Supplementary materials

Supplementary information 1. DNA sequences used for qPCR standards and primers

Table S1. Synthetic fragments used to obtain qPCR standard curves for the 16S rRNA, *intI1*, *sul1* and *sul2* genes.

Gene	Sequence
16S rRNA	CGGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCG GGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGG ATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTG CTACAATGG
intI1	GATCGGTCGAATGCGTGTGCTGCGCAAAAACCCAGAACCACGGCCA GGAATGCCCGGCGCGCGGATACTTCCGCTCAAGGGCGTCGGGAAGC GCAACGCCGCTGCGGCCCTCGGCCTGGTCCTTCAGCCACCATGCCC GTGCACGCGACAGCTGCTCGCGCAGGCTGGGTGCCAAGCTCTCGGG TAACATCAAGGC
sul1	CGCACCGGAAACATCGCTGCACGTGCTGTCGAACCTTCAAAAGCTG AAGTCGGCGTTGGGGCTTCCGCTATTGGTCTCGGTGTCGCGGAAAT CCTTCTTGGGCGCCACCGTTGGCCTTCCTGTAAAGGATCTGGGTCCA GCGAGCCTTGCGGCGGAACTTCA
sul2	TGGAGGCCGGTATCTGGCGCCAGACGCAGCCATTGCGCAGGCGCGT AAGCTGATGGCCGAGGGGGCAGATGTGATCGACCTCGGTCCGGCAT CCAGCAATCCCGACGCCGCGCCTGTTTCGTCCGACACAGAAATCGC GCGTATCGCGCCGGTGCTGGACGCGCTCAAGGCAGATGGCATTCCC G

Table S2. Sets of primers used for qPCR analyses

Gene	*Forward Primer (5' → 3')*	*Reverse Primer (5' → 3')*
16S rRNA	CGGCAACGAGCGCAACCC	CCATTGTAGCACGTGTGTAGCC
intI1	GATCGGTCGAATGCGTGT	GCCTTGATGTTACCCGAGAG
sul1	CGCACCGGAAACATCGCTGCAC	TGAAGTTCCGCCGCAAGGCTCG
sul2	TCCGGTGGAGGCCGGTATCTGG	CGGGAATGCCATCTGCCTTGAG

Supplementary information 2. Mathematical modelling simulations

Simulations of SMX biodegradation and effect on ordinary heterotrophic organisms

The simulation conditions were related to the experimental conditions of the first and second sets of batches conducted in this study. The input parameters for the simulations run in Aquasim are given for the acetate substrate (**Table S3a**), sulfamethoxazole antibiotic (**Table S3b**), and the biomass of ordinary heterotrophic organisms (OHOs) (**Table S3c**). Simulations were run over 200 h (*i.e.*, > 1 week) at low SMX concentrations (1-10 mg SMX L^{-1}) and over 10'000 h (*i.e.*, > 1 year) for the highest SMX concentration (150 mg SMX L^{-1}) to account for short- and long-term effects of these concentrations on biomass growth and inhibition and on the resulting SMX biodegradation profiles. Parameters were implemented in mol-based and hour time units (highlighted in grey in the **Tables S3a-c** hereafter).

The first set of batches was conducted by exposing a high biomass concentration (4.4 g VSS L^{-1}) to low SMX concentrations (0 and 1 mg SMX L^{-1}), with a lower acetate loading (1200 mg COD L^{-1}), and without adjusting nitrogen and phosphorus requirements for growth. The second set of batches exposed a low biomass concentration (0.5 g VSS L^{-1}) to low and high SMX concentrations (0, 1, 10 and 150 mg SMX L^{-1}) and supplied with a higher concentration of acetate (3 g COD L^{-1}) while adjusting the nutrient requirements to a COD:N:P ratio of 100:13:1.3 m/m/m.

Table S3a. Input parameters of the model simulations: **acetate substrate (Ac)**; simulation time (Δt), initial concentration of acetate (S_Ac_init), affinity constant for acetate (K_Ac).

Material		**Acetate ($C_2H_3O_2^-$)**			
MM (mg/mmol)		59.044			
γ (mmol e-/mmol i)		8			
ThOD (mg COD/mmol i)		64			
Parameter	**Δt**	**S_Ac_init**		**K_Ac**	
Units	h	mg COD/L	mmol Ac/L	mg COD/L	mmol Ac/L
First set of batch experiments					
Simulation 1.0	200	**1200**	18.750	10	0.156
Simulation 1.1	200	**1200**	18.750	10	0.156
Second set of batch experiments					
Simulation 2.0	200	**3000**	46.875	10	0.156
Simulation 2.1	200	**3000**	46.875	10	0.156
Simulation 2.2	200	**3000**	46.875	10	0.156
Simulation 2.3	10000	**3000**	46.875	10	0.156

Table S3b. Input parameters of the model simulations: **sulfamethoxazole antibiotic (SMX)**; initial concentration of SMX (S_SMX_init), pseudo first-order biodegradation rate constant (k_SMX), minimum inhibitory concentration (K_SMX_MIC).

Material		**Sulfamethoxazole SMX ($C_{10}H_{11}N_3O_3S$)**					
MM (mg/mmol)		253.729					
γ (mmol e-/mmol i)		142					
ThOD (mg COD/mmol i)		1136					
Parameter	**Δt**	**S_SMX_init**		**k_SMX**		**K_SMX_MIC**	
Units	h	mg SMX/L	mmol SMX/L	L/d/g VSS	L/h/mmol X	mg SMX/L	mmol SMX/L
First set of batch experiments							
Simulation 1.0	200	**0**	0.004	3	0.003	8	0.032
Simulation 1.1	200	**1**	0.004	3	0.003	8	0.032

Second set of batch experiments							
Simulation 2.0	200	**0**	0.004	3	0.003	8	0.032
Simulation 2.1	200	**1**	0.004	3	0.003	8	0.032
Simulation 2.2	200	**10**	0.039	3	0.003	8	0.032
Simulation 2.3	10'000	**150**	0.591	3	0.003	8	0.032

Table S3c. Input parameters of the model simulations: **biomass of ordinary heterotrophic organisms (OHOs)**; initial concentration of biomass (X_OHO_init), biomass-specific maximum growth rate (mu_max_OHO), biomass-specific decay rate (b_OHO), yield of biomass growth on organic substrate (Y_X/S), yield of acetate consumption per biomass production (-Y_Ac/X).

Material		**Biomass of ordinary heterotrophic organisms OHOs ($C_1H_{1.8}O_{0.5}N_{0.2}$)**								
MM (mg/mmol)		24.6								
γ (mmol e-/mmol i)		4.2								
ThOD (mg COD/mmol i)		33.6								
Parameter	**Δt**	**X_OHO_init**		**mu_max_OHO**		**b_OHO**		**Y_X/S**		**-Y_Ac/X**
Units	h	g VSS/L	mmol X/L	1/d	1/h	1/d	1/h	g COD X /g COD Ac	mmol X /mmol Ac	mmol Ac /mmol X
First set of batch experiments										
Simulation 1.0	200	**4.4**	0.179	5	0.208	0.275	0.011	0.5	0.952	-1.050
Simulation 1.1	200	**4.4**	0.179	5	0.208	0.275	0.011	0.5	0.952	-1.050
Second set of batch experiments										
Simulation 2.0	200	**0.5**	0.020	5	0.208	0.275	0.011	0.5	0.952	-1.050
Simulation 2.1	200	**0.5**	0.020	5	0.208	0.275	0.011	0.5	0.952	-1.050
Simulation 2.2	200	**0.5**	0.020	5	0.208	0.275	0.011	0.5	0.952	-1.050
Simulation 2.3	10'000	**0.5**	0.020	5	0.208	0.275	0.011	0.5	0.952	-1.050

The profiles of acetate consumption, biomass growth and inhibition, and SMX biodegradation are provided for the first set (**Figure S1a**) and second set (**Figures S1b**) of batch experiments operated at the different nutrient and SMX concentrations.

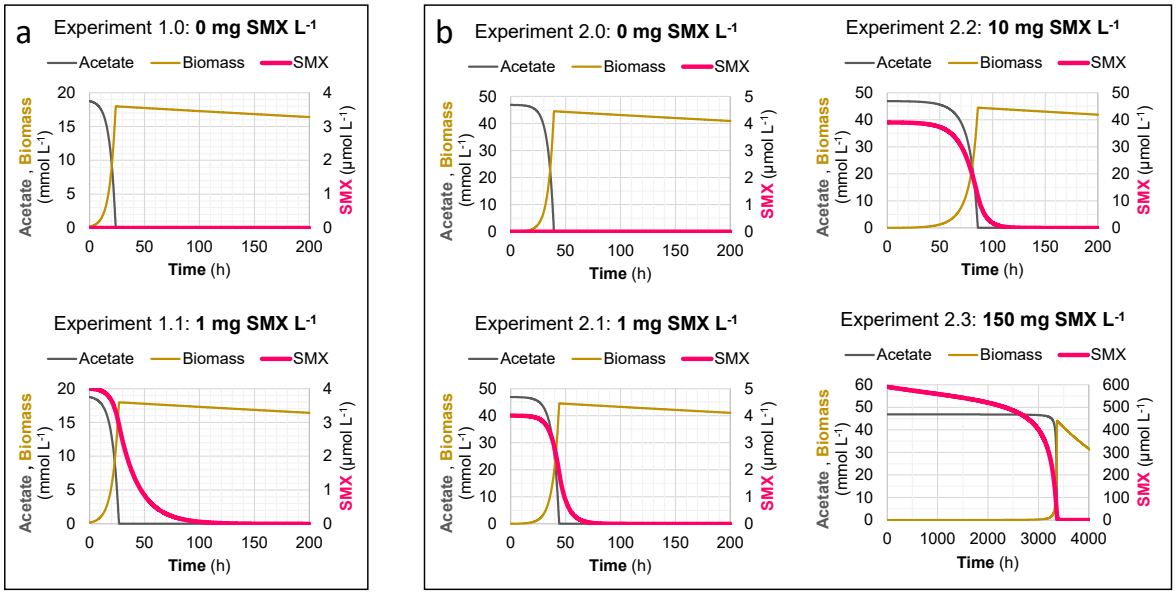

Figure S1. Evolutions of the acetate substrate, OHO biomass, and SMX antibiotic simulated under the conditions of the first set and second set of batch experiments. (**a**) The first batches were conducted at an initial concentration of acetate of 1200 mg COD L^{-1} without balancing the nitrogen requirement for growth (residual nitrogen present in the activated sludge), by exposing a concentrated biomass (4.4 g VSS L^{-1}) to antibiotic concentrations of 0 (control) and 1 mg SMX L^{-1}. (**b**) The second batches were conducted under balanced COD:N:P conditions for microbial growth by adjusting the initial concentrations of acetate (3000 mg COD L^{-1}), ammonium (400 mg $N\text{-}NH_4^+$ L^{-1}) and phosphate (40 mg $P\text{-}PO_4^{3-}$ L^{-1}), while exposing a low-concentrated biomass (0.5 g VSS L^{-1}) to antibiotic concentrations of 0 (control), 1, 10 and 150 mg SMX L^{-1}. Simulations were performed using mol-based and hour time units. The simulation time was adapted in function of SMX concentrations to reveal the full biodegradation profiles. High concentration of SMX results in a prolonged bacteriostatic inhibition of the biomass.

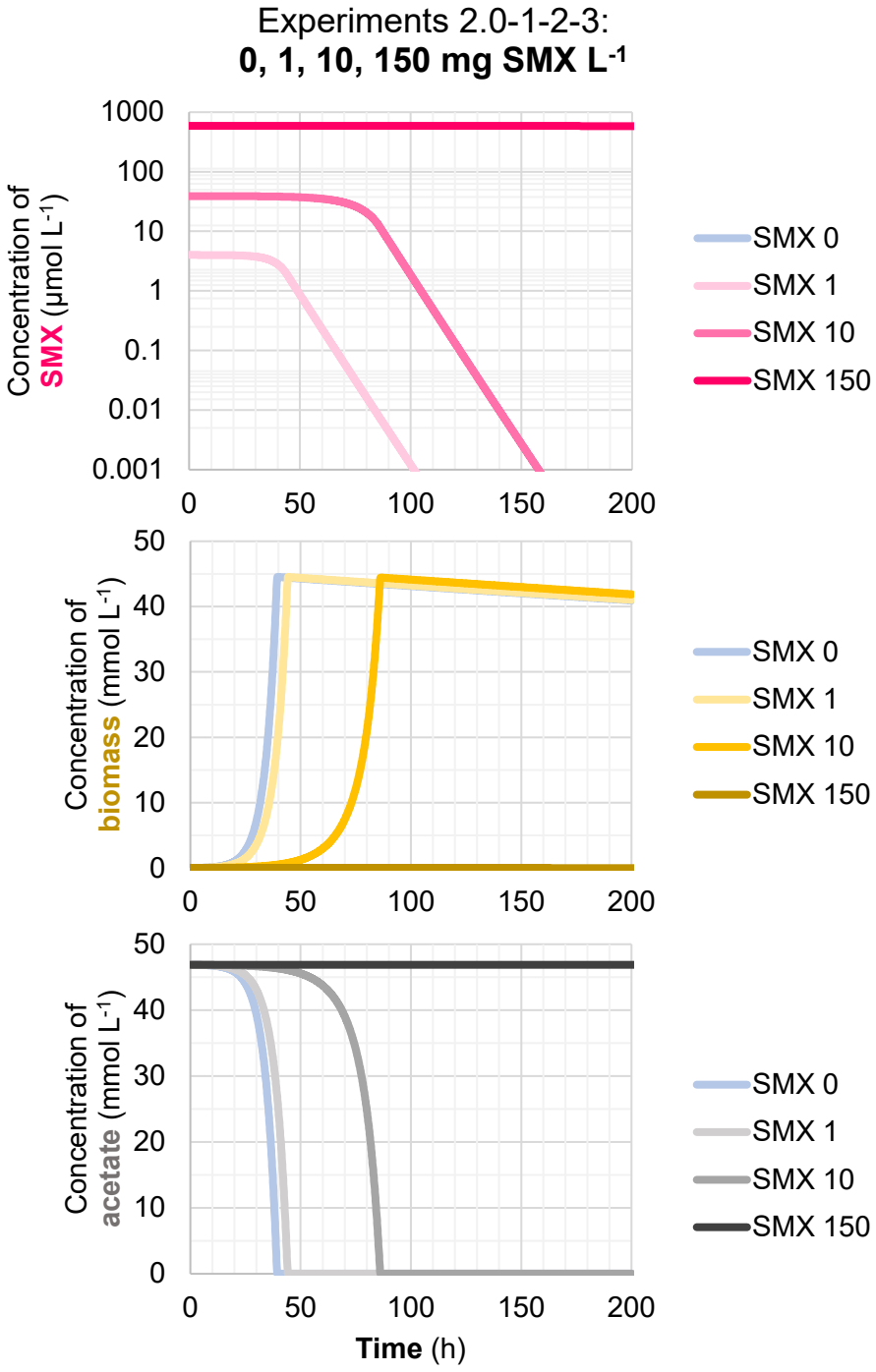

Figure S2. Comparison of the profiles of SMX biodegradation, biomass growth and inhibition, and acetate consumption simulated under the conditions of the second set of batch experiments conducted at antibiotic concentrations of 0 (control), 1, 10 and 150 mg SMX L^{-1}.