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ROLE OF MICELLE-CLAY COMPLEXES AND QUATERNARY AMINE CATIONS IN REMOVAL OF BACTERIA FROM WATER: ADSORPTION, BIOSTATIC, AND BIOCIDAL EFFECTS

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Abstract—The present report is a review of uses of quaternary ammonium cations (QACs) as free monomers or immobilized in micelle-clay complexes in bacteria removal from water. The removal of bacteria from water by filtration through a bed of a granulated QAC-clay micelle was improved by minute concentrations of QAC that were released from the complex during filtration, which exerted biostatic or biocidal effects on the bacteria that emerged from the filter. The relationships between antibacterial activity (minimum inhibition concentration, MIC; minimum lethal concentration, MLC) and structural parameters of the QACs (head group size and alkyl chain length) are discussed. The antibacterial activity of QACs in aqueous phases is mainly due to the free monomeric species. Bacterial inactivation is enhanced by QACs with longer alkyl chains. In most recorded cases, however, minimum MIC and MLC values occurred at $n = 14-16$ and mostly at $n = 16$, where n is the number of C atoms in the alkyl chain. This outcome is explained by the combination of two antagonistic effects: (i) An increase in alkyl chain length (*i.e.*, QAC hydrophobicity) enhances QAC binding, penetration, and destabilization of bacterial membranes; and (ii) an increase in alkyl chain length lowers the critical micelle concentration (CMC) of QACs and, thus, reduces QAC monomer concentrations, which more efficiently inactivate bacteria than the micelles. The octadecyltrimethylammonium (ODTMA, $n = 18$) MLC value (0.25 μm) for the cyanobacterium genus *Aphanizomenon* is significantly lower than the CMC (300 μm) value. Hence, a test to determine the minimum MLC value at $n = 16$ is of interest. Removal of bacteria from water by filtration is expected to be made more efficient by small increases in the ODTMA/clay ratio in the complex, which will act to increase the concentrations of ODTMA cations released during filtration.

Key Words—Antimicrobial Activity, *Aphanizomenon*, Biostatic and Biocidal Effects, *Escherichia coli*, Filtration and Biocidal Effects, *Microcystis, Pseudomonas aeruginosa*, Quaternary Ammonium Cations (QAC), *Staphylococcus aureus*.

INTRODUCTION

The efficient removal of pathogenic microorganisms (*e.g.*, bacteria) from water by minimal amounts of disinfectants is a primary health issue. The micelle, liposome, and polymer species of quaternary ammonium compound (QAC) clay composites have been shown to be promising materials for this task (Shtarker-Sassi *et al*. 2013; Undabeytia *et al*. 2014). Kalfa *et al*. (2017) examined the removal of *Escherichia coli* and reductions in the total bacteria counts in water using granulated micelle-bentonite complexes. Kalfa *et al*. (2017) showed that a QAC/clay complex based on benzyldimethylhexadecylammonium (BDMHDA) yielded larger filtered water volumes without bacteria than the QAC/clay complex based on octadecyltrimethylammonium (ODTMA) despite the capture of similar amounts of bacteria in the two filters. This effect was explained by biocidal or biostatic effects, which were exerted by minute BDMHDA concentrations released during filtration that were larger than the ODTMA concentrations. The granulated ODTMA-bentonite complex yielded efficient removal of cyanobacteria (filamentous *Aphanizomenon* and single cell *Microcystis*) from water by filtration (Sukenik *et al*. 2017). During the time of filtration, the cells seemingly retained integrity and no chlorophyll release was observed. Complete loss of photosynthetic activity, however, was observed in a suspension that included $5{\cdot}10^6$ colony forming units (CFU) per mL of *Microcystis* or $2{\cdot}10^3$ CFU/mL of *Aphanizomenon* cells incubated for 20 h with a 10 g/L suspension of an ODTMA-bentonite complex. Analysis indicated that this loss in photosynthetic activity, which amounts to cell death because the main metabolic activity of the cells was abolished, was not due to the interactions of the cells with the QAC/clay complex. Rather, the loss in photosynthetic activity was due to the biocidal action of the released QAC cations. In parallel experiments, the addition of 30 mg/L of ODTMA cations completely abolished the photosynthetic activity of *Aphanizomenon* cells within 5 to 10 min, but the addition of 75 μg/L of ODTMA cations was sufficient to achieve the same outcome within 20 to 40 min (Table 1). We note that Table 1 includes data in the same units used in the original articles (*e.g.*, mg/L or mM). For the sake of completeness, we have added the

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Table 1. Toxicities of QACs to bacteria.

QA cation (counter ion)	Bactericidal parameters	Reference
	Total bacteria count (TBC)	
Octadecyltrimethyl ammonium	MIC = 40 mg/L (0.13 mM)	Kalfa *et al*. (2017)
Benzyldimethylhexadecyl ammonium	MIC = 40 mg/L (0.12 mM)	
	Escherichia coli	
Decyltrimethyl ammonium (Br)	MIC = 3000 μM	Inacio *et al*. (2016)
Dodecyltrimethyl ammonium (Br)	MIC = 300 μM	Inacio *et al*. (2016)
Tetradecyltrimethyl ammonium (Br)	MIC = 150 μM	Inacio *et al*. (2016)
Hexadecyltrimethyl ammonium (Br)	MIC = 80 μM	Inacio *et al*. (2016)
Trimethyl-(2-hydroxyethyl) ammonium (Br)	MIC > 2000 mg/ L; (>11.0 mM) (MLC > 2000 mg/L (>11.0 mM)	Thorsteinsson *et al*. (2003)
Trimethyl-(2-ethyldecylate) ammonium (Br)	MIC = 64 mg/L; (0.18 mM) MLC = 64 mg/L; (0.18 mM)	Thorsteinsson *et al*. (2003)
Benzyldimethyloctyl ammonium (Cl)	MLC = 1000 mg/L (3.7 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyldecyl ammonium (Cl)	MLC = 400 mg/L (1.3 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyldodecyl ammonium (Cl)	MIC = 60 μM;	Inacio *et al*. (2016)
Benzyldimethyldodecyl ammonium (Cl)	MLC = 25 mg/L (0.08 mM) in 10 min. at 25ºC	Jono *et al*. (1986)
Benzyldimethyltetradecyl ammonium (Cl)	MLC = 25 mg/L (0.07 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethylhexadecyl ammonium (Cl)	MLC = 25 mg/L (0.07 mM) in 10 min at 25ºC; MIC = 45 mg/L (0.13 mM)	Jono *et al*. (1986) Shtarker-Sasi *et al*. (2013)
Benzyldimethyloctadecyl ammonium (Cl)	MLC = 100 mg/L (0.24 mM) in 10 min at 25ºC	Jono *et al*. (1986)
	Enterococcus faecalis	
Trimethyl-(2-hydroxyethyl) ammonium (Br)	MIC > 2000 mg/L (>11.0 mM); MLC > 2000 mg/L (>11.0 mM)	Thorsteinsson *et al*. (2003)
Trimethyl-(2-ethyldecylate) ammonium (Br)	MIC = 16 mg/L (440 μM); MLC = 64 mg/L (175.8 μM)	Thorsteinsson *et al*. (2003)
	Staphylococcus aureus	
Trimethyl-(2-hydroxyethyl) ammonium (Br)	MIC >2000 mg/L (> 11 mM); MLC > 2000 mg/L (>11 mM)	Thorsteinsson *et al*. (2003)
Trimethyl-(2-ethyldecylate) (Br) ammonium	MIC = 16 mg/L (0.04 mM); MLC = 16 mg/L (0.04 mM)	Thorsteinsson *et al*. (2003)
Benzyldimethyloctyl ammonium (Cl)	MLC = 1000 mg/L (3.7 mM) n 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyldecyl ammonium (Cl)	MLC = 500 mg/L (1.67 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyldodecyl ammonium (Cl)	MLC = 50 mg/L (0.15 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyltetradecyl ammonium (C14, BZK) (Cl)	MLC = 25 mg/L (0.07 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethylhexadecyl ammonium (Cl)	MLC = 12.5mg/L (0.03 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyloctadecyl ammonium (Cl)	MLC = 25 mg/L (0.06 mM) in 10 min at 25ºC	Jono *et al*. (1986)

values in molar units in parentheses when needed. It follows from these examples that bacterial removal from water can be optimized by utilizing both the adsorption of bacteria by the filter matrix and by the biostatic or biocidal effects of released or added free QAC cations. The antibacterial activity of QACs was reviewed in several studies (*e.g*. Cooper, 1988; Kenawy *et al*., 2007; Madaan and Tyagi, 2008; Buffet-Bataillon *et al*., 2012; and Zhang *et al*., 2015).

When referring to bacteria, the biocidal/biostatic activity of QAC compounds is characterized by the following stages (Maris, 1995; Maillard, 2002; Ferreira *et al*. 2011; Carmona-Ribeiro and Carrasco 2013): (i) Adsorption and penetration of the agent into the cell wall and interactions with outer cell components; (ii) interaction with the cytoplasmic membrane (lipid or protein) followed by membrane disorganization; (iii) disruption of the cytoplasmic membrane and

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QA cation (counter ion)	Bactericidal parameters	Reference
	Pseudomonas aeruginosa	
Trimethyl-(2-hydroxyethyl) ammonium (Br)	MIC = 2000 (11.0) mg/L; MLC >2000 mg/L (11.0)	Thorsteinsson *et al*. (2003)
Trimethyl-(2-ethyldecylate) ammonium (Br)	MIC = 250 mg/L (0.69 mM); MLC = 250 mg/L (0.69 mM)	Thorsteinsson *et al*. (2003)
Benzyldimethyloctyl ammonium (Cl)	MLC = 1000 mg/L (3.7 mM) in 10 min. at 25ºC	Jono *et al*. (1986)
Benzyldimethyldecyl ammonium (Cl)	MLC = 500 mg/L (1.7 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyldodecyl ammonium (Cl)	MLC = 50 mg/L (0.15 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyltetradecyl ammonium (Cl)	MLC = 50 mg/L (0.14 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethylhexadecyl ammonium (Cl)	MLC = 100 mg/L (0.26 mM) in 10 min at 25ºC	Jono *et al*. (1986)
Benzyldimethyloctadecyl ammonium	MLC > 400 mg/L (>0.1 mM) in 10 min at 25ºC	Jono *et al*. (1986)
	Cyanobacteria genus: *Aphanizomenon* and *Microcystis*	
Octadecyltrimethyl ammonium	*Aphanizomenon* MLC = 75 μg/L (0.25 μM); (20–40 min); 30 mg/L (0.1 mM) (5 min) *Microcystis* MLC = 6 mg/L (0.02 mM) (20–40 min); 60 mg/L (0.2 mM) (5–10 min)	Sukenik *et al*. (2017)

leakage of intracellular, low molecular weight material; (iv) degradation of proteins and nucleic acids; and (v) cell wall lysis caused by autolytic enzymes. Inacio *et al.* (2016) suggested three concentration-dependent mechanisms for the antibacterial activity of QACs: (1) Impairment of cell reproduction at low QAC concentrations; (2) membrane permeabilization at intermediate QAC doses; and (3) disruption of bacterial membranes at higher QAC concentrations. Knauf *et al*. (2018) showed that *Acinetobacter baumannii* protein aggregation is associated with cell death at low concentrations of benzalkonium chloride (alkylbenzyldimethyl ammonium chloride, BZK).

The attachment and adsorption of QACs to negatively charged sites, such as acidic phospholipids in bacterial membranes, is mainly governed by the QAC head groups. Once this occurs, the long hydrophobic alkyl tail of the QACs is integrated into the core of the bacteria hydrophobic membrane.

This mini review has largely discussed the factors that affect the biocidal or biostatic effects of QACs similar to ODTMA or BDMHDA. The factors that were reviewed and discussed include the effects of QAC concentration and chain length, time of incubation, and the wide range of bacterial susceptibilities to QAC exposure.

SURVEY OF LITERATURE RESULTS AND DISCUSSION

The USEPA (1988) clustered QACs into 4 groups according to similarity in chemical structure (the polar head group and the hydrophobic long alkyl group) and the effects on toxicity: (I) Alkyl or hydroxyl (straight chain) substituted; (II) non-halogenated benzyl substituted; (III) di- and tri-chlorobenzyl substituted; and (IV) QACs with unusual substituents (charged heterocyclic compounds). The antimicrobial activities of the QACs on a variety of bacteria in the survey were presented as the minimal concentration for complete growth inhibition (MIC) and/or the minimal lethal concentration of QACs (MLC), *i.e*., the lowest concentration of an antimicrobial that kills all the cells of a particular bacterium.

Effect of chain length, headgroup, bacterial species, and contact time

The structural parameters influence both the hydrophobicity and the CMC values of QACs (Buckingham *et al*., 1993; Tan and Birge, 1996).

Effect of chain length. Gilbert and Al-Taae (1985) investigated the antimicrobial activity (MIC values) of some QACs toward representative strains of microorganisms. The QACs were of the n-alkyltrimethylammonium bromide salt type (USEPA group I). The QAC cations had n-alkyl chain lengths that ranged from $n = 5$ to $n = 22$ and the microorganisms included

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Staphylococcus aureus, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*. Gilbert and Al-Taae (1985) found a dramatic increase in antimicrobial activity (*i.e.*, a reduction in MIC values) of the order of three log units (1000×) as the QAC substituent chain lengths were increased from n = 10 to n = 12. Minimal MIC values (*i.e.*, maximum biocidal/biostatic activities) were achieved for chain lengths of around n = 12 to n = 16. Gilbert and Al-Taae (1985) suggested that separate dependencies existed for short (n < 10) and long (n > 10) chain substituents. They interpreted the results in terms of distinct mechanisms of action, binding sites, and/or physicochemical properties for extreme members of the series.

Inacio *et al.* (2016) (Table 1) reported MIC values that monotonically decreased (from 3000 μM to 80 μM) for members of the same series (*i.e.*, QACs similar to ODTMA) for *E. coli* with an increase in alkyl chain length from 10 to 16 C. Jono *et al.* (1986) reported MLC values (Table 1) for the non-halogenated benzyl substituted QACs (group II). For *E. coli*, the MLC values decreased from 1000 to 25 mg/L for an alkyl chain length increase from 8 to 16 (BDMHDA). This pattern was inverted, however, for n = 18 because the MLC value increased to 100 mg/L. For *S. aureus*, a similar pattern was obtained (Table 1; Jono *et al.*, 1986) and MLC values decreased from 1000 to 12.5 mg/L for a chain length increase from 8 to 16, but the MLC value increased to 25 mg/L for a QAC with an n = 18 alkyl group. For *P. aeruginosa* (Table 1; Jono *et al.*, 1986), the MLC value was 100 mg/L for n = 16, whereas the MLC value for n = 18 was >400 mg/L. Note that when the MIC values for *E. coli* (Table 1, Inacio *et al.*, 2016) after units conversion for n-alkyltrimethylammonium bromide salts are compared to the MLC values for non-halogenated benzyl substituted QACs (Table 1; Jono *et al.*, 1986), the MIC values for n = 10 to 14 for QACs were several times larger than the MLC values. The MLC values, however, were comparable for n = 16 QACs (*i.e.* 80 *vs.* 70 μM after unit conversion).

Effect of incubation time. The MLC values reported by Jono et al. (1986) (Table 1) were obtained for a 10 min contact time between QACs and bacteria. Kalfa *et al.* (2017) demonstrated that the addition of OTDMA or BDMHDA at equal concentrations of 6–15 mg/L to water, which had a total bacteria count of more than 57,000 CFU/mL, reduced the CFU/mL values from 0 to 3 within about 2 h. Complete abolishment of reproduction was achieved by ODTMA with an average concentration of 40 mg/L (Table 1), but a biocidal effect could not be ruled out. Shtarker-Sasi et al. (2013) showed that addition of 45 mg/L of ODTMA to *E. coli* (gram-negative, Table 1), or *Bacillus megaterium* (gram-positive) bacteria caused growth inactivation within a 10 min incubation.

In a study on cyanobacteria, Sukenik *et al.* (2017) showed complete inhibition of the photosynthetic activity (*i.e.*, cell death) upon exposure of *Aphanizomenon* cells to 75 μg/L of ODTMA within 20 to 40 min or by exposure to 30 mg/L of the ODTMA cation within 5 to 10 min. In the case of *Microcystis*, 6 mg/L of ODTMA caused complete cell death within 20 to 40 min or within 5 min for 60 mg/L ODTMA. A prolonged incubation time likely could result in complete death of these cyanobacteria cells by smaller ODTMA concentrations than reported above (Table 1). This effect of prolonged incubation times on reducing the MLC values of ODTMA (n = 18) was more pronounced than for *E. coli* MIC values (see figure 2a in Inacio *et al.*, 2016). In the latter example, incubation times were between 10 and 60 min and the QAC was C_{12} pyridinium.

Effect of head group. Thorsteinsson *et al.* (2003) reported on this subject. For example, QACs with a small head group, such as trimethyl-(2-ethyldecylate) ammonium Br exhibited a MLC value of 1760 μM when tested against *Enterococcus faecalis*. In contrast, the MLC value measured by exposing the same type of bacteria to a similar, but more bulky pyridine derivative was 3200 μg/mL (*i.e.* 9800 μM).

Variations in toxicity of QACs to different cells

Table 1 is arranged to show MIC and MLC values for several QACs. For total bacteria count and cyanobacteria in Table 1, the ODTMA cation was chosen and the ranges in MIC or MLC values for different cells were presented. For the total bacteria count of samples incubated for >2h, the MIC value of ODTMA was 40 mg/L (Kalfa *et al.*, 2017) and the MLC value for *Aphanizomenon* incubated for 20–40 min was 75 μg/L (Sukenik *et al.*, 2017). This range of values spans a factor of 530, but when the incubation times and the differences between the MIC and MLC values are considered, the realistic span in the values is probably several thousand.

Inacio *et al.* (2016) focused on QACs with alkyl chain lengths of 12 in inactivating bacteria. These authors found that two strains of *Neisseria gonorrhoeae* were more susceptible to n-dodecylpyridinium bromide (C_{12}PB) than *E. coli*.

The antibacterial activity of the USEPA group IV QACs was investigated by Thorsteinsson *et al.* (2003) for 25 pyridine derivative QACs that differed in the type of the attached long alkyl chain. The respective MIC and MCL values of hexadecylpyridinium chloride (cetylpyridinium) for *E. coli, Enterococcus faecalis*, *S. aureus*, and *P. aeruginosa were* <0.5 and 8, <0.5 and 4, 16 (just MIC), and 500 and 1000 mg/L, respectively.

Zhao and Sun (2008) employed four 4-aminopyridinium derivative QACs in antimicrobial tests against both gram-negative and gram-positive bacteria (*E. coli* and

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S. aureus). The four QACs had the same long alkyl chain, but differed in the hydrophobic group substitutions at the 4-amino position. All of the quaternary pyridinium salts exhibited significant antimicrobial activities, but to different extents according to the hydrophobicity at the 4-amino position. The QACs that had larger hydrophobic groups were significantly more effective than the QACs with smaller hydrophobic groups. The Zhao and Sun (2008) study showed that in addition to the alkyl chains, which have been recognized to be a key structural feature in QACs in providing a powerful biocidal effect against both gram-negative and gram-positive bacteria, other hydrophobic groups can also improve the antimicrobial power of QACs. Alptuzun *et al.* (2006) investigated the antibacterial and antifungal activity of pyridinium salts, which were mainly ether derivatives of pyridinium oxime. The 1-(3-phenylpropyl)-3-[([(naphthyl-1-il)methoxy]imino)-methyl]pyridinium bromide QAC exhibited the highest antimicrobial activity against *P. aeruginosa, E. coli, Enterococcus faecali*, and *S. aureus* with MIC values of 312.5, 39.1, 9.8, and 9.8 mg/L, respectively.

Discussion: Effect of chain length and head group size

The immobilization of QAC micelles by binding to a clayey substrate and the formation of a granulated micelle-clay complex favors QACs with larger alkyl chains, such as BDMHDA (n = 16) and ODTMA (n = 18). This strategy has a distinct advantage because the micelle-clay complex provides a durable antibacterial preparation that limits leaching of the active monomeric QAC species.

Though the antibacterial activity (MIC and MLC values) depends on the exposure conditions (bacteria type, QAC concentration, incubation duration, and temperature), it is also governed by the chemical structure of the QAC, such as type of head group and length of the alkyl chain. The positively charged QAC head group plays the main role in QAC attachment to negative sites at the bacterial membrane (*e.g.*, acidic phospholipids and glycoproteins) and a greater activity is attained by QACs with smaller head groups.

With regard to QAC alkyl chain length, separate dependencies may exist for short- ($n < 10$) and long- ($n > 10$) chained substituents. For short-chained QACs ($n < 10$), the antimicrobial activity is solely dependent on a positively charged ammonium group. Indeed, QACs with n-alkyl chain lengths of $n < 4$ are virtually inactive (Gilbert and Moore 2005). The microbial killing effect of QACs that have a long alkyl chain ($n > 10$) stems from both the positive charges and the penetration of the hydrophobic chain into the bacterial membrane, which causes physical disruption. In this range of alkyl chain *n*-values, the biocidal activity of QACs frequently approximates a parabolic function of the compound lipophilicity (*i.e.* n-alkyl chain length). For gram-positive bacteria and yeast, the biocidal activity of QACs is greatest for alkyl chain lengths of $n = 12-14$, while for gram-negative bacteria, the optimal biocidal activity is achieved by compounds with an alkyl chain length of $n = 14-16$ (Gilbert and Moore, 2005; see also Table 1). Further increases in alkyl chain length (18 and higher) were reported to result in decreased antibacterial efficacy.

The parabola-like relationship between growth inhibitory activity and n-alkyl chain length of QACs can be explained by the two competing trends. On one hand, because the action against bacterial cells generally involves a perturbation of lipid bilayer membranes, an increase in the lipophilicity of the QAC is beneficial. Note, however, that an increase in alkyl chain length implies enhanced binding of the QAC. This also stems from the enhanced interactions between bulk water molecules when a larger lipophilic QAC molecule binds to a bacterial surface. On the other hand, an increase in lipophilicity lowers the concentration of active QAC monomeric cations in the aqueous phase due to the lower CMC values. In other words, the CMC values are lower due to the increased fraction of QAC aggregates, micelles, and liposome species for QACs with two alkyl chains.

Li *et al.* (2013) added another explanation for the decreased biocide activity of QACs with alkyl chain lengths of $n \geqslant 18$. They suggested that when QAC alkyl chains are too long, the alkyl chain might possibly bend or curl. Hence, the alkyl groups might partly cover the positively charged quaternary ammonium groups. This might block electrostatic interactions with the bacterial surface and diminish antibacterial potency. QACs with $n = 16$ alkyl groups might be the maximum size for alkyl chains that can extend without masking the quaternary ammonium sites. A QAC with an alkyl chain length of $n = 18$ might be too long and might bend to form barriers between the N of positively charged QACs and bacterial surfaces.

Mishael *et al.* (2002) demonstrated that ODTMA, an alkylammonium cation with $n = 18$, had a significantly larger binding affinity to montmorillonite (a negatively charged surface) than a similar QAC cation with $n = 16$. This disagrees with the suggestion of Li *et al.* (2013) that QAC activity decreases for QACs with alkyl chain lengths of $n \geqslant 18$, at least for USEPA (1988) group I QACs.

In the following sentences, the observed increase in MIC and/or MLC values for QAC alkyl chain lengths from $n = 16$ to $n = 18$ will be examined in relation to the CMC values. The CMC value of dissolved ODTMA ($n = 18$) is 0.3 mM, whereas the CMC for $n = 16$ is 1 mM. The CMC of BDMHDA ($n = 16$) is 0.6 mM. For *P. aeruginosa*, the MLC value for the benzyldimethyloctadecyl ammonium ($n = 18$) QAC (Jono *et al.*, 1986, Table 1) is > 1.2 mM (> 466 mg/L), whereas the MLC value for a QAC with $n = 16$ is 0.3 mM or half of the CMC value. Here, an MLC value that exceeds the CMC

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value implies a reduced solution concentration of the free QAC, because a significant fraction of the QAC cations resides in micelles, which are less toxic. In the case of *S. aureus*, however, the MLC was 0.073 mM (Jono *et al.* 1986, Table 1) for a QAC from the same family (n = 18). The MLC value for a QAC with n = 16, however, was two times smaller (0.036 mM) or 16 times smaller than the CMC (0.6 mM). A similar pattern emerged for other QACs in Table 1 (Jono *et al.* 1986; Inacio *et al*. 2016). This pattern can still be reconciled with the suggestion that an increase in chain length can lower the active QAC concentration. This is because QAC solution concentrations are expected to be significantly higher at the negatively charged surfaces of bacteria and can form surface micelles at the expense of QAC monomers. Please note in passing that for the cyanobacteria *Aphanizomenon* and *Microcystis*, the ODTMA MLC values were 0.25 and 20 μM, respectively. These MLC values are 1200 and 15 times smaller than the CMC (300 μM) values. It is of interest to test the prediction that the MLC values of QACs similar to ODTMA but with n = 16 alkyl groups will be larger than those for n = 18 for *Aphanizomenon*, which is not like all the other cases in Table 1. A determination of the MLC values for QACs with n = 16 alkyl groups for *Microcystis* may also be instructive. Knauf *et al*. (2018) found that high concentrations of BZK act primarily through membrane damage, but the disruption of cellular protein homeostasis associated with cell death occurs at low concentrations.

A detailed explanation of the mechanism(s) of cell inactivation by QACs is beyond the scope of the present review, but citation and discussion of previous studies may be helpful in the future. One focal point of interest is how to optimize the removal of bacteria and other pathogenic microorganisms from water during filtration by utilizing the effect of released QACs.

Biocidal and/or biostatic effects during adsorption of bacteria in suspension or during filtration

Shtarker-Sassi *et al*. (2013) removed material from the center of a filter filled with a powdered BDMHDA-montmorillonite complex (mixed with excess sand at a 1:100 w/w ratio) after it was close to saturation with *E. coli* K-12 bacteria (K12 is a strain of *E. coli*). No colonies of bacteria were detected on LB agar plates supplemented with nutrients (LB is a common broth used for bacteria). Hence, filtration using the BDMHDA-montmorillonite complex might at least exert a biostatic effect on the bacteria. Kalfa *et al.* (2017) used *E. coli* S17 bacteria and found the same result in filters which contained granulated ODTMA or BDMHDA QAC cation complexes mixed with sand at a 1:22 w/w ratio (results not reported) (S17 is a strain of *E. coli*).

Respiration tests demonstrated that *E. coli* K-12 cells adsorbed to a suspended BDMHDA-montmorillonite micelle complex lost viability (Shtarker-Sassi *et al.* 2013) (*i.e*., adsorption of bacteria by the powdered complex produced a biocidal effect). It was not possible, however, to conclude in these cases whether the biostatic and biocidal effects were due to direct interactions of the complex with bacteria or whether it was due to small concentrations of dissolved QAC cations in the filter and in suspension because the QAC cations alone inhibited bacterial reproduction (Table 1, section 2).

Possible optimization of granulated micelle-clay complex design

The powdered (Mishael *et al.*, 2002, Polubesova *et al*., 2005) and granulated (Nir *et al*., 2015) micelle-clay complexes had an excess of positive charge, which corresponds to one half of the cation exchange capacity of the montmorillonite or bentonite sample. Under this condition, most of the added organic cations were adsorbed and their release into suspension either initially (Zadaka *et al*., 2005) or during filtration (Nir *et al*., 2015) was minimal. Note that the complex based on BDMHDA was significantly inferior to that based on ODTMA in perchlorate removal during filtration (Nir *et a*l., 2015), which contrasts with the effective bacteria removal (Kalfa *et al.* 2017). As Zadaka *et al*. (2005) and Kalfa *et al*. (2017) found, BDMHDA release from the complex was significantly greater than ODTMA release. The enhanced biostatic/biocidal effect produced by the larger number of free BDMHDA cations was advantageous in the removal of bacteria. In contrast for perchlorate removal, the free QAC cations might interact with perchlorate anions to form small binary or oligomeric complexes, which are not retained by the filter.

Removal of bacteria from water by filtration is expected to be more efficient when the (relatively small) concentrations of the released QAC cations are increased. A small increase in the amounts of ODTMA used during preparation of the granular micelle complex is expected to increase the ODTMA fraction in the complex and the concentrations of ODTMA cations released. The overall design assumes that the micelle-clay filter includes a bottom layer of activated C or is followed by a filter filled with activated C to capture the ODTMA cations that remain in solution.

The removal of cyanobacteria (Sukenik *et al.*, 2017) from water by filtration and the biocidal effect of small concentrations of ODTMA cations were demonstrated. In this case, however, disruption of the cyanobacteria cell integrity releases the toxins, which must be removed from the water. The ODTMA complex was shown to adsorb the *Microcystis* toxins efficiently. In general, however, it would be advantageous to estimate the conditions under which bacteria with toxins can be disrupted. Whenever no bacterial toxins are present or the toxins can be accounted for, one may deduce from recent results that it may be advantageous to prepare a

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granulated ODTMA-bentonite complex with a somewhat enlarged ODTMA/bentonite ratio beyond that currently used. The ordinary micelle-clay complex yields a positive charge which is 50% greater than the bentonite cation exchange capacity.

In recent preliminary results (D. Margalit and S. Nir, personal communication), a granulated ODTMA-clay micelle complex was prepared using 10% more ODTMA than the regular complex described in Nir *et al*. (2015). This granulated ODTMA-clay micelle complex yielded a significantly better reduction in total bacteria count (TBC) than the regular ODTMA-clay complex. During passage of the first 720 L through each of the 4 filters (duplicates of the two ODTMA-clay complexes), the removal of bacteria was practically identical for all filters and was close to 0 CFU/mL of emerging bacteria. The flow was then stopped for 2.5 days. After flow through was renewed, an additional 180 L was passed through each filter and the average number of emerging bacteria from the filters with the new complex was 4 CFU/mL *versus* 800 CFU/mL from the filters with the regular ODTMA complex. The flow was halted after 3 h for 21 h, then started again. After renewal of the flow, the average number of emerging bacteria from the filters with the new complex was 4 CFU/mL (as before) *vs.* 21,000 CFU/mL from the filters with the regular complex.

CONCLUSIONS

1. Bacteria can be efficiently removed from water by a combination of (i) filtration through a bed containing a granulated QAC-clay micelle complex and (ii) by the minute QAC concentrations released from the complex during filtration, which exert biostatic or biocidal effects on all or a part of the bacteria that emerge from the filter.

2. Another mode of removing bacteria from water is by just adding QACs in small concentrations. The QACs from USEPA (1988) groups I, II, and IV were shown to inactivate various types of bacteria with MIC and MLC values that spanned 4 orders of magnitude from >1000 mg/L to 75 μg/L due to differences in the QACs, the bacteria, and the incubation time.

3. Smaller MIC and MLC values are produced by QACs with a smaller head group.

4. The QACs with longer alkyl chains enhance bacterial inactivation but, in most recorded cases, the minimum MIC and MLC values were produced by QACs with n = 16 alkyl groups. This trend is explained by: (i) An increase in alkyl chain length of QACs enhances binding to bacteria, penetration into bacterial membranes, and membrane destabilization. (ii) an increase in QAC alkyl chain length lowers the CMC and, thus, reduces the concentrations of QAC monomers, which more efficiently inactivate bacteria than micelles. The ODTMA (n = 18) MLC value for the *aphanizomenon* (0.25 μM) cyanobacterium was significantly lower than the CMC (300 μM) value. This may suggest that QACs with an n = 16 alkyl group chain length will yield minimum MLC values.

5. A small increase in the ODTMA to clay ratio in comparison to the current ODTMA-clay complex preparation method (ODTMA equivalent to 150% of the clay cation exchange capacity) will increase the concentrations of released ODTMA cations during filtration. Consequently, bacteria removal from water by filtration will be more efficient.

REFERENCES

Alptuzun V., Tasli, H., and Erciyas, E. (2006) Synthesis and antimicrobial activity of some pyridinium salts. *Journal of the Faculty of Pharmacy of Ankara University*, **35**, 177–188.

Buckingham, S.C., Christopher, J.G., and Warr, G.G. (1993) Effect of head group size on micellization and phase behavior in quaternary ammonium surfactant system. *The Journal of Physical Chemistry*, **97**, 10236–10244.

Buffet-Bataillon, S., Tattevin, P., Bonnaure-Mallet, M., and Jolivet-Gougeon, A. **(**2012**)** Emergence of resistance to antibacterial agents: The role of quaternary ammonium compounds – A critical review. *International Journal of Antimicrobial Agents*, **39**, 381–389.

Carmona-Ribeiro, A.M. and de Melo Carrasco, L.M. (2013) Cationic antimicrobial polymers and their assemblies. *International Journal of Molecular Sciences*, **14**, 9906–9946.

Cooper, J.C. (1988) Review of the environmental toxicity of quaternary ammonium halides. *Ecotoxicology and Environmental Safety*, **16**, 65–71.

Ferreira, C., Pereira, A.M., Pereira, M.C., Melo, L.F., and Simões, M. (2011) Physiological changes induced by the quaternary ammonium compound benzyldimethyldodecylammonium chloride on *Pseudomonas fluorescens*. *Journal of Antimicrobial Chemotherapy*, **66**, 1036–1043.

Gilbert, P. and Al-Taae, A. (1985) Antibacterial activity of some alkyltrimethylammonium bromides. *Letters in Applied Microbiology*, **1**, 101–104.

Gilbert, P. and Moore, L.E. (2005) Cationic antiseptics: Diversity of action under a common epithet. *Journal of Applied Microbiology*, **99**, 703–715.

Inacio, A.S., Domingues, S.N., Nunes, A., Martins, P.T., Moreno, M.J., Estronca, L.M., Fernandes, R., Moreno, A.J.M., Borrego, M.J., Gomes, J.P., Vaz, W.L.C., and Vieira, O.V. (2016) Quaternary ammonium surfactant structure determines selective toxicity towards bacteria: Mechanisms of action and clinical implications in antibacterial prophylaxis. *Journal of Antimicrobial Chemotherapy*, **71**, 641–654.

Jono, K., Takayama, T., Kuno, M., and Higashide, E. (1986) Effect of alkyl chain length of benzalalkonium chloride on the bactericidal activity and binding to organic materials. *Chemical and Pharmaceutical Bulletin*, **34**, 4215–4224.

Kalfa, A., Rakovitsky, N., Tavassi, M., Ryskin, M., Ben-Ari, J., Etkin, H., Shuali, U., and Nir, S. (2017) Removal of *Escherichia coli* and total bacteria from water by granulated micelle-clay complexes: Filter regeneration and modeling of filtration kinetics. *Applied Clay Science*, **147**, 63–68.

Kenawy, E.R., Worley, S.D., and Broughton, R. (2007) The chemistry and applications of antimicrobial polymers: A state of the art review. *Biomacromolecules*, **8**, 1359–1384.

Knauf, G.A., Cunnigham, A.L., Kazi, M.I., Riddington, I.M., Cattoir, V., Trent, M.S., and Davies, B.W. (2018) Exploring the antimicrobial action of quaternary amines against

https://doi.org/10.1346/CCMN.2018.064116 Published online by Cambridge University Press

Acinetobater baumannii. *American Society for Microbiology*, **9**, 1–13. DOI:10.1288/mBio.02394–17.

Li, F., Weir, M.D., and Xu, H.H.K. (2013) Effects of quaternary ammonium chain length on antibacterial bonding agents. *Journal of Dental Research*, **92**, 932–938.

Madaan, P. and Tyagi, V.K. (2008) Quaternary pyridinium salts: A review. *Journal of Oleo Science*, **57**, 197–215.

Maillard, J.Y. (2002) Bacterial target sites for biocide action. *Journal of Applied Microbiology Symposium Supplement*, **92**, 16s–27s.

Maris, P. (1995) Modes of action of disinfectants. *Scientific and Technical Review of the Office International des Epizooties (Paris)*, **14**, 47–55.

Mishael, Y.G., Undabeytia, T., Rytwo, G., Papahadjopoulos-Sternberg, B., Rubin, B., and Nir, S. (2002) Sulfometuron adsorption *via* alkylammonium cations adsorption as monomers and micelles on montmorillonite. *Journal of Agricultural and Food Chemistry*, **50**, 2856–2863.

Nir, S., Brook, I., Anavi, Y., Ryskin, M., Ben-Ari, J., Shveky-Huterer, R., Etkin, H., Zadaka-Amir, D., and Shuali, U. (2015) Water purification from perchlorate by a micelle-clay complex: Laboratory and pilot experiments. *Applied Clay Science*, **114**, 151–156.

Polubesova, T., Nir, S., Zadaka, D., Rabinovitz, O., Serban, C., Groisman, L., and Rubin, B. (2005) Water purification from organic contaminants by optimized micelle-clay systems. *Environmental Science & Technology*, **39**, 2343–2348.

Shtarker-Sasi, A., Castro Sowinski, S., Matan, O., Kagan, T., Nir, S., Okon, Y., and Nasser, A. (2013) Removal of bacteria and cryptosporidium from water by micelle-clay complexes. *Desalination and Water Treatment*, **51**, 7672–7680.

Sukenik, A., Viner-Mozzin, Y., Tavassi, M., and Nir, S. (2017) Removal of cyanobacteria and cyanotoxins from lake water by composites of bentonite with micelles of the cation octadecyltrimethyl ammonium (ODTMA). *Water Research*, **120**, 165–173.

Tan, E.H.L. and Birge, R.R. (1996) Correlation between surfactant/micelle structure and stability of bacteriorhodopsin in solution, *Biophysical Journal*, **70**, 2385–2395.

Thorsteinsson, T., Masson, M., Kristinsson, K.G., Hjalmarsdottir, M.A., Hilmarsson, H., and Loftsson, T. (2003) Soft antibacterial agents: Synthesis and activity of labile environmentally friendly long chain quaternary ammonium compounds. *Journal of Medicinal Chemistry*, **46**, 4173–4181.

Undabeytia, T., Posada, R., Nir, S., Galindo, I., Laiz, L., Saiz-Jimenez C., and Morillo, E. (2014) Removal of waterborne microorganisms by filtration using clay-polymer complexes. *Journal of Hazardous Materials*, **279**, 190–198.

USEPA, United States Environmental Protection Agency. (1988) Clustering of Quaternary Ammonium Compounds. *PR Notice* 88-2.

Zadaka, D., Polubesova, T., Mishael,Y.G., Spitzy, A., Koehler, H., Wakshal, E., Rabinovitz, O., and Nir, S. (2005) Determination of release of organic cations from micelle-clay complexes and their re-adsorption in sand/clay columns. *Applied. Clay Science.* **29**, 282–286.

Zhang, C., Cui, F., Zeng, G.M., Yang, Z.Z., Yu, Z.G., Zhu, M.Y., and Shen, L.Q. (2015) Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment. *Science of the Total Environment*, **518–519**, 352–362.

Zhao, T. and Sun, G. (2008) Hydrophobicity and antimicrobial activities of quaternary pyridinium salts. *Journal of Applied Microbiology*, **104**, 824–830.

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