pH dependency of 2,4-chlorophenol dechlorination by acclimated anaerobic granules

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Abstract

The pH dependency of anaerobic dechlorination of 2,4-dichlorophenol by acclimated anaerobic granules was investigated. The results showed that the dechlorination could be performed by these granules at a wide pH range of 6.8 to 7.9, but dechlorination rate increased as pH value went up. The data analysis considering the effect of sorption indicated that the increase of the dechlorination rate was due to pH dependence of metabolic activity of the acclimated anaerobic granules.

Introduction

Reductive dechlorination is considered the initial step in anaerobic biodegradation of chlorinated phenols. Although the dechlorination mechanism and pathway have been extensively investigated, little attention has been paid to the pH dependency of this biodegradation process. Information on the effect of pH on reductive dechlorination was diverse, and sometimes conflicting. Mohn and Kennedy (1992) reported the reductive dechlorination of 2,3,5-trichlorophenol by a dechlorinating bacterial strain, *Desulfomonile tiedje*, at a media pH of 7.5. A *Clostridium*-like endospore forming bacterium was identified to be involved in anaerobic dechlorination at a media pH of 7.0 to 7.1 (Madsen and Aamand, 1992). Zhang and Wiegel (1990) successfully used a pH 8.5 buffer in their study on sequential anaerobic degradation of 2,4-dichlorophenol (2,4-DCP) in freshwater sediments. Armenante et al. (1993) and Togna et al. (1995) observed the rapid dehalogenation of 2,4,6-trichlorophenol at alkaline pH (8.0 to 9.0) by an unidentified anaerobic enrichment culture. Recently, Kennes et al. (1996) reported that dechlorination of 2,4,6-trichlorophenol was achieved by methanogenic PCP-degrading granules at pH 7.0 to 7.2. A strictly anaerobic bacterium that can grow at an optimum pH 7.2 to 7.8 by reductive dechlorination of ortho-chlorinated phenols was also reported (Gerritse et al., 1996). Comparatively, information on pH dependency of dechlorination in anaerobic reactors is less available. In anaerobic treatment of a simulated high-strength industrial waste water containing acetate, phenol, and 2-chlorophenol (2-CP), Suidan et al (1996) indicated that the effluent phenol and 2-CP concentration decreased slightly when pH was raised from 7.0 to 7.5. Anaerobic dechlorination of pentachlorophenol (PCP) at pH 7.0 to 7.5 in upflow anaerobic sludge blanket (UASB) reactors was observed by Hendriksen et al. (1992). An earlier study also indicated that reductive dechlorination of CPs proceeded well at the normal range of pH (7.0 to 7.5) in anaerobic bioreactors (Woods, 1985).

In this study, pH dependency of anaerobic dechlorination of 2,4-DCP by acclimated anaerobic granules was investigated to better understand the pH dependence. 2,4-DCP was used because it is a major intermediate product of highly chlorinated phenols and other phenolic compounds in anaerobic dechlorination, while anaerobic granules are representative of the immobilised anaerobic organisms in UASB reactors.

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Materials and methods

Acclimated anaerobic granules

The 2,4-DCP acclimated anaerobic granules were cultivated in a laboratory-scale continuous UASB reactor with an effective volume of 5.5 ℓ (Ning, 1997). The seed anaerobic sludge was obtained from Lake Utopia Paper Ltd., Canada. This sludge was used to treat a chemi-thermal mechanical pulp (CTMP) waste water from hydrogen peroxide bleaching processes and had never been acclimated or exposed to waste water containing chlorinated phenols. The average granular size and the VSS/SS ratio of this sludge were 0.5 ± 0.3 mm and 0.908 ± 0.001, respectively. The feed waste water for the reactor is given in Table 1. The operating temperature of the reactors was controlled at 35 ± 2°C and pH in the effluent varied between 7.06 and 7.30 during acclimation. The hydraulic retention time (HRT) and specific organic loading rate (SOLR) for the reactor were maintained at 16 h and 0.45 g COD/g VSS·d, respectively. Before the sludge was harvested to be used in batch experiments, the UASB reactor had been continuously operated for 9 months. The physical properties for the granular sludge were: average diameter, 2.1 ± 0.6 mm; VSS/SS ratio, 0.940 ± 0.001.

TABLE 1
COMPOSITION OF FEED FOR THE UASB REACTOR

Substances	Concentration (g/ℓ)
Sucrose	3.00
Acetic acid	3.00
NH_4HCO_3	1.20
$NaHCO_3$	3.00
$KHCO_3$	3.75
$(NH_4)_2SO_4$	0.30
K_2HPO_4	0.12
KH_2PO_4	0.16
Yeast abstract	0.06
2,4-DCP	0.01

2,4-DCP

The 2,4-DCP used in this investigation was from Aldrich Chemicals with 99% purity. The solution was made up in 0.01 M NaOH with distilled/deionised Milli-Q water, adjusted to pH 7.5 using H_2SO_4 and stored in the dark at 2~4°C before use.

Batch dechlorination tests

Dechlorination of 2,4-DCP was conducted in batch tests using 60-mℓ serum bottles. Sample preparation and dechlorination test procedures are as follows:

Preparation of sample in serum bottles. Components in prepared samples for all experiments consisted of anaerobic sludge granules (14.7 g VSS/ℓ), dilution solution, phosphate buffer (0.06 M), Na_2S (100 mg/ℓ); resazurin (1.0 mg/ℓ) and 2,4-DCP (30, 60 and 90 mg/ℓ). Three phosphate buffer solutions, with pHs at 6.5, 7.5 and 8.1, respectively, were used. The dilution solution used contained the following components (per liter): NH_4Cl, 0.5 g; $MgCl_2$, 0.1 g; $CaCl_2$, 0.1 g; $KHCO_3$, 3.0 g; $FeCl_2 \cdot 4H_2O$, 1.5 mg; $CoCl_2 \cdot 6H_2O$, 10.0 mg; $MnCl_2 \cdot 4H_2O$, 0.03 mg; $NiSO_4 \cdot 6H_2O$, 0.03 mg; $(NH_4)_6MO_7O_{24} \cdot 4H_2O$, 2.0 mg; H_3BO_3, 0.3 mg; $ZnSO_4 \cdot 7HO_2$, 0.1 mg; $AlK(SO_4)_2 \cdot 12H_2O$, 0.1 mg. Dilution solution pH was adjusted to a specific pH for the tests using 0.1M H_2SO_4 before use. Since anaerobic granules were too large in size to be quantitatively transferred by pipette into serum bottles, filtered granules without supernatant were added into serum bottles based on wet weight. Procedures for preparation of the serum bottles are as follows:

- add filtered granules into serum bottle and purge head space in serum bottle with nitrogen gas 1 min;
- add deoxygenated dilution water, phosphate buffer solution;
- bubble the solution in serum bottle with nitrogen gas 1 min;
- add sodium sulfide and resazurin solution, and cap the bottle with rubber cap and aluminum seal when purging head space of serum bottle with nitrogen gas;
- agitate the serum bottles for 72 h at 100 r/min and 35 ± 1°C to ensure complete oxygen depletion.

All samples were prepared in duplicate or triplicate. Control bottles were also prepared according to the above steps, except without addition of anaerobic biomass (blank control) or without 2,4-DCP solution to be injected (positive control). It was observed during experiments that the reduced conditions (mixed liquor colorless) could be achieved within 30 min after the serum bottles were agitated.

Degradation tests. At the initial time of the batch degradation tests, deoxygenated 2,4-DCP solution was injected into serum bottles. Serum bottles were then agitated at 100 r/min and 35± 1°C. 1 mℓ samples were taken from serum bottles at time intervals of about 1, 3, 9, 21, and 30 h for analyses of 2,4-DCP and 4-monochlorophenol (4-MCP). The final pH in the serum bottles was also measured. The degradation tests showed that 4-MCP was the major product of 2,4-DCP dechlorination (Ning, 1997).

Analytical methods

2,4-DCP and 4-MCP. Chlorophenol concentrations were determined using a Hewlett Packard (model 1090) high performance liquid chromatograph (HPLC) with a Hypersil-ODS C18 column. The wavelength of the diode array detector was set at 280 nm. The column was maintained at 40°C and the flow rate of the mobile phase was set at 0.3 mℓ/min. For the mobile phase, a mixture of HPLC grade methanol (60%) and 0.05M sodium acetate (40%) at pH 4.7 was applied. Accuracy limits for 2,4-DCP and 4-MCP in the measurements were 0.5 mg/ℓ.

Suspended solids (SS), volatile suspended solids (VSS), and pH. The SS and VSS of samples were performed according to *Standard Methods*, 1989). The pH of samples was determined with a Fisher Scientific Accumet pH meter.

Results and analyses

The experimental results are presented in Fig. 1. The final pH values measured in the serum bottles for the three initial pHs (6.5, 7.5 and 8.1) were 6.79 ± 0.05, 7.52 ± 0.14 and 7.90 ± 0.15, respectively. Note that the pH values indicated in the figure are the final pH and are rounded up to one decimal. The removal of 2,4-DCP in the liquid phase of the media was shown to be a sorption-dechlorination coupled process since the 2,4-DCP concentration in the liquid phase in the bottle decreased significantly in the first 3 h but production of 4-MCP was only about 1 mg/ℓ (Fig. 2). From Fig. 1 and 2, it can be seen that, in the first 3 h reaction time, sorption dominates the removal, and after 3 to 9 h, dechlorination dominates. From the slopes of the process curves after 3 to 9 h for each initial 2,4-DCP dosage condition in the two figures, it appears that the 2,4-DCP dechlorination rate increased as the reaction media pH increased.

Since 2,4-DCP is ionisable (pKa = 7.89), both sorption and dechlorination of chlorophenols by anaerobic granules are pH dependent and highly correlated. Figure 1 shows that overall removal of 2,4-DCP from the liquid phase in the batch dechlorination tests depends on both sorption and dechlorination. For the initial 2,4-DCP dosages of 60 and 90 mg/ℓ, the highest removal occurred at pH 6.8 due to the higher sorption capacity at the lower pH condition, although the dechlorination rate became slower at that pH (indicated by the slope of the process curves after 3 to 9 h).

However, it was observed that, for the same initial dose, 2,4-DCP concentration in the liquid phase was higher when the media pH was higher, which is because the ionisation of 2,4-DCP increases as pH increases according to pKa, and the 2,4-DCP phenolate ion has a lower sorption tendency than its molecular form. From Fig. 1, it is not clear whether the high dechlorination rate under the high pH condition was due to the increased dechlorination activity of the anaerobic granules, or due to the relatively high concentration of 2,4-DCP in the liquid phase.

Previous investigations indicated that sorption of 2,4-DCP to anaerobic granules could reach equilibrium conditions at 3 to 5 h, and after attaining sorption equilibrium developed, sorption followed linear sorption isotherm (Kennedy et al., 1992; Ning, 1997). Using this sorption linearity that is valid only after the sorption equilibrium has been achieved, the dechlorination rate can be determined using the differential equation below:

$$\bar{r}_{DCP} = \frac{(C_{DCP,1} - C_{DCP,2})\,(1 + K_{d,DCP}X)}{(t_2 - t_1)X} \qquad (1)$$

where:

$\bar{r}_{DCP}$ is the average degradation rate (mg/g VSS/h)

C_{DCP} is the measured 2,4-DCP concentration in the liquid phase that includes both ionised and unionised forms of 2,4-

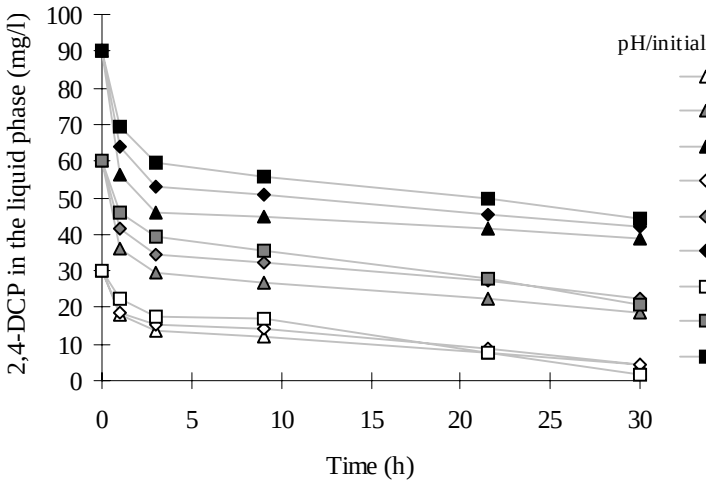

Figure 1
Effect of pH on 2,4-DCP removal under different initial dosage conditions.

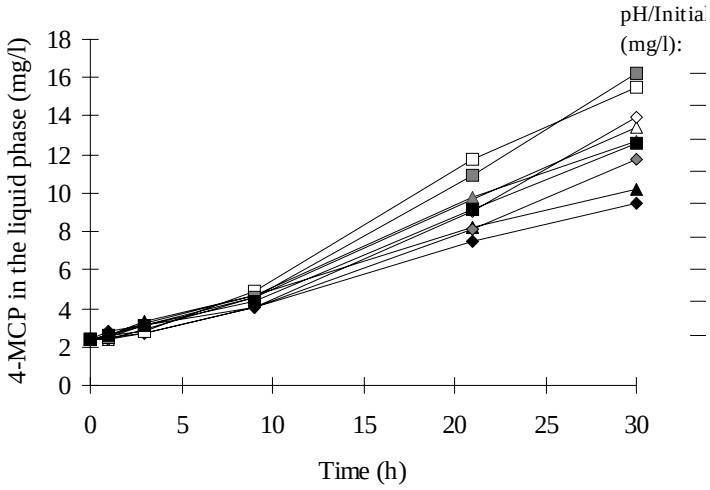

Figure 2
Effect of pH on 4-MCP production under different initial dosage conditions

DCP (mg/ℓ)
X is the granule concentration in reaction media (g VSS/ℓ)
t is reaction time (h). By using Eq. (1), the real dechlorination rate is determined.

Before using Eq. (1) to determine the dechlorination rate, the sorption coefficient values, $K_{d,DCP}$, at the experimental pH have to be defined. It was observed in the tests that only about 1.0 mg/ℓ of 4-MCP appeared in the liquid phase at the reaction time of 3 h at which the sorption had reached equilibrium (Fig. 2). Since the 2,4-DCP loss due to dechlorination at 3 h is negligible compared with sorption (< 4% on average), the data for 2,4-DCP concentrations in the liquid phase at 3 h were used to determine the values of $K_{d,DCP}$. The values determined for $K_{d,DCP}$ are 0.067, 0.059 and 0.049 ℓ/g VSS at the media pH of 6.8, 7.5 and 7.9, respectively.

Consequently, the relations between the dechlorination rates and 2,4-DCP concentrations in the liquid phase under the three pH conditions are obtained (Fig. 3). Data in Fig. 3 show that dechlorination metabolic activity of the acclimated anaerobic granules is pH dependent. The Haldane type degradation kinetics is used to fit the data in Fig. 3.

$$r_{DCP} = \frac{1}{X}\frac{dC_{DCP}}{dt} = -\frac{kC_{DCP}}{K_s + C_{DCP} + C^2_{DCP}/K_I} \qquad (2)$$

where k, K_s and K_I are the maximum specific dechlorination rate, saturation constant and inhibition constant. The model fitting results indicate that the maximum specific dechlorination rates at the media pH of 6.8, 7.5 and 7.9 are 0.07, 0.08 and 0.11 mg DCP/g VSS/h, respectively.

Discussion

pH dependency of 2,4-DCP dechlorination by acclimated anaerobic granules enriched with sucrose/acetic acid-based waste water was observed in this study. The results show that microbial dechlorination can be performed by these granules at a wide pH range of 6.8 to7.9, but the rate of dechlorination increased as the pH value increased. This observation could be useful for the operation of anaerobic reactors treating chlorophenol containing waste water.

According to the variation of pH dependency of dechlorination observed in this study and reported in other investigations, it seems reasonable to consider that organisms involved in reductive dehalogenation are quite diverse and different strains, and each has a different pH preference, although only a few of the strains have been isolated. As a result, the pH dependency in dechlorination for a specific anaerobic reactor may vary from system to system. However, the pH dependency of dechlorination remains important in system operation.

Since the removal of chlorinated phenols in anaerobic reactors is related to sorption and degradation, the effect of pH on both sorption and degradation needs to be considered in optimising

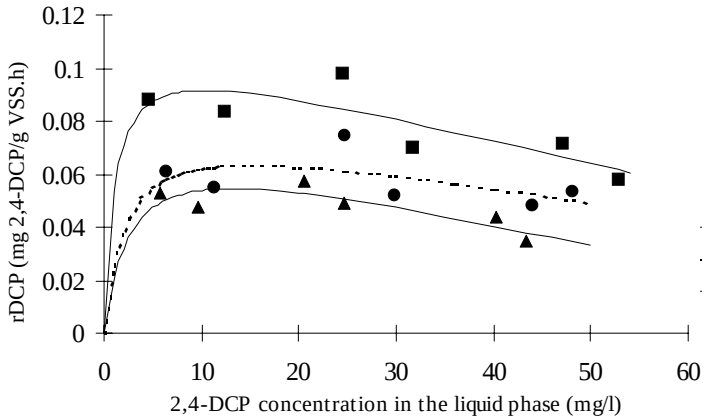

Figure 3
Effect of pH on 2,4-DCP dechlorination rate

system operation. As indicated in Fig. 1, sorption could have a significant influence on removal when the initial 2,4-DCP concentration is high. For a continuous anaerobic reactor, the effect of pH on dechlorination is more critical since direct removal of chlorophenols by sorption in the system is not so significant as in batch tests shown in this study.

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