Supplementary information of: Removal rate constants are not necessarily constant: the case of organic micropollutant removal in sewage treatment plants

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SI 1. Michaelis-Menten theory and (pseudo) first order rate constants

According to Michaelis-Menten, the reaction speed is dependent on substrate concentration and is calculated according to equation S1:

$$V = \frac{Vmax * [S]}{[S] + Km} \qquad \text{(Equation S1)}$$

where V is the reaction rate (mol L^{-1} h^{-1}), V_{max} is the maximum reaction rate (mol L^{-1} h^{-1}), [S] is the substrate concentration (mol L^{-1}), and K_m is the substrate concentration where V is half of V_{max} (mol L^{-1}). When studying OMPs in very low concentrations (ng/L - µg/L), it is assumed that the substrate concentration is far below (Figure S1A, grey box $k_m < 1$). In this case, the reaction rate is not decreasing with increasing substrate, but instead the reaction rate depends linearly on the substrate concentration.

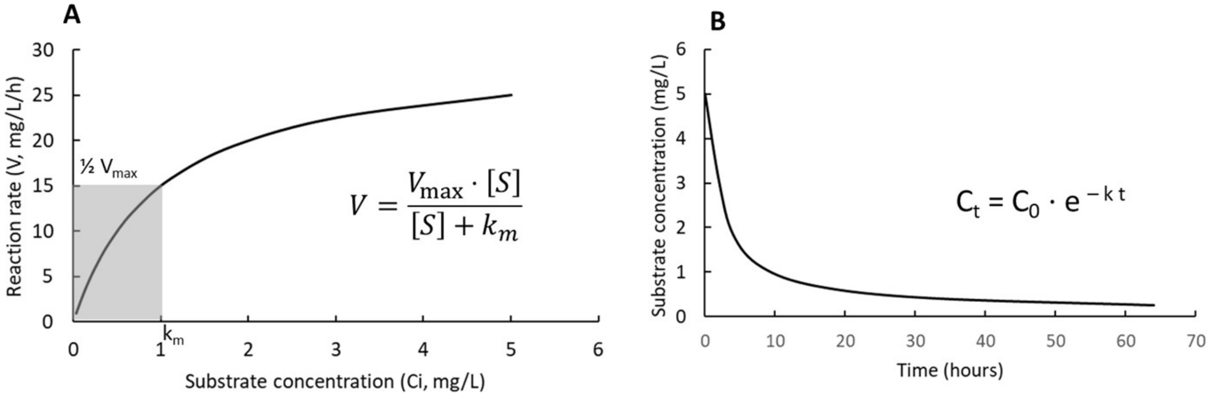

Figure S1. A) Michaelis-Menten theory; B) pseudo-first order kinetics.

When the reaction rate depends linearly on the substrate concentration, *k* can be calculated from a decrease in concentration over time via pseudo-first order biotransformation kinetics (equation 2, figure 1B, Schwarzenbach et al. 2005, Simkins and Alexander 1984):

$$C_t = C_0 * e^{-k\,t} \qquad \text{(equation S2)}$$

where C_t is the concentration at time t (mol L^{-1}), C_0 is the concentration at time 0 (mol L^{-1}), and k is the rate constant (h^{-1}).

Table S1. VIF values for each variable after excluding collinear variables (in case VIF > 3).

variable	**VIF**
$\log K_{ow}$	1.9
pK_a	1.2
log HBA	2.2
v	1.8
E_{LUMO}	1.9
$\Delta E_{L\text{–}H}$	1.6
H_f	1.7
log C_i	1.9
log SRT	1.4
log Q	1.5
T	1.1
pH	1.2
HRT	1.8

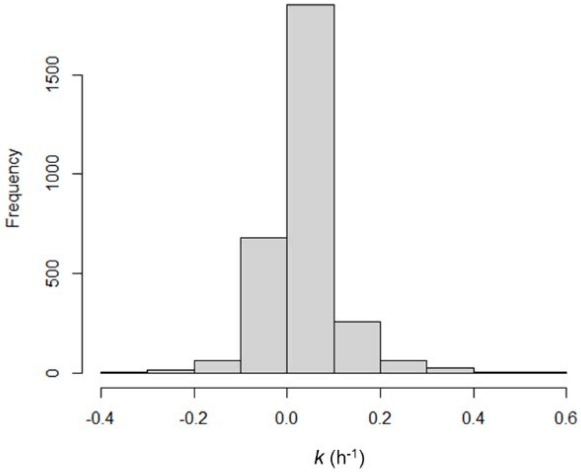

Figure S2. Histogram of removal rate constants *k* (h^{-1}) and the frequency of values.

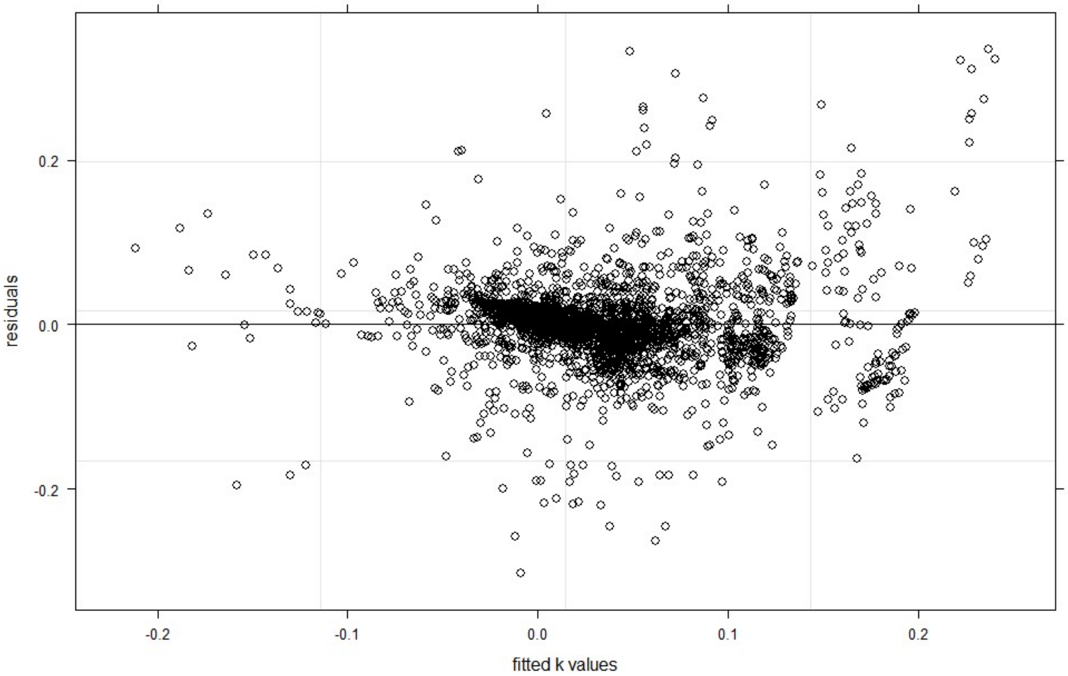

Figure S3. Residuals plotted against modelled k values.

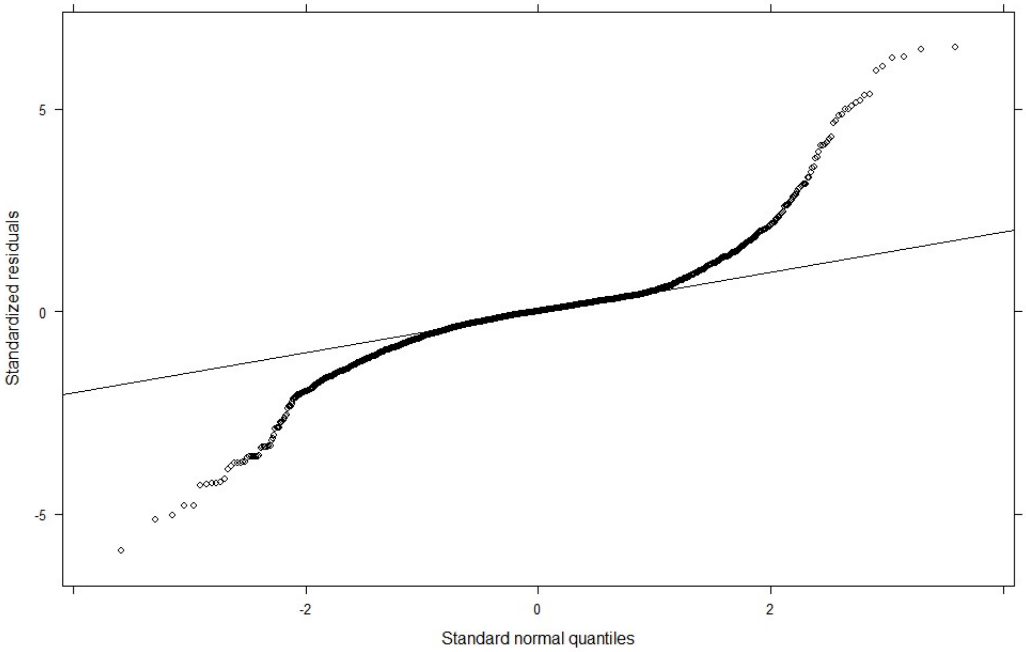

Figure S4. Standardized residuals plotted against standard normal quantiles.

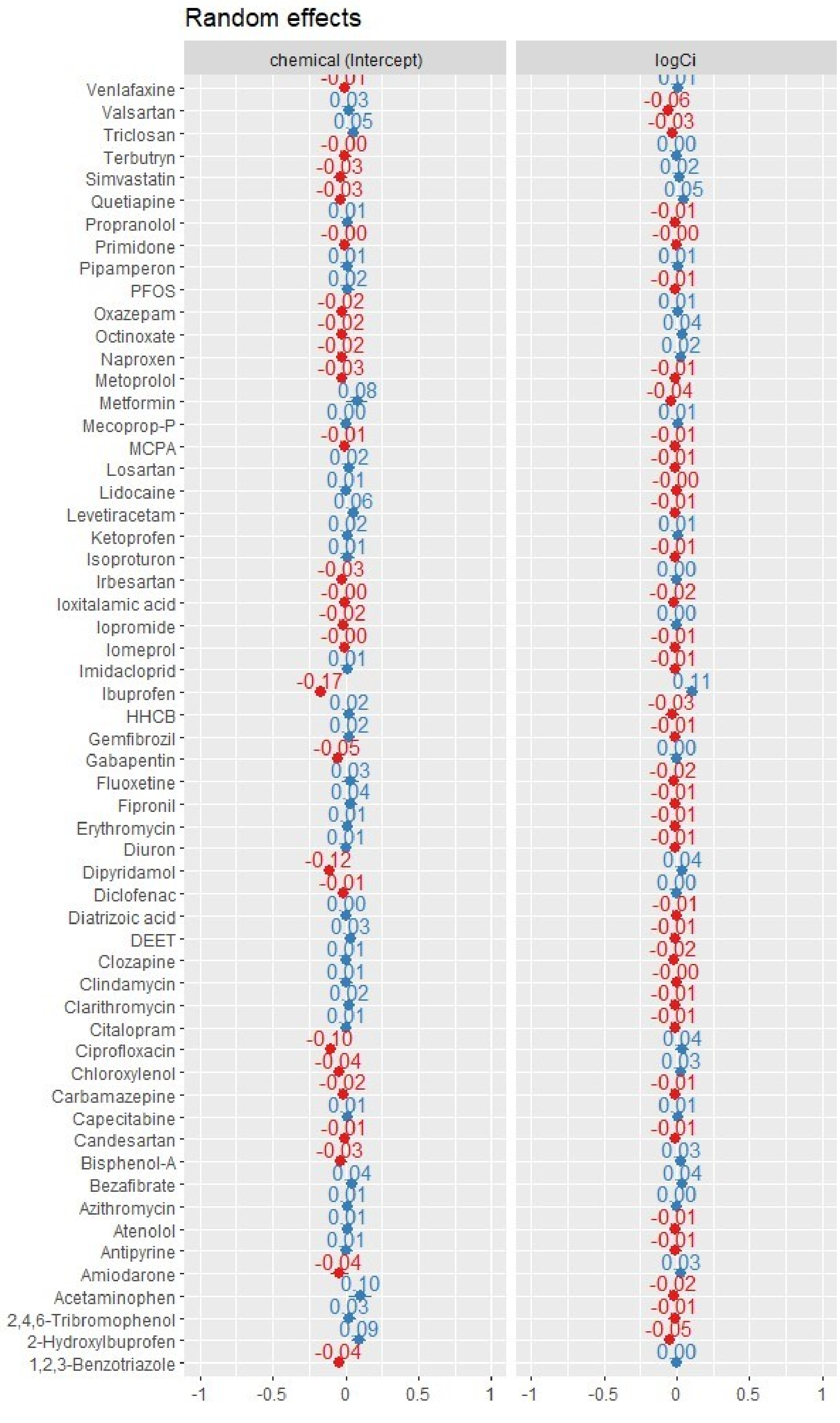

Figure S5. Deviations of intercept (left) and slope (right) from the overall model (equation 4), caused by random effect OMP. This affects the relationship between influent concentration and *k* for each OMP. Red dots represent a negative intercept or slope, while dots represent a positive intercept or slope.

Figure S6. Deviations of intercept from overall model (equation 4) by random effect WWTP. Red dots represent a negative intercept, while dots represent a positive intercept.

Table S2. Mode of action (MoA) per OMP, number of observations (n), minimum and maximum influent concentration (C_i) in wastewater (µmol L^{-1}), and the intercept and slope of the relationship between C_i and *k* per OMP. We collected MoAs primarily via a dataset reported by Posthuma et al. (2019), supplemented with other literature studies. Abbreviations: AAA = Analgesics, Anti-inflammatory Drugs and Antipyretics; ARB = angiotensin II receptor blocker, Cardiovasc. drugs = cardiovascular drugs; Psychoth. Drugs = physicotherapeutic drugs; Phosph. inhibitor = Phosphodiesterase inhibitor; NA includes OMPs for which no MoA was available.

OMP	**Mode of action**	**n**	**min**	**max**	**intercept**	**slope**
1,2,3-Benzotriazole	NA	78	0.010	0.193	-0.008	0.045
2,4,6-Tribromophenol	Narcosis	3	0.001	0.002	0.062	0.031
2-Hydroxylbuprofen	NA	77	0.016	0.265	0.126	-0.005
Acetaminophen	Analgesics, Anti-inflammatory Drugs and Antipyretics (AAA)	77	0.549	4.895	0.138	0.021
Amiodarone	Class III antiarrhythmic	1	0.000	0.000	-0.006	0.074
Antipyrine	Analgesics, Anti-	3	0.000	0.002	0.043	0.037

	inflammatory Drugs and Antipyretics (AAA)					
Atenolol	Cardiovascular Drugs	78	0.000	0.005	0.050	0.039
Azithromycin	Bactericides	74	0.000	0.003	0.048	0.047
Bezafibrate	Cardiovascular Drugs	56	0.000	0.002	0.077	0.082
Bisphenol-A	Narcosis	19	0.009	0.307	0.006	0.071
Candesartan	Angiotensin II receptor blocker (ARB)	58	0.000	0.001	0.028	0.030
Capecitabine	Nucleoside metabolic inhibitor	42	0.000	0.002	0.048	0.054
Carbamazepine	Anticonsulvant	83	0.001	0.005	0.016	0.034
Chloroxylenol	Narcosis	75	0.001	0.070	-0.006	0.077
Ciprofloxacin	Bactericides	9	0.003	0.006	-0.064	0.083
Citalopram	Psychotherapeutic drugs	74	0.000	0.001	0.040	0.037
Clarithromycin	Bactericides	76	0.000	0.001	0.056	0.035
Clindamycin	Bactericides	7	0.000	0.000	0.043	0.040
Clozapine	Psychotherapeutic drugs	49	0.000	0.001	0.042	0.028
DEET	Insect repellents	78	0.001	0.094	0.067	0.035
Diatrizoic acid	Contrast media	6	0.000	0.002	0.040	0.040
Diclofenac	Analgesics, Anti-inflammatory Drugs and Antipyretics (AAA)	79	0.001	0.006	0.024	0.047
Dipyridamol	Phosphodiesterase inhibitor	59	0.003	0.030	-0.080	0.083
Diuron	Phenylurea	7	0.000	0.004	0.042	0.038
Erythromycin	Bactericides	49	0.000	0.002	0.047	0.034
Fipronil	Pyrazole	78	0.000	0.000	0.071	0.034
Fluoxetine	Psychotherapeutic drugs	1	0.000	0.000	0.067	0.025
Gabapentin	Anticonsulvant	78	0.006	0.099	-0.014	0.047
Gemfibrozil	Cardiovascular Drugs	72	0.000	0.010	0.056	0.034
HHCB	NA	78	0.005	0.026	0.059	0.013
Ibuprofen	Analgesics, Anti-inflammatory Drugs and Antipyretics (AAA)	154	0.011	0.451	-0.133	0.154
Imidacloprid	Neurotoxicant	38	0.000	0.001	0.047	0.032
Iomeprol	Contrast media	59	0.001	0.129	0.035	0.031
Iopromide	Contrast media	35	0.000	0.042	0.018	0.048
Ioxitalamic acid	Contrast media	54	0.000	0.009	0.031	0.024
Irbesartan	angiotensin II receptor blocker (ARB)	78	0.001	0.010	0.009	0.046
Isoproturon	Phenylurea	3	0.000	0.001	0.050	0.035
Ketoprofen	Analgesics, Anti-inflammatory Drugs and Antipyretics (AAA)	42	0.000	0.002	0.054	0.051
Levetiracetam	Antiepiletics	72	0.008	0.100	0.091	0.037

Lidocaine	local anesthetic	53	0.000	0.002	0.041	0.040
Losartan	angiotensin II receptor blocker (ARB)	78	0.001	0.007	0.060	0.037
MCPA	Phenoxyacetic	15	0.000	0.009	0.027	0.038
Mecoprop-P	Phenoxypropanoic	14	0.000	0.002	0.038	0.056
Metformin	Antidiabetics	78	0.178	1.936	0.113	0.009
Metoprolol	Cardiovascular Drugs	78	0.001	0.024	0.006	0.034
Naproxen	Analgesics, Anti-inflammatory Drugs and Antipyretics (AAA)	78	0.009	0.078	0.010	0.069
Octinoxate	UV filter	77	0.002	0.083	0.010	0.084
Oxazepam	Psychotherapeutic drugs	78	0.001	0.007	0.015	0.052
PFOS	Surfactant	6	0.000	0.001	0.053	0.034
Pipamperon	Psychotherapeutic drugs	13	0.000	0.000	0.048	0.054
Primidone	Anticonsulvant	10	0.000	0.000	0.034	0.043
Propranolol	Cardiovascular Drugs	72	0.000	0.001	0.048	0.032
Quetiapine	Psychotherapeutic drugs	28	0.000	0.001	0.002	0.095
Simvastatin	HMG-CoA reductase inhibitors	3	0.000	0.001	0.003	0.063
Terbutryn	Narcosis	3	0.000	0.001	0.035	0.048
Triclosan	Narcosis	61	0.001	0.008	0.086	0.019
Valsartan	angiotensin II receptor blocker (ARB)	78	0.001	0.046	0.060	-0.011
Venlafaxine	Psychotherapeutic drugs	78	0.000	0.002	0.030	0.050

Table S3. Recent studies of biofilm reactors and bottle incubations that reported an effect of concentration (C) on the removal rate constant (*k*), removal rate/percentage or removal efficiency (RE) of OMPs.

study	**system**	**sample**	**Spiking concentration range (order of magnitude)**	**OMPs**	**Concentration relationship**
Wang et al. (2020)	WWTPs	Influent and effluent	-	169 OMPs	Positive relationships between concentration and RE for clusters of OMPs, a negative relationship for the trimethoprim cluster
Nolte et al. (2020)	WWTPs	Influent and effluent	-	28 OMPs	OMPs with a higher C also had a higher *k*
Rios-Miguel et	Reactor	Activated sludge	10^0- 10^2	diclofenac, metoprolol,	C proportionally increased the removal rate of each

al. (2021)		from reaeration tank of the WWTP		metformin, carbamazepine and fluoxetine	compound; removal percentage and C were not correlated
van Bergen et al. (2021)	Bottle incubation	Activated sludge from reaeration tank of the WWTP	0–10^{-2} µg L^{-1}	Metformin & Metoprolol	Increase in *k* with increase in C
Svendsen et al. (2020)	Moving bed biofilm reactor	WWTP effluent	0–10^{2} µg L^{-1}	Citalopram, metoprolol, sulfamethizole, ac-sulfadiazine, clarithromycin, iohexol, iopromide, iomeprol	*k* initially increased; C > environmentally relevant concentration decreased
			0–10^{2} µg L^{-1}	Ibuprofen, sotalol, trimethoprim, erythromycin, atenolol, diclofenac	Decrease in *k* with decrease in C
Birch et al. (2021)	Bottle incubation	WWTP effluent	10^{-2} – 10^{4} µg L^{-1}	linalool	*k* was highest at intermediate C
			10^{-2} – 10^{4} µg L^{-1}	Geraniol, citronellol, 4-tert-butylcyclohexyl acetate, 2-ethylhexyl-4-methoxycinnamate, tert-butyl-4-methoxyphenol	Decrease in *k* with increase in C
			10^{-2} – 10^{4} µg L^{-1}	α-isomethylionone	Decrease in *k* with increase in C, but *k* was lower at highest C than at second-highest C.
			10^{-2} – 10^{4} µg L^{-1}	naphthalene	Increase in *k* with increase in C
			10^{-2} – 10^{4} µg L^{-1}	phenanthrene	*k* was highest at intermediate C
Wei et al. (2019)	Bottle incubation	Activated sludge from reaeration tank of the WWTP	10^{3}– 10^{5} µg L^{-1}	Metronidazole, bezafibrate, ibuprofen, sulfamethoxazole	Decrease in *k* with increase in C

Table S4. Best fitted models (AIC delta < 2), fitted with dredge (MuMIn), and the corresponding AIC value, calculated based on maximum likelihood.

Model structure	**AIC**
k = 0.035 – 0.017 HRT + 0.044 logC_i + (logC_i | OMP) + (1 | WWTP)	-8892.6
k = 0.035 – 0.017 HRT + 0.044 logC_i + 0.001 T_i + (logC_i | OMP) + (1 | WWTP)	-8892.5
k = 0.035 – 0.018 HRT + 0.044 logC_i – 0.003 logQ + (logC_i | OMP) + (1 | WWTP)	-8891.8
k = 0.034 – 0.018 HRT + 0.044 logC_i – 0.003 logQ + 0.001 T_i + (logC_i | OMP) + (1 | WWTP)	-8891.8
k = 0.034 – 0.011 H_f – 0.016 HRT + 0.044 logC_i – 0.011 logHBA + 0.002 T_i + (logC_i | chemical) + (1 | wwtp)	-8891.6
k = 0.034 – 0.011 H_f – 0.016 HRT + 0.045 logC_i – 0.01 logHBA + (logC_i | OMP) + (1 | WWTP)	-8891.5
k = 0.034 – 0.005 H_f – 0.016 HRT + 0.044 logCi + (logC_i | OMP) + (1 | WWTP)	-8891.3
k = 0.034 – 0.006 H_f – 0.016 HRT + 0.044 logC_i + 0.001 T_i + (logC_i | chemical) + (1 | wwtp)	-8891.3
k = 0.035 – 0.017 HRT + 0.045 logC_i – 0.005 logHBA + (logC_i | OMP) + (1 | WWTP)	-8891.2
k = 0.035 – 0.016 HRT + 0.044 logC_i – 0.005 logHBA + 0.001 T_i + (logC_i | OMP) + (1 | WWTP)	-8891.2
k = 0.035 – 0.017 HRT + 0.044 logC_i – 0.004 v + (logC_i | OMP) + (1 | WWTP)	-8891.1
k = 0.035 – 0.016 HRT + 0.044 logC_i + 0.001 T_i – 0.004 v + (logC_i | OMP) + (1 | WWTP)	-8891.1
k = 0.035 – 0.005 pKa – 0.017 HRT + 0.044 logC_i + (logC_i | OMP) + (1 | WWTP)	-8891.1
k = 0.035 – 0.005 pKa – 0.016 HRT + 0.044 logC_i + 0.001 T_i + (logC_i | OMP) + (1 | WWTP)	-8891.1
k = 0.034 – 0.011 H_f – 0.018 HRT + 0.044 logC_i – 0.011 logHBA – 0.003 logQ + 0.002 T_i + (logC_i | OMP) + (1 | WWTP)	-8890.8
k = 0.035 + 0.003 $\Delta E_{L\text{–}H}$ – 0.017 HRT + 0.045 logCi + (logC_i | OMP) + (1 | WWTP)	-8890.8
k = 0.035 – 0.016 HRT + 0.045 logC_i – 0.002 logSRT + (logC_i | OMP) + (1 | WWTP)	-8890.8
k = 0.035 + 0.002 $\Delta E_{L\text{–}H}$ – 0.016 HRT + 0.044 logC_i + 0.001 T_i + (logC_i | OMP) + (1 | WWTP)	-8890.8
k = 0.034 – 0.01 H_f – 0.018 HRT + 0.044 logC_i – 0.011 logHBA – 0.003 logQ + (logC_i | OMP) + (1 | WWTP)	-8890.7
k = 0.035 – 0.016 HRT + 0.044 logCi – 0.002 logSRT + 0.001 Ti + (logC_i | OMP) + (1 | WWTP)	-8890.7
k = 0.035 – 0.017 HRT + 0.045 logC_i + 0.002 logK_{ow} + (logC_i | OMP) + (1 | WWTP)	-8890.7
k = 0.035 – 0.016 HRT + 0.044 logCi + 0.002 logK_{ow} + 0.001 T_i + (logC_i | OMP) + (1 | WWTP)	-8890.6
k = 0.035 – 0.017 HRT + 0.045 logC_i – 0.0003pH + (logC_i | OMP) + (1 | WWTP)	-8890.6
k = 0.035 – 0.016 HRT + 0.044 logC_i + 0.0004 pH + 0.002 T_i + (logC_i | OMP) + (1 | WWTP)	-8890.6
k = 0.035 + $9.2 \cdot 10^{-6}$ E_{lumo} – 0.017 HRT + 0.045 logC_i + (logC_i | OMP) + (1 | WWTP)	-8890.6

References

Birch, H., Sjøholm, K.K., Dechesne, A., Sparham, C., van Egmond, R. and Mayer, P. (2021) Biodegradation Kinetics of Fragrances, Plasticizers, UV Filters, and PAHs in a Mixture– Changing Test Concentrations over 5 Orders of Magnitude. Environmental Science & Technology 56(1), 293-301. DOI: 10.1021/acs.est.1c05583.

Nolte, T.M., Chen, G., van Schayk, C.S., Pinto-Gil, K., Hendriks, A.J., Peijnenburg, W.J.G.M. and Ragas, A.M.J. (2020) Disentanglement of the chemical, physical, and biological processes aids the development of quantitative structure-biodegradation relationships for aerobic wastewater treatment. Science of The Total Environment 708, 133863. DOI: 10.1016/j.scitotenv.2019.133863.

Posthuma, L., van Gils, J., Zijp, M.C., van De Meent, D. and de Zwart, D. (2019) Species sensitivity distributions for use in environmental protection, assessment, and management of aquatic ecosystems for 12 386 chemicals. Environmental Toxicology and Chemistry 38(4), 905-917.

Rios-Miguel, A.B., Jetten, M.S.M. and Welte, C.U. (2021) Effect of concentration and hydraulic reaction time on the removal of pharmaceutical compounds in a membrane bioreactor inoculated with activated sludge. Microb Biotechnol 14(4), 1707-1721. DOI: 10.1111/1751-7915.13837.

Schwarzenbach, R., Gschwend, P. and Imboden, D. (2005) pp. 387-458. Environmental organic chemistry, USA. Wiley.

Simkins, S. and Alexander, M. (1984) Models for mineralization kinetics with the variables of substrate concentration and population density. Applied and Environmental Microbiology 47(6), 1299-1306.

Svendsen, S.B., El-Taliawy, H., Carvalho, P.N. and Bester, K. (2020) Concentration dependent degradation of pharmaceuticals in WWTP effluent by biofilm reactors. Water Research 186, 116389. DOI: 10.1016/j.watres.2020.116389.

van Bergen, T.J.H.M., Rios-Miguel, A.B., Nolte, T.M., Ragas, A.M.J., van Zelm, R., Graumans, M., Scheepers, P.T.J., Jetten, M.S.M., Hendriks, A.J. and Welte, C.U. (2021) Do initial concentration and activated sludge seasonality affect pharmaceutical biotransformation rate constants? Applied Microbiology and Biotechnology 105(16), 6515-6527. DOI: 10.1007/s00253-021-11475-9.

Wang, Y., Fenner, K. and Helbling, D.E. (2020) Clustering micropollutants based on initial biotransformations for improved prediction of micropollutant removal during conventional activated sludge treatment. Environmental Science: Water Research & Technology 6(3), 554-565. DOI: 10.1039/C9EW00838A.

Wei, Z., Li, W., Zhao, D., Seo, Y., Spinney, R., Dionysiou, D.D., Wang, Y., Zeng, W. and Xiao, R. (2019) Electrophilicity index as a critical indicator for the biodegradation of the pharmaceuticals in aerobic activated sludge processes. Water research 160, 10-17.