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Prolonged exposure of mixed aerobic cultures to low temperature and benzalkonium chloride affect the rate and extent of nitrification

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HIGHLIGHTS

- Inhibition of nitrification in enriched nitrifying culture occurred at >5 mg BAC/L.
- The non-competitive inhibition coefficient at 22–24 °C was 1.5 ± 0.9 mg BAC/L.
- Maintenance of a heterotrophic/nitrifying culture at 10 °C led to slow nitrification.
- BAC inhibition of nitrification exacerbated by prolonged low temperature conditions.
- BAC degradation rate by heterotrophs decreased with a temperature decrease to 10 °C.

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ABSTRACT

The combined effect of benzalkonium chloride (BAC) and prolonged exposure to low temperature on nitrification was investigated. Ammonia oxidation at 22–24 °C by an enriched nitrifying culture was inhibited at increasing BAC concentrations and ceased at 15 mg BAC/L. The non-competitive inhibition coefficient was 1.5 ± 0.9 mg BAC/L. Nitrification tests were conducted without and with BAC at 5 mg/L using an aerobic, mixed heterotrophic/nitrifying culture maintained at a temperature range of 24–10 °C. Maintaining this culture at 10 °C for over one month in the absence of BAC, resulted in slower nitrification kinetics compared to those measured when the culture was first exposed to 10 °C. BAC was degraded by the heterotrophic population, but its degradation rate decreased significantly as the culture temperature decreased to 10 °C. These results confirm the negative impact of quaternary ammonium compounds on the nitrification process, which is further exacerbated by prolonged, low temperature conditions.

1. Introduction

Quaternary ammonium compounds (QACs) are organic compounds that contain four functional groups attached covalently to a positively charged central nitrogen atom (R_4N^+). The functional groups (R) include at least one long chain alkyl group and the rest are either methyl, long chain alkyl, or benzyl groups (Tezel and Pavlostathis, 2012). QACs are cationic compounds massively produced throughout the world and used extensively in domestic, agricultural, health care, and industrial applications as surfactants, emulsifiers, fabric softeners, disinfectants, pesticides, corrosion inhibitors, paint additives, cosmetics and personal care products (Tezel and Pavlostathis, 2012). Such widespread use of QACs has resulted in their release to wastewater treatment plants and accumulation in aquatic environments (Martinez-Carballo et al., 2007a,b; Li and Brownawell, 2010; Ismail et al., 2010).

The fate of QACs in aerobic biological treatment systems has been studied. QAC biodegradation has been reported in aerobic, activated sludge systems; however, the extent of biodegradation varies depending on the QAC concentration, structure, microbial acclimation and presence of QAC resistant/degrading microorganisms (Zhang et al., 2011; Hajaya and Pavlostathis, 2012, 2013; Tezel and Pavlostathis, 2012). Certain microorganisms that are resistant to QACs and capable of QAC degradation have been reported (van Ginkel et al., 1992; Nishihara et al., 2000; Al-Ahmad et al., 2000; Patrauchan and Oriel, 2003; Tezel et al., 2012;

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Bergero and Lucchesi, 2013; Oh et al., 2013, 2014; Tandukar et al., 2013).

Previous research has shown that QACs in wastewater treatment systems are biodegraded under aerobic conditions (Boethling, 1994; van Ginkel, 2004; Zhang et al., 2011; Tezel and Pavlostathis, 2012). However, only limited research has been performed to investigate the effect of QACs on specific aerobic biological treatment processes, especially on nitrification (Boethling, 1994; Hajaya and Pavlostathis, 2012, 2013). We have previously reported that the QAC benzalkonium chloride (BAC) did not affect the rate and extent of degradation of a mixture of dextrin and peptone and the production of ammonia (ammonification) in a mixed, aerobic culture which degraded BAC up to 50 mg BAC/L; after a significant lag period, complete nitrification of the resulting ammonia took place at 20 mg BAC/L, but complete inhibition of nitrification was observed at 50 mg BAC/L (Yang, 2007).

It is well known that temperature has a significant effect on the nitrification process, which is a two-step process: ammonia oxidation to nitrite, followed by nitrite oxidation to nitrate (Rittmann and McCarty, 2001; Tchobanoglous et al., 2014). Although the maximum net growth rate of nitrite oxidizing bacteria (NOB) is slightly higher than that of ammonia oxidizing bacteria (AOB), the effect of temperature on their growth rate is very similar (about 59% decrease for a temperature drop from 25 to 10 °C; Fig. S1, Supplementary data). However, the possible compounded effect of QAC toxicity and low temperature has not been evaluated before in spite of the fact that such conditions are common (e.g., poultry processing wastewater treatment plants). The impetus for the work reported here was the dramatic loss of nitrification at several poultry processing facilities in the Southeastern US after a period of unseasonal, prolonged low temperatures (below 15 °C), during which increased ammonia levels were observed in effluent wastewater (Pavlostathis et al., 2008). It is also possible that the observed negative effect of low temperature on nitrification may have been exacerbated by disinfectants, more likely associated with episodic QAC releases in these facilities.

The objectives of this study were to: (a) systematically assess the effect of a BAC concentration range on nitrification at room temperature (22–24 °C) using a highly enriched nitrifying culture; and (b) investigate the combined effect of BAC and prolonged exposure to low temperature on nitrification by an heterotrophic and nitrifying mixed culture.

2. Methods

2.1. Benzalkonium chloride

Due to its extensive use among all QACs, alkyl benzyl dimethyl ammonium chloride, also referred to as benzalkonium chloride (BAC), was selected for this study. The chemical formula of BAC is $C_{n+9}H_{2n+14}NCl$, where n refers to the number of carbons in the alkyl chain (typically 12, 14, or 16). The commercial sanitizer Barquat MB-80™, which was used in this study, is a mixture of BACs with C_{12}, C_{14}, and C_{16} alkyl group length, ethanol, and water as shown in Table 1. BAC stock solutions of 10,000 mg/L of active ingredient were prepared in deionized (DI) water and further diluted as needed in the various parts of this study.

Table 1
Characteristics of the BAC mixture (Barquat MB-80™) used in this study.

Parameter	BAC homologues		
	C_{12}-BAC	C_{14}-BAC	C_{16}-BAC
Composition (% w/w)[a]	32	40	8
Molecular formula	$C_{21}H_{38}NCl$	$C_{23}H_{42}NCl$	$C_{25}H_{46}NCl$
Molecular weight	339.99	368.04	396.09
ThOC (g C/g BAC)	0.74	0.75	0.76
DOC (mg/L)[b]	173.2 ± 0.8		
ThOD (g O_2/g BAC)	2.72	2.83	2.87
COD (mg/L)[b]	629.5 ± 27.1		

[a] Commercial mixture contains ethanol and water (10% each, w/w).
[b] In a 200 mg/L BAC mixture solution.

2.2. Cultures

2.2.1. Control mixed nitrifying culture

A mixed nitrifying culture was developed with mixed liquor from the activated sludge reactor of the RM Clayton wastewater treatment plant in Atlanta, GA, USA. The culture was enriched and maintained in a 2-L glass reactor (1.5 L culture volume), magnetically mixed, and its pH was controlled at 7.5 ± 0.3 with a pH controller and a pump connected to a 0.5 N $NaHCO_3$ solution. Pre-humidified air was supplied through a fine pore diffuser. The culture was maintained at room temperature (22–24 °C) and was fed twice a week as follows: the culture was allowed to settle for more than 30 min, 1.35 L of culture supernatant was wasted and replaced with medium and 100 mg N/L NH_4Cl. The composition of the culture medium was as follows (in mg/L): K_2HPO_4, 600; KH_2PO_4, 335; $CaCl_2{\cdot}2H_2O$, 67.5; $MgCl_2{\cdot}6H_2O$, 135; $MgSO_4{\cdot}7H_2O$, 267.5; $FeCl_2{\cdot}4H_2O$, 67.5. An aliquot of a trace metal stock solution was also used (0.7 mL per L of medium), which had the following composition (in mg/L): $ZnCl_2$, 25; $MnCl_2{\cdot}4H_2O$, 15; H_3BO_3, 150; $CoCl_2{\cdot}6H_2O$, 100; $CuCl_2{\cdot}2H_2O$, 5; $NiSO_4{\cdot}6H_2O$, 10; $NaMoO_4{\cdot}2H_2O$, 15. pH, total and volatile suspended solids (TSS, VSS), ammonia, nitrite, and nitrate were periodically measured. This culture was not exposed to BAC and served as the control. Based on oxygen uptake measurements using NH_4Cl and glucose as substrates, it was shown that the heterotrophic population size was insignificant after 85 days of culture enrichment (Fig. S2). The steady-state biomass concentration of the culture was 290 ± 30 mg VSS/L (mean ± standard deviation).

2.2.2. Control mixed heterotrophic/nitrifying culture

Another, aerobic heterotrophic and nitrifying culture was developed with mixed liquor from the aerobic and nitrifying reactor of a poultry processing facility. The culture was maintained at room temperature (22–24 °C) and its pH was controlled at 7.5 ± 0.3 with a pH controller and a pump connected to a 0.5 N $NaHCO_3$ solution. The culture was fed periodically with dextrin (500 mg COD/L) and 100 mg N/L NH_4Cl following the same procedure as for the nitrifying culture (see Section 2.2.1, above). This culture was not exposed to BAC and served as the control. The steady-state biomass concentration of this culture was 900 ± 30 mg VSS/L. The following analyses were periodically performed: pH, TSS, VSS, soluble chemical oxygen demand (COD), ammonia, nitrite, and nitrate.

2.3. Nitrification assays

2.3.1. Short-term nitrification assay

The effect of BAC at 22–24 °C and at a concentration range from 0 to 20 mg/L was assessed by conducting a batch assay as follows. An aliquot of 200 mL of the mixed nitrifying culture described in Section 2.2.1, above, was removed immediately after the replacement of the culture supernatant with fresh medium and the addition of 100 mg N/L NH_4Cl. This culture sample was then transferred to an Erlenmeyer flask where it was continuously aerated and mixed. An aliquot of a BAC stock solution was then added to the culture resulting in an initial BAC concentration of 20 mg/L. Four more culture series with initial BAC concentrations of 2, 5, 10, and 15 mg/L were prepared in the same manner. For all five culture series, the concentration of nitrogen species and BAC were