held at 100°C for 2 min, and increased to 180°C, and held for 5 min.

GC-MASS spectrometry methods

The identification by GC retention times of intermediates from PCP and 2,4,6-TCP degradation was confirmed by GC-MASS spectrometry. Samples were extracted as described previously. Extracts were analyzed with a GC-MASS (HP GC/MS system 5988A), interfaced with a data system with matching library data base (HP-1000 E/series system). The GC was equipped with a DB-5 capillary column. The column temperature was held at 90°C for 4 min and increased to 280°C and held for 12 min. Helium served as the carrier gas at a flow rate of 1 ml min^{-1}. The injection temperature was 90°C. The impact detector was operated at 70 eV, and the scanning rate was about 2/s.

RESULTS

2,4,6-TCP Degradation and Identification of Metabolites

The degradation of 2,4,6-TCP at different concentrations is shown in Figure 1. At the concentration of 0.5, 5 and 50 μg ml^{-1}, 2,4,6-TCP was completely dechlorinated by 2,4-DCP adapted communities within 8, 12 and 18 weeks. A slow loss of 2,4,6-TCP occurred over the following weeks. Only the 2,4-DCP intermediate was identified quantitatively.

The degradation pathway was dependent on the anaerobic environment studied. Very similar intermediates were obtained at different concentrations. The compounds

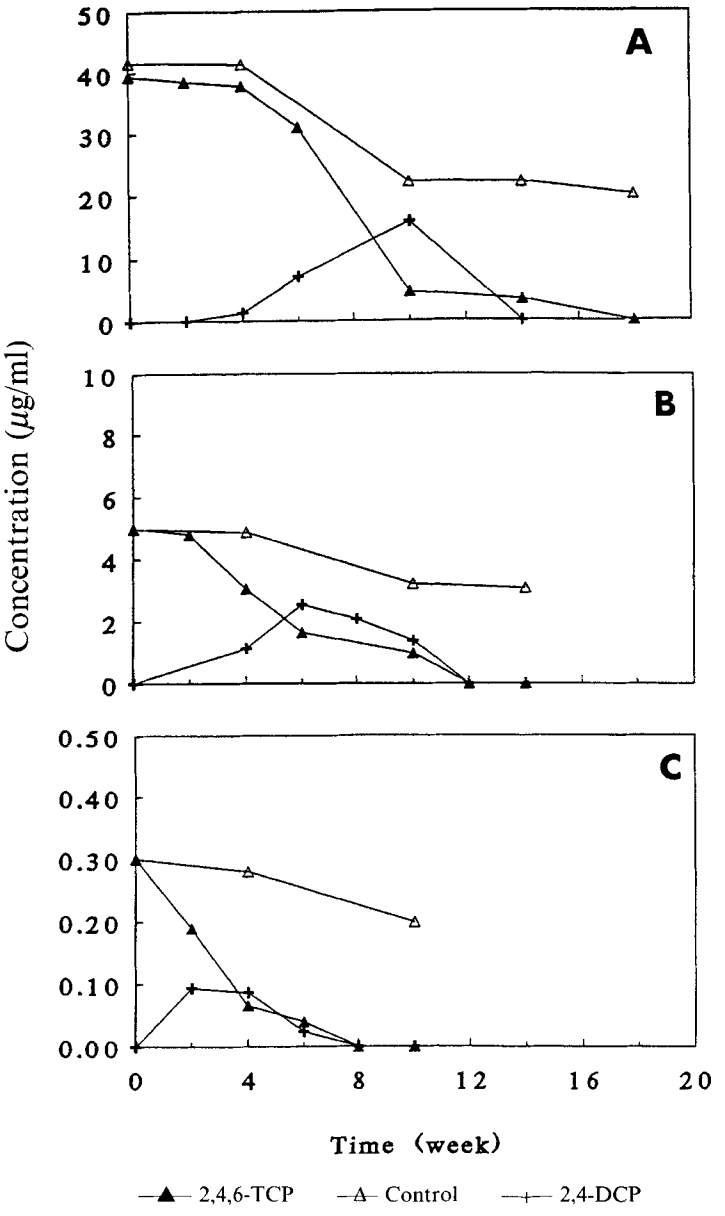

Figure 1 Reductive dechlorination of 2,4,6-TCP at different concentrations. (A) 50 μg/ml; (B) 5 μg/ml; (C) 0.5 μg/ml.

were identified as 2,4-DCP, phenol, benzoate and hexanoic acid by comparing with authentic samples exhibiting the same spectrum reaction in mass spectrometry (Figure 2). The pathway started with ortho cleavage following by para and meta cleavage. The disappearance of 2,4,6-TCP occurs via 2,4-DCP, phenol, benzoate and hexanoic acid. This led to a proposed degradation pathway (Figure 3).

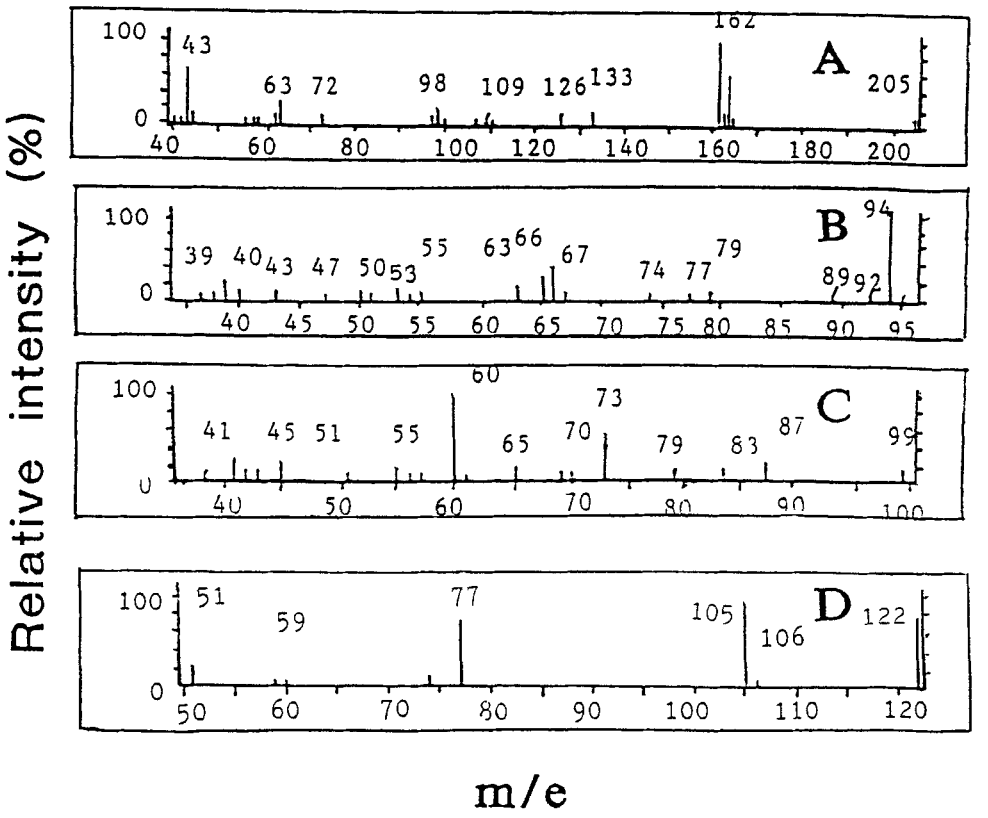

Figure 2 Mass spectra of authentic samples of 2, 4-DCP (A), phenol (B), hexanoic acid (C) and benzoate (D).

PCP Degradation and Identification of Metabolites

Reductive dechlorination of PCP at different concentrations is showed in Figure 4. Initially, the loss of PCP (50 μg ml^{-1}) occurred after a lag phase of 4 weeks, whereas 0.5 and 5 μg ml^{-1} of PCP were removed without a lag phase. PCP (at 5 and 50 μg ml^{-1}) was stoichiometrically converted to 3,5-DCP after 20 weeks of incubation. The 3,5-DCP produced during this time maintained its concentration and other products were below detection limit. PCP (at 0.5 μg ml^{-1}) was converted to 2,4,6-TCP. The 2,4,6-TCP produced during this time subsequently decreased in concentration, and a concomitant increase in 2,4-DCP could be detected. The disappearance of PCP and 2,4,6-TCP is assumed to be due to biodegradation, volatilization, and sorption on to microbial surfaces.

The intermediate products of PCP (at 0.5 μg ml^{-1}) degradation were further identified in a second experiment. In an extract of the culture supernatant, a new peak of 2,4-DCP and 2,4,6-TCP with retention times of 14.7 and 17.5 min were detected by GC-ECD. In a spiking experiment, the addition of an authentic sample of 2,4-DCP and 2,4,6-TCP to the extract resulted, as expected, in an increase in the peak height at the same retention times of 14.7 and 17.5 min in the gas chromatograph. The compounds were identified as 2,4-DCP, 2,4,6-TCP and phenol, benzoate and

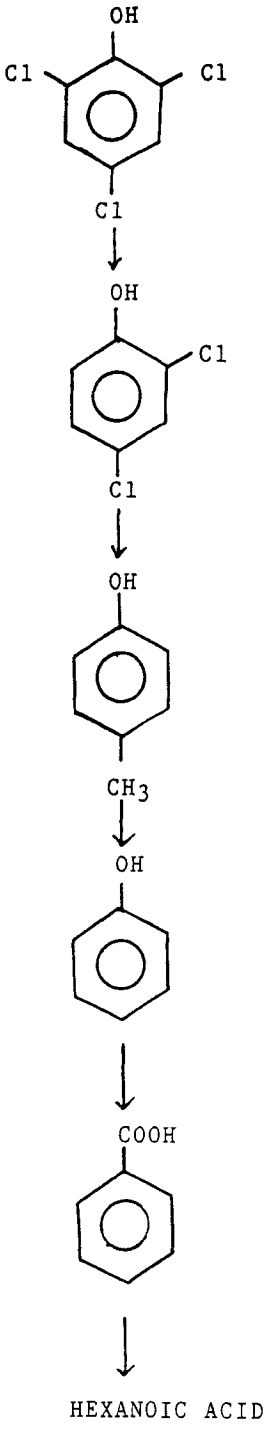

Figure 3 Proposed pathway for the degradation of 2,4,6-TCP.

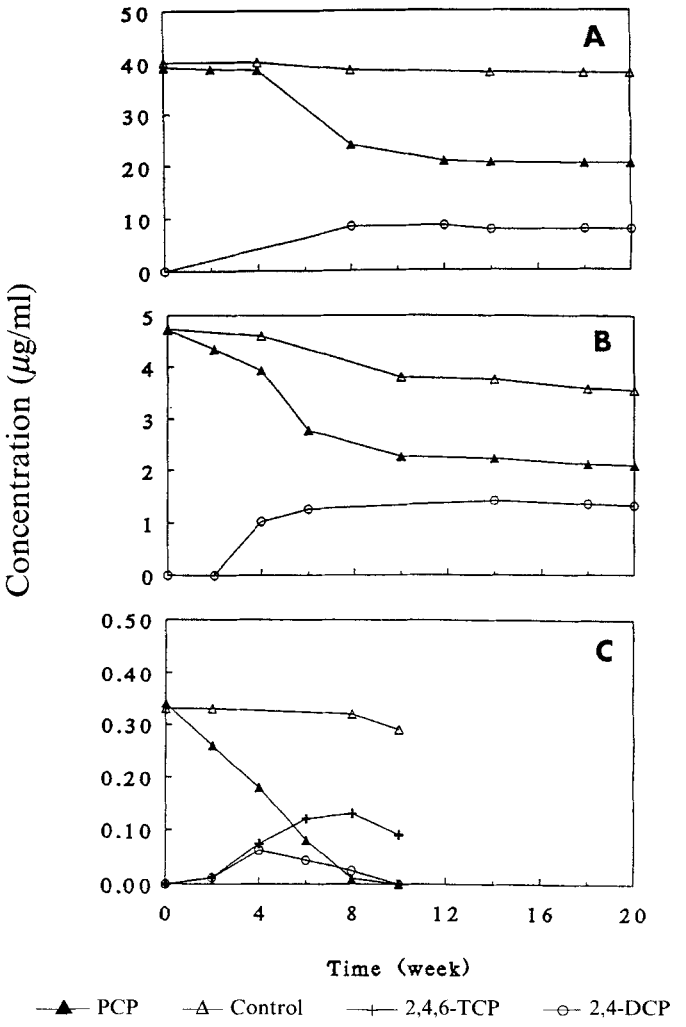

Figure 4 Reductive dechlorination of PCP at different concentrations. (A) 50 μg/ml; (B) 5 μg/ml; (C) 0.5 μg/ml.

hexanoic acid by GC/MS analysis. In an experiment similar to those described above, the intermediate products of PCP (5 and 50 μg ml^{-1}) degradation were identified as 3,4,5-TCP, 3,5-DCP, phenol, benzoate, and hexanoic acid. The 2,4-DCP adapted microbial communities initially removed the ortho-chlorine from PCP (at 5 and 50 μg ml^{-1}), whereas these communities removed the para-chlorine from PCP at lower concentration (0.5 μg ml^{-1}) (Figure 5).

Effect of Supplements on PCP and 2,4,6-TCP Degradation

The anaerobic degradation of PCP and 2,4,6-TCP (50 μg ml^{-1}) was tested with acetate, pyruvate and sulphate. The results showed that dechlorination of PCP and

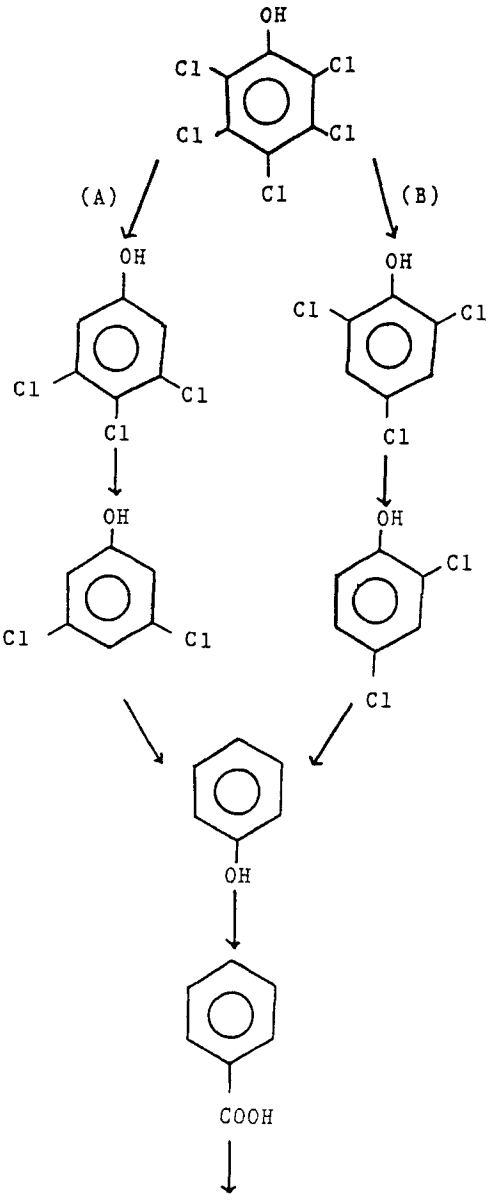

Figure 5 Proposed pathway for the degradation of PCP. (A) 5 and 50 μg/ml; (B) 0.5 μg/ml

2,4,6-TCP amended compounds with supplements occurred without a lag phase. The amendment had a significantly stimulating effect for PCP and 2,4,6-TCP within the first 8 weeks, but had an inhibiting effect for PCP after 8 weeks (Figure 6). Supplements, including acetate, pyruvate and sulphate, all had similar results.

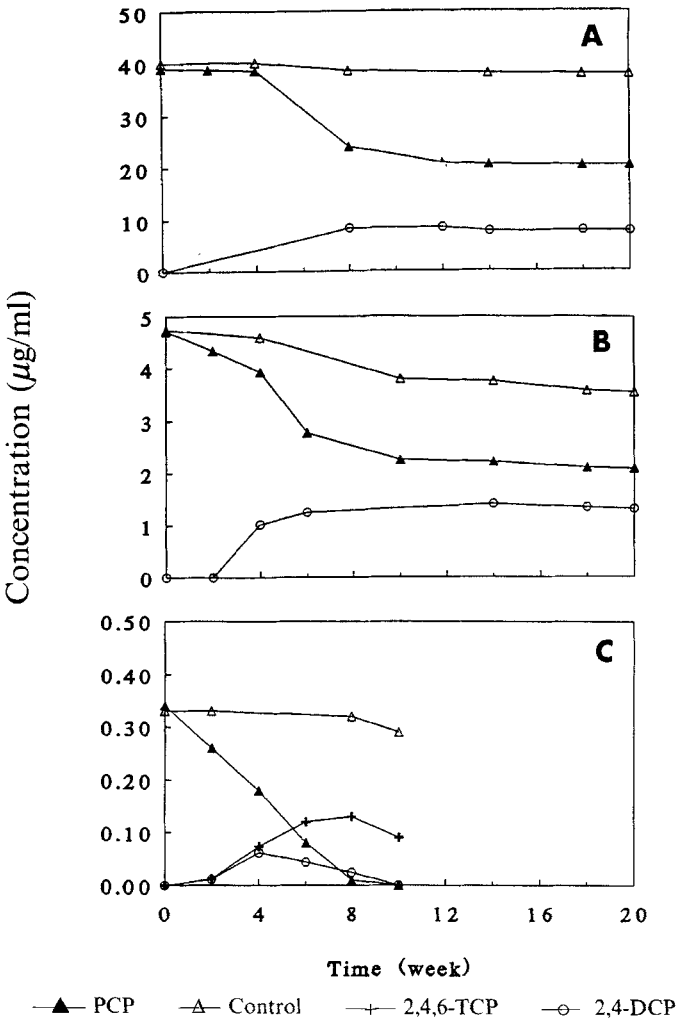

Figure 6 Effect of supplements on reductive dechlorination; acetate (A), pyruvate (B), sulphate (C).

DISCUSSION

From our experiments, we proposed that the degradation of PCP occurred via the pathway depicted in Figure 5. One pathway for dechlorination of PCP at concentrations of 5 and 50 $\mu g\ ml^{-1}$ proceeded through two ortho cleavage steps and then by a para cleavage. For PCP at a concentration of 0.5 $\mu g\ ml^{-1}$, the other pathway started with two meta cleavage steps and continued through an ortho cleavage. This indicates that more than a single microorganism is involved in the primary dechlorination at different concentrations during the two pathways above.

It is shown that the higher the concentration of chlorophenols, the slower is their degradation. Reduction dechlorinations of PCP with concentrations of 5 and 50 $\mu g\ ml^{-1}$ had a longer lag phase than that at a concentration of 0.5 $\mu g\ ml^{-1}$. After 18 weeks'

incubation, 2,4,6-TCP had disappeared completely and the residues of PCP were 0, 44.46, 51.96% at 0.5, 5 and 50 $\mu g\ ml^{-1}$, respectively (Figure 7). Complete degradation at 0.5 $\mu g\ ml^{-1}$ occurred within about 55 days without an initial lag phase. In our study, this may be too short a time for acclimation of microbial communities to degrade at higher concentrations.

The 2,4-DCP adapted communities initially removed the ortho-chlorine from PCP (5 and 50 $\mu g\ ml^{-1}$), with dechlorination following ortho > para > meta order of chlorine removal, whereas the 2,4-DCP adapted communities initially moved the para-chlorine from PCP at lower concentration (0.5 $\mu g\ ml^{-1}$). The communities vary in their regiospecificity of chlorine removal as well as in the range of chlorinated phenols that can be dechlorinated. The DCP-adapted communities were consistent with the findings of Bryant *et al.* (1991), initially removing the ortho-chlorine, then the para-chlorine from PCP. The products phenol and benzoate were as previously reported by Knoll and Winter (1989).

In this experiment, we used Na-thioglycollate as reducing agent; the reduction potential is about –200 mV, and the mixed communities might be sulphidogenic bacteria, such as *Desulphonema* sp., *Desulphobacter* sp., *Desulphobulbus* sp., *Desulphococcus* sp., and *Desulphonsarcina* sp. (Zinder, 1988). The addition of terminal electron donors such as acetate, pyruvate and sulphate stimulated dechlorination within 4 weeks. However, volatile organic compounds such as acetate, propinate, butyrate were accumulated and lead to feedback inhibition.

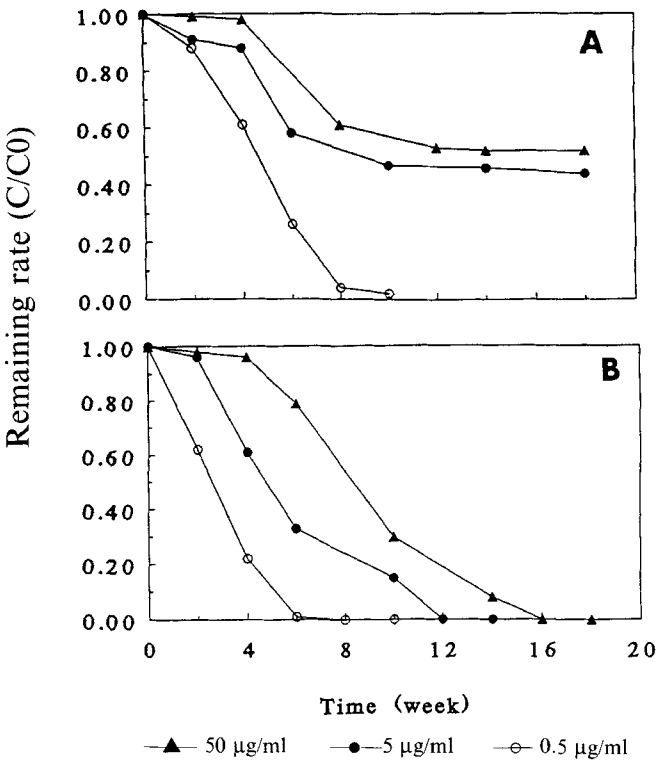

Figure 7 Comparison of reductive dechlorination of PCP (A) and TCP (B).

Acknowledgements

This work was supported by grants from the National Science Council, R.O.C. (NSC 81-0211-B031-05).

References

Bryant, F.O., Hale, D.D. and Rogers, J.E. (1991) Regiospecific dechlorination of pentachlorophenol by dichlorophenol-adapted microorganism in fresh anaerobic sediment slurries. *Appl. Environ. Microbiol.* **57**, 2293–2301.

Guthrie, M.A., Kirsch, E.J. Wudasch, R.F. and Grady, C.P.L. Jr. (1984) Pentachlorophenol biodegradation-II. *Water Res.* **18**, 451–461.

Haggblom, M.M., Nohynek, L.J. and Salkinoja-Salonen, M.S. (1988) Degradation and O-methylation of chlorinated phenolic compounds by *Rodococcus* and *Mycobacterium* strains. *Appl. Environ. Microbiol.* **54**, 3034–3052.

Holliger, C., Schraa, G., Stams, A.J.M., and Zehnder, A.J.B. (1992) Enrichment and properties of an anaerobic mixed culture reductively dechlorinating 1,2,3-trichlorobenzene to 1,3-dichlorobenzene. *Appl. Environ. Microbiol.* **58**, 1636–1644.

Katner, M. (1991) Reductive dechlorination of tri- and tetrachloroethylenes depends on transition from aerobic to anaerobic conditions. *Appl. Environ. Microbiol.* **57**, 2039–2046.

Keith, L.H., and Telliard, W.A. (1979) Priority pollutants. I.A perspective view. *Environ. Sci. Technol.* **13**, 416–423.

Knoll, G., and Winter, J. (1989) Degradation of phenol via carboxylation to benzoate by a defined, obligate syntrophic consortium of anaerobic bacteria. *Appl. Microbiol. Biotechnol.* **30**, 318–324.

Kohring, G.M., Zhang, X., and Wiegel, J. (1989) Anaerobic dechlorination of 2,4-dichlorophenol in freshwater sediments in the presence of sulfate. *Appl. Environ. Microbiol.* **55**, 2735–2737.

Kuhn, E.P., Townsend, G.T. and Sulflita, J.M. (1990) Effect of sulfate and organic carbon supplements on reductive dehalogenation chlorination of chloroanilines in anaerobic aquifer slurries. *Appl. Environ. Microbiol.* **56**, 2630–2637.

Larsen, S., Hendriksen, H.V., and Ahring, B.K. (1991) Potential for thermophilic (50∞C) anaerobic dechlorination of pentachlorophenol in different ecosystems. *Appl. Environ. Microbiol.* **57**, 2085–2090.

Mikesell, M.D., and Boyd, S.A. (1985) Reductive dechlorination of the pesticides 2,4-D, 2,4,5-T and pentachlorophenol in anaerobic sludges. *J. Environ. Qual.* **14**, 337–340.

Mikesell, M.D., and Boyd, S.A. (1986) Complete reductive dechlorination and mineralization of pentachlorophenol by anaerobic microorganisms. *Appl. Environ. Microbiol.* **52**, 861–865.

Mikesell, M.D., and Boyd, S.A. (1988) Enhancement of pentachlorophenol degradation soil through induced anaerobiosis and bioaugmention with anaerobic sewage sludge. *Environ. Sci. Technol.* **22**, 1411–1414.

Saber, D.L., and Crawford, R.L. (1985) Isolation and characterization of *Flavobacterium* strains that degrade pentachlorophenol. *Appl. Environ. Microbiol.* **50**, 1512–1518.

Stanlake, G.J., and Finn, R.K. (1982) Isolation and characterization of a pentachlorophenol-degrading bacterium. *Appl. Environ. Microbiol.* **44**, 1421–1427.

Topp, E., Crawford, R.L. and Hanson, R.S. (1988) Influence of readily metabolizable carbon on pentachlorophenol metabolism by pentachlorophenol-degrading *Flavobacterium* sp. *Appl. Environ. Microbiol.* **54**, 2452–2459.

Valo, R., and Salkinoja-Salonen, M. (1986) Bioreclamation of chlorophenol-contaminated soil by composting. *Appl. Microbiol. Biotechnol.* **25**, 68–75.

Yokoyama, M.T., Johnson, K.A., and Gierzak, J. (1988) Sensitivity of ruminal microorganisms to pentachlorophenol. *Appl. Environ. Microbiol.* **54**, 2619–2624.

Yuan, S.Y., Jaung, W.Z., Pan, T.M., and Chang, B.V. (1991) Fates of chlorophenols in the environment. *Chemistry* **49**, 316–322.

Zinder, A.J.B. (1988) Biology anaerobic microorganisms. In: *Microbiology and Ecology of Sulfur-reducing Bacteria*. Ed. by Friedrich, John Wiley and Sons, New York, pp. 469–586.