

Comparative antimicrobial activity of chlorine-based disinfectants for use within point-of-use drinking water systems

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Abstract:

Point-of-use [POU] drinking water treatment systems can provide alternative solutions to communities where access to centralised facilities are not available. This study compared the antimicrobial activity of three chlorine-based disinfectants (electrochemically activated solutions [ECAS], NaOCl and HOCl) using standard chemical disinfectant assays, at standardised equivalent free chlorine concentrations, at a contact time of 5 minutes, with varying organic loading. This study also assessed the antimicrobial of the disinfectants against established *Pseudomonas aeruginosa* biofilms. At free chlorine concentrations > 50 mg L⁻¹ ECAS exhibited the greatest antimicrobial activity, and the activity of the all disinfectants reduced as organic loading increased. ECAS exhibited the greatest antimicrobial activity against established biofilms. This study demonstrated ECAs had comparable antimicrobial activity compared to NaOCl and HOCl.

Keywords: Antimicrobial activity; Electrochemically activated solutions; Point-of-use drinking water production

Introduction

For the past century, chlorine has been critical in ensuring the production of biologically safe drinking water, and is the most widely used disinfectant for drinking water treatment. Alternative disinfection techniques that can be implemented as part of point-of-use [POU] drinking water treatment systems to ensure biological safe water production require investigation. Electrochemically activated solutions [ECAS] are generated by passing a weak NaCl solution (e.g. 1% [w/v]) through an electrochemical cell containing separate anodic and cathodic chambers. Anodic solutions are highly oxidative [> + 1000 mV], resulting in the generation of hydroxyl radicals and transient oxidative functional groups which are highly reactive and exhibit rapid antimicrobial kinetics (e.g. between 2 – 10 seconds) (Robinson et al., 2011).

This study investigated the antimicrobial activity of ECAS against *Escherichia coli* ATCC 10536 compared to NaOCl and HOCl, through standardised equivalent free chlorine [FC] concentrations. This study also investigated the antimicrobial activity of NaOCl, HOCl and ECAS at reducing established *Pseudomonas aeruginosa* ATCC 15442 biofilms (48 hours) through direct disinfection, standardised equivalent FC concentrations.

Methods

Three chlorine-based disinfectants were used throughout this study; sodium hypochlorite [NaOCl], hypochlorous acid [HOCl] and electrochemically activated solutions [ECAS].



Two standard planktonic bactericidal assays (British Standards Institution, 2005a, 2005b) assessed the antimicrobial activity of three disinfectants in the absence and presence of an inhibitory solution, bovine serum albumen [BSA], against a *Escherichia coli* ATCC 10536. Antimicrobial activity was compared through standardised equivalent FC concentrations: 25, 50, 100 and 150 mg L⁻¹.

Pseudomonas aeruginosa ATCC 15442 biofilms were established on polycarbonate substrates within a Centre for Disease Control Biofilm reactor. Standardised FC concentrations of 5, 25, 50, 75, 100 and 150 mg L⁻¹ were used as treatment regimens for each of the test disinfectants, with a 5-minute contact time.

Results and Discussion:

The antimicrobial activity of the disinfectants with no inhibitory solution present (sterile deionised water) resulted in comparable reductions for each of the disinfectants tested (Figure 1.1 A). However, at 25 mg L⁻¹ FC, NaOCl was significantly less efficacious than HOCl and ECAS, and HOCl exhibited the greatest antimicrobial activity of the three disinfectants tested. Introducing an inhibitory solution (0.3 g L⁻¹ BSA) resulted in ECAS and HOCl maintaining significantly greater activity compared to NaOCl at 25 and 50 mg L⁻¹ FC (Figure 1.1 B). At 25 mg L⁻¹ FC, ECAS was the only disinfectant to reduce the *E. coli* load by \geq 5 log₁₀ CFU mL⁻¹ (6.077 ± 1.441 log₁₀ CFU mL⁻¹). Further increasing the inhibitory solution concentration to 3.0 g L⁻¹ BSA, the antimicrobial activity of the disinfectants decreased (Figure 1.1 C). However, HOCl reduced *E. coli* by 7.282 ± 0.013 log₁₀ CFU mL⁻¹ at 100 and 150 mg L⁻¹ FC concentrations. Whereas, neither NaOCl and or ECAS reduced *E. coli* by >5 log₁₀ CFU mL⁻¹ for any of the free chlorine concentrations tested.

Direct disinfection of established *P. aeruginosa* biofilms on polycarbonate coupons resulted in comparable log reductions between NaOCl and HOCl at all FC concentrations (Figure 1.2). However, ECAS exhibited a significantly antimicrobial effect at FC concentrations $\geq 50 \text{ mg L}^{-1}$. The reduction in biofilm density, compared to NaOCl and HOCl, could be a result of transient antimicrobial species present at the point of ECAS generation, which rupture cell membranes inhibiting crucial microbial functions.

This study demonstrated that ECAS exhibits comparable antimicrobial activity to NaOCl and HOCl against planktonic *E. coli*. ECAS also exhibited comparable antimicrobial activity to NaOCl and HOCl in reducing established *P. aeruginosa* biofilms, at equivalent standardised free chlorine concentrations. This study demonstrated that both ECAS and HOCl should be considered as alternative disinfectants to typically used chlorine solutions (e.g. NaOCl) for point-of-use drinking water treatment applications.





Figure 1.1 Antimicrobial efficacy of ECAS $[\bigcirc]$, HOCl $[\square]$ and NaOCl $[\triangle]$ when free chlorine matched at a range of standardised concentrations against *E. coli* ATCC 10536 with [A] no interfering solution, interfering solutions of [B] 0.3 g L⁻¹ BSA and [C] 3.0 g L⁻¹ (British Standards Institution,2009). Dotted line represents the minimum log reduction (5 log CFU mL-1) required to demonstrate basic bactericidal activity under the experimental conditions of the assay (n = 3 ± SD).





Figure 1.2 Antimicrobial activity of NaOCl (\triangle), HOCl (\Box) and ECAS (\bigcirc) with standardised FC concentrations against *Pseudomonas aeruginosa* ATCC 15442 biofilm (n = 9 ± SD). Untreated biofilm density (-) refers to the mean CFU coupon⁻¹ recovered from control treatment (0 mg L⁻¹); n = 18.

REFERENCES

- British Standards Institution, 2005a. Chemical disinfectants and antiseptics Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics — Test method and requirements (phase 1), European Committee for Standardization.
- British Standards Institution, 2005b. Chemical disinfectants and antiseptics Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics — Test method and requirements (phase 1), European Committee for Standardization.
- Robinson, G.M., Tonks, K.M., Thorn, R.M.S., Reynolds, D.M., 2011. Application of Bacterial Bioluminescence To Assess the Efficacy of Fast-Acting Biocides. Antimicrob. Agents Chemother. 55, 5214–5219. doi:10.1128/AAC.00489-11