Supplementary Material

for

Ammonia as an in-situ sanitizer: influence of virus genome type on inactivation.

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Number of pages : 9

Number of figures : 3

Number of tables : 5

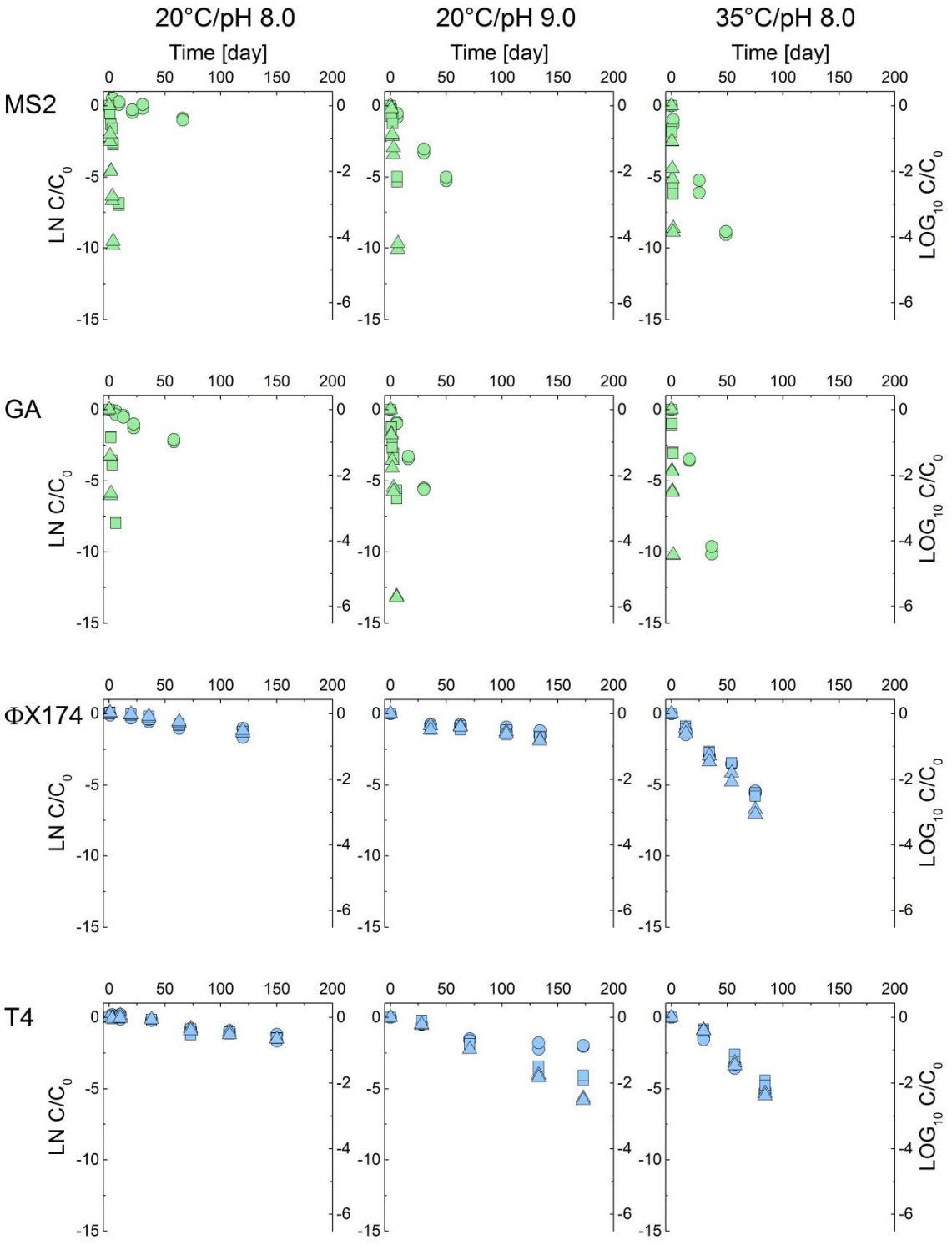

FIG S1 Kinetics of phage inactivation. Circles, squares and triangles represent PCa and AmCa with an approximate {NH_3(aq)} of 0, 20 and 40 mmol L^{-1} respectively. The actual, measured {NH_3(aq)} is listed in Table S1.

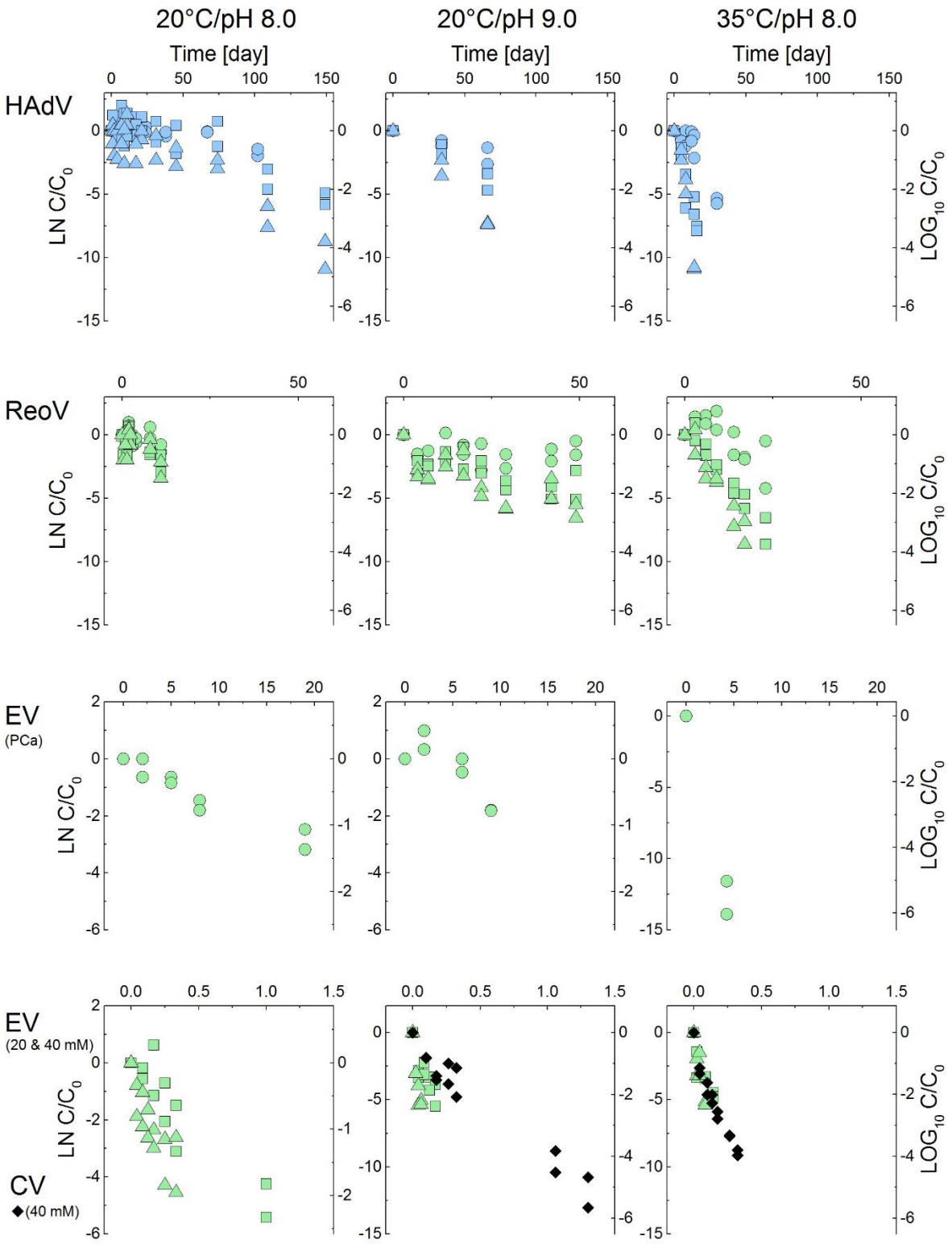

FIG S2 Kinetics of mammalian virus inactivation. Circles, squares and triangles represent PCa and AmCa with an approximate {NH_3(aq)} of 0, 20 and 40mmol L^{-1} respectively. The actual, measured {NH_3(aq)} is listed in Table S1.

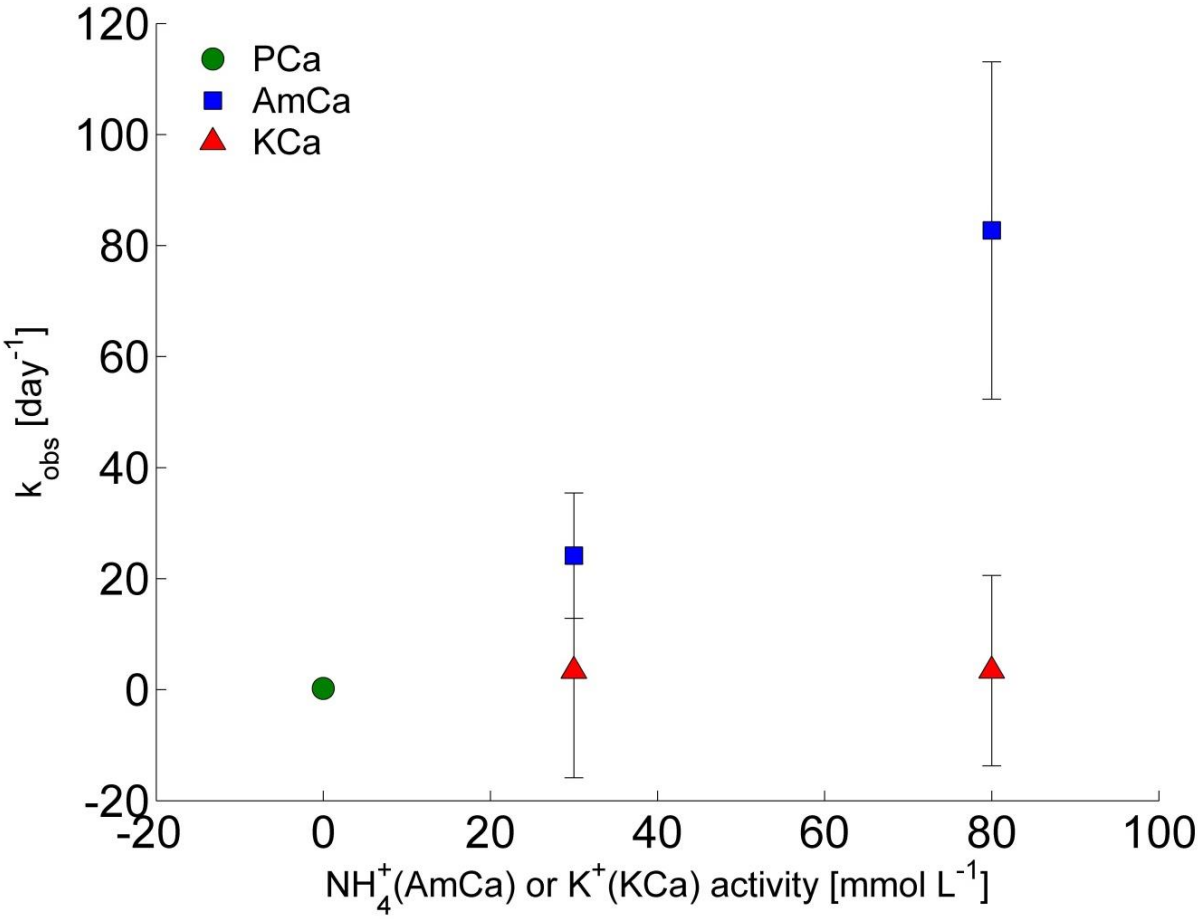

FIG S3. Influence of NH_4^+ and K^+ activity on EV inactivation at pH 9/20°C. The influence of NH_4^+ and K^+ activity were was examined in AmCa and KCa buffer, respectively. The error bars depict the 95% confidence intervals associated with k_{obs}.

TABLE S1 Composition of working solutions.

T		pH[a]	EC	*Buffer composition (added substances)*								{$NH_3(aq)$}[e]
				Na_2CO_3[c]	$NaHCO_3$[c]	NH_4Cl[b]	$NaH_2PO_4*2H_2O$[c]	HCl[c]	NaOH[c]	NaCl[c]	KCl[c]	
			[mS cm^{-1}]	[mmol L^{-1}]	[mmol L^{-1}]	[mmol L^{-1}]	[mmol L^{-1}]	[mmol L^{-1}]	[mmol L^{-1}]	[mmol L^{-1}]	[mmol L^{-1}]	[mmol L^{-1}]
20°C	PCa	7.86	10.4	50.0			60.0		5.7			0.0
	PCaH	7.87	127.0	50.0			60.0		12.0	1770.0		0.0
	AmCa 20	8.03	91.6	50.0		920.4		26.3				22.7
	AmCa 40	7.95	174.2	50.0		1976.8			4.9			37.6
20°C	PCa	8.86		50.0			45.0		1.0			0.0
	AmCa 20[d]	8.93		50.0		54.9		23.5				10.8
	AmCa 40[d]	8.94		50.0		147.0		1.2				28.4
	KCa 30[d]	9.00		50.0				44.3			44.0	0.0
	KCa 80[d]	9.00		50.0				43.8			113.0	0.0
35°C	PCa	7.92		50.0			60.0		7.0			0.0
	AmCa 20	7.98		50.0		295.1		28.1				19.5
	AmCa 40	8.03		50.0		652.9		5.6				44.3
50°C	PCa	8.00		50.0			60.0		8.8			0.0
60°C	PCa	8.00		50.0			60.0		10.6			0.0
20°C		10.00	0.45	4.5	5.5					10.0		0.0
20°C		11.00	0.57	9.8	0.2					10.0		0.0
20°C		11.50	0.65				10.0		15.1	10.0		0.0
20°C		12.00	0.95				10.0		24.0	10.0		0.0

[a] Measured at experimental temperature.

[b] Total ammonia measured by IC (see experimental method).

[c] Concentration determined from the amount of the compound added at the beginning of the experiment. The concentration was calculated with PHREEQC (Pitzer database), see material and methods.

[d] Correspond respectively to NH_4^+ activity of 30 and 80 mM and K^+ activity of 30 and 80 mM.

[e] NH_3 activities determined from the buffer composition (see material and method).

TABLE S2 Kinetic parameters associated with phage inactivation: k_{obs} and coefficient of determination (R^2).

Phage	pH	T		k_{obs} [day^{-1}]	IC 95%	R^2
MS2	8	20°C	PCa	0.02	0.01	0.75
			PCaH	0.11	0.01	0.98
			AmCa 20	0.78	0.05	0.99
			AmCa 40	2.50	0.23	0.99
	9	20°C	PCa	0.10	0.01	0.99
			AmCa 20	0.86	0.11	0.98
			AmCa 40	1.47	0.14	0.99
	8	35°C	PCa	0.18	0.03	0.98
			AmCa 20	3.18	0.32	0.99
			AmCa 40	5.32	0.58	0.99
	8	50°C	PCa	5.51	0.34	0.99
		60°C	PCa	294.01	42.69	0.97
	10	20°C		0.44	0.06	0.98
	11	20°C		5.16	0.23	0.99
	11.5	20°C		37.76	4.12	0.98
	12	20°C		11623	5384	0.99
GA	8	20°C	PCa	0.04	0.01	0.96
			PCaH	0.14	0.01	0.99
			AmCa 20	1.35	0.13	0.99
			AmCa 40	3.33	0.08	0.99
	9	20°C	PCa	0.19	0.02	0.99
			AmCa 20	1.02	0.11	0.98
			AmCa 40	2.30	0.14	0.99
	8	35°C	PCa	0.28	0.04	0.99
			AmCa 20	1.84	0.19	0.99
			AmCa 40	6.10	0.22	0.99
PhiX174	8	20°C	PCa	0.0114	0.0029	0.88
			PCaH	0.0115	0.0012	0.98
			AmCa 20	0.0117	0.0016	0.96
			AmCa 40	0.0110	0.0013	0.97
	9	20°C	PCa	0.0088	0.0034	0.81
			AmCa 20	0.0108	0.0044	0.79
			AmCa 40	0.0124	0.0044	0.84
	8	35°C	PCa	0.0686	0.0094	0.97
			AmCa 20	0.0726	0.0078	0.98
			AmCa 40	0.0893	0.0085	0.99
	8	50°C	PCa	1.1280	0.1548	0.97
		60°C	PCa	265.62	26.25	0.99
	11	20°C		0.0101	0.0068	0.67
	12	20°C		0.0669	0.0128	0.93
T4	8	20°C	PCa	0.0104	0.0018	0.93
			PCaH	0.0079	0.0023	0.83
			AmCa 20	0.0106	0.0018	0.93
			AmCa 40	0.0108	0.0014	0.96
	9	20°C	PCa	0.0119	0.0038	0.86
			AmCa 20	0.0263	0.0030	0.98
			AmCa 40	0.0336	0.0021	0.99
	8	35°C	PCa	0.0628	0.0049	0.99
			AmCa 20	0.0560	0.0097	0.97
			AmCa 40	0.0663	0.0118	0.97
	8	50°C	PCa	0.8331	0.0883	0.98
	8	60°C	PCa	196.93	35.54	0.95
	10	20°C		0.0315	0.0067	0.93
	11	20°C		0.9625	0.1295	0.99
	11.5	20°C		22198	14815	0.98

TABLE S3 Kinetic parameters associated with mammalian virus inactivation: k_{obs} and coefficient of determination (R^2).

Virus	pH	T		k_{obs} [day^{-1}]		IC 95%	R^2
HAdV	8	20°C	PCa	0.018		0.008	0.62
			PCaH	0.003	*	0.007	0.04
			AmCa 20	0.037		0.009	0.71
			AmCa 40	0.060		0.011	0.83
	9	20°C	PCa	0.030		0.019	0.80
			AmCa 20	0.061		0.025	0.91
			AmCa 40	0.111		0.026	0.97
	8	35°C	PCa	0.185		0.056	0.81
			AmCa 20	0.468		0.120	0.90
			AmCa 40	0.786		0.205	0.94
ReoV	8	20°C	PCa	0.040	*	0.123	0.04
			PCaH	0.048	*	0.124	0.05
			AmCa 20	0.186		0.103	0.52
			AmCa 40	0.174		0.131	0.37
	9	20°C	PCa	0.015	*	0.028	0.09
			AmCa 20	0.069		0.031	0.59
			AmCa 40	0.089		0.040	0.58
	8	35°C	PCa	0.154		0.085	0.56
			AmCa 20	0.352		0.041	0.97
			AmCa 40	0.470		0.067	0.96
EV	8	20°C	PCa	0.15		0.03	0.92
			PCaH	0.25		0.06	0.91
			AmCa 20	4.97		1.51	0.84
			AmCa 40	10.24		4.08	0.71
	9	20°C	PCa	0.22		0.15	0.66
			AmCa 20	24.15		11.28	0.75
			AmCa 40	82.72		30.38	0.87
	8	35°C	PCa	3.00		0.87	0.98
			AmCa 20	27.73		13.98	0.79
			AmCa 40	57.78		24.31	0.84
CV	9	20°C	AmCa 40	8.40		1.19	0.95
	8	35°C	AmCa 40	25.31		3.89	0.94

* not significantly different from zero.

TABLE S4 Kinetic parameters associated with virus and phage inactivation: k_{NH3} and coefficient of determination (R^2).

Phage/Virus	pH	T	k_{NH3} [b] [$day^{-1}M^{-1}$]	IC 95%	R^2
MS2	8	20°C	63.4	38.3	0.89
	9	20°C	46.7	16.6	0.96
	8	35°C	114.8	82.7	0.97
GA	8	20°C	85.2	35.2	0.95
	9	20°C	74.3	1.4	0.99
	8	35°C	133.3	110.8	0.96
PhiX174	8	20°C	-0.009 *	0.031	0.20
	9	20°C	0.122	0.041	0.96
	8	35°C	0.480 *	1.690	0.93
T4	8	20°C	0.009	0.001	0.99
	9	20°C	0.729	0.423	0.90
	8	35°C	0.090 *	0.930	0.16
HAdV	8	20°C	1.1	0.4	0.97
	9	20°C	2.9	0.0	1.00
	8	35°C	13.6	2.1	0.99
ReoV	8	20°C	3.8	3.4	0.79
	9	20°C	2.4	1.8	0.85
	8	35°C	7.0	6.6	0.95
EV	8	20°C	264.0	66.3	0.98
	9	20°C	2944.3	519.7	0.99
	8	35°C	1236.1	72.8	0.99
f2[a]	8	20°C	35.3	5.4	0.99
Poliovirus[a]	8	20°C	235.0	91.3	0.97

[a] Determined from Burge et al. (1983) data.

[b] According to Eq.2 (see material and methods).

* Not significantly different from zero.

TABLE S5 Comparison of DNA, RNA and protein cleavage rate constants.

	Spontaneous DNA hydrolysis (Gates, 2009)			
A	**Spontaneous hydrolysis of phosphodiester backbone (Schroeder, 2006)**			
				[day^{-1}]
		pH 7.0	25°C	8.64E-11
B	**Hydrolytic deamination of DNA base --> mutagenesis, no evidence of cleavage (Gates, 20**			
				[day^{-1}]
	dsDNA	pH 7.4	37°C	3.79E-08
	ssDNA	pH 7.4	37°C	9.49E-06
C	**Hydrolysis of phosphodiester backbone through abasic site (C1 and C2 are the rate limitin**			
C1.a	*Depurination/depyrimidation (Lindhal, 1972 & 1973) --> acid-catalyzed reaction*			
				[day^{-1}]
	pyrimidine (CT)	pH 7.4	37°C	1.30E-07
	purine (AG)	pH 7.4	37°C	2.59E-06
C1.b	*Depurination (An, 2014) --> acid-catalyzed reaction*			
				[day^{-1}]
	Adenine	pH 7.1	37°C	1.90E-05
	Guanine	pH 7.1	37°C	2.16E-05
C2	*Self catalyzed (site specific) DNA depurination (Amosova, 2005)*			
				[day^{-1}]
		pH 7.0	37°C	1.01E-02
C3	*Chain breakage at abasic site (alkaline-catalyzed reaction)(Gates, 2009)(Küpfer, 2007)*			
				[day^{-1}]
		pH 7.4	37°C	1.20E-02-1.25E-01
	in 0.1 M NaOH			2.61E+02
	Spontaneous RNA hydrolysis (Li, 1999)			
				[day^{-1}]
		pH 7.0	37°C	1.73E-04
		pH 11.0	37°C	1.48E+00
	Spontaneous peptide bond hydrolysis (Smith, 1998)			
				[day^{-1}]
		pH 7.0	37°C	4.02E-06
		pH 11.0	37°C	1.49E-04
	Protein denaturation			
	Ricin denaturation (Levy, 1950)			
				[day^{-1}]
		pH 11.0	37°C	5.97E-01
	Penicilin Acylase denaturation (Guranda, 2004)			
				[day^{-1}]
		pH 11.0	37°C	4.67E+03

(1–12)

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