Effect of bromide on the degradation kinetics of antibiotic resistance genes during water disinfection with chlorine

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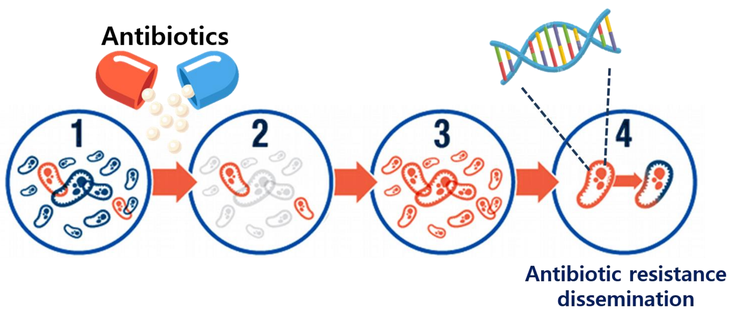
Introduction

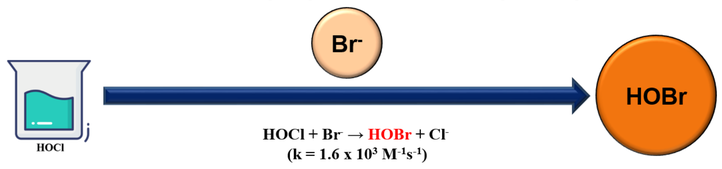
Environmental spread of antibiotic resistance



❖ Misuse and overuse of antibiotics selectively increases the proportion of antibiotic resistant bacteria in the bacterial population

❖ Antibiotic resistance is encoded in antibiotic resistant gene which can be disseminated by horizontal gene transfer pathways

Known effect of Bromide (Br^-) on Chlorine (HOCl)



❖ Br^- is ubiquitous in natural waters and wastewaters at concentrations ranging from a few tens $\mu g \cdot L^{-1}$ to $mg \cdot L^{-1}$.

❖ Free available chlorine (FAC) ($HOCl/OCl^-$) is widely used as a disinfectant for drinking water and wastewater.

❖ Free available bromine (FAB) ($HOBr/OBr^-$) is generated during HOCl treatment of Br^--containing water. Bromine usually exhibits higher reactivity than chlorine toward (in)organic compounds

❖ The effect of Br^- on HOCl disinfection and the degradation kinetics of ARG during HOBr disinfection are poorly understood

Research objectives

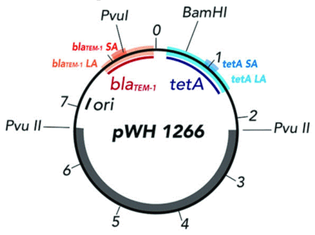
❖ To determine and compare the degradation kinetics of antibiotic resistant genes (ARGs) during HOBr and HOCl disinfection

❖ To quantitatively understand the effect of Br^- during HOCl disinfection

❖ To elucidate the effect of pH during HOCl disinfection in the presence of Br^-

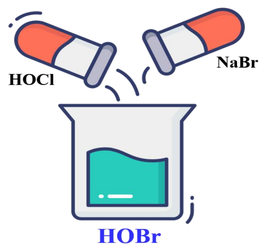
Methods

pWH1266 plasmid



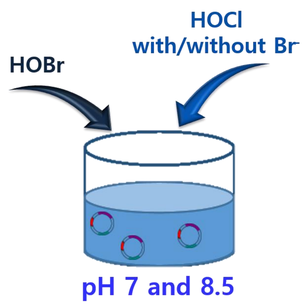
❖ The pWH1266 plasmid encodes an ampicillin resistance gene (*bla*TEM-1) and a tetracycline resistance gene (*tet*A)

Reagent preparation



❖ HOBr was prepared by mixing HOCl and NaBr for at least 3 days

HOBr, HOCl with/without Br^- treatment



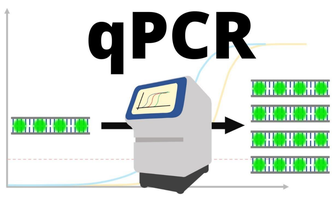
❖ pWH1266 was treated with 12.5 μM of HOBr, 60 μM of HOCl, under homogeneous mixing conditions at pH 7 and 8.5 in a phosphate buffer (PB)

❖ The treatments were performed with and without Br^- presence (concentration range of 6-12 μM)

❖ At each sampling point, the residual concentration of HOBr and HOCl was measured using ABTS colorimetric methods

❖ After the predetermined time intervals, samples were gathered and immediately quenched with sodium thiosulfate

Analytical methods



❖ **Quantitative polymerase chain reaction (qPCR)**
Molecular biological measurement of gene degradation

❖ **Four types of amplicons were used to investigate the correlation between gene type, amplicon length, and gene degradation.**
Short amplicon: *tet*A_216bp, *bla*TEM-1_209bp
Long amplicon: *tet*A_1136bp, *bla*TEM-1_829bp

Results & Discussion

Degradation kinetics of ARGs during exposure to HOBr and HOCl)

Symbols are the experimental data, and the dashed lines represent the linear regression for HOBr data, or two-step reaction model fits for HOCl data.
[pWH1266]=10^{11} copies/ml (1.5 μM), [HOBr]=12.5 μM, [HOCl]=60 μM,, room temperature, pH=7 and 8.5 (phosphate buffer)

❖ In all cases, faster degradation of ARG amplicons was achieved for longer amplicon length, due to increased reaction sites in the amplicon

<Two-step reaction model for DNA and FAC>

1) $Amp + FAC \underset{Na_2S_2O_3}{\overset{k_{FAC,Amp}}{\rightleftharpoons}} Amp_{N\text{-}Cl}$

2) $Amp_{N\text{-}Cl} + FAC \xrightarrow{k_{FAC,AmpN\text{-}Cl}} Product$

3) $\frac{d[product]}{dt} = k_{FAC,\,Amp_{N\text{-}Cl}}[FAC][Amp_{N\text{-}Cl}] = k_{FAC,\,N\text{-}Cl\ bp}\left(\frac{\#N\text{-}Cl\ bp}{Amp_{N\text{-}Cl}}\right)[FAC][Amp_{N\text{-}Cl}]$

❖ At pH 7, the degradation of ARGs by HOCl was initially slow but accelerated with increased chlorine exposure, exhibiting accelerating kinetics. These observed kinetics could be described by the sequential, two-step reaction model proposed by He et al. (2019) (see left)

❖ The rate constants of N-chlorination were $3.3(\pm0.2)\times10^3$ $M^{-1}s^{-1}$ for *tetA* 216 bp and $2.9(\pm0.2)\times10^3$ $M^{-1}s^{-1}$ for $bla_{TEM\text{-}1}$ 209 bp, and increased to 1.6 $(\pm0.1)\times10^4$ $M^{-1}s^{-1}$ for *tetA* 1136 bp and $1.1(\pm0.07)\times10^4$ $M^{-1}s^{-1}$ for $bla_{TEM\text{-}1}$ 829 bp. The rate constant ($k_{FAC,\,N\text{-}Cl\ bp}$) for C-chlorination of individual base pairs of 2.9×10^{-1} $M^{-1}s^{-1}$ was used

❖ During HOBr treatment, the accelerating kinetics was not observed, which was different from the case of chlorination. The logarithmic degradation of ARGs was linear with respect to HOBr exposure, indicating second-order kinetics. The resulting second-order rate constants for the degradation of ARGs by HOBr were $4.9(\pm0.3)\times10^2$ $M^{-1}s^{-1}$ for *tetA* 216 bp, $4.0(\pm0.2)\times10^2$ $M^{-1}s^{-1}$ for $bla_{TEM\text{-}1}$ 209 bp, $1.6(\pm0.1)\times10^3$ $M^{-1}s^{-1}$ for *tetA* 1136 bp, and $1.1(\pm0.07)\times10^3$ $M^{-1}s^{-1}$ for $bla_{TEM\text{-}1}$ 829 bp

❖ The base pair normalized rate constant for HOBr was 2.3 $M^{-1}s^{-1}$ for *tetA* 216 bp, 1.9 $M^{-1}s^{-1}$ for $bla_{TEM\text{-}1}$ 209 bp, 1.4 $M^{-1}s^{-1}$ for *tetA* 1136 bp, and 1.3 $M^{-1}s^{-1}$ for $bla_{TEM\text{-}1}$ 829 bp. These values are larger than the rate constant for C-chlorination of individual base pair of 0.29 $M^{-1}s^{-1}$ by factors of 4.6 – 7.8. This indicates that formation of HOBr during chlorination can enhance the degradation of ARGs

❖ At pH 8.5, gene degradation rates were slower for both HOCl and HOBr treatments, showing a ratio of about 0.6 compared to that at pH 7, likely due to the decreased concentration of HOCl/HOBr, which are known to be more oxidizing than OCl^-/OBr^- as the pH increases

Degradation kinetics of ARGs during chlorination without and with Br^-

Symbols are the experimental data, and the dashed lines represent the model simulation.
[pWH1266]=10^{11} copies/ml (1.5 μM), [HOCl]=60 μM, [Br^-]=6, 9, 12 μM, room temperature, pH=7 and 8.5 (phosphate buffer).

❖ As the Br^- concentration increased from 6 to 12μM, compared to HOCl treatment alone, the ARG was additionally degraded from 1.1 to 4.6-log at pH 7 and from 0.6 to 1.9-log at pH 8.5, showing that the rate of gene degradation increased with the increase of concentration of Br^-

❖ The reaction rate constant between HOCl and Br^- is 1600 $M^{-1}S^{-1}$, indicating rapid HOBr formation. Additionally, as observed in previous result, the generated HOBr demonstrated a higher reactivity with DNA than that of HOCl

❖ At pH 8.5, a less increase in gene degradation was observed for the same concentration of Br^- compared to that at pH 7

❖ This is thought to be due to the decreased concentration of the more reactive HOBr as pH increases, resulting in reduced reactivity between DNA and FAB

❖ Another contributing factor is that the pKa of FAC is 7.5, meaning that at pH 8.5, only approximately 10% of FAC is in the more reactive HOCl form. This implies that a lower concentration of HOBr was generated at pH 8.5 than at pH 7

< Key reactions for the degradation of ARGs during chlorination of Br-containing water >

4) $HOCl + Br^- \rightarrow HOBr + Cl^-$ (k=1550 $M^{-1}s^{-1}$)

5) $Amp + HOBr \rightarrow Product$
(e.g. k=1600 $M^{-1}s^{-1}$ for *tet*A 1136bp)

*Reactions 1 – 3 (i.e., two-step DNA/FAC reaction model) were also included in the model

❖ The reaction model was constructed based on formation of HOBr from the reaction of HOCl with Br-, reaction of bromine with ARGs, and the two-step reactions for the reaction of chlorine with ARGs (see left)

❖ The developed model could reasonably well simulate the degradation kinetics of ARGs

Conclusions

❖ Bromine exhibited high reactivity toward DNAs with second-order rate constants of 4.0×10^2 $M^{-1}s^{-1}$ – 1.6×10^3 $M^{-1}s^{-1}$ for the investigated ARGs

❖ During chlorination, the degradation of ARGs was significantly enhanced in the presence of Br^- (6 – 12 μM) due to a formation of HOBr and its reaction with ARGs

❖ The increase in gene degradation rate due to the effect of Br^- during chlorination decreases as the pH increases due to the reduced concentration of the more reactive HOCl/HOBr in the FAC/FAB

❖ The developed two-step reaction model could well describe the kinetic behavior of ARG degradation during chlorination in the presence of Br^-

❖ The effect of Br^- on the degradation of ARG during chlorination needs to be tested in real water matrices, as real water matrix components such as DOM can affect the fate of HOBr, the key reactive intermediate

Acknowledgement

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