Supporting information

Chloride enhances DNA reactivity with chlorine at conditions relevant to water treatment

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References
**Text S1. qPCR assay descriptions.**

qPCR assays for bacteriophage T3 (regions A + B) dsDNA and bacteriophage φX174 (Regions A + B) ssDNA were adapted from Qiao et al.\textsuperscript{1} 10 μL qPCR assays were used for each region, consisting of: 5 μL Fast EvaGreen qPCR Master Mix (Biotium), 2.075 μL of nuclease free water (IDT), 1 μL of ROX reference dye (Biotium), 0.8 μL of 5 μM forward and reverse primer mixture (IDT), 0.125 μL of 50 mg/mL ultrapure bovine serum albumin (BSA) (LifeTechnologies), and 1 μL of template. All assays were run on a QuantStudio 3 thermocycler (Applied Biosystems). For T3 Regions A and B, thermocycling conditions included a 95°C initial denaturation step for 2 minutes, followed by 40 cycles of 95°C denaturation step for 15 sec, 60°C annealing step for 30 sec, and 72°C extension step for 45 sec. A melt curve analysis performed at 0.1°C/s at a temperature range from 60°C to 95°C showed that a single target amplicon was amplified during each reaction. For φX174 Regions A and B, the thermocycling conditions included a 95°C initial denaturation step for 5 minutes, followed by 40 cycles of 95°C denaturation step for 20 sec, 55°C annealing step for 20 sec, and 72°C extension for 20 sec. A melt curve analysis performed at 0.1°C/s at a temperature range from 55°C to 95°C showed that a single target amplicon was amplified during each reaction. For both T3 and φX174, DNA extracted from purified bacteriophage stocks was used for standard curves, with a working range of ~10\textsuperscript{2} to ~10\textsuperscript{8} gc/mL. No template controls did not amplify during the runs. The regions targeted, region size, primers used, assay efficiency and R2 value corresponding to the assay standard curve as recommended in the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE)\textsuperscript{2} are listed in Table S1.

**Text S2. Trace contaminant effect on dsDNA FAC reactivity**

We tested whether trace contaminants present in salts affect FAC reactivity with DNA. To do so, we measured FAC reactivity with dsDNA (pH = 9.0) in the presence of 10 mM trace metal grade perchlorate or nitrate salts. We compared the FAC reactivity to that observed with 10 mM ACS grade perchlorate salt used throughout the manuscript (Figure S7). The observed second order rate constants were within 10% of each other regardless of salt used (Table S6). Statistical tests comparing the second order rate constants showed no differences between the conditions (Table S13). When we used statistical tests to compare samples collected at the longest reaction time, we found that the Region A amplicon sample in the trace metal grade perchlorate condition was statistically different from the Region A amplicon sample in the ACS grade perchlorate condition (Table S14). However, given the lack of statistical significance in all other comparisons, it is highly unlikely that trace contaminants in salts affect FAC reactivity with DNA in our study.
**Text S3.** Estimating the contribution of HOCl, OCl\(^-\), Cl\(_2\)O and Cl\(_2\) to DNA FAC reactivity.

We used the variable-chloride and variable-pH data sets (Table S6; 10 mM added salts) to estimate the contribution of chlorine species to DNA FAC reactivity.

Given that 5 mg Cl\(_2\)/L of free active chlorine (FAC) was added to each reaction, and less than 10% of FAC was lost throughout the reactions, we assumed a constant, 5 mg Cl\(_2\)/L (0.071 mM), FAC concentration. Four FAC species, HOCl, OCl\(^-\), Cl\(_2\)O and Cl\(_2\) were considered as potential reactive chlorinating agents in our kinetic models.

We used the following equilibrium equations to construct a chlorine speciation diagram (Fig S10):

\[
\begin{align*}
\text{HOCl} & \rightleftharpoons \text{OCl}^- + \text{H}^+ \quad \text{pK}_{a,\text{HOCl}} = 7.54 \quad (\text{eq. S1}) \\
\text{HOCl} + \text{Cl}^- + \text{H}^+ & \rightleftharpoons \text{Cl}_2(aq) + \text{H}_2\text{O} \quad \log K_{\text{Cl}_2} = 2.72 \quad (\text{eq. S2}) \\
2 \text{HOCl} & \rightleftharpoons \text{Cl}_2\text{O}(aq) + \text{H}_2\text{O} \quad \log K_{\text{Cl}_2\text{O}} = -2.06 \quad (\text{eq. S3}) 
\end{align*}
\]

Our experiments were conducted under pseudo-first-order conditions, and the change in DNA concentration over time was expressed as:

\[
- \frac{d[DNA]}{dt} = k_{\text{obs}}[DNA] \quad (\text{eq. S4})
\]

where [DNA] is the concentration of either ssDNA or dsDNA, and \(k_{\text{obs}}\) is the observed pseudo-first-order rate constant calculated from the apparent second-order rate constants reported in Table S6. \(k_{\text{obs}}\) can be rewritten as a function of second-order reaction rate constants:

\[
k_{\text{obs}} = k_{\text{HOCl}}[\text{HOCl}] + k_{\text{OCl}^-}[\text{OCl}^-] + k_{\text{Cl}_2\text{O}}[\text{Cl}_2\text{O}] + k_{\text{Cl}_2}[\text{Cl}_2] \quad (\text{eq. S5})
\]

where [HOCl], [OCl\(^-\)], [Cl\(_2\)O] and [Cl\(_2\)] are the concentrations of HOCl, OCl\(^-\), Cl\(_2\)O and Cl\(_2\) in the system. Equilibrium speciation of FAC was determined by simultaneously solving eqs S1 – S3, in conjunction with a mass balance equation for FAC (eq. S6):

\[
[FAC] = [\text{HOCl}] + [\text{OCl}^-] + 2[\text{Cl}_2\text{O}] + [\text{Cl}_2] \quad (\text{eq. S6})
\]

Scientist 3.0 was used to conduct all regression analyses based on the following algorithms:

**dsDNA:**

Using the variable-chloride data set (n = 8; Table S6), second-order rate constants for Cl\(_2\) and HOCl were fit simultaneously while assuming a negligible contribution from OCl\(^-\). These results gave good fits to the data collected as a function of pH (10 mM NaCl; Table S6), which serve as robust, independent validation of the calculated second-order rate constants. Inclusion of a second-order rate constant for OCl\(^-\) or Cl\(_2\)O did not improve model fits across conditions for dsDNA samples. When OCl\(^-\) was included for dsDNA data, the overall error, driven by error at
pH = 6.0-7.5 increased. Hence, we did not include OCl\(^-\) as a reactant for dsDNA data. We assume that OCl\(^-\) and Cl\(_2\)O do not contribute appreciably to the reactivity of dsDNA in these systems.

**ssDNA:**
1. Using the variable-chloride data set (n = 8; Table S6), second-order rate constants for Cl\(_2\) and HOCl were fit simultaneously while assuming a negligible contribution from OCl\(^-\).
2. Using the variable pH data (10 mM added NaCl; Table S6), second-order rate constant for OCl\(^-\) was fit while assuming fixed values for the second-order rate constant for HOCl and Cl\(_2\) (determined in the previous step).
3. Using the variable-chloride data set (n = 8), second-order rate constants for Cl\(_2\) and HOCl were fit simultaneously while assuming a fixed value for the second-order rate constant for OCl\(^-\) (determined in the previous step).
4. Steps 2 and 3 were repeated iteratively until second-order rate constants for all active chlorine species changed by <1% upon subsequent iteration.

Inclusion of a second-order rate constant for Cl\(_2\)O did not improve model fits. Therefore, we assume that Cl\(_2\)O does not contribute appreciably to the reactivity of ssDNA in these systems.

The resulting values for \(k_{HOCl}\), \(k_{OCl^-}\), and \(k_{Cl_2}\) for ssDNA and dsDNA are listed in Table 1 in the main manuscript. Using these values, we calculated the contribution of HOCl, OCl\(^-\), and Cl\(_2\) to the reactivity of the ssDNA and dsDNA genomes (Figure S11).
Text S4. Analysis of salts and solutions for bromide

Six nominally bromide-free salts were analyzed for bromide following the method of Dias et al.\(^3\) The six examined salts included NaCl (ACS grade), NaCl (trace-metal grade), NaClO\(_4\) (ACS grade), NaClO\(_4\) (trace-metal grade), NaNO\(_3\) (ACS grade), and NaH\(_2\)PO\(_4\) (ACS grade). Solutions containing ~1 M of each salt were prepared in ultrahigh-purity (Miliq) water. Solutions of phosphate-buffered saline (PBS) and ultrahigh-purity water (solvent blank) were also analyzed. NaOCl was added to aliquots of each salt solutions and reagent blank to oxidize any bromide present into HOBr. NaOCl-amended aliquots were allowed to stand at room temperature for 2 min prior to addition of 1,3,5-trimethoxybenzene (TMB, methanolic spike) to derivatize HOBr into 1-bromo-2,4,6-TMB (Br-TMB). Quenched samples were extracted into toluene and were analyzed for Br-TMB by gas chromatography with a quadrupole mass analyzer. For complete methodological details, see Dias et al.\(^3\)

Bromide was detected in ACS and trace-metal grade NaCl at 58 and 11.3 mg/kg (Table S15). These levels of bromide as an impurity in NaCl samples are on par with those reported by Dias et al.\(^3\) All other examined salts had concentrations of bromide less than 2 mg/kg.
Table S1. qPCR assay parameters for T3 and φX174 genomes.

| Bacteriophage | Genome type | Genome size | Regions analyzed | Primers (5’ to 3’) | Region location (bases or base pairs) | Region size | Efficiency | R²     |
|---------------|-------------|-------------|------------------|--------------------|---------------------------------------|-------------|------------|--------|
| T3            | dsDNA       | 38.2 kbp    | Region A         | F: AACGCAAAGGTCAAACGCATC R: CTGCATGACCTGAATGTCG F: GAGACGACACGTCACCCCCCTTT R: TCTCTCTCTCGGCGCAGTGTAA | 1678-2186 | 509 bp     | 0.90-0.95 | > 0.99 |
|               |             |             | Region B         |                    | 11825-12324                        | 499 bp     | 0.92-0.97 | > 0.99 |
| φX174         | ssDNA       | 5.4 kbp     | Region A         | F: GACGCTGAGCATTGTTGGA R: ATCTGACCAGCGAAGGAGCC F: GGGCTTATATCTCCTGCGCAT R: CCACTGCAAACAATGACGGG | 571-1074  | 504 bp     | 0.87-0.92 | > 0.99 |
|               |             |             | Region B         |                    | 1717-2269                          | 493 bp     | 0.88-0.92 | > 0.99 |
Table S2. p-values comparing T3 dsDNA genome degradation at elevated chloride concentrations. Statistical differences between conditions were determined by comparing observed second order rate constants modelled from the pseudo-first order portions of the reactions. p-values < 0.05 are bolded.

|          | 0 mM       | 10 mM      | 50 mM      | 100 mM     |
|----------|------------|------------|------------|------------|
|          | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0 mM     |        |        |        |        |        |        |        |        |
| Reg A    | 0.35  | -     | 0.19  | -     | -     | -     | -     | -     |
| Reg B    |        | -     | 0.18  | -     | -     | -     | 0.0075| -     |
| 10 mM    |        |        |        |        |        |        |        |        |
| Reg A    | 0.19  | -     | -     | 0.19  | -     | -     | 0.0062| -     |
| Reg B    | 0.18  | -     | 0.19  | -     | -     | -     | -     | -     |
| 50 mM    |        |        |        |        |        |        |        |        |
| Reg A    | 0.0099| -     | -     | 0.0062| -     | -     | 0.26  | -     |
| Reg B    | 0.0075| 0.0052| -     | 0.0052| 0.26  | -     | 0.016 | -     |
| 100 mM   |        |        |        |        |        |        |        |        |
| Reg A    | 0.048 | -     | 0.0034| -     | -     | 0.016 | -     | 0.52  |
| Reg B    | 0.012 | -     | 0.013 | -     | -     | -     | -     | -     |

Table S3. p-values comparing T3 dsDNA genome degradation at elevated chloride concentrations. Given the deviation from linear kinetics for select reaction conditions, statistical differences between conditions were determined by comparing samples collected at the longest reaction times. p-values < 0.05 are bolded.

|          | 0 mM       | 10 mM      | 50 mM      | 100 mM     |
|----------|------------|------------|------------|------------|
|          | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0 mM     |        |        |        |        |        |        |        |        |
| Reg A    | 0.99  | -     | 0.031 | -     | 0.019 | -     | 0.014 | -     |
| Reg B    |        | 0.99  | -     | 0.00034| -     | 0.0019| -     | 0.0017|
| 10 mM    |        |        |        |        |        |        |        |        |
| Reg A    | 0.031 | -     | -     | 0.0034| 0.40  | -     | 0.0089| -     |
| Reg B    | -     | 0.00034| -     | 0.40  | -     | 0.019 | 0.015 | -     |
| 50 mM    |        |        |        |        |        |        |        |        |
| Reg A    | 0.019 | -     | 0.0089| -     | 0.019 | 0.76  | 0.022 | -     |
| Reg B    | 0.0019| -     | 0.0019| -     | -     | 0.0079| -     | 0.25  |
| 100 mM   |        |        |        |        |        |        |        |        |
| Reg A    | 0.014 | -     | 0.0015| -     | 0.014 | 0.0079| -     | 0.25  |
| Reg B    | 0.0017| -     | 0.014 | -     | 0.0079| -     | -     | -     |
Table S4. p-values comparing φX174 ssDNA genome degradation at elevated chloride concentrations. Statistical differences between conditions were determined by comparing observed second order rate constants modelled from the pseudo-first order portions of the reactions. p-values < 0.05 are bolded.

|       | 0 mM |     | 100 mM |     |
|-------|------|-----|---------|-----|
|       | Reg A | Reg B | Reg A | Reg B |
| 0 mM  | -    | 0.39 | 0.64   | -   |
| 100 mM| 0.64 | -    | -      | 0.51|

Table S5. p-values comparing φX174 ssDNA genome degradation at elevated chloride concentrations. Given the deviation from linear kinetics for select reaction conditions, statistical differences between conditions were determined by comparing samples collected at the longest reaction times. p-values < 0.05 are bolded.

|       | 0 mM |     | 100 mM |     |
|-------|------|-----|---------|-----|
|       | Reg A | Reg B | Reg A | Reg B |
| 0 mM  | -    | 0.61 | 0.81   | -   |
| 100 mM| 0.81 | -    | -      | 0.66|
Table S6. Based normalized apparent second order reaction rate constants for the reaction of T3 dsDNA genome and φX174 with FAC (5 mg-CI2/L; 0.071 mM). Reaction rate constants were calculated using data where pseudo-first order kinetics held (where the r^2 for the log-linear fit was greater than 0.97). Asterisks mark reaction rate constants that were modelled through a subset of the collected data set (where the r^2 for the log-linear fit was greater than 0.97). For rate constants that are not marked with an asterisk, pseudo-first-order kinetics held through the entire collected data set (the r^2 was greater than 0.97 for the log-linear fit for all the data points collected). Error bars indicate the range of experimental duplicates.

| pH = 7.5 | k (M^-1 s^-1 b^-1) | r^2 |
|---------|---------------------|-----|
|          | T3                  | φX174 | T3 | φX174 |
| pH = 7.5 |                     |       |    |       |
| 5 mM phosphate | 100 mM salt | Region A | 0.106 ± 4.5 x 10^-3 | 10.37 ± 8.5 x 10^-2 | 0.99 | 0.98 |
|          |                    | Region B | 0.113 ± 3.8 x 10^-3 | 10.65 ± 2.4 x 10^-1 | 0.99 | 0.97 |
|          |                    | Region A | 0.116 ± 2.3 x 10^-3 | - | - | - |
|          |                    | Region B | 0.122 ± 2.3 x 10^-3 | - | - | - |
|          |                    | Region A | 0.156 ± 2.3 x 10^-3 | - | - | - |
|          |                    | Region B | 0.160 ± 1.5 x 10^-3 | - | - | - |
|          |                    | Region A | 0.191 ± 3.8 x 10^-3 | 10.51 ± 2.4 x 10^-1 | 0.99 | 0.97 |
|          |                    | Region B | 0.198 ± 8.5 x 10^-3 | 11.01 ± 5.8 x 10^-1 | 0.99 | 0.97 |
| pH = 7.5 |                     |       |    |       |
| 5 mM phosphate | 10 mM salt | Region A | 0.077 ± 1.4 x 10^-3 | 9.30 ± 1.9 x 10^-1 | 0.98 | 0.99 |
|          |                    | Region B | 0.080 ± 7.1 x 10^-4 | 9.16 ± 2.9 x 10^-2 | 0.99 | 0.98 |
|          |                    | Region A | 0.085 ± 7.0 x 10^-4 | - | - | - |
|          |                    | Region B | 0.085 ± 2.1 x 10^-3 | - | - | - |
|          |                    | Region A | 0.092 ± 7.0 x 10^-4 | - | - | - |
|          |                    | Region B | 0.091 ± 2.1 x 10^-3 | - | - | - |
|          |                    | Region A | 0.102 ± 2.1 x 10^-3 | 9.02 ± 2.8 x 10^-2 | 0.99 | 0.99 |
|          |                    | Region B | 0.105 ± 2.9 x 10^-3 | 9.16 ± 1.7 x 10^-1 | 0.99 | 0.97 |
| pH = 6.0 |                     |       |    |       |
| 5 mM phosphate | 10 mM salt | Region A | 0.229 ± 7.0 x 10^-4 | 12.85 ± 4.4 x 10^-1 | 0.99 | 0.99 |
|          |                    | Region B | 0.221 ± 1.4 x 10^-3 | 13.77 ± 4.8 x 10^-1 | 0.99 | 0.99 |
|          |                    | Region A | 0.476 ± 3.2 x 10^-2 | 14.38 ± 4.8 x 10^-1 | - | - |
|          |                    | Region B | 0.452 ± 1.8 x 10^-2 | 15.41 ± 2.2 x 10^-1 | - | - |
|          |                    | Region A | 0.673 ± 3.5 x 10^-3 | 17.68 ± 3.8 x 10^-1 | - | - |
|          |                    | Region B | 0.701 ± 3.1 x 10^-2 | 18.96 ± 2.7 x 10^-1 | - | - |
|          |                    | Region A | 0.869 ± 1.4 x 10^-3 | 23.15 ± 4.3 x 10^-2 | * | * |
|          |                    | Region B | 0.795 ± 5.7 x 10^-3 | 25.06 ± 1.5 x 10^-2 | 0.99 | 0.99 |
| pH = 6.8 |                     |       |    |       |
| 5 mM phosphate | 10 mM salt | Region A | 0.085 ± 5.6 x 10^-3 | 6.69 ± 2.0 x 10^-1 | 0.99 | 0.99 |
|          |                    | Region B | 0.082 ± 3.4 x 10^-3 | 5.82 ± 8.7 x 10^-2 | 0.99 | 0.99 |
|          |                    | Region A | 0.157 ± 2.1 x 10^-3 | 9.23 ± 2.7 x 10^-1 | 0.99 | 0.98 |
|          |                    | Region B | 0.161 ± 7.1 x 10^-4 | 9.09 ± 1.3 x 10^-1 | 0.99 | 0.99 |
| pH = 7.5 |                     |       |    |       |
| 5 mM phosphate | 10 mM salt | Region A | 0.077 ± 1.4 x 10^-3 | 9.30 ± 1.9 x 10^-1 | 0.99 | 0.99 |
|          |                    | Region B | 0.080 ± 7.1 x 10^-4 | 9.16 ± 2.9 x 10^-2 | 0.99 | 0.99 |
|          |                    | Region A | 0.102 ± 2.1 x 10^-3 | 9.02 ± 2.9 x 10^-2 | 0.99 | 0.98 |
|          |                    | Region B | 0.105 ± 2.9 x 10^-3 | 9.16 ± 1.7 x 10^-1 | 0.99 | 0.99 |
| pH = 9.0 |                     |       |    |       |
| 5 mM phosphate | 10 mM salt | Region A | 0.047 ± 7.0 x 10^-4 | 17.54 ± 7.1 x 10^-2 | * | 0.98 |
|          |                    | Region B | 0.047 ± 1.4 x 10^-3 | 17.18 ± 7.7 x 10^-1 | * | 0.97 |
|          |                    | Region A | 0.042 ± 1.4 x 10^-3 | - | - | - |
|          |                    | Region B | 0.042 ± 1.4 x 10^-3 | - | - | - |
|          |                    | Region A | 0.042 ± 2.0 x 10^-3 | - | - | - |
|          |                    | Region B | 0.042 ± 8.9 x 10^-4 | - | - | - |
|          |                    | Region A | 0.032 ± 1.4 x 10^-3 | 16.54 ± 1.1 x 10^1 | 0.97 | 0.97 |
|          |                    | Region B | 0.032 ± 7.0 x 10^-4 | 17.18 ± 1.2 x 10^-1 | 0.98 | 0.98 |
**Table S7.** p-values comparing T3 dsDNA genome degradation at chloride concentrations relevant to water treatment. Statistical differences between conditions were determined by comparing observed second order rate constants modelled from the pseudo-first order portions of the reactions. p-values < 0.05 are bolded.

|       | 0 mM      | 3 mM      | 7 mM      | 10 mM     |
|-------|-----------|-----------|-----------|-----------|
|       | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0 mM  |        |        |        |        |        |        |        |        |        |        |
|       | 0.15  | -     | 0.038 | -     | 0.011 | -     | 0.0096 | -     |
| 3 mM  | 0.038 | -     | 0.99  | 0.019 | 0.019 | -     | 0.015  | -     |
| 7 mM  | 0.011 | -     | 0.020 | 0.59  | 0.041 | -     | 0.015  | -     |
| 10 mM | 0.0096 | -     | 0.041 | -     | 0.049 | 0.51  | -       | -     |

**Table S8.** p-values comparing T3 dsDNA genome degradation at chloride concentrations relevant to water treatment. Given the deviation from linear kinetics for select reaction conditions, statistical differences between conditions were determined by comparing samples collected at the longest reaction times. p-values < 0.05 are bolded.

|       | 0 mM      | 3 mM      | 7 mM      | 10 mM     |
|-------|-----------|-----------|-----------|-----------|
|       | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0 mM  |        |        |        |        |        |        |        |        |        |        |
|       | 0.93  | -     | 0.031 | -     | 0.0075 | -     | 0.0029 | -     |
| 3 mM  | 0.031 | -     | 0.97  | 0.089 | -     | 0.12  | 0.031  | -     |
| 7 mM  | 0.0075 | -     | 0.97  | 0.97  | -     | 0.039 | -       | 0.050 |
| 10 mM | 0.0029 | -     | 0.050 | -     | 0.0041 | 0.93  | -       | -     |
**Table S9.** p-values comparing φX174 ssDNA genome degradation at varying pHs. Statistical differences between conditions were determined by comparing observed second order rate constants modelled from the pseudo-first order portions of the reactions. p-values < 0.05 are bolded.

| pH     | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM |
|--------|------|-------|------|-------|------|-------|------|-------|------|-------|
| pH = 6.0 |     |       |      |       |      |       |      |       |      |       |
| 0 mM    | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 29      | 0.0018 | 0.0020 | 34   | 0.020 | 31   | 0.020 | 31   | 0.020 | 31   | 0.020 |
| pH = 6.8 |     |       |      |       |      |       |      |       |      |       |
| 0 mM    | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0.057   | 0.017  | 0.0023 | 34   | 0.020 | 31   | 0.020 | 31   | 0.020 | 31   | 0.020 |
| pH = 7.5 |     |       |      |       |      |       |      |       |      |       |
| 0 mM    | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0.036   | 0.027  | 0.050  | 34   | 0.020 | 31   | 0.020 | 31   | 0.020 | 31   | 0.020 |
| pH = 9.0 |     |       |      |       |      |       |      |       |      |       |
| 0 mM    | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0.036   | 0.027  | 0.050  | 34   | 0.020 | 31   | 0.020 | 31   | 0.020 | 31   | 0.020 |

**Table S10.** p-values comparing φX174 ssDNA genome degradation at varying pHs. Given the deviation from linear kinetics for select reaction conditions, statistical differences between conditions were determined by comparing samples collected at the longest reaction times. p-values < 0.05 are bolded.

| pH     | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM |
|--------|------|-------|------|-------|------|-------|------|-------|------|-------|
| pH = 6.0 |     |       |      |       |      |       |      |       |      |       |
| 0 mM    | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0.036   | 0.027  | 0.050  | 34   | 0.020 | 31   | 0.020 | 31   | 0.020 | 31   | 0.020 |
| pH = 6.8 |     |       |      |       |      |       |      |       |      |       |
| 0 mM    | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0.036   | 0.027  | 0.050  | 34   | 0.020 | 31   | 0.020 | 31   | 0.020 | 31   | 0.020 |
| pH = 7.5 |     |       |      |       |      |       |      |       |      |       |
| 0 mM    | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0.036   | 0.027  | 0.050  | 34   | 0.020 | 31   | 0.020 | 31   | 0.020 | 31   | 0.020 |
| pH = 9.0 |     |       |      |       |      |       |      |       |      |       |
| 0 mM    | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 0.036   | 0.027  | 0.050  | 34   | 0.020 | 31   | 0.020 | 31   | 0.020 | 31   | 0.020 |

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Table S11. p-values comparing T3 dsDNA genome degradation at varying pHs. Statistical differences between conditions were determined by comparing observed second order rate constants modelled from the pseudo-first order portions of the reactions. p-values < 0.05 are bolded.

| pH   | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM |
|------|------|-------|------|-------|------|-------|------|-------|------|-------|
|      | A    | B     | A    | B     | A    | B     | A    | B     | A    | B     |
| 6.0  |      |       | Reg A | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
| 6.8  |      |       | Reg A | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
| 7.5  |      |       | Reg A | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
| 9.0  |      |       | Reg A | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |

Table S12. p-values comparing T3 dsDNA genome degradation at varying pHs. Given the deviation from linear kinetics for select reaction conditions, statistical differences between conditions were determined by comparing samples collected at the longest reaction times. p-values < 0.05 are bolded.

| pH   | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM | 0 mM | 10 mM |
|------|------|-------|------|-------|------|-------|------|-------|------|-------|
|      | A    | B     | A    | B     | A    | B     | A    | B     | A    | B     |
| 6.0  |      |       | Reg A | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
| 6.8  |      |       | Reg A | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
| 7.5  |      |       | Reg A | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
| 9.0  |      |       | Reg A | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
Table S13. p-values comparing T3 dsDNA genome degradation when ACS grade ClO₄⁻, trace metal grade ClO₄⁻, ACS grade NO₃⁻, and ACS grade Cl⁻ were added to samples at pH = 9.0. Statistical differences between conditions were determined by comparing observed second order rate constants modelled from the pseudo-first order portions of the reactions. p-values < 0.05 are bolded.

|                  | 10 mM ACS grade ClO₄⁻ | 10 mM trace metal grade ClO₄⁻ | 10 mM ACS grade NO₃⁻ | 10 mM Cl⁻ |
|------------------|------------------------|--------------------------------|----------------------|---------|
|                  | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B | Reg A | Reg B |
| 10 mM ACS grade ClO₄⁻ | 0.011 | 0.012 | 0.034 | 0.033 | 0.057 | 0.014 | 0.45 | - |
| 10 mM trace metal grade ClO₄⁻ | - | - | 0.019 | - | 0.30 | 0.098 | 0.0029 | - |
| 10 mM ACS grade NO₃⁻ | Reg A | Reg B | 0.29 | - | 0.23 | 0.27 | - | 0.043 |
|                  | Reg B | Reg B | 0.419 | - | 0.23 | - | 0.76 | - |
|                  | Reg A | Reg B | 0.30 | 0.27 | 0.76 | 0.84 | 0.029 | - |
|                  | Reg B | Reg B | 0.000030 | - | 0.019 | - | 0.13 | - |
| 10 mM Cl⁻ | Reg A | Reg B | 0.0029 | - | 0.043 | - | 0.029 | - |

Table S14. p-values comparing T3 dsDNA genome degradation when ACS grade ClO₄⁻, trace metal grade ClO₄⁻, ACS grade NO₃⁻, and ACS grade Cl⁻ were added to samples at pH = 9.0. Given the deviation from linear kinetics for select reaction conditions, statistical differences between conditions were determined by comparing samples collected at the longest reaction times. p-values < 0.05 are bolded.

Table S15. Measured concentrations of bromide as an impurity in salts and solutions. Uncertainties denote 95% confidence intervals determined from triplicate samples. Method limit of detection = 0.0014 mg Br⁻/kg salt. Method limit of quantitation = 0.005 mg Br⁻/kg salt.
| Salt (grade)          | [Br\(^-\)] (mg/kg) |
|----------------------|---------------------|
| NaCl (ACS)           | 58 ± 3              |
| NaCl (Trace metal)   | 11.3 ± 0.5          |
| NaClO\(_4\) (Trace Metal) | 1.74 ± 0.05       |
| NaClO\(_4\) (ACS)    | 0.46 ± 0.04         |
| NaNO\(_3\) (ACS)     | 0.52 ± 0.01         |
| NaH\(_2\)PO\(_4\) (ACS) | 0.37 ± 0.02      |

| Solution              | [Br\(^-\)] (mg/L)  |
|-----------------------|---------------------|
| Phosphate buffered saline (Gibco) | 0.43 ± 0.03 |
| Miliq water (Solvent blank)        | 0.03 ± 0.003      |

![Graph A](image1.png)

![Graph B](image2.png)
**Figure S1.** Reactivity of (A) region A and (B) region B of T3 dsDNA genome with FAC (5 mg-Cl₂/L, pH = 7.5) in samples augmented with 0 mM or 10 mM chloride. Genomes were extracted with either a Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Maxwell) or QIAmp® UltraSens® Virus Kit (Qiagen). Experimental duplicates are shown for each condition. Statistical tests showed no difference in degradation kinetics between genomes extracted by the different kits within each experimental condition.

**Figure S2.** Reactivity of (A) T3 dsDNA genome (Region B) or (B) φX174 ssDNA genome (Region B) with free chlorine (5 mg-Cl₂/L, pH = 7.5) at increasing chloride (Cl⁻) concentrations. Unfilled markers with downward arrows represent data that was below the detection limit. Experimental duplicates are shown for each condition.
Figure S3. Reactivity of T3 dsDNA genome (Region B) with free chlorine (5 mg-Cl₂/L, pH = 7.5) at a constant ionic strength representative of drinking water conditions (5 mM phosphate buffer; 10 mM salts, [ClO₄⁻] + [Cl⁻] = 10 mM) at increasing chloride (Cl⁻) concentrations. Experimental duplicates are shown.
Figure S4. Reactivity of T3 dsDNA genome (Region A) with free chlorine (5 mg-C\textsubscript{2}L/L) at (A) pH = 6.0, (B) pH = 6.8, (C) pH = 7.5 and (D) pH = 9.0. in samples augmented with 0 mM or 10 mM chloride. Unfilled markers with downward arrows represent data that was below the detection limit. Experimental duplicates are shown.
Figure S5. Reactivity of T3 dsDNA genome (Region B) with free chlorine (5 mg-Cl/L) at (A) pH = 6.0, (B) pH = 6.8, (C) pH = 7.5 and (D) pH = 9.0 in samples augmented with 0 mM or 10 mM chloride. Unfilled markers with downward arrows represent data that was below the detection limit. Experimental duplicates are shown.
Figure S6. Reactivity of ϕX174 ssDNA genome (Region B; qPCR) with free chlorine (5 mg-Cl₂/L) at (A) pH = 6.0, (B) pH = 6.8, (C) pH = 7.5 and (D) pH = 9.0 in samples augmented with 0 mM or 10 mM chloride. Unfilled markers with downward arrows represent data that was below the detection limit. Experimental duplicates are shown.
Figure S7. Reactivity of $\phi$X174 ssDNA genome (Region A (panel A); Region B (panel B)) with free chlorine (5 mg-Cl$_2$/L). 10 mM of NaClO$_4$ (ACS reagent grade), NaClO$_3$ (trace metal grade), or NaNO$_3$ (ACS reagent grade) salts were used. Experimental duplicates are shown.
Figure S8. Impact of 100 mM chloride addition on qPCR quantification of T3 dsDNA bacteriophage (Region A). Genomes were suspended in 5 mM phosphate buffer (pH = 7.5) and augmented with 100 mM chloride or not augmented with chloride. Experimental duplicates are shown for each condition.
Figure S9. Impact of FAC + Tris-HCl quenching buffer on (A) T3 dsDNA bacteriophage and (B) φX174 ssDNA bacteriophage genome stability during storage of samples. Genomes were suspended in 5 mM phosphate buffer (pH = 7.5) augmented with 10 mM chloride. FAC + Tris-HCl samples were additionally supplemented with 5 mg/Cl₂/L FAC and 50 mM Tris-HCl (final concentration) and stored on ice for two hours mimicking experimental setup conditions. Region A was quantified for both dsDNA and ssDNA.
Figure S10. Chlorine speciation diagram for the reaction system used in this study. A 0.071 mM total chlorine concentration and a 10 mM chloride concentration were assumed.
**Figure S11.** The overall observed FAC pseudo-first-order rate constant and calculated contribution of HOCl (HOCl calculated; $k_{\text{HOCl}[\text{HOCl}]}$), OCl$^-$ (OCl$^-$ calculated; $k_{\text{OCl}^-[\text{OCl}^-]}$), and Cl$_2$ (Cl$_2$ calculated; $k_{\text{Cl}_2[\text{Cl}_2]}$) to overall FAC reactivity for (A) ssDNA samples augmented with 10 mM chloride, (B) dsDNA samples augmented with 10 mM chloride, (C) ssDNA samples not augmented with chloride (0.17 mM chloride concentration assumed$^4$), and (D) dsDNA samples not augmented with chloride (0.17 mM chloride concentration assumed$^4$). Observed FAC reaction rate constants for Region A and Region B amplicons is shown.
Figure S12. Inactivation of (A) T3 dsDNA bacteriophage or (B) ϕX174 ssDNA bacteriophage with FAC (2 mg-Cl₂/L, pH = 7.5) at increasing chloride (Cl⁻) concentrations. Unfilled markers with downward arrows represent data that was below detection limit. Experimental duplicates/triplicates are shown for each condition.
Figure S13. The overall observed FAC pseudo-first-order rate constant and calculated contribution of HOCl (HOCl calculated; $k_{\text{HOCl}}$), OCl$^-$ (OCl$^-$ calculated; $k_{\text{OCl^-[OCl^-]}}$), and Cl$^-$ (Cl$^-$ calculated; $k_{\text{Cl^-[Cl^-]}}$) to overall FAC reactivity for (A) ssDNA samples at pH = 7.5 and (B) dsDNA samples at pH = 7.5. For samples not augmented with chloride, a 0.17 mM chloride concentration was assumed. Observed FAC reaction rate constants for Region A and Region B amplicons is shown.
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