

Simultaneous anaerobic-aerobic biodegradation of halogenated phenolic compound under oxygen-limited conditions

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Abstract: The successful application of co-immobilized aerobic-anaerobic biomass under limited aeration in wastewater treatment systems would eliminate the problems associated with the intermediates mono-chlorophenol(MCP) and di-chlorophenol(DCP) accumulations. With low initial pentachlorophenol(PCP) concentration, all PCP could be completely removed under oxygen-limited strict anaerobic conditions, and the removal efficiencies with different initial headspace oxygen percentage(IHOP) were not obviously different from each other. While at high initial PCP concentration, under strictly anaerobic conditions PCP and their intermediates were clearly higher than that under other conditions, and produced obvious accumulation, the highest PCP reduction was achieved by the system receiving 30 IHOP, oxygen-limited system also exhibited lower residual TOC concentration and lower concentration of metabolic intermediates MCP and DCP. These results suggested that under strictly anaerobic condition the reductive dechlorination of low chlorinated compounds became rate limiting in the reductive dechlorination pathway, less chlorinated compounds be more amenable to aerobic degradation, and the aerobes of outer layers could function under limited oxygen. The co-immobilized aerobic-anaerobic biomass for methanogenesis under limited-aeration for chlorophenol degradation might be an attractive and efficient alternative for the sequential anaerobic/aerobic system to achieve mineralization of a broad range of recalcitrance highly chlorinated organics and low final TOC concentrations.

Keywords: PCP; oxygen-limited; immobilized cell

Introduction

Chlorinated phenolic compounds are widely used in many industries including petrochemical, oil refinery, plastic, pulp and insulation material. These compounds have become of environmental concern due to their acute toxicity and resistance to degradation. The recalcitrance of chlorophenols due to the carbon-halogen bond, which is cleaved with great difficulty and the stability of their aromatic structure, is resulting in their accumulation in nature. Mono- and di-chlorophenols could be easily metabolized by aerobic microorganisms, but the rate of reductive dechlorination of chlorophenols under anaerobic conditions is more effective for heavier chlorinated compounds, whereas progressively slower as the chlorophenol molecules become more dechlorinated. A possible strategy to degrade higher chlorinated phenols is to use a combined process in which the target substance were first attacked anaerobically to reductively dechlorinate them to the point where they can be effectively mineralized aerobically in a subsequent step(Chen, 2004).

In conventional biological systems anaerobic and aerobic conditions are separated, or temporarily separated in phases in sequencing batch reactors(Atuanya, 2000). It is a good strategy that both reductive and oxidative biotransformations might occur concomitantly to complete mineralization highly substituted compound under micro-aeration. Under oxygen-limited conditions, methanogenesis and oxygen reduction with sucrose as the primary substrate was achieved concurrently in a single mixed culture maintained without pre-culture precaution using digester and activated sludge as inoculums, methanogenesis was sustainable in oxygen-limited cultures(Zitomer, 1998). Even though dissolved oxygen was present in the bulk liquid at all times, methane was still produced and methanogenic activity could be significant. Due to the formation of reduced micro-niches in immobilized calcium alginate beads, anaerobic transformation of PCE into lower chlorinated compounds was coupled with aerobic

dechlorination of these intermediates in oxygen-limited condition was also documented(Tartakovsky, 1998; 2003). Instead of calcium alginate beads, anaerobic granular sludge could be also used as a carrier material for both anaerobic and aerobic microniches(Tartakovsky, 2003; Chen, 2003).

The objective of this investigation was to study in detail a concomitant methanogenesis and oxygen reduction process under oxygen-limited condition to completely degrade pentachlorophenol(PCP), chosen here as the model target compound.

1 Materials and methods

1.1 Chemicals

PCP, 2,4,6-TCP, 2,4-DCP and 4-CP were purchased from Geel, Belgium (New Jersey, UAS), all the other chemicals were of analytical grade.

1.2 Analytical methods

The volatile suspended solids(VSS) were determined as described in Standard Methods(APHA, 1995); the inorganic chloride content released was measured following a mercuric thiocyanate method(APHA, 1995); total organic carbon(TOC) was analyzed on filtered samples(0.45 μm) with a Model 100 Benchtop TOC Analyzer using the combustion/non-dispersive infrared gas analysis method.

Chlorophenol were analyzed using a gas chromatograph, coupled to an electron capture detector(Autosystem XL, Perkin-Elmer). 3 ml of sample was taken and immediately acidified to pH 2 with 6 mol/L H_2SO_4 to quench the biodegradation reaction. The acidified samples were extracted with dichloromethane, the extracts were injected into a DB-5 capillary column(15 m \times 0.32 mm i. d.)(J&W Scientific, Folsom, CA). The temperature program was started at 80 $^\circ\text{C}$ for 1 min, then ramped at 20 $^\circ\text{C}/\text{min}$ to 150 $^\circ\text{C}$, maintained at 150 $^\circ\text{C}$ for 2 min, then increased to 300 $^\circ\text{C}$ at 5 $^\circ\text{C}/\text{min}$. The injector temperature was 280 $^\circ\text{C}$, the detector temperature was 350 $^\circ\text{C}$ and the carrier gas was nitrogen. Injections of pure chlorophenol were used to identify degradation intermediates.

1.3 Cell immobilization

Aerobic bacteria were immobilized onto granular sludge is given elsewhere (Chen, 2003), microbial cells entrapped in the bead were then activated in inorganic media containing a mixed solution of 5 mg/L 4-CP, 2, 3-DCP (dichlorophenol), 2, 4-DCP and 2, 4, 6-TCP (trichlorophenol).

1.4 Serum bottle studies

The assay medium was prepared by adding 250 ml serum bottles with 75 ml of coimmobilized beads containing entrapped microbial cells, PCP, nutrient stock solution, which was composed of acetic acid of 2 g/L, propionic acid of 0.5 g/L and butyric acid of 0.5 g/L, and basal medium to complete a final volume of 150 ml. The pH was adjusted to 7.0 and nitrogen gas was bubbled up into each bottle and sealed with rubber septa in order to remove air from the head space. Pure oxygen was added by first removing the gas from the bottle and replacing it with the same amount of oxygen using syringes fitted with needles to arrange an initial headspace oxygen percentage (IHOP), excess headspace gas was wasted daily using a 100 ml glass syringe with a wetted barrel. Samples were collected at a fixed interval and the bottles were placed in a shaker bath, microorganisms were

acclimated for about 30 d before the data were collected. All culture conditions were maintained on a shaker table.

The basal medium contained the following in tap water: $\text{Fe}(\text{NH}_4)_2 \cdot 6\text{H}_2\text{O}$ (2 mg/L); $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (4 mg/L); $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (4 mg/L); $(\text{NH}_4)_2 \text{HPO}_4$ (60 mg/L); $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (60 mg/L) and Na_2HCO_3 (4000 mg/L).

In addition to oxygen-limited reactors, a strictly anaerobic and a blank control system was operated in parallel for comparison. All assays were performed in triplicate.

2 Results and discussion

2.1 Decomposition of low and high initial PCP concentration

Three oxygen-limited condition (10, 30, 50 IHOP), an aerobic condition (100 IHOP), a strictly anaerobic condition (0 IHOP) and a blank control (containing autoclaved immobilized bead) serum bottle cultures were operated at low and high initial PCP concentration (28 and 87 $\mu\text{mol/L}$). The removal of PCP and their intermediates metabolites by immobilized beads in five IHOP conditions are shown in Fig. 1 and 2 respectively, and all the results were the average of three parallel batch experiment.

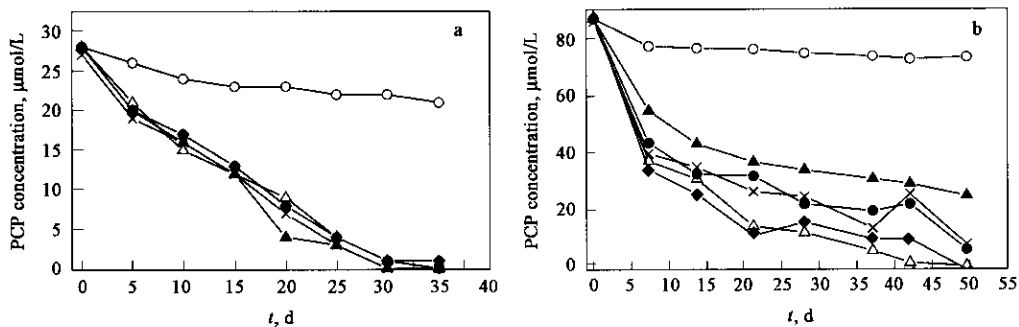


Fig. 1 PCP versus time at initial 28 $\mu\text{mol/L}$ (a) and 87 $\mu\text{mol/L}$ (b) with various IHOP
 -○- blank; -▲- 0 IHOP; -●- 10 IHOP; -△- 30 IHOP; -◆- 50 IHOP; -x- 100 IHOP

Fig. 1 is time courses of PCP exposed to different IHOP at 28 (a) and 87 (b) $\mu\text{mol/L}$ PCP. As shown in Fig. 1, biodegradation began immediately by all systems and no lag phase was observed, irrespective of the initial oxygen and PCP concentration. At low initial PCP concentration (Fig. 1a), the removal efficiencies with different IHOP were not obvious different from each other, and all PCP had been transformed. While at high initial PCP concentration (Fig. 1b), the residual PCP concentrations were relatively high, the highest PCP reduction was achieved by the system receiving 30 IHOP and 50 IHOP. The results obtained with

blanks containing dead bacterial mass showed that adsorption as well as evaporation of PCP affected the experimental results.

The intermediates of initial 28 and 87 $\mu\text{mol/L}$ PCP after 34 and 49 d batch assay are shown in Fig. 2 that unable to detect any TCPs in all batch assay.

At low initial PCP concentration (28 $\mu\text{mol/L}$ PCP, Fig. 2a), the removal efficiencies with different IHOP were not obviously different from each other, all PCP had been transformed at the day 34, we only detect intermediates MCP (mono-chlorophenol) and DCP (di-chlorophenol) in all batch

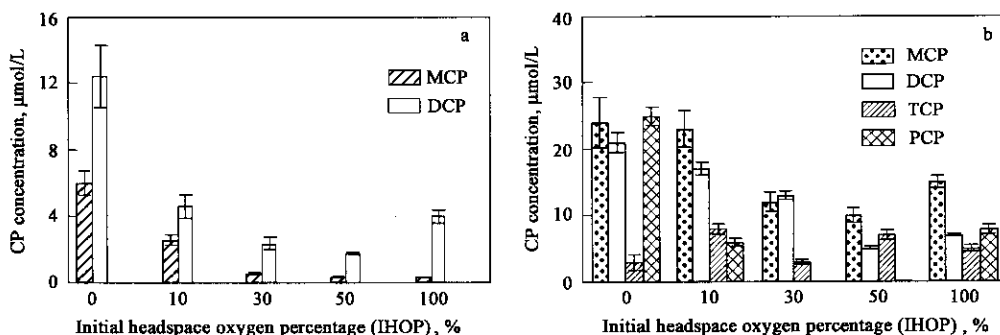


Fig. 2 PCP intermediates metabolites distribution at initial PCP of 28 $\mu\text{mol/L}$ (a) and 87 $\mu\text{mol/L}$ (b) with various IHOP

assay, but their concentrations were different at various IHOP. The intermediates concentration decreased with IHOP, and reached lowest at 50 IHOP, then increased thereafter.

At high initial PCP concentration (Fig. 2b), the strictly anaerobic system (0 IHOP) intermediates and PCP were clearly higher than that under other conditions, MCP and DCP were the major intermediates, and produced obvious accumulation, while in the case of oxygen limited system (30, 50 IHOP), PCP were nearly completely removed, MCP and DCP were less than strictly anaerobic system. These results suggested that in strictly anaerobic condition that reductive dechlorination of low chlorinated compounds became rate limiting in the reductive dechlorination pathway, less chlorinated compounds be more amenable to aerobic degradation, and the aerobes of outer layers could function under limited oxygen.

The intermediates MCP and DCP might exert substrate toxicity on oxygenase expressing cultures (cultures which produce oxygenase enzymes), would not only inhibit oxidation of the cell's primary substrate, but also its own degradation. However in oxygen-limited condition, the high dechlorination efficiency might be due to the co-metabolism MCP and DCP by aerobic degradation bacteria.

2.2 TOC removal

Fig. 3 plots the time profiles for medium TOC at initial 87 $\mu\text{mol/L}$ PCP concentration. The oxygen-limited system exhibited lower residual TOC concentration, whereas the anaerobic reactor exhibited a little higher residual TOC concentration. The lower residual TOC concentrations in oxygen-limited condition probably in part due to the lower concentrations of intermediates in oxygen limited condition compared with strictly anaerobic condition, which most likely reduces degradation rates of carbon source as well as itself, it was also possible that carbon source such as propionic acid, acetic acid might had been oxidized directly by aerobic or micro-aerophilic processes, aerobic oxidation of carbon source such as propionic acid, acetic acid, and hydrogen occurred. The possibility of lower concentrations of intermediates (such as MCP, DCP) in the more toxic environments might also explain the relatively high activity of methanogens in cultures that received the most oxygen. Elevated concentrations of these intermediates might potentially inhibit methane production.

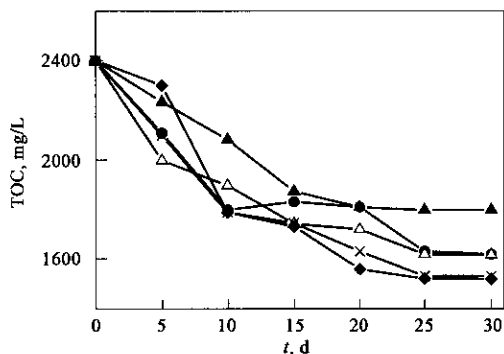


Fig. 3 TOC versus time in various oxygen concentration conditions with initial 87 $\mu\text{mol/L}$ PCP

The symbols were same as Fig. 1

From TOC and PCP degradation, we could also see that simultaneous utilization of the toxic substrate PCP and carbon source.

3 Conclusions

The successful application of co-immobilized aerobic-anaerobic biomass under limited aeration in wastewater treatment systems could eliminate the problems associated with the intermediates MCP and DCP accumulations. Under low initial PCP concentration, all PCP could be completely removed by oxygen-limited and strictly anaerobic conditions, and the removal efficiencies with different IHOP were not obviously different from each other.

While at high initial PCP concentration, the residual PCP concentrations and their intermediates were correlated highly with the IHOP, MCP and DCP were the major intermediates, under strictly anaerobic conditions PCP and their intermediates were clearly higher than that under other condition, and produced obvious accumulation, the highest PCP reduction was achieved by the system receiving 30 IHOP.

Oxygen-limited system exhibited lower residual TOC concentration and metabolic intermediates MCP and DCP. The lower residual TOC concentrations in oxygen-limited condition probably in part due to the lower concentrations of intermediates in oxygen limited condition compared with strictly anaerobic condition, which most likely reduces degradation rates of carbon source as well as itself, it was also possible that carbon source such as propionic acid, acetic acid might had been oxidized directly by aerobic or micro-aerophilic processes, aerobic oxidation of carbon source such as propionic acid, acetic acid, and hydrogen occurred.

All these results indicated that the co-immobilized aerobic-anaerobic biomass for methanogenesis under limited-aeration for chlorophenol degradation might be an attractive efficient alternative for the sequential anaerobic/aerobic system to achieve mineralization of a broad range of recalcitrance highly chlorinated organics and low final TOC concentrations.

References:

- Atuanya E I, Purohit H J, Chakrabarti T, 2000. Anaerobic and aerobic biodegradation of chlorophenols using UASB and ASC bioreactors[J]. *World Journal of Microbiology and Biotechnology*, 16: 95–98.
- APHA, AWWA, WEF, 1995. Standard methods for the examination of water and wastewater [M]. 19th ed. Washington, DC: American Public Health Association.
- Chen G, 2004. Reductive dehalogenation of tetrachloroethylene by microorganisms: current knowledge and application strategies [J]. *Appl Microbiol Biotechnol*, 63: 373–377.
- Chen Y, Zhan H, Chen Z, 2003. Coupled anaerobic/aerobic biodegradation of halogenated phenolic compounds[J]. *Journal of Environmental Science*, 15 (4): 469–474.
- Tartakovsky B, Manuel M F, Guiot S R, 2003. Trichloroethylene degradation in a coupled anaerobic aerobic reactor oxygenated using hydrogen peroxide [J]. *Environ Sci Technol*, 37: 5823–5828.
- Tartakovsky B, Miquez C B, Petti L *et al.*, 1998. Tetrachloroethylene dechlorination using a consortium of co-immobilized methanogenic and methanotrophic bacteria[J]. *Enz Micro-biol Technol*, 22: 255–260.
- Zitomer D H, Shrout J D, 1998. Feasibility and benefits of methanogenesis under oxygen-limited conditions[J]. *Waste Management*, 18: 107–116.

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