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Influence of quaternary ammonium compounds on the microbial reductive dechlorination of pentachloroaniline

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ABSTRACT

The inhibitory effect of two widely used quaternary ammonium compounds (QACs) – alkyl benzyl dimethyl (AB) and hexadecyl trimethyl (HD) ammonium chloride – on fermentation, methanogenesis and pentachloroaniline (PCA) dechlorination was assessed using a mixed, methanogenic, PCA-dechlorinating culture amended with AB or HD at a concentration range from 5 to 70 μM. PCA dechlorination was inhibited at 5 μM AB and was completely inhibited at 25 or 5 μM by AB or HD, respectively. However, the PCA dechlorination pathway was the same in both the QACs-free and QACs-amended culture series. Fermentation (acidogenesis) and methanogenesis were inhibited by both AB and HD at and above 25 μM but to a lesser degree than PCA dechlorination. Overall, HD resulted in a more severe inhibition of the mixed culture than AB. Adsorption of both QACs to the mixed culture biomass followed the Freundlich isotherm model. The adsorption affinity of HD for the mixed culture biomass was significantly higher than that of AB, which may be related to the observed higher inhibitory effects of HD compared to AB. Both AB and HD were not degraded in the mixed, dechlorinating culture used in this study.

1. Introduction

Pentachloroaniline (PCA) is the main anoxic/anaerobic (bio) transformation product of pentachloronitrobenzene (PCNB), a widely used fungicide all over the world (Klupinski et al., 2004; Okutman Tas and Pavlostathis, 2005, 2007, 2008; Okutman Tas et al., 2006). PCNB and PCA have been frequently detected in soil and surface water at concentrations ranging from parts per trillion (ppt) to low parts per million (ppm) (Charizopoulos and Papadopoulou-Mourkidou, 1999; Metcalfe et al., 2008; Xu et al., 2007). PCA is not easily degraded under aerobic conditions, but is microbially dechlorinated to less chlorinated anilines under anoxic/anaerobic conditions (Okutman Tas and Pavlostathis, 2005; Susarla et al., 1996). The persistence of

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these compounds in the environment, along with their toxicity and carcinogenicity, pose a threat to human and environmental health.

Remediation of sites contaminated with chlorinated compounds has been studied widely. A key challenge in enhancing the activity of dechlorinating microorganisms at field sites is obtaining intimate contact between microorganisms, the contaminant, and the electron donor that is usually added (McGuire and Hughes, 2003). Surfactants can increase the contaminant mass transfer to the aqueous phase resulting in increased biodegradation rates (Tiehm et al., 1997; Van Hoof and Jafvert, 1996). However, inhibition of microbial activity brought about by interaction of surfactant with the cell membrane or by interaction with membrane-bound proteins and enzymes vital to cell function, has also been observed (Tsomides et al., 1995; Cort and Bielefeldt, 2000).

Quaternary ammonium compounds (QACs) are cationic surfactants extensively used in domestic and industrial applications (Garcia et al., 1999; Patrauchan and Oriel, 2003). QACs strongly adsorb to a variety of materials such as activated sludge and wastewater solids in wastewater systems (Boethling, 1994; Ismail et al., 2010). QACs degrade under aerobic conditions in biological treatment systems (Al-Ahmad et al., 2000; Nishihara et al., 2000; Patrauchan and Oriel, 2003; Tezel et al., 2012; Tezel and Pavlostathis, 2012; Van Ginkel et al., 1992; Zhang et al., 2011). However, QACs sorption is faster than biodegradation in aerobic systems leading to their transfer to anoxic/anaerobic compartments, such as anaerobic digesters and aquatic sediments (Li and Brownawell, 2010; Martinez-Carballo et al., 2007; Sutterlin et al., 2007). The transformation of benzalkonium chloride, a widely used QAC, under anoxic, nitrate reducing conditions was recently reported by means of an abiotic nitrite nucleophilic substitution reaction (modified Hofmann reaction) producing alkyl dimethyl amines (tertiary amines) (Tezel and Pavlostathis, 2009, 2012). Under anaerobic conditions, there is no evidence of mineralization of QACs that contain alkyl or benzyl groups (Battersby and Wilson, 1989; Federle and Schwab, 1992; Garcia et al., 1999, 2000; Tezel et al., 2006, 2007; Tezel and Pavlostathis, 2012). QACs are also used in agricultural formulations as active ingredients as well as auxiliary chemicals (adjuvants) to enhance the solubility, rain fastness and penetration of pesticides and they are applied together with the pesticides (Pesticide Action Network). Typical QAC concentrations in agrochemical tank-mixed sprays range from 0.05 to 0.5% v/v (Gustavsson, 2001). QACs can also be used to enhance the degradation of pesticides in contaminated areas (Badawi and Ahmed, 2007).

Co-existence of pesticides and surfactants is expected at pesticide formulation sites, on- or off-farm disposal sites for left-over agrochemicals, agricultural fields where pesticides are applied, as well as surface and groundwater resulting from accidental spills and other activities. In recent years, on-farm biopurification systems (i.e., biobeds) have been explored as a safe way to dispose of left-over pesticide mixtures and equipment cleaning wastewater in order to avoid pesticide point source contamination of soil and water resources (Castillo et al., 2008; Karanasios et al., 2012; Marinozzi et al., 2013; Vischetti et al., 2008). Concentrations of pesticides as well as other admixtures in excess of 50 ppm are encountered in biobeds. As a result, PCNB/PCA and QACs may co-occur in biobeds, agricultural soils treated with fungicides, or in water bodies receiving agricultural run-off.

The effects of nonionic surfactants on the anaerobic reductive dechlorination of chlorinated benzenes and ethenes have been investigated (Amos et al., 2007; Yeh et al., 1999; Yeh and Pavlostathis, 2001). However, little information is available regarding the effect of cationic surfactants such as QACs on the microbial reductive dechlorination process. The objective of this research was to evaluate the effect of two structurally different QACs on PCA dechlorination in a mixed, methanogenic and PCA-dechlorinating culture.

2. Materials and methods

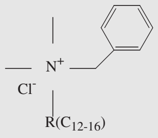
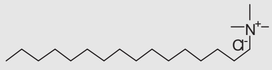
2.1. *Quaternary ammonium compounds (QACs)*

Alkyl benzyl dimethyl (AB) and hexadecyl (C_{16}) trimethyl (HD) ammonium chloride ($C_{19}H_{42}NCl$) were used in this study. AB was a commercial mixture of (w/w) 40% C_{12} ($C_{21}H_{38}NCl$), 50% C_{14} ($C_{23}H_{42}NCl$) and 10% C_{16} ($C_{25}H_{46}NCl$). Stock solutions (10 g/L) of QACs were prepared in de-ionized (DI) water taking into account the active ingredients purity. The structure and properties of AB and HD are shown in Table 1. The critical micelle concentration (CMC) of the AB mixture was determined as described in Section 2.5 below.

2.2. *Dechlorinating culture*

A dechlorinating enriched culture was developed from a contaminated sediment as previously reported (Okutman Tas

Table 1 – Properties of alkyl benzyl dimethyl (AB) and hexadecyl trimethyl (HD) ammonium chloride.

Properties	AB	HD
Molecular weight (g/mol)	359.61[a]	320.00
Purity (%)	80	100
pH[b]	5.33	4.41
Log K_{ow}	0.97[d]	1.50 ± 0.06[e]
CMC (mM)	1.82 ± 0.22[c]	1.31 ± 0.03[e]
COD (mg/L)[b]	629.5 ± 27.1	532.5 ± 32.7
Molecular structure		

[a] Weighted average of 40% C_{12}, 50% C_{14} and 10% C_{16} (w/w) alkyl benzyl dimethyl ammonium chloride mixture.

[b] For a 200 mg/L solution in DI water.

[c] Mean ± standard deviation ($n = 3$).

[d] Weighted average of the AB mixture based on measured values for each homolog as follows: C_{12}-AB, 0.59 ± 0.04; C_{14}-AB, 1.67 ± 0.02; C_{16}-AB, 2.97 ± 0.03 (Tezel, 2009).

[e] Values from Tezel (2009).