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## Supporting information for:

# Integrated Shortcut Nitrogen and Biological Phosphorus Removal from Mainstream

# Wastewater: Process Operation and Modeling

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#### S1. Methods

#### S1.1 Process Modeling



Figure S1. Process representation in the Simba# 3.0 software.

Modeled specific growth rates for AOO, NOO, and PAOs were quantified throughout the SBR cycles with rate equations and parameter values from the Simba# inCTRL ASM matrix. Rate equations and parameters values (at 20°C) discussed in the text are as follows:

$$net specific growth rate of AOO (d^{-1}) = \mu_{AOO} \\ = \hat{\mu}_{AOO} \frac{S_{NHx}}{S_{NHx} + K_{NHx,AOO}} \frac{S_{O2}}{S_{O2} + K_{O2,AOO}} \frac{S_{PO4}}{S_{PO4} + K_{PO4,ANO}} \frac{S_{ALK}}{S_{ALK} + K_{ALK,AOO}} \\ - \hat{b}_{AOO,O2} \frac{S_{O2}}{S_{O2} + K_{O2,AOO}} - \hat{b}_{AOO,NOx} \frac{S_{NO3} + S_{NO2}}{S_{NO3} + S_{NO2} + K_{NOx,ANO}} \frac{K_{O2,AOO}}{S_{O2} + K_{O2,AOO}} \\ - \hat{b}_{AOO,ANA} \frac{K_{NOx,ANO}}{S_{NO3} + S_{NO2} + K_{NOx,ANO}} \frac{K_{O2,AOO}}{S_{O2} + K_{O2,AOO}}$$

Where:

$$\begin{split} \hat{\mu}_{AOO} &= maximum \ specific \ growth \ rate \ of \ AOO \ (d^{-1}) = 0.9 \\ S_{NHx} &= concentration \ of \ NH_4^+ + NH_3 \ \left(\frac{mgN}{L}\right) \\ K_{NHx,AOO} &= AOO \ half \ saturation \ coefficient \ for \ (NH_4^+ + NH_3) \ \left(\frac{mgN}{L}\right) = 0.7 \\ S_{O2} &= concentration \ of \ dissolved \ O_2 \ \left(\frac{mgO_2}{L}\right) \\ K_{O2,AOO} &= AOO \ half \ saturation \ coefficient \ for \ dissolved \ O_2 \ \left(\frac{mgO_2}{L}\right) = 0.25 \end{split}$$

$$\begin{split} S_{PO4} &= \text{concentration of } PO_4^{3-} \left(\frac{mgP}{L}\right) \\ K_{PO4,ANO} &= \text{nitrifier nutrient half saturation coefficient for } PO_4^{3-} \left(\frac{mgP}{L}\right) \\ &= 0.001 \\ S_{ALK} &= \text{concentration of alkalinity } \left(\frac{meq}{L}\right) \\ K_{ALK,AOO} &= AOO \text{ half saturation coefficient for alkalinity } \left(\frac{meq}{L}\right) = 0.5 \\ \hat{b}_{AOO,O2} &= \text{maximum specific aerobic decay rate of } AOO (d^{-1}) = 0.17 \\ \hat{b}_{AOO,NOx} &= \text{maximum specific anoxic decay rate of } AOO (d^{-1}) = 0.1 \\ S_{NO3} &= \text{concentration of } NO_3^{-} \left(\frac{mgN}{L}\right) \\ S_{NO2} &= \text{concentration of } NO_2^{-} \left(\frac{mgN}{L}\right) \\ K_{NOx,ANO} &= \text{nitrifier half saturation for anoxic conditions } \left(\frac{mgN}{L}\right) = 0.03 \\ \hat{b}_{AOO,ANA} &= \text{maximum specific anaerobic decay rate of } AOO (d^{-1}) = 0.05 \end{split}$$

$$\begin{array}{l} \textit{net specific growth rate of NOO} \ (d^{-1}) = \mu_{NOO} \\ = \ \hat{\mu}_{NOO} \frac{S_{NO2}}{S_{NO2} + K_{NO2,NOO}} \frac{S_{O2}}{S_{O2} + K_{O2,NOO}} \frac{S_{NHx}}{S_{NHx}} + \frac{S_{PO4}}{S_{PO4} + K_{PO4,ANO}} \frac{S_{ALK}}{S_{ALK} + K_{ALK,NOO}} \\ - \ \hat{b}_{NOO,O2} \frac{S_{O2}}{S_{O2} + K_{O2,NOO}} - \ \hat{b}_{AOO,NOx} \frac{S_{NO3} + S_{NO2}}{S_{NO3} + S_{NO2} + K_{NOx,ANO}} \frac{K_{O2,NOO}}{S_{O2} + K_{O2,NOO}} \\ - \ \hat{b}_{NOO,ANA} \frac{K_{NOx,ANO}}{S_{NO3} + S_{NO2} + K_{NOx,ANO}} \frac{K_{O2,NOO}}{S_{O2} + K_{O2,NOO}} \end{array}$$

Where (in addition to above):

 $\hat{\mu}_{NOO} = maximum \ specific \ growth \ rate \ of \ NOO \ (d^{-1}) = 0.7 \\ K_{NO2,NOO} = NOO \ half \ saturation \ coefficient \ for \ (NO_2^-) \left(\frac{mgN}{L}\right) = 0.1 \\ K_{O2,NOO} = NOO \ half \ saturation \ coefficient \ for \ dissolved \ O_2 \ \left(\frac{mgO_2}{L}\right) = 0.1 \\ K_{NHx,ANO} = Nitrifier \ nutrient \ half \ saturation \ coefficient \ for \ (NH_4^+ + NH_3) \ \left(\frac{mgN}{L}\right) = 0.001 \\ K_{ALK,NOO} = NOO \ half \ saturation \ coefficient \ for \ alkalinity \ \left(\frac{meq}{L}\right) = 0.5 \\ \hat{b}_{NOO,O2} = maximum \ specific \ aerobic \ decay \ rate \ of \ NOO \ (d^{-1}) = 0.15$ 

 $\hat{b}_{NOO,NOx} = maximum$  specific anoxic decay rate of NOO  $(d^{-1}) = 0.07$  $\hat{b}_{NOO,ANA} = maximum$  specific anaerobic decay rate of NOO  $(d^{-1}) = 0.04$ 

AOO and NOO washout SRT calculation

The modeled SRT to avoid washout for NOO was calculated by taking the inverse of average modeled  $\mu_{NOO}$  values (as shown above, calculated approximately every minute) over one cycle, i.e.:

washout 
$$SRT_{NOO} = \frac{1}{mean(\mu_{NOO})}$$

A similar calculation was done for AOO to affirm that modeled SRT was sufficiently high to retain AOO. The aerobic fraction of the resulting SRT for AOO and NOO was then calculated by assuming that 48% of the intermittently aerated react phase was aerobic – see Section 2.1 for details.

$$SRT_{AER} = SRT * \frac{0.48(t_{AER})}{t_{AN} + t_{AER}} = SRT * 0.399$$

Where:

 $SRT_{AER} = aerobic SRT$   $t_{AER} = length of modeled intermittently aerated react phase (minutes)$  = 222 (variable in the actual reactor)  $t_{AN} = length of anaerobic react phase (minutes)$ = 45 (same in the actual reactor)

## **PAO-Related Rate Equations**

specific growth rate of PAOs on PHA and 
$$O_2(d^{-1}) = \mu_{PAO,O2}$$
  
=  $\hat{\mu}_{PAO} \frac{\frac{X_{PHA}}{X_{PAO}}}{\frac{X_{PHA}}{X_{PAO}} + K_{PHA}} \frac{S_{O2}}{S_{O2} + K_{O2,OHO}} \frac{S_{NHx}}{S_{NHx} + K_{NHx,OHO}} \frac{S_{PO4}}{S_{PO4} + K_{PO4,PAO}} \frac{S_{ALK}}{S_{ALK} + K_{ALK}}$ 

Where (in addition to above):

$$\begin{split} \hat{\mu}_{PAO} &= maximum \ specific \ growth \ rate \ of \ PAOs \ (d^{-1}) = 0.95 \\ X_{PHA} &= concentration \ of \ polyhydroxyalkanoates - PHAs \ \left(\frac{mgCOD}{L}\right) \\ X_{PAO} &= concentration \ of \ PAOs \ \left(\frac{mgCOD}{L}\right) \\ K_{PHA} &= half \ saturation \ coefficient \ for \ PHA \ \left(\frac{mgCOD}{L}\right) = 0.1 \\ K_{O2,OHO} &= \ OHO \ and \ PAO \ half \ saturation \ coefficient \ for \\ dissolved \ O_2 \ \left(\frac{mgO_2}{L}\right) = 0.05 \\ K_{NHx,OHO} &= \ OHO \ and \ PAO \ nutrient \ half \ saturation \ coefficient \ for \ (NH_4^- + NH_3) \ \left(\frac{mgN}{L}\right) = 0.001 \\ K_{PO4,PAO} &= \ PAO \ half \ saturation \ coefficient \ for \ PO_4^{3-} \ \left(\frac{mgP}{L}\right) = 0.15 \end{split}$$

 $K_{PO4,PAO} = PAO \text{ half saturation coefficient for } PO_4^o \left(\frac{L}{L}\right) = 0.15$  $K_{ALK} = PAO \text{ half saturation coefficient for alkalinity } \left(\frac{meq}{L}\right) = 0.1$  specific growth rate of PAOs on PHA and  $NO_2^-(d^{-1}) = \mu_{PAO,NO2}$ 

$$= \hat{\mu}_{PAO} \eta_{anox,PAO} \frac{\frac{X_{PHA}}{X_{PAO}}}{\frac{X_{PHA}}{X_{PAO}} + K_{PHA}} \frac{S_{NO2}}{S_{NO2} + K_{NO2,OHO}} \frac{K_{O2,OHO}}{S_{O2} + K_{O2,OHO}} \frac{S_{NHx}}{S_{NHx} + K_{NHx,OHO}}$$

$$\frac{S_{PO4}}{S_{PO4} + K_{PO4,PAO}} \frac{S_{ALK}}{S_{ALK} + K_{ALK}}$$

Where (in addition to above):

 $\eta_{anox,PAO} = PAO$  anoxic growth factor = 0.33  $K_{NO2,OHO} = OHO$  and PAO half saturation coefficient for  $NO_2^-\left(\frac{mgN}{r}\right) = 0.05$ 

specific growth rate of PAOs on PHA and  $NO_3^-(d^{-1}) = \mu_{PAO,NO3}$ 

 $= \hat{\mu}_{PAO} \eta_{anox,PAO} \frac{\frac{X_{PHA}}{X_{PAO}}}{\frac{X_{PHA}}{X_{PAO}}} \frac{S_{NO3}}{S_{NO3} + K_{NO3,OHO}} \frac{K_{NO2,OHO}}{S_{NO2} + K_{NO2,OHO}} \frac{K_{O2,OHO}}{S_{O2} + K_{O2,OHO}}$ 

 $\frac{S_{NHx}}{S_{NHx} + K_{NHx.OHO}} \frac{S_{PO4}}{S_{PO4} + K_{PO4.PAO}} \frac{S_{ALK}}{S_{ALV} + K_{ALV}}$ 

Where (in addition to above):

$$K_{NO3,OHO} = OHO$$
 and PAO half saturation coefficient for  $NO_3^-\left(\frac{mgN}{L}\right) = 0.1$ 

#### **Additional Equations Governing PHA in the Model**

While the three equations above govern PAO growth on PHA associated with P uptake, there are three additional equations that govern PAO growth in PO<sub>4</sub><sup>3-</sup>-limited conditions. These three equations are identical to those listed above (i.e. one each for aerobic, NO2<sup>-</sup> and NO3<sup>-</sup>) aside from the following changes:

Substitute  $\hat{\mu}_{PAO}$  with  $\hat{\mu}_{PAO,LIM}$  = maximum specific growth rate of PAOs,  $P - limited (d^{-1}) = 0.42$ 

Substitute *K*<sub>PHA</sub> with *K*<sub>PHA.lim</sub>

= half saturation coefficient for PHA, P - limited  $\left(\frac{mgCOD}{I}\right) = 0.05$ 

And add the following Monod term:  $\frac{X_{PP,LO}}{X_{PP,LO}+K_{PP,lim}}$ 

Where:  $X_{PP,LO} = releasable stored polyphosphate \left(\frac{mg^P}{I}\right)$ 

$$K_{PP,lim} = polyphosphate \ limitation \ half - saturation \ \left(rac{mgP}{L}
ight) = 0.001$$

PHA production is modeled via the following equation:

#### Specific PHA storage rate from VFAs by PAOs $(d^{-1})$ : $X_{PPIO}$

$$= q_{PAO,PHA} \frac{\frac{1}{X_{PAO}}}{\frac{X_{PP,LO}}{X_{PAO}} + K_{PP,LO}} \frac{S_{VFA}}{S_{VFA} + K_{STORE,VFA}} \frac{S_{ALK}}{S_{ALK} + K_{ALK}}$$

Where (in addition to above):

$$q_{PAO,PHA} = PHA$$
 storage rate by PAOs  $(d^{-1}) = 6$   
 $K_{PP,LO} = polyphosphate half - saturation for storage  $\left(\frac{mgP}{mgCOD}\right) = 0.01$   
 $S_{VFA} = concentration of volatile fatty acids  $\left(\frac{mgCOD}{L}\right)$   
 $K_{STORE,VFA} = VFA half - saturation for storage  $\left(\frac{mgCOD}{L}\right) = 1$$$$ 

Aside from the equations above, modeling of PHA in the inCTRL ASM matrix is affected only by the aerobic, anoxic and anaerobic PAO decay rate equations, which causes PHA release proportional to the PHA content of the PAO biomass.

#### S1.2. Solids Retention Time (SRT) Control

SRT was controlled via timed mixed liquor wasting after the aerated react period and before settling. A maximum wasting pump time was set on the PLC, and the actual pumping time for each cycle varied depending on the length of the aerated react phase. For example, if the maximum wasting pump time was set to 1 minute, the maximum aeration time was set to 300 minutes, and the actual aeration time for a given cycle was 150 minutes, the actual pumping time would be 1 minute  $\times \frac{150 \text{ minutes}}{300 \text{ minutes}} = 0.5 \text{ minutes}$ . Because the aeration time varied on a cycle-by-cycle basis according to the influent strength and the target effluent NH<sub>4</sub><sup>+</sup> level, the dynamic SRT value was calculated for each individual cycle, as adapted from (Laureni et al., 2019) and Takács et al., (2008). SRT for each cycle was calculated according to the equation below (Laureni et al., 2019).

$$SRT_{t+\Delta t} = SRT_t \left(1 - \frac{X_E V_E + X_R V_W}{X_R V_R}\right) + \Delta t$$

Where:

 $\begin{array}{ll} SRT_{t+\Delta t} &= \text{Solids retention time of cycle under analysis (days)} \\ SRT_t &= \text{Solids retention time of previous cycle (days)} \\ V_R &= \text{Volume of reactor (L)} \end{array}$ 



- = Effluent volume for the cycle under analysis (L)
- $X_R$  = Reactor MLVSS concentration for the cycle under analysis (mg/L)
- $V_W$  = Mixed liquor wasting volume for the cycle under analysis (L)
- $\Delta t$  = React time of the cycle under analysis, not including settling and decant (days)



**Figure S2**. Total and aerobic dynamic SRT over time in the SBR. The average total and aerobic SRT during Phase 1 was  $11 \pm 7$  and  $4.5 \pm 3.0$  days, and the average total and aerobic SRT during Phase 2 was  $9.2 \pm 1.8$  and  $3.6 \pm 0.9$  days, respectively. \*Mixed liquor wasting was suspended from days 158 - 195 to recover AOO activity.

### S1.3. 16S rRNA Gene Amplicon Sequencing

16S rRNA gene amplicon library preparations were performed using a two-step PCR protocol using the Fluidigm Biomark: Multiplex PCR Strategy as previously described (Griffin and Wells, 2017). In the first round of PCR, each 20 uL reaction contained 10  $\mu$ L of FailSafe PCR 2X PreMix F (Epicentre, Madison, WI), 0.63 units of Expand High Fidelity PCR Taq Enzyme (Sigma-Aldrich, St. Louis, MO), 0.4  $\mu$ M of forward primer and reverse primer modified with Fluidigm common sequences at the 5' end of each primer, 1  $\mu$ L of gDNA (approximately 100 ng) and the remaining volume molecular biology grade water. The V4-V5 region of the 16S rRNA gene was amplified in duplicate from 10 samples collected over the course of reactor operation using the 515F-Y (5'-GTGYCAGCMGCCGCGGTAA-3') and 926R (5'-CCGYCAATTYMTTTRAGTTT-3') (Parada et al., 2016) primer set. Thermocycling conditions for the 515F-Y/926R primer set were 95°C for 5 minutes, then 28 cycles of 95°C for 30 seconds, 50°C for 45 seconds, and 68°C for 30 seconds, followed by a final extension of 68°C for 5 minutes. Specificity of amplification was checked for all samples via agarose gel electrophoresis.

Samples were then barcoded by sample via a second stage PCR amplification using Access Array Barcodes (Fluidigm, South San Francisco, CA) (Griffin and Wells, 2017). Each 20 uL PCR reaction consisted of 10  $\mu$ L of FailSafe PCR 2X PreMix F, 0.63 units of Expand High Fidelity PCR Taq Enzyme, 2  $\mu$ L of template from the first round of PCR, 4  $\mu$ L of sample-specific barcode primers and the remaining volume molecular biology grade water. The conditions for the second round of PCR were 95°C for 5 minutes, then 8 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 68°C for 30 seconds. Agarose gel electrophoresis was run again after the second round of PCR to verify correct amplification. Sequencing was performed on an Illumina Miseq sequencer (Illumina, San Diego, CA) using Illumina V2 (2x250 paired end) chemistry.

For amplicon sequence analysis, sequence quality control was performed through DADA2 (Callahan et al., 2016) integrated in QIIME2 version qiime2-2018.8 (Bolyen et al., 2018), which included quality-score-based sequence truncation, primer trimming, merging of paired-end reads, and removal of chimeras. Taxonomy was assigned to each individual sequence variation using the Silva database, release 132.

#### **S1.4 qPCR supermix and reaction conditions**

Bio-Rad iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) containing 50 U/ml iTaq DNA polymerase, 0.4 mM dNTPs, 100 mM KCl, 40 mM Tris-HCl, 6 mM MgCl2, 20 mM fluorescein, and stabilizers was used for two qPCR assays. Target genes included ammonia oxidizing bacterial *amoA* via the *amoA*-1F and *amoA*-2R primer set (Rotthauwe et al., 1997) and total bacterial (universal) 16S rRNA genes via the Eub519/Univ907 primer set (Burgmann et al., 2011). The final volume of the reaction mix for each PCR and qPCR reaction was 20  $\mu$ l, in which the DNA template was ~1 ng, and the primer concentrations were 0.2  $\mu$ M. All assays were performed in triplicate. For each assay, triplicate standard series were generated by tenfold serial dilutions (10<sup>2</sup>-10<sup>8</sup> gene copies/ $\mu$ l).

### S2. Process Modeling Reproduces Key Elements of Process Performance

Agreement between the process model and our experimental results suggest that the trends in N and P removal from mainstream wastewater that we observed are likely generally applicable to other locations. By closely modeling the influent (primary effluent), reactor control, aeration control and SRT (model SRT 9.5 days, reactor SRT 9.2  $\pm$  1.8 days) from Phase 2, the resulting model performance closely matched that of the reactor (Figure 5): modeled HRT was 7.2 hours (reactor HRT 6.8  $\pm$  2.8 hours), modeled VSS was 1,245 mg/L (reactor VSS 1,344  $\pm$  226 mg/L) and Figure 2, Figure 4, and Table 3 demonstrate that both in-cycle nutrient dynamics and effluent concentrations were well-matched between the model and reactor performance. Importantly, this was done via a commercially available wastewater process modeling software without modification to the inCTRL ASM matrix.

### **S3.** Supporting Table and Figures

**Table S1.** Sampling frequency and target analytes (per APHA, 2005) for daily composite samples. All samples listed are of reactor influent and effluent except NO<sub>2</sub><sup>-</sup>-N (effluent only) and mixed liquor TSS and VSS (sampled in-reactor).

	Samples
Analyte	per week

Total COD	3
Filtered COD (1.2 µm filter)	3
Alkalinity	3
Total Kjeldahl Nitrogen	3
NH4 <sup>+</sup> -N	3
NO <sub>X</sub> <sup>-</sup> -N <sup>1</sup>	3
NO <sub>2</sub> <sup>-</sup> -N (effluent only)	3
Total Phosphorus	3
Ortho-Phosphate	2
TSS <sup>2</sup> & VSS <sup>3</sup>	1
Mixed Liquor TSS <sup>2</sup> & VSS <sup>3</sup>	2
$^{1}NO_{X} - N = NO_{2} - N + NO_{3} - N$	
$^{2}TSS = total suspended solids$	
$^{3}VSS = volatile suspended solids$	

Table S2. Influent (primary effluent) C	COD fractionation and COD-to-nutrient ratios.
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	Pr Ef	imaı flue	As percent of total COD	
Total COD $(mgCOD/L)^a$	164.4	±	46.2	
Particulate COD (mgCOD/L)	61.7	±	23.8	37%
Colloidal COD (mgCOD/L)	28.6	±	18.1	17%
Soluble COD not including VFA (mgCOD/L)	56.4	±	19.4	34%
VFA (mgCOD/L)	18.8	±	8.9	11%
$\text{COD:TP}^{b}(\text{gCOD/gP})$	6	57:1		
COD:TKN <sup>b</sup> (gCOD/gN)	8	.3:1		

<sup>*a*</sup>Primary effluent COD fractionation was performed weekly from days 114 - 515 (n = 50). <sup>*b*</sup>COD:Nutrient ratios are taken from average of all samples from days 27 - 519 (n = 192).

Day of cycle tested	N2O emitted/ influent TKN	N2O emitted/ TIN removed	influent TKN (mgN/L)	influent COD (mg/L)	COD/ TKN	Effluent NO2 <sup>-</sup> (mgN/L)	Average temp (°C)
414	3.8%	11.4%	23	206	9	2.9	20.5
426	6.2%	12.0%	20	204	10	2.7	20.3
428	1.0%	2.3%	12	140	12	1.2	20.5
475	1.0%	2.6%	13	64	5	2.0	20.4
489	2.2%	4.3%	19	183	10	2.4	20.3
503	0.2%	0.2%	14	160	11	0.4	19.4
517	0.8%	1.6%	21	147	7	1.9	19.4
531	1.56%	7.36%	13	144	11	2.1	19.4

3.0 Phase 1 Phase 2 NH<sub>4</sub><sup>+</sup> & TIN removal rate (mgN/gVSS/hr) 2.5 ●NH₄<sup>+</sup> removal rate 2.0 1.5 TIN removal rate 1.0 0.5 0.0 200 300 . 400 500 . 600 100 0 25 PO<sub>4</sub><sup>3-</sup> release & uptake rate (mgP/gVSS/hr) Anaerobic P 20 release Ī 15 Aerobic P uptake 10 5 0 200 300 400 Day of Operation 600 0 100 500

**Figure S3.** In-cycle N and P removal rates from least-squares regression of the linear portions of in-cycle grab samples for  $NH_4^+$ ,  $NO_2^-$ ,  $NO_3^-$ , and  $PO_4^{3-}$ . Error bars represent standard errors of the slopes.

Table S3. N <sub>2</sub> O emissions test	results for 8 cycl	es during Phase 2.
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**Figure S4.** Relative *Accumulibacter*, *Tetrasphaera*, and *Competibacter* abundance through the first 421 days of reactor operation according to 16S rRNA gene sequencing. Day "0" represents the inoculum, which was sampled before reactor operation began.



**Figure S5.** Ammonia oxidizing bacterial *amoA* gene abundance normalized to total bacterial 16S rRNA genes through the first 421 days of reactor operation according to qPCR.



Figure S6. Reactor influent and effluent alkalinity concentrations from composite sampling



Figure S7. Reactor influent and effluent total phosphorus concentrations from composite sampling.



Figure S8. Reactor mixed liquor TSS and VSS concentrations.



**Figure S9.** Reactor influent and effluent filtered COD from composite sampling. Samples were filtered through a  $1.2 \,\mu$ m pore size membrane.



Figure S10. Reactor influent and effluent total COD concentrations from composite sampling.



Figure S11. Reactor influent and effluent TKN concentrations from composite sampling.

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#### Fractions Name Description Unit S<sub>VFA</sub> VFAs g COD/m^3 $S_{\rm F}$ Fermentable substrate g COD/m<sup>3</sup> S<sub>MEOL</sub> Methanol g COD/m<sup>3</sup> C<sub>B</sub>Colloidal biodegradable substrate g COD/m<sup>3</sup> X<sub>B</sub> Slowly biodegradable substrate a COD/m^3 $S_{\rm U}$ Soluble unbiodegradable organics g COD/m^3 C<sub>U</sub>Colloidal unbiodegradable organics g COD/m<sup>3</sup> $X_{\rm U}$ Particulate unbiodegradable organics g COD/m<sup>3</sup> X<sub>PHA</sub> Stored PHA g COD/m^3 $X_{\rm E}$ Endogenous decay products g COD/m^3 X<sub>OHO</sub> Ordinary heterotrophs g COD/m<sup>3</sup> $X_{\rm MEOLO}$ Anoxic methanol utilizers q COD/m<sup>3</sup> X<sub>PAO</sub> Phosphorus accumulating organisms g COD/m^3 $X_{AOO}$ Aerobic ammonia oxidizers g COD/m<sup>3</sup> $X_{\rm NOO}$ Nitrite oxidizing organisms a COD/m^3 $S_{\rm NHx}$ Total ammonia q N/m^3 $S_{\rm NO2}$ Nitrite g N/m^3 S<sub>NO3</sub> Nitrate g N/m^3 S<sub>N2</sub> Dissolved Nitrogen a N/m^3 $S_{\rm N,F}$ Soluble biodegradable organic N (from S\_F) g N/m^3 $X_{\rm N,B}$ Particulate biodegradable organic N g N/m^3 S<sub>N,U</sub>Soluble unbiodegradable organic N a N/m^3 $X_{\rm N,U}$ Particulate unbiodegradable organic N g N/m^3 S<sub>PO4</sub> Orthophosphate g P/m^3 *X*<sub>PP,LO</sub> Releasable stored polyphosphate g P/m^3 *X*<sub>PP,HI</sub>Non-releasable stored polyphosphate g P/m^3 $S_{P,F}$ Soluble biodegradable organic P (from S F) g P/m^3 $X_{P,B}$ Particulate biodegradable organic P g P/m^3 $S_{P,U}$ Soluble unbiodegradable organic P content g P/m^3 X<sub>P,U</sub> Particulate unbiodegradable organic P content g P/m<sup>3</sup> $S_{O2}$ Dissolved oxygen a O2/m^3 $X_{\rm IG}$ Inorganic suspended solids g TSS/m^3 $X_{\rm MeOH}$ Metal hydroxide compounds g Me/m^3 X<sub>MeP</sub> Metal phosphate compounds g Me/m^3 S<sub>ALK</sub> Alkalinity eq/m^3

From Inflaent Fractionation	Norre	Default	Description	UNI
	NO NO	1.8	Xb XCOD/VS9 ratio Xu XCOD/VS9 ratio	g COD/g VSS g COD/g VSS
	Aca form	8.85 8.45	N content of biodegradable colloids N content of unbiodegradable velicide	g Ng COD g Ng COD
	40.0	0.01	P content of Unbiologitable colloids	g Pig COD
Heterotrophic kinetics	4000	0.005	P content of unbiodegradable colloids	g Pig COD
	FITEMORD	3.2	Maximum specific growth rate of OHOs Fernentation rate coefficient (anserobic OHO growth)	1/3
	K <sub>N.K.FIBM</sub>	1	Alkalinity half saturation coefficient for fermentation	eqim"3
	K <sub>SF</sub>	3	Substrate half seturation for OHOs	g COD/W/3
	K <sub>BUANA</sub>	5	Substrate half saturation during fermentation Methoded half saturation for DHDs (accordin)	g COD/H/3
	K <sub>01.0ED</sub>	0.05	Q2 half saturation for QHOs	g 02/m*3
	ABRUDED ASJMDOL	1	Answe growth reduction for CHOs Growth reduction for CHOs on 8_MEOL	
	KNOLORD KNULORD	0.1	NO3 half saturation for OHOs NO2 half saturation for OHOs	g NW/3 g NW/3
	KNOA,ORD	8.85	NOx half saturation for OHOs	g N/m*3
	KPOLOBO	0.062	PO4 half saturation for OHOs	g Pin*3
	Annance	0.4	Aerobic decay rate coefficient for OHOs Anonic decay rate coefficient for OHOs	1/0
Methylatioph kinetics	Auto, and	0.2	Anaerobic decay rate coefficient for OHOs	1/8
	PMERED KNOCK	13	Methylotroph maximum specific growth rate Methylotroph half suburbline coefficient	1/8 0.0003/13
	KAIK MILLO	0.1	Alkalinity half saturation coefficient for Methylotrophs	eqim"3
	hectosos	4.45	Aerobic decay rate coefficient for methylotrophs Anceic decay rate coefficient for methylotrophs	1/4
Ammonia oxidizer kinetics	PRECEDENT	4.42	Anaerabic decay rate coefficient for methylotrophs	1/d
	Kapp App	0.9 0.7	Maximum specific growth rate of ADOs Anamonia half saturation for ADOs	1/d g N/m*3
	F02,400	8.25	Oxygen helf saturation for AOOs fearblin denses rate coefficient for AOOs	g O2/m*3
	PARSARK	0.1	Anonic decay rate coefficient for ADOs	1/8
	KALK, ADD	0.5	Anaerobic decay rate coefficient for ADOs Alkalinity half saturation for ADOs	nd eqim*3
Nitrite axidizer kinetios	Anno	0.T	Maximum specific growth rate of NOOs	1/8
	K <sub>01,000</sub>	0.15	Oxygen half saturation for NOOs NO2 half saturation for NOOs	g 02/m*5 g N/m*3
	A500000	8.15	Aerobic decay rate coefficient for NOOs	1/d
	7400,50x /900,454	8.87 8.94	Anaerobic decay rate coefficient for NOOs Anaerobic decay rate coefficient for NOOs	1/8
Närifer känelica	KALK-NOO	0.3	Alkalinity half saturation for NOOs	egin/3
	Knozano Knozano	8.81 0.1	NO2 half saturation for answir conditions NO3 half saturation for answir conditions	g N/m^3 g N/m^3
	K <sub>NDLAND</sub>	8.83	Half saturation for anoxic conditions	g Nwr3
	K <sub>POLAND</sub>	0.000	Nutrient POH half auturation for nitrifiers	g Nim*3 g Pim*3
Phosphorus accumulating (	unetics Frad	0.95	Maximum specific growth rate of PAOs	14
	PROIN bytoav	8.42 8.83	Maximum specific growth rate of PAOs, P limited Anserobic maintenance PP desvece	1/d 1/d
	Keen	0.1	FHA half saturation coefficient	g 000/m/3
	+950,5m Next240	#:45 8.33	PAO anoxio growth factor	,
	STADJEA K <sub>ETORENTA</sub>	6	PHA storage rate VFA half substation for storage	1/d g 000/m*3
	K <sub>PELD</sub>	8.81	PP-low half saturation for storage POA half saturation for PKO*	g Pitn#3 g Pitn#3
	- Xoran Ketan	0.001	PP Imitation as nutrient	g Pinns
	Макадов Макадов	0.2	Access secury rate coefficient for PROs Anonic decay rate coefficient for PROs	14
Conversion kinetics	hino,ana	8.82	Anaerobic decay rate coefficient for PAOs	1/8
	Tali Kabi	0.15	Maximum specific adsorption rate of colloidal matter Threshold ratio of adsorbed colloidal matter to OHOs	refsig COD* g COD/g COD
	683D	2	Hydrolysis rate coefficient Martinistic half ant ratios coefficient	1/8
	AUD'NOV	4.28	Ancelic hydrolysis factor	
	TAMMON	8.14	Anaerstiis tydrolysis factor Ammonification rate coefficient	1/8
	Stokener Stadi	0.5	Phosphate release rate coefficient Endogenous residue conversion rate coefficient	1/4
	\$ASSD4	1	Assimilative NHx production rate coefficient	1/4
	K <sub>INDLASSIM</sub>	0.001	Assimilative NO2 helf saturation	g NWP3
	KOROLANDA KOROLANDA	0.000	Assimilative NO3 half saturation Assimilative CHO half saturation	g Nan's g COD/m*s
	WHEEC VERSION	1 0.6	Precipitation rate coefficient Precipitate dissolution rate coefficient	1/d 1/d
Antenios coefficients	KALK	0.1	Alkalinity half saturation	egin/3
	8 <sub>80,080</sub>	1.072	Arthenius coefficient	
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Variables		
Pre-defined variables	riable Description	Unit
<u>V</u>	Volume	m^3
External variables	oxygen input	ka/d
T	Temperature	degC
Instrumental variables		
$EEQ_{N2} = 3 \frac{MO}{2 M_N}$	Electron equivalence of N2	g COD/g N
$EEQ_{NO2} = 3 \frac{M_O}{M_N}$	Electron equivalence of NO2	g COD/g N
$EEQ_{\rm NO3} = 4 \frac{M_{\rm O}}{M_{\rm N}}$	Electron equivalence of NO3	g COD/g N
$EEQ_{\rm NO2,NO3} = \frac{M_{\rm O}}{M_{\rm N}}$	Electron equivalence of NO2 to NO3	g COD/g N
$EEQ_{N2,NO2} = 3 \frac{M_O}{2 M_N}$	Electron equivalence of N2 to NO2	g COD/g N
$CH_{\rm VFA} = \frac{-1}{4 M_{\rm O}}$	Charge of VFA	e-/g COD
$CH_{\rm NO2} = \frac{-1}{M_{\rm N}}$	Charge of NO2	e-/g N
$CH_{\rm NO3} = \frac{-1}{M_{\rm N}}$	Charge of NO3	e-/g N
$CH_{\rm NHx} = -\frac{1}{M_{\rm N}}$	Charge of NHx	e-/g N
$CH_{\rm PO4} = \frac{-1.5}{M_{\rm P}}$	Charge of PO4	e-/g P
$X_{\rm BIO} = X_{\rm OHO} + X_{\rm PAO} + X_{\rm MEOLO} + X_{\rm AOO} + X_{\rm NOO}$	COD of all biomasses	g COD/m^3
$X_{\text{HETO}} = X_{\text{OHO}} + X_{\text{PAO}} + X_{\text{MEOLO}}$	COD of all heterotrophic biomasses	g COD/m^3
$X_{\rm COD} = X_{\rm BIO} + X_{\rm B} + X_{\rm U} + X_{\rm PHA} + X_{\rm E}$	Particulate COD	g COD/m^3
$X_{\text{VSS,BIO}} = \frac{X_{\text{OHO}} + X_{\text{PAO}} + X_{\text{MEOLO}} + X_{\text{AOO}} + X_{\text{NOO}}}{i_{\text{CV,BIO}}}$	VSS of all biomasses	g VSS/m^3
$X_{\text{VSS}} = X_{\text{VSS,BIO}} + \frac{X_{\text{B}}}{2} + \frac{X_{\text{U}}}{2} + \frac{X_{\text{PHA}}}{2} + \frac{X_{\text{E}}}{2} + \frac{X_{\text{E}}}{2} + \frac{f_{\text{H2O,Me}}}{2} + f_{\text{H2O,Me$	VSS	g VSS/m^3
$^{I}CV,B$ $^{I}CV,U$ $^{I}CV,PHA$ $^{I}CV,E$	) 700	
$\Delta_{\text{TSS}} = \Delta_{\text{VSS}} + \Delta_{\text{IG}} + J_{\text{MeOOH,Me}} + \Delta_{\text{MeOH}} + J_{\text{MeP,Me}} + \Delta_{\text{MeP}} + \eta_{\text{ISS,PP}} + (\Delta_{\text{PP,LO}} + \Delta_{\text{IF}})$	P,HI ) 188	g 188/m/3
$\mu_{\text{OHO},1}  \mu_{\text{OHO},1}  \mu_{\text{OHO},1}  (\sigma_{\text{mu},\text{OHO}}, 1, 1, 1, 2, 3, 2, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3,$		1/d
$h_{\text{FERM},\text{OHO},1} = h_{\text{FERM},\text{OHO},2} + T_{\text{FERM},\text{OHO},1} + T_{\text{FERM},1} + T_{FERM$		1/d
bolio Nor $T = bolio Nor Arth (\theta_b olio Nor T, Thase)$		1/d
$b_{OHO, ANA, T} = b_{OHO, ANA} Arth (\theta_{b, OHO, ANA, T}, Tbase)$		1/d
$\mu_{\text{MEOLOT}} = \mu_{\text{MEOLO}} Arrh (\theta_{\text{mm}} \text{ MEOLO}, T, Tbase)$		1/d
$b_{\text{MEOLO,O2,T}} = b_{\text{MEOLO,O2}} Arrh (\theta_{\text{MEOLO,O2}}, T, Tbase)$		1/d
$b_{\text{MEOLO,NOX,T}} = b_{\text{MEOLO,NOX}} Arrh (\theta_{b,\text{MEOLO,NOX}}, T, Tbase)$		1/d
$b_{\text{MEOLO,ANA,T}} = b_{\text{MEOLO,ANA}} Arrh \left( \theta_{b,\text{MEOLO,ANA}}, T, T base \right)$		1/d
$\mu_{\text{PAO},\text{T}} = \mu_{\text{PAO}} Arrh \left( \theta_{\text{mu,PAO}}, T, T base \right)$		1/d
$\mu_{\text{PAO,LIM,T}} = \mu_{\text{PAO,LIM}} \operatorname{Arrh} \left( \theta_{\text{mu,PAO,LIM}}, T, T \right)$		1/d
$q_{\text{PAO,PHA,T}} = q_{\text{PAO,PHA}} Arrh \left( \theta_{q,\text{PAO,PHA}}, T, T base \right)$		1/d
$b_{\text{PAO},O2,T} = b_{\text{PAO},O2} Arrh (\theta_{b,\text{PAO},O2}, T, Tbase)$		1/d
$b_{\text{PAO,NOx,T}} = b_{\text{PAO,NOx}} Arrh \left( \theta_{b,\text{PAO,NOx}}, T, T base \right)$		1/d
$b_{\text{PAO,ANA,T}} = b_{\text{PAO,ANA}} \operatorname{Arrh} \left( \theta_{b,\text{PAO,ANA}}, T, T base \right)$		1/d
$b_{\text{PPLO,ANA,T}} = b_{\text{PPLO,ANA}} Arrh (\theta_{b,\text{PPLO,ANA}}, T, Tbase)$		1/d
$\mu_{AOO,T} = \mu_{AOO} Arrh \left( \theta_{mu,AOO}, T, Tbase \right)$		1/d
$b_{AOO,O2,T} = b_{AOO,O2} Arrh \left( \theta_{b,AOO,O2}, T, Tbase \right)$		1/d
$b_{AOO,NOx,T} = b_{AOO,NOx} Arrh (\theta_{b,AOO,NOx}, T, Tbase)$		1/d
$b_{AOO,ANA,T} = b_{AOO,ANA} Arrh (\theta_{b,AOO,ANA}, T, Tbase)$		1/d
$\mu_{\text{NOO,T}} = \mu_{\text{NOO}} Arrh \left( \theta_{\text{mu,NOO}}, T, T \right)$		1/d
$b_{\text{NOO,O2,T}} = b_{\text{NOO,O2}} Arrh \left( \theta_{b,\text{NOO,O2}}, T, T base \right)$		1/d
$b_{\text{NOO,NOx,T}} = b_{\text{NOO,NOx}} \operatorname{Arrh} \left( \theta_{b,\text{NOO,NOx}}, T, T base \right)$		1/d
$b_{\text{NOO,ANA,T}} = b_{\text{NOO,ANA}} \operatorname{Arrh} \left( \theta_{b,\text{NOO,ANA}}, T, T \right)$		1/d
$q_{ads,T} = q_{ads} Arrh \left( \theta_{q,ads}, T, T base \right)$	Maximum specific adsorption rate at liquid ter	mperature 1/d
$q_{\rm HYD,T} = q_{\rm HYD} Arrh \left( \theta_{q,\rm HYD}, T, T base \right)$		1/d
$q_{\text{AMMON,T}} = q_{\text{AMMON}} Arrh \left( \theta_{q,\text{AMMON}}, T, T \right)$		1/d
$q_{\text{PO4conv,T}} = q_{\text{PO4conv}} Arrh \left( \theta_{q,\text{PO4conv}}, T, T base \right)$		1/d
$q_{\text{EtoB,T}} = q_{\text{EtoB}} Arrh \left( \theta_{q,\text{EtoB}}, T, T base \right)$		1/d
$q_{\text{ASSIM},T} = q_{\text{ASSIM}} Arrh \left( \theta_{q,\text{ASSIM}}, T, T base \right)$		1/d