Supporting Information for

Modeling the effect of external carbon source addition under different electron acceptor conditions in biological nutrient removal activated sludge systems

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Model limitations

Even though, the developed model is complex, there are still a few important issues that may be incorporated in further expansions or modifications:

(1) The competition between PAOs and GAOs was ignored due to a negligible activity of the GAOs (confirmed experimentally). However, in the EBPR systems with a substantial competition between PAOs and GAOs, the metabolism of GAOs cannot be ignored.

(2) When abundant in municipal wastewater, propionate may be incorporated in a model as a separate state variable in order to describe better the response of PAOs to different carbon sources (acetate and propionate) and the competition between PAOs and GAOs.

(3) Since the nitrite effect on PAOs has not ultimately been clarified, the actual inhibitory effect of nitrite or FNA in BNR systems requires further investigation.

(4) Two-step denitrification is considered. The growing concern of greenhouse gas (GHG) emissions from WWTPs may require further extensions by including four steps ($NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$).

Organization of the modeling study procedure

The modelling study followed the consecutive steps presented in SI Figure S1. First (step 1), the original ASM2d was calibrated/validated under dynamic conditions with results of both batch tests with the settled wastewater (without the addition of external carbon) and 96-hour measurement campaign in the full-scale MUCT bioreactor.[28] Next, sensitivity analysis using a one-variable-at-a-time approach was performed to establish number of parameters that indeed influence the model outputs (step 2). Considering the most influential parameters, the expanded model was calibrated (step 3) based on experimental data of the selected two-phase batch tests carried out under anaerobic/aerobic and anaerobic/anoxic conditions with acetate (S_A) and ethanol ($S_{A,1}$) as two different types of external carbon sources. For model calibration, a special GPS-X utility called "Optimizer" was used. Parameters were estimated based on the Nelder-Mead simplex method with the maximum likelihood as an objective function. The 95% confidence intervals for the estimated parameters were calculated from the parameter estimation error covariance matrix. Furthermore, a correlation matrix was developed in step 4 for each calibration step to evaluate the degree of correlation between pairs of the adjusted parameters and determine if a change in the value of one parameter could be compensated by a change in the value of another parameter (to avoid potential overparametrization). The details on the computational methods can be found in the GPS-X "Technical Reference" (Hydromantis, Canada). The expanded model was validated based on results of the remaining one- and two-phase batch experiments (step 5). In order to evaluate the goodness of fit between observed data and model predictions, the absolute and relative standard errors as well as correlation coefficients of determination (R^2) were determined for each measured variable.

Finally (step 6), the long-term acclimation experiment with addition of the $S_{A,1}$ type of external carbon source (fusel oil) to the anoxic zone of a bench-scale pilot system was simulated with the expanded ASM2d.

The biomass composition for setting the initial conditions was determined based on steady-state and dynamic simulations of the full-scale activated sludge system at the studied plant. This procedure was described by Swinarski et al.[28] Specifically, the original ASM2d model was first calibrated/validated under steady-state and dynamic simulations in the full-scale plant to determine each soluble and particulate fraction in the biomass. Then the initial biomass concentrations in each batch experiment were calculated based on the ratio of MLVSS concentration in the batch reactor and the MLVSS concentration in the sampling point at the full-scale plant.

Parameter estimation, confidence intervals and correlation matrix

The nominal range sensitivity analysis using a one-variable-at-a-time approach was performed to establish number of parameters that indeed influence the model outputs (Table S3 in SI).[14] Based on those results, 13 sensitive parameters were identified in the expanded ASM2d model, excluding the parameters which were directly adopted from the study of Swinarski et al.[28]

Parameter estimation was performed based on data derived from the selected batch experiments, including two-phase tests with the different external carbon sources (acetate, ethanol) and electron acceptors (DO, NO_2-N, NO_3-N). Results of parameter estimation are shown in SI Table S5 along with uncertainty of the parameter estimates, quantified as the 95% confidence intervals. Several new parameters revealed relatively large confidence intervals, which implied rather poor parameter estimation in those cases e.g. K_{IOPHA} = 3.60±1.135 or K_{IPHA} = 0.02±0.138. Therefore, a correlation matrix between the new estimated parameters was developed and analyzed in order to explain the large confidence intervals. Correlations between the parameters estimated in step 3.1 and steps 3.2-3.4 are shown in SI Table S6 and Table S7, respectively.

Strong correlations (values close to 1) were found between the parameters K_{IPHA} and K_{MAX1} (0.98), and the parameters Y_{H1} and μ_{H1} (0.96) in step 3.1 and 3.2, respectively. The strong correlation implied that a change in the value of one parameter could be compensated by a change in the value of another parameter. This could make it difficult to find unique estimates for those new parameters. Nevertheless, all optimization runs using different initial parameter estimates converged to the same optimal parameter set. The low standards errors listed in SI Table S4 and high coefficients of determination R^2 (the average value of approximately 0.92) confirmed the goodness of fit with respect to the estimated parameters for each batch experiment.

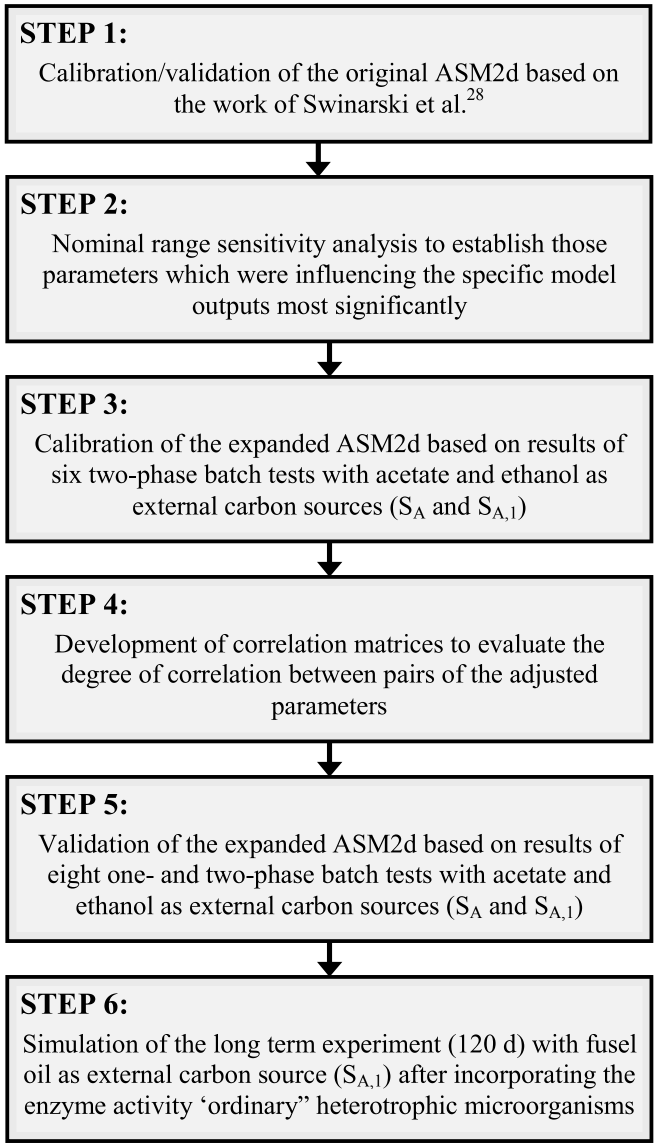

Figure S1. Calibration/validation procedure of the expanded ASM2d for predicting the effects of external carbon sources in BNR activated sludge systems.

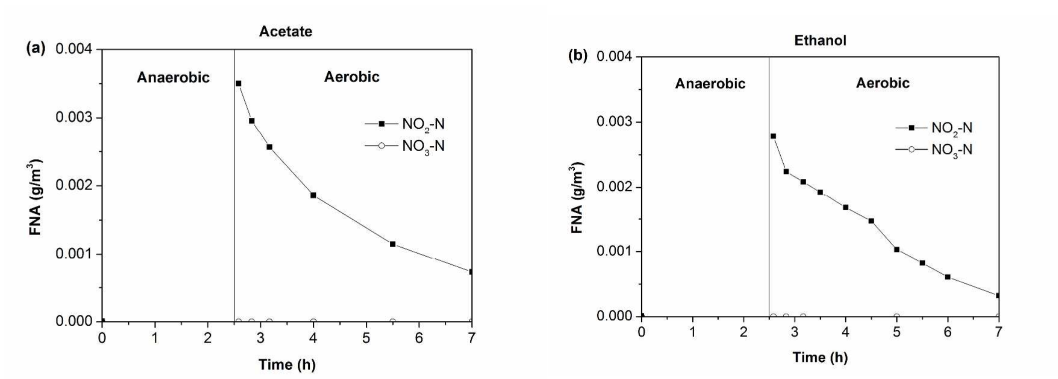

Figure S2. FNA concentrations measured in two-phase anaerobic-anoxic experiments with different external carbon sources and different electron acceptors: (a) acetate, NO_3-N and NO_2-N, (b) ethanol, NO_3-N and NO_2-N.

Table S1. Stoichiometric matrix for the expanded ASM2d (only new and modified processes)

Process \ Component		1 S_O	2 S_F	3 S_A	4 S_{A1}	5 S_{NH4}	6 S_{NO2}	7 S_{NO3}	8 S_{PO4}	9 S_I	10 S_{ALK}
Growth of heterotrophic organisms (X_H)											
1	Aerobic growth of X_H on $S_{A,1}$	$1-\frac{1}{Y_{H1}}$			$-\frac{1}{Y_{H1}}$	$\nu_{1,NH4}$			$\nu_{1,PO4}$		
2	Anoxic growth on S_F, denitrification (S_{NO2})		$-\frac{1}{Y_H}$			$\nu_{2,NH4}$	$-\frac{1-Y_H}{1.71Y_H}$		$\nu_{2,PO4}$		
3	Anoxic growth on S_F, denitrification (S_{NO3})		$-\frac{1}{Y_H}$			$\nu_{3,NH4}$		$-\frac{1-Y_H}{2.86Y_H}$	$\nu_{3,PO4}$		
4	Anoxic growth on S_A, denitrification (S_{NO2})			$-\frac{1}{Y_H}$		$\nu_{4,NH4}$	$-\frac{1-Y_H}{1.71Y_H}$		$\nu_{4,PO4}$		
5	Anoxic growth on S_A, denitrification (S_{NO3})			$-\frac{1}{Y_H}$		$\nu_{5,NH4}$		$-\frac{1-Y_H}{2.86Y_H}$	$\nu_{5,PO4}$		
6	Anoxic growth on S_{A1}, denitrification (S_{NO2})				$-\frac{1}{Y_{H1}}$	$\nu_{6,NH4}$	$-\frac{1-Y_{H1}}{1.71Y_{H1}}$		$\nu_{6,PO4}$		
7	Anoxic growth on S_{A1}, denitrification (S_{NO3})				$-\frac{1}{Y_{H1}}$	$\nu_{7,NH4}$		$-\frac{1-Y_{H1}}{2.86Y_{H1}}$	$\nu_{7,PO4}$		
Activity of Phosphorus Accumulating Organisms (PAO) (X_{PAO})											
8	Storage of X_{PHA} by PAOs			-1					Y_{PO4}		

	Process \ Component	1 S_O	2 S_F	3 S_A	4 S_{A1}	5 S_{NH4}	6 S_{NO2}	7 S_{NO3}	8 S_{PO4}	9 S_I	10 S_{ALK}
9	Aerobic storage of X_{pp} by PAOs on X_{PHA}	$-Y_{PHA}$							-1		
10	Anoxic storage of X_{pp}, denitrification (S_{NO2})						$v_{10,NO2}$		-1		
11	Anoxic storage of X_{pp}, denitrification (S_{NO3})							$v_{11,NO3}$	-1		
12	Aerobic storage of X_{pp} by PAO on S_A	$-Y_{SA}$		$-Y_{SA}$					-1		
13	Anoxic storage of X_{pp} on S_A, denitrification (S_{NO2})			$-Y_{SA}$			$v_{13,NO2}$		-1		
14	Anoxic storage of X_{pp} on S_A, denitrification (S_{NO3})			$-Y_{SA}$				$v_{14,NO3}$	-1		
15	Aerobic storage of X_{pp} by PAO on $S_{A,1}$	$-Y_{SA1}$			$-Y_{SA1}$				-1		
16	Anoxic storage of X_{pp} on $S_{A,1}$, denitrification (S_{NO2})				$-Y_{SA1}$		$v_{16,NO2}$		-1		
17	Anoxic storage of X_{pp} on $S_{A,1}$, denitrification (S_{NO3})				$-Y_{SA1}$			$v_{17,NO3}$	-1		
18	Aerobic growth of X_{PAO} on X_{PHA}	$v_{18,O}$				$v_{18,NH4}$			$-i_{P,BM}$		
19	Anoxic growth of $X_{PAO,}$ denitrification (S_{NO2})					$v_{19,NH4}$	$v_{19,NO2}$		$-i_{P,BM}$		
20	Anoxic growth of $X_{PAO,}$ denitrification (S_{NO3})					$v_{20,NH4}$		$v_{20,NO3}$	$-i_{P,BM}$		
21	Aerobic growth of X_{PAO} on S_A	$v_{21,O}$		$-\frac{1}{Y_{PAO,S}}$		$v_{21,NH4}$			$-i_{P,BM}$		
22	Anoxic growth of X_{PAO} on $S_{A,}$ denitrification (S_{NO2})			$-\frac{1}{Y_{PAO,S}}$		$v_{22,NH4}$	$v_{22,NO2}$		$-i_{P,BM}$		
23	Anoxic growth of X_{PAO} on $S_{A,}$ denitrification (S_{NO3})			$-\frac{1}{Y_{PAO,}}$		$v_{23,NH4}$		v_{23NO3}	$-i_{P,BM}$		
24	Aerobic growth of X_{PAO} on $S_{A,1}$	$v_{24,O}$			$-\frac{1}{Y_{PAO,S}}$	$v_{24,NH4}$			$-i_{P,BM}$		
25	Anoxic growth of X_{PAO} on $S_{A,1,}$ denitrification (S_{NO2})				$-\frac{1}{Y_{PAO,S}}$	$v_{25,NH4}$	$v_{25,NO2}$		$-i_{P,BM}$		
26	Anoxic growth of X_{PAO} on $S_{A,1,}$ denitrification (S_{NO3})				$-\frac{1}{Y_{PAO,S}}$	$v_{26,NH4}$		v_{26NO3}	$-i_{P,BM}$		

	Process \ Component	1 S_O	2 S_F	3 S_A	4 S_{A1}	5 S_{NH4}	6 S_{NO2}	7 S_{NO3}	8 S_{PO4}	9 S_I	10 S_{ALK}
Hydrolysis processes											
27	Anoxic hydrolysis of X_S with nitrite (S_{NO2})		$1-f_{SI}$			$\nu_{27,NH4}$			$\nu_{27,PO4}$	f_{SI}	
28	Anoxic hydrolysis of X_S with nitrate (S_{NO3})		$1-f_{SI}$			$\nu_{28,NH4}$			$\nu_{28,PO4}$	f_{SI}	
Activity of the denitrification enzyme for the external carbon source ($S_{A,1}$)											
29	Enzyme synthesis										
30	Enzyme decay										

Table S1. (continued) Stoichiometric matrix for the expanded ASM2d (only new and modified processes)

	Process \ Component	11 X_S	12 X_H	13 X_{PAO}	14 X_{PP}	15 X_{PHA}	16 E_{sat}
Growth of heterotrophic organisms (X_H)							
1	Aerobic growth of X_H on $S_{A,1}$		1				
2	Anoxic growth on S_F, denitrification (S_{NO2})		1				
3	Anoxic growth on S_F, denitrification (S_{NO3})		1				
4	Anoxic growth on S_A, denitrification (S_{NO2})		1				
5	Anoxic growth on S_A, denitrification (S_{NO3})		1				
6	Anoxic growth on S_{A1}, denitrification (S_{NO2})		1				
7	Anoxic growth on S_{A1}, denitrification (S_{NO3})		1				
Activity of Phosphorus Accumulating Organisms (PAO) (X_{PAO})							
8	Storage of X_{PHA} by PAOs				$-Y_{PO4}$	1	
9	Aerobic storage of X_{pp} by PAOs on X_{PHA}				1	$-Y_{PHA}$	
10	Anoxic storage of X_{pp}, denitrification (S_{NO2})				1	$-Y_{PHA}$	

Process \ Component		11 X_S	12 X_H	13 X_{PAO}	14 X_{PP}	15 X_{PHA}	16 E_{sat}
11	Anoxic storage of X_{pp}, denitrification (S_{NO3})				1	$-Y_{PHA}$	
12	Aerobic storage of X_{pp} by PAO on S_A				1		
13	Anoxic storage of X_{pp} on S_A, denitrification (S_{NO2})				1		
14	Anoxic storage of X_{pp} on S_A, denitrification (S_{NO3})				1		
15	Aerobic storage of X_{pp} by PAO on $S_{A,1}$				1		
16	Anoxic storage of X_{pp} on $S_{A,1}$, denitrification (S_{NO2})				1		
17	Anoxic storage of X_{pp} on $S_{A,1}$, denitrification (S_{NO3})				1		
18	Aerobic growth of X_{PAO} on X_{PHA}			1		$-\frac{1}{Y_{PAO}}$	
19	Anoxic growth of $X_{PAO,}$ denitrification (S_{NO2})			1		$-\frac{1}{Y_{PAO}}$	
20	Anoxic growth of $X_{PAO,}$ denitrification (S_{NO3})			1		$-\frac{1}{Y_{PAO}}$	
21	Aerobic growth of X_{PAO} on S_A			1			
22	Anoxic growth of X_{PAO} on $S_{A,}$ denitrification (S_{NO2})			1			
23	Anoxic growth of X_{PAO} on $S_{A,}$ denitrification (S_{NO3})			1			
24	Aerobic growth of X_{PAO} on $S_{A,1}$			1			
25	Anoxic growth of X_{PAO} on $S_{A,1,}$ denitrification (S_{NO2})			1			
26	Anoxic growth of X_{PAO} on $S_{A,1,}$ denitrification (S_{NO3})			1			
Hydrolysis processes							
27	Anoxic hydrolysis of X_S with nitrite (S_{NO2})	-1					
28	Anoxic hydrolysis of X_S with nitrate (S_{NO3})	-1					
Activity of the denitrification enzyme for the external carbon source ($S_{A,1}$)							
29	Enzyme synthesis						1
30	Enzyme decay						-1

Table S2. Process rates for the expanded ASM2d (only new and modified processes)

	Process	Process rate, ρ_j
Growth of heterotrophic organisms (X_H)		
1	Aerobic growth of X_H on $S_{A,1}$	$\mu_{H1} \cdot \frac{S_{O,H}}{K_{O,H}+S_O} \cdot \frac{S_{A1}}{K_{SA1,H}+S_{A1}} \cdot \frac{S_{NH4,H}}{K_{NH4,H}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,H}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,H}+S_{ALK}} \cdot X_H$
2	Anoxic growth on S_F, denitrification (S_{NO2})	$\mu_H \cdot \eta_{NO2,H} \cdot \frac{K_{O,H}}{K_{O,H}+S_O} \cdot \frac{S_F}{K_{SF,H}+S_F} \cdot \frac{S_F}{S_F+S_A} \cdot \frac{S_{NO2}}{K_{NO2,H}+S_{NO2}} \cdot \frac{S_{NH4,H}}{K_{NH4,H}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,H}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,H}+S_{ALK}} \cdot X_H \cdot E_{Sat}$
3	Anoxic growth on S_F, denitrification (S_{NO3})	$\mu_H \cdot \eta_{NO3,H} \cdot \frac{K_{O,H}}{K_{O,H}+S_O} \cdot \frac{S_F}{K_{SF,H}+S_F} \cdot \frac{S_F}{S_F+S_A} \cdot \frac{S_{NO3}}{K_{NO3,H}+S_{NO3}} \cdot \frac{S_{NH4,H}}{K_{NH4,H}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,H}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,H}+S_{ALK}} \cdot X_H \cdot E_{Sat}$
4	Anoxic growth on S_A, denitrification (S_{NO2})	$\mu_H \cdot \eta_{NO2,H} \cdot \frac{K_{O,H}}{K_{O,H}+S_O} \cdot \frac{S_A}{K_{SA,H}+S_A} \cdot \frac{S_A}{S_F+S_A} \cdot \frac{S_{NO2}}{K_{NO2,H}+S_{NO2}} \cdot \frac{S_{NH4,H}}{K_{NH4,H}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,H}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,H}+S_{ALK}} \cdot X_H \cdot E_{Sat}$
5	Anoxic growth on S_A, denitrification (S_{NO3})	$\mu_H \cdot \eta_{NO3,H} \cdot \frac{K_{O,H}}{K_{O,H}+S_O} \cdot \frac{S_A}{K_{SA,H}+S_A} \cdot \frac{S_A}{S_F+S_A} \cdot \frac{S_{NO3}}{K_{NO3,H}+S_{NO3}} \cdot \frac{S_{NH4,H}}{K_{NH4,H}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,H}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,H}+S_{ALK}} \cdot X_H \cdot E_{Sat}$
6	Anoxic growth on S_{A1}, denitrification (S_{NO2})	$\mu_{H1} \cdot \eta_{NO2,H1} \cdot \frac{K_{O,H}}{K_{O,H}+S_O} \cdot \frac{S_{A1}}{K_{SA1,H}+S_{A1}} \cdot \frac{S_{NO2}}{K_{NO2,H}+S_{NO2}} \cdot \frac{S_{NH4,H}}{K_{NH4,H}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,H}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,H}+S_{ALK}} \cdot X_H \cdot E_{Sat}$
7	Anoxic growth on S_{A1}, denitrification (S_{NO3})	$\mu_{H1} \cdot \eta_{NO3,H1} \cdot \frac{K_{O,H}}{K_{O,H}+S_O} \cdot \frac{S_{A1}}{K_{SA1,H}+S_{A1}} \cdot \frac{S_{NO3}}{K_{NO3,H}+S_{NO3}} \cdot \frac{S_{NH4,H}}{K_{NH4,H}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,H}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,H}+S_{ALK}} \cdot X_H \cdot E_{Sat}$
Activity of Phosphorus Accumulating Organisms (PAO) (X_{PAO})		
8	Storage of X_{PHA} by X_{PAO}	$q_{PHA} \cdot \frac{S_A}{K_{SA,PAO}+S_A} \cdot \frac{K_{IOPHA}}{K_{IOPHA}+S_O} \cdot \frac{K_{INO2,PAO}}{K_{INO2,PA0}+S_{NO2}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP}+X_{PP}/X_{PAO}} \cdot \frac{K_{MAX1}-X_{PHA}/X_{PAO}}{K_{IPHA}+K_{MAX1}-X_{PHA}/X_{PAO}} X_{PAO}$
9	Aerobic storage of X_{pp} by PAO on X_{PHA}	$q_{PP} \cdot \frac{S_O}{K_{O,PAO}+S_O} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
10	Anoxic storage of X_{pp} on X_{PHA}, denitrification (S_{NO2})	$q_{PP} \cdot \eta_{NO2,PAO} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_{NO2}}{K_{NO2,PAO}+S_{NO2}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
11	Anoxic storage of X_{pp} on X_{PHA}, denitrification (S_{NO3})	$q_{PP} \cdot \eta_{NO3,PAO} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_{NO3}}{K_{NO3,PAO}+S_{NO3}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$

Process		Process rate, ρ_j
12	Aerobic storage of X_{pp} by PAO on S_A	$q_{PPSA} \cdot \frac{S_O}{K_{O,PAO}+S_O} \cdot \frac{S_A}{K_{SA,PAO}+S_A} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
13	Anoxic storage of X_{pp} on S_A, denitrification (S_{NO2})	$q_{PPSA} \cdot \eta_{NO2,PAOSA} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_A}{K_{SA,PAO}+S_A} \cdot \frac{S_{NO2}}{K_{NO2,PAO}+S_{NO2}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
14	Anoxic storage of X_{pp} on S_A, denitrification (S_{NO3})	$q_{PPSA} \cdot \eta_{NO3,PAOSA} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_A}{K_{SA,PAO}+S_A} \cdot \frac{S_{NO3}}{K_{NO3,PAO}+S_{NO3}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
15	Aerobic storage of X_{pp} by PAO on S_{A1}	$q_{PPSA1} \cdot \frac{S_O}{K_{O,PAO}+S_O} \cdot \frac{S_{A1}}{K_{SA1,PAO}+S_{A1}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
16	Anoxic storage of X_{pp} on S_{A1}, denitrification (S_{NO2})	$q_{PPSA1} \cdot \eta_{NO2,PAOSA1} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_{A1}}{K_{SA1,PAO}+S_{A1}} \cdot \frac{S_{NO2}}{K_{NO2,PAO}+S_{NO2}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
17	Anoxic storage of X_{pp} on S_{A1}, denitrification (S_{NO3})	$q_{PPSA1} \cdot \eta_{NO3,PAOSA1} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_{A1}}{K_{SA1,PAO}+S_{A1}} \cdot \frac{S_{NO3}}{K_{NO3,PAO}+S_{NO3}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
18	Aerobic growth of X_{PAO} on X_{PHA}	$\mu_{PAO} \cdot \frac{S_O}{K_{O,PAO}+S_O} \cdot \frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot X_{PAO}$
19	Anoxic growth of X_{PAO} on X_{PHA}, denitrification (S_{NO2})	$\mu_{PAO} \cdot \eta_{NO2,PAO} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_{NO2}}{K_{NO2,PAO}+S_{NO2}} \cdot \frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot X_{PAO}$
20	Anoxic growth of X_{PAO} on X_{PHA}, denitrification (S_{NO3})	$\mu_{PAO} \cdot \eta_{NO3,PAO} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_{NO3}}{K_{NO3,PAO}+S_{NO3}} \cdot \frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot X_{PAO}$
21	Aerobic growth of X_{PAO} on S_A	$\mu_{PAOSA} \cdot \frac{S_O}{K_{O,PAO}+S_O} \cdot \frac{S_A}{K_{SA,PAO}+S_A} \cdot \frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot X_{PAO}$
22	Anoxic growth of X_{PAO} on S_A, denitrification (S_{NO2})	$\mu_{PAOSA} \cdot \eta_{NO2,PAOSA} \cdot \frac{K_{O,PAO}}{K_{O,PAO}+S_O} \cdot \frac{S_A}{K_{SA,PAO}+S_A} \cdot \frac{S_{NO2}}{K_{NO2,PAO}+S_{NO2}} \cdot \frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}} \cdot X_{PAO}$

Process		Process rate, ρ_i
23	Anoxic growth of X_{PAO} on S_A, denitrification (S_{NO3})	$\mu_{PAOSA}\cdot\eta_{NO3,PAOSA}\cdot\frac{K_{O,PAO}}{K_{O,PAO}+S_O}\cdot\frac{S_A}{K_{SA,PAO}+S_A}\cdot\frac{S_{NO3}}{K_{NO3,PAO}+S_{NO3}}\cdot\frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}}\cdot\frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}}\cdot\frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}}\cdot X_{PAO}$
24	Aerobic growth of X_{PAO} on $S_{A,1}$	$\mu_{PAOSA1}\cdot\frac{S_O}{K_{O,PAO}+S_O}\cdot\frac{S_{A1}}{K_{SA1,PAO}+S_{A1}}\cdot\frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}}\cdot\frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}}\cdot\frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}}\cdot X_{PAO}$
25	Anoxic growth of X_{PAO} on $S_{A,1}$, denitrification (S_{NO2})	$\mu_{PAOSA1}\cdot\eta_{NO2,PAOSA1}\cdot\frac{K_{O,PAO}}{K_{O,PAO}+S_O}\cdot\frac{S_{A1}}{K_{SA1,PAO}+S_{A1}}\cdot\frac{S_{NO2}}{K_{NO2,PAO}+S_{NO2}}\cdot\frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}}\cdot\frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}}\cdot\frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}}\cdot X_{PAO}$
26	Anoxic growth of X_{PAO} on $S_{A,1}$, denitrification (S_{NO3})	$\mu_{PAOSA1}\cdot\eta_{NO3,PAOSA1}\cdot\frac{K_{O,PAO}}{K_{O,PAO}+S_O}\cdot\frac{S_{A1}}{K_{SA1,PAO}+S_{A1}}\cdot\frac{S_{NO3}}{K_{NO3,PAO}+S_{NO3}}\cdot\frac{S_{NH4}}{K_{NH4,PAO}+S_{NH4}}\cdot\frac{S_{PO4}}{K_{PO4,PAO}+S_{PO4}}\cdot\frac{S_{ALK}}{K_{ALK,PAO}+S_{ALK}}\cdot X_{PAO}$
Hydrolysis processes		
27	Anoxic hydrolysis of X_S with nitrite (S_{NO2})	$k_h\cdot\eta_{NO2,HYD}\cdot\frac{K_{O,H}}{K_{O,H}+S_O}\cdot\frac{S_{NO2}}{K_{NO2,HYD}+S_{NO2}}\cdot\frac{X_S/X_H}{K_X+X_S/X_H}\cdot X_H$
28	Anoxic hydrolysis of X_S with nitrate (S_{NO3})	$k_h\cdot\eta_{NO3,HYD}\cdot\frac{K_{O,H}}{K_{O,H}+S_O}\cdot\frac{S_{NO3}}{K_{NO3,HYD}+S_{NO3}}\cdot\frac{X_S/X_H}{K_X+X_S/X_H}\cdot X_H$
Activity of the denitrification enzyme for the external carbon source ($S_{A,1}$)		
29	Enzyme synthesis	$k_{S,ENZ}\cdot(\frac{S_{NO3}}{K_{NO3,ENZ}+S_{NO3}}\cdot\frac{S_{NO3}}{S_{NO2}+S_{NO3}}+\frac{S_{NO2}}{K_{NO2,ENZ}+S_{NO2}}\cdot\frac{S_{NO2}}{S_{NO2}+S_{NO3}})\cdot\frac{K_{O,ENZ}}{K_{O,ENZ}+S_O}\cdot(1-E_{Sat})$
30	Enzyme decay	$k_{D,ENZ}\cdot E_{Sat}$

Table S3. List of sensitivity coefficients calculated for the adjusted parameters during the calibration of the extended ASM2d model

Symbol	Anaerobic		Anoxic				Aerobic	
	COD	PO_4-P	COD	PO_4-P	NO_3-N	NO_2-N	COD	PO_4-P
Original kinetic parameters in ASM2d								
Heterotrophic organisms (X_H):								
μ_H	-0.046	-0.029	**-0.371**	-0.160	**-1.062**	**-0.466**	**-0.304**	**0.407**
Phosphorus Accumulating Organisms (X_{PAO}):								
q_{PHA}	**-0.498**	**0.510**	-0.092	0.217	**0.503**	0.004	-0.045	0.125
q_{PP}	-0.001	0.004	-0.061	**-0.336**	**-0.714**	0.000	0.091	**-1.464**
K_{IPP}	0.003	-0.026	0.017	**0.280**	0.220	0.002	0.016	**0.539**
K_{PHA}	-0.003	-0.014	0.007	0.049	0.175	-0.004	-0.006	**0.259**
Hydrolysis of particulate substrate (X_S):								
K_h	0.082	0.061	0.217	0.213	**-0.882**	-0.080	0.103	0.039

Symbol	Anaerobic		Anoxic				Aerobic	
	COD	PO_4-P	COD	PO_4-P	NO_3-N	NO_2-N	COD	PO_4-P
η_{fe}	**0.270**	0.220	0.008	0.078	-0.033	-0.005	0.005	0.062
K_X	0.142	0.237	-0.062	-0.075	**0.380**	0.037	-0.034	-0.005
New kinetic parameters in expanded ASM2d								
Heterotrophic organisms (X_H):								
μ_{H1}	-0.025	0.016	-0.061	0.018	0.123	-0.118	**-0.520**	**1.072**
$\eta_{NO2,H}$	-0.001	0.000	**-0.332**	0.035	0.094	**-0.462**	0.000	0.001
$\eta_{NO3,H}$	-0.042	-0.030	**-0.371**	-0.158	**-1.064**	-0.002	0.012	-0.024
$\eta_{NO2,H1}$	-0.001	0.000	-0.053	0.009	0.000	-0.193	0.012	-0.015
$\eta_{NO3,H1}$	-0.001	0.000	-0.061	0.018	-0.111	0.008	0.011	-0.014
$K_{SA,H}$	0.001	0.001	0.014	0.020	0.080	0.025	0.012	-0.036
$K_{SA1,H}$	-0.001	0.000	0.008	-0.002	0.012	0.013	0.012	-0.010
$K_{NO2,H}$	0.001	0.000	0.000	0.001	-0.002	0.013	-0.011	0.014
$K_{NO3,H}$	0.020	0.015	0.008	0.040	**0.604**	0.012	0.010	-0.010
Phosphorus Accumulating Organisms (X_{PAO}):								
q_{PPSA}	-0.001	0.008	-0.016	-0.058	-0.140	-0.001	-0.008	**-0.263**
q_{PPSA1}	0.000	0.000	-0.006	-0.122	-0.019	0.008	0.013	**-0.851**
μ_{PAOSA}	0.009	-0.001	-0.018	-0.068	-0.188	-0.001	-0.074	**-0.286**
μ_{PAOSA1}	-0.013	0.008	-0.009	-0.135	-0.093	-0.093	**-1.156**	**-0.434**
$\eta_{NO2,PAO}$	0.001	0.001	0.000	-0.227	0.000	0.109	-0.011	0.014
$\eta_{NO3,PAO}$	0.005	0.009	-0.044	-0.133	**-0.481**	0.000	0.001	0.002
$\eta_{NO2,PAOSA}$	0.000	0.001	-0.090	-0.192	0.000	0.202	0.000	0.000
$\eta_{NO3,PAOSA}$	-0.005	0.021	-0.053	-0.134	**-0.463**	0.000	-0.003	-0.006
$\eta_{NO2,PAOSA1}$	0.000	0.001	-0.105	-0.198	0.000	0.113	0.000	0.000
$\eta_{NO3,PAOSA1}$	-0.022	0.045	-0.092	-0.149	-0.144	0.008	-0.031	0.025
$K_{SA,PAO}$	0.044	-0.035	0.027	-0.020	0.026	0.037	0.007	0.031
$K_{SA1,PAO}$	0.002	-0.001	0.001	0.004	0.005	0.000	0.033	0.136
$K_{NO2,PAO}$	-0.001	0.001	-0.124	0.191	0.000	0.000	0.002	-0.001
$K_{NO3,PAO}$	0.001	-0.015	0.007	0.024	**0.270**	0.000	0.000	0.002
$K_{O,PAO}$	-0.001	0.002	0.000	0.000	0.000	0.000	-0.022	0.373
K_{IPHA}	0.000	0.000	-0.012	0.000	-0,013	0.000	0.000	**0.610**
K_{IOPHA}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**-0.525**
K_{max1}	0.000	0.000	0.006	0.049	0.053	0.000	**-0,629**	**1.073**
Hydrolysis of particulate substrate (X_S):								
$\eta_{NO2,HYD}$	0.001	0.001	0.192	0.018	-0.003	-0.075	0.000	0.000
$\eta_{NO3,HYD}$	0.018	0.015	0.029	0.093	**-0.843**	0.008	0.012	-0.005
$K_{NO2,HYD}$	-0.001	-0.001	-0.005	-0.002	0.003	0.001	-0.012	0.016
$K_{NO3,HYD}$	-0.014	-0.009	-0.012	-0.011	0.204	0.000	0.002	-0.008
Denitrification enzymes								
$k_{S,ENZ}$	-	-	-0.032	-0.004	-0.082	-0.054	-	-
$k_{D,ENZ}$	-	-	0.000	0.000	0.002	0.000	-	-
$K_{NO2,ENZ}$	-	-	0.000	0.000	0.000	0.000	-	-
$K_{NO3,ENZ}$	-	-	0.000	0.000	0.000	0.000	-	-
$K_{O,ENZ}$	-	-	0.000	0.000	0.000	0.000	-	-
Original stoichiometric parameters in ASM2d								
Heterotrophic organisms (X_H):								
$\mathbf{Y_H}$	**-0.529**	**-0.411**	**0.728**	**-0.663**	**1.910**	**3.974**	0.076	**-0.993**
Phosphorus Accumulating Organisms (X_{PAO}):								
Y_{PO4}	0.008	**0.963**	-0.009	**0.820**	**-0.266**	0.008	-0.016	**1.895**
New stoichiometric parameters in expanded ASM2d								
Heterotrophic organisms (X_H):								
Y_{H1}	0.001	0.000	0.065	-0.051	**0.684**	**0.560**	-0.002	0.002
Phosphorus Accumulating Organisms (X_{PAO}):								
Y_{PAOSA}	0.024	-0.101	0.046	-0.073	**0.669**	-0.001	0.011	**-0.258**

Symbol	Anaerobic		Anoxic				Aerobic	
	COD	PO_4-P	COD	PO_4-P	NO_3-N	NO_2-N	COD	PO_4-P
Y_{PAOSA1}	0.002	-0.015	0.079	-0.060	**0.878**	-0.010	**0.730**	-0.198
Y_{SA1}	0.000	0.000	-0.008	0.008	-0.085	0.000	-0.023	0.000

Table S4. Calibration/validation procedure for the expanded ASM2d based on results of the batch tests, list of the adjusted parameters, and evaluation of simulation results against experimental data

Batch Test	Electron Acceptor	External Carbon	Target Variable	Standard Error		R^2	Adjusted parameters
				Relative	Absolute		
Model calibration							
Step 3.1							
2 phase	DO	acetate	OUR	0.054	0.130	0.838	Y_H
			COD	0.003	0.172	0.906	
			PO_4-P	0.108	0.132	0.967	K_{IPP}, K_{PHA}, $K_{IO,PHA}$, K_{IPHA}, K_{max1}
Step 3.2							
2 phase	DO	ethanol	OUR	0.153	0.163	0.925	Y_{H1}
			COD	0.147	0.202	0.934	$\mu_{H1,}$
			PO_4-P	0.199	0.243	0.987	q_{PPSA1}
Step 3.3							
2 phase	NO_3-N	no external carbon	NO_3-N	0.007	0.022	0.993	$\eta_{NO3,H}$
	NO_2-N		NO_2-N	0.015	0.032	0.965	$\eta_{NO2,H}$
Step 3.4							
2 phase	NO_3-N	ethanol	NO_3-N	0.035	0.088	0.997	$\eta_{NO3,H1}$
	NO_2-N		NO_2-N	0.012	0.049	0.967	$\eta_{NO2,H1}$
Model validation with other tests							
Step 5							
1 phase	DO	acetate	OUR	0.104	0.146	0.925	
			COD	0.056	0.086	0.972	
			PO_4-P	0.004	0.038	0.995	
1 phase	DO	ethanol	OUR	0.0029	0.116	0.936	
			COD	0.0156	0.036	0.984	
			PO_4-P	0.120	0.315	0.978	
2 phase	NO_3-N	no external carbon	COD	0.025	0.096	0.952	
			PO_4-P	0.142	0.155	0.972	
2 phase	NO_2-N	no external carbon	COD	0.026	0.113	0.958	
			PO_4-P	0.222	0.255	0.897	
1 phase	NO_3-N	acetate	COD	0.011	0.092	0.848	
			NO_2-N	0.047	0.085	0.888	
			NO_3-N	0.178	0.304	0.792	
			PO_4-P	0.228	0.245	0.810	

1 phase	NO_2-N	acetate	COD	0.142	0.142	0.965
			NO_3-N	0.087	0.090	0.988
			PO_4-P	0.089	0.013	0.942
2 phase	NO_3-N	ethanol	COD	0.036	0.091	0.966
			PO_4-P	0.007	0.082	0.952
2 phase	NO_2-N	ethanol	COD	0.0125	0.109	0.972
			PO_4-P	0.035	0.079	0.957
2 phase	NO_3-N	acetate	COD	0.071	0.144	0.895
			NO_3-N	0.279	0.279	0.883
			PO_4-P	0.061	0.092	0.922
2 phase	NO_2-N	acetate	COD	0.052	0.006	0.960
			NO_2-N	0.024	0.097	0.892
			PO_4-P	0.091	0.136	0.862
1 phase	NO_3-N	ethanol	COD	0.063	0.068	0.862
			NO_3-N	0.062	0.064	0.884
			PO_4-P	0.011	0.083	0.956
1 phase	NO_2-N	ethanol	COD	0.091	0.112	0.873
			NO_2-N	0.056	0.098	0.948
			PO_4-P	0.029	0.064	0.943

Table S5. Kinetic and stoichiometric parameters adjusted in the ASM2d and new parameters in the expanded ASM2d

Symbol	Definition	Unit	Defaulted value	Calibrated value		
				Swiniarski et al. (2012)	This study	95% confidence intervals
Kinetic parameters in the original ASM2d						
Heterotrophic organisms (X_H):						
μ_H	Maximum growth rate of X_H on S_A and S_F	1/d	6.00	3.00	3.00	
Phosphorus Accumulating Organisms (X_{PAO}):						
q_{PHA}	Rate constant for storage of X_{PHA}	1/d	3.00	6.00	6.00	
q_{PP}	Rate constant for storage of X_{PP}	1/d	1.50	4.50	4.50	
K_{IPP}	Inhibition coefficient for X_{PP} storage	g P/g COD	0.02	0.10	0.13	±0.114
K_{PHA}	Saturation coefficient for X_{PHA}	g COD/g COD	0.01	0.15	0.10	±0.029
Hydrolysis of particulate substrate (X_S):						
k_h	Hydrolysis rate constant	1/d	3.00	2.50	2.50	
η_{fe}	Anaerobic hydrolysis reduction factor	-	0.40	0.10	0.10	
K_X	Saturation coefficient for particulate COD	g COD/g COD	0.10	0.20	0.20	
New kinetic parameters in the expanded ASM2d						
Heterotrophic organisms (X_H):						
μ_{H1}	Maximum growth rate of X_H on $S_{A,1}$	1/d		0.40	0.26	±0.042

Symbol	Definition	Unit	Defaulted value	Calibrated value		
				Swiniarski et al. (2012)	This study	95% confidence intervals
$\eta_{NO2,H}$	Reduction factor for denitrification (S_{NO2})	-			0.32	±0.054
$\eta_{NO3,H}$	Reduction factor for denitrification (S_{NO3})	-	0.80	0.80	0.35	±0.138
$\eta_{NO2,H1}$	Reduction factor on $S_{A,1}$ for denitrification (S_{NO2})	-			0.06	±0.028
$\eta_{NO3,H1}$	Reduction factor on $S_{A,1}$ for denitrification (S_{NO3})	-		0.80	0.21	±0.029
$K_{SA1,H}$	Saturation coefficient for growth of X_H on $S_{A,1}$	g COD/m^3		4.00	4.00	
$K_{NO2,H}$	Saturation coefficient for growth of X_H (S_{NO2})	g N/m^3			0.50	
Phosphorus Accumulating Organisms (X_{PAO}):						
q_{PPSA}	Rate constant for storage of X_{PP} on S_A	1/d		1.00	1.00	
q_{PPSA1}	Rate constant for storage of X_{PP} on $S_{A,1}$	1/d		4.50	5.00	±1.115
μ_{PAOSA}	Maximum growth rate of X_{PAO} on S_A	1/d		1.00	1.00	
μ_{PAOSA1}	Maximum growth rate of X_{PAO} on $S_{A,1}$	1/d		1.00	1.00	
$\eta_{NO2,PAOSA}$	Reduction factor for anoxic activity on S_A (S_{NO2})	-			0.00	
$\eta_{NO3,PAOSA}$	Reduction factor for anoxic activity on S_A (S_{NO3})	-		0.60	0.60	
$\eta_{NO2,PAOSA1}$	Reduction factor for anoxic activity on $S_{A,1}$ (S_{NO2})	-			0.00	
$\eta_{NO3,PAOSA1}$	Reduction factor for anoxic activity on $S_{A,1}$ (S_{NO3})	-		0.60	0.60	
$K_{SA,PAO}$	Saturation coefficient for storage of X_{PP} and X_{PAO} growth on S_A	g COD/m^3		4.00	4.00	
$K_{SA1,PAO}$	Saturation coefficient for storage of X_{PP} and X_{PAO} growth on $S_{A,1}$	g COD/m^3		4.00	4.00	
$K_{NO2,PAO}$	Saturation coefficient for storage of X_{PP} and X_{PAO} growth (S_{NO2})	g N/m^3			3.00	
$K_{O,PAO}$	Saturation coefficient for oxygen for storage of X_{pp} and X_{PAO} growth	g O_2/m^3		0.20	0.20	
K_{IOPHA}	Inhibition coefficient for X_{PHA}	g O_2/m^3			3.60	±1.135
K_{MAX1}	Maximum ratio of X_{PHA}/X_{PAO}	g COD/g COD			0.34	±0.126
K_{IPHA}	X_{PHA} storage inhibition coefficient	g COD/g COD			0.02	±0.138
Hydrolysis of particulate substrate (X_S):						
$\eta_{NO2,HYD}$	Anoxic hydrolysis reduction factor (S_{NO2})	-			0.6	
$K_{NO2,HYD}$	Saturation coefficient for hydrolysis (S_{NO2})	g N/m^3			0.5	
Denitrification enzymes						
$k_{S,ENZ}$	Synthesis rate constant for denitrification enzymes	1/d			30.00	

Symbol	Definition	Unit	Defaulted value	Calibrated value		
				Swiniarski et al. (2012)	This study	95% confidence intervals
$k_{D,ENZ}$	Decay rate constant for denitrification enzymes	1/d			4.00	
$K_{NO2,ENZ}$	Nitrite half saturation coefficient for denitrification enzymes	g N/m^3			0.50	
$K_{NO3,ENZ}$	Nitrate half saturation coefficient for denitrification enzymes	g N/m^3			0.50	
$K_{O,ENZ}$	Oxygen half saturation coefficient for denitrification enzymes	g O_2/m^3			0.10	
Stoichiometric parameters in the original ASM2d						
Heterotrophic organisms (X_H):						
Y_H	Yield coefficient for S_A	g COD/g COD	0.63	0.67	0.82	±0.016
Phosphorus Accumulating Organisms (X_{PAO}):						
Y_{PO4}	X_{PP} requirement per X_{PHA} stored (S_{PO4} release)	g P/g COD	0.40	0.34	0.40	
New stoichiometric parameters in the expanded ASM2d						
Heterotrophic organisms (X_H):						
Y_{H1}	Yield coefficient of X_H for $S_{A,1}$	g COD/g COD		0.70	0.43	±0.049
Phosphorus Accumulating Organisms (X_{PAO}):						
Y_{PAOSA}	Yield coefficient of X_{PAO} for S_A	g COD/g COD		0.625	0.625	
Y_{PAOSA1}	Yield coefficient of X_{PAO} for $S_{A,1}$	g COD/g COD		0.625	0.625	
Y_{SA1}	$S_{A,1}$ requirement for storage of X_{PP}	g COD/g P		0.20	0.20	

Table S6. Approximate correlation matrix of parameter estimates using experimental data for step 3.1 of model calibration

	Parameter	Unit	Correlation matrix					
			Y_H	K_{MAX1}	K_{IPP}	K_{IPHA}	K_{PHA}	K_{IOPHA}
Step 3.1	Y_H	g COD/ g COD	1.00					
	K_{MAX1}	g P/ g COD	0.05	1.00				
	K_{IPP}	g P/g COD	-0.70	0.11	1.00			
	K_{IPHA}	g P/g COD	0.12	0.98	0.08	1.00		
	K_{PHA}	g COD/ g COD	-0.58	-0.10	0.13	-0.16	1.00	
	K_{IOPHA}	g O_2/m^3	0.34	-0.55	-0.55	-0.54	-0.07	1.00

Table S7. Approximate correlation matrix of parameter estimates using experimental data for steps 3.2-3.4 of model calibration

	Parameter	Unit	Correlation matrix						
			Y_{H1}	μ_{H1}	$q_{PP,SA1}$	$\eta_{NO3,H}$	$\eta_{NO2,H}$	$\eta_{NO3,H1}$	$\eta_{NO2,H1}$
Step 3.2	Y_{H1}	g COD/ g COD	1.00						
	μ_{H1}	1/d	0.96	1.00					
	$q_{PP,SA1}$	1/d	-0.02	0.01	1.00				
Step 3.3	$\eta_{NO3,H}$	-				1			
	$\eta_{NO2,H}$	-				-0.51	1		
Step 3.4	$\eta_{NO3,H1}$	-						1	
	$\eta_{NO2,H1}$	-						-0.62	1