Supplementary Material

Pilot Plant Demonstration of Stable and Efficient High Rate Biological Nutrient Removal with Low Dissolved Oxygen Conditions

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1. Tables

Table S1. Average operational parameters for the pilot- and full-scale plants

	Average DO Concentrations (mg O_2/L)			Solids Retention Time (days)	Total Hydraulic Retention Time (hours)	Aerobic Hydraulic Retention Time (hours)
Plant	**Tank 1**	**Tank 2**	**Tank 3**			
Pilot phase 1	0.51 ± 0.28	0.72 ± 0.44	1.1 ± 0.28	10 ± 3.4	9.3 ± 0.96	6.0 ± 0.62
Pilot phase 2	0.41 ± 0.20	0.54 ± 0.30	0.67 ± 0.30			
Pilot phase 3	0.23 ± 0.13	0.48 ± 0.12	0.28 ± 0.25			
Full-scale, Plant 3	**Basin 1**	**Basin 2**	**Basin 3**	10	17	13
	0.40	0.83	4.20			

Table S2. Energy savings summary table, including modeled basin DO, calculated total energy usage, percent energy savings and change in annual energy costs based on $0.0835/kWh. Shaded rows represent the current scenario and the low-DO scenario that corresponds to the stable low-DO operation of the pilot plant.

DO Concentrations (mg O_2/L)						
Basin 1	**Basin 2**	**Basin 3**	**Calculated Total Energy Use (kWh/yr)**	**Percent Aeration Energy Reduction (%)**	**Change in Energy Use (kWh/yr)**	**Change in Annual Energy Costs ($)**
0.90	2.90	4.30	12,400,000	0.0%	0	$0
2.00	2.00	2.00	11,200,000	9.6%	-1,190,000	-$99,700
1.50	1.50	1.50	10,600,000	14.9%	-1,850,000	-$155,000
1.00	1.00	1.00	10,000,000	19.6%	-2,440,000	-$204,000
0.60	0.60	0.60	9,570,000	23.1%	-2,870,000	-$240,000
0.50	0.50	0.50	9,470,000	23.9%	-2,970,000	-$248,000
0.40	0.40	0.40	9,370,000	24.7%	-3,070,000	-$256,000
0.33	0.33	0.33	9,300,000	25.2%	-3,140,000	-$262,000
0.20	0.20	0.20	9,170,000	26.3%	-3,270,000	-$273,000

2. Figures

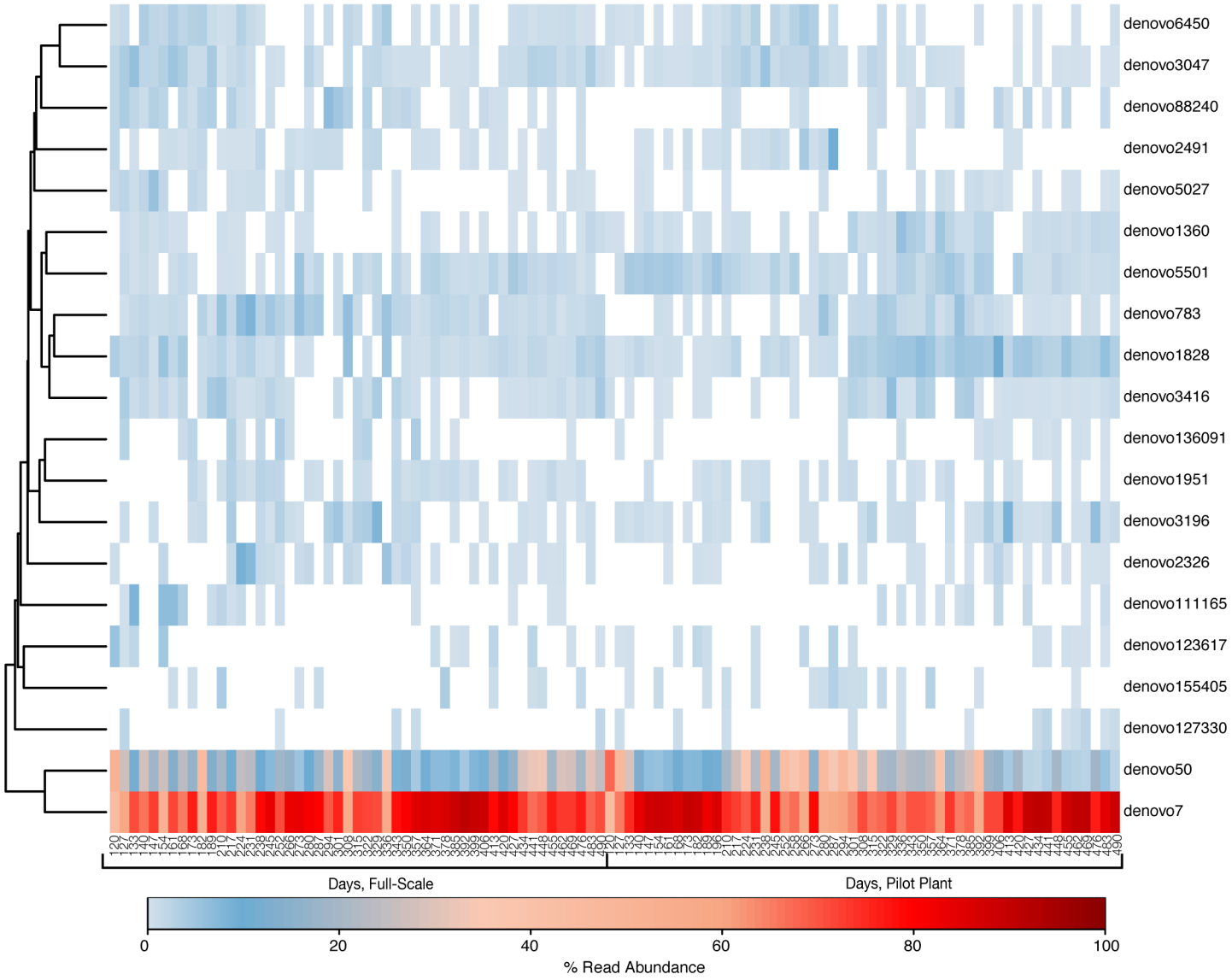

Figure S1. Heatmap dendrogram demonstrating changes in the relative abundance of OTUs classified as *Candidatus* Accumulibacter in the full-scale and pilot-scale plants. Note that the read abundance was calculated using the total number of reads in the subset of *Ca. Accumulibacter* OTUs, not the whole community.

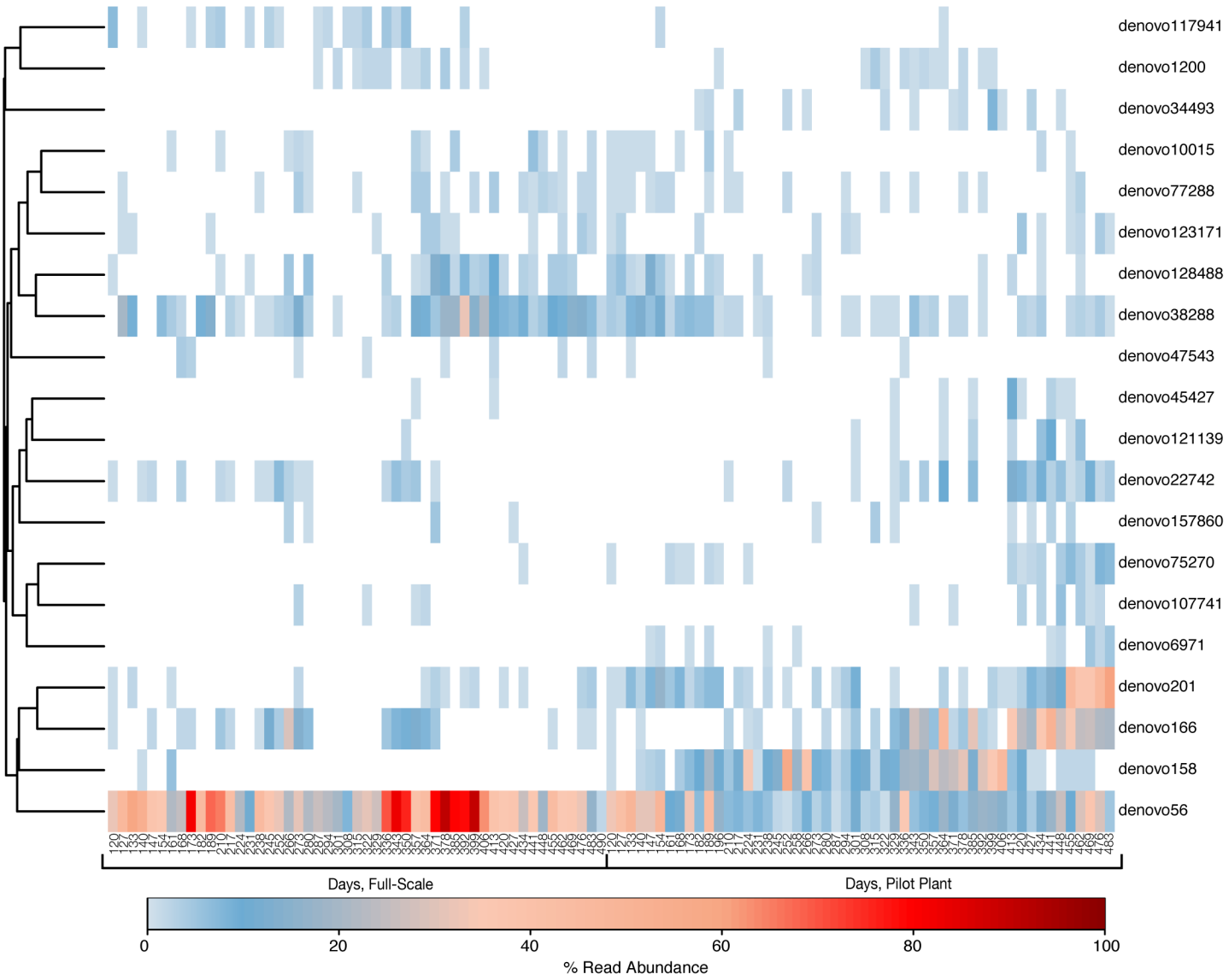

Figure S2. Heatmap dendrogram demonstrating changes in the relative abundance of OTUs classified as *Nitrosomonas* in the full-scale and pilot-scale plants. Note that the read abundance was calculated using the total number of reads in the subset of *Nitrosomonas* OTUs, not the whole community.

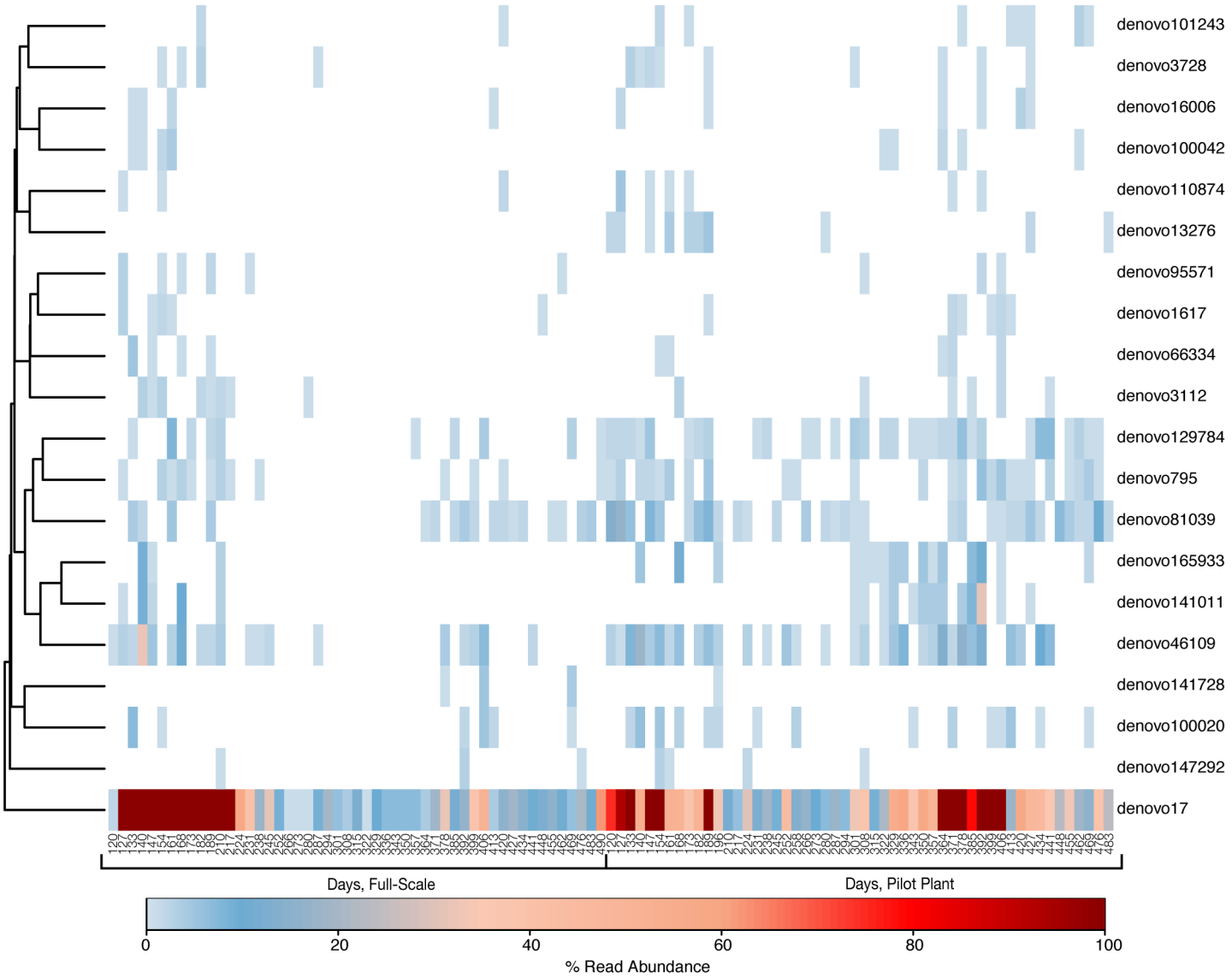

Figure S3. Heatmap dendrogram demonstrating changes in the relative abundance of OTUs classified as *Nitrospira* in the full-scale and pilot-scale plants. Note that the read abundance was calculated using the total number of reads in the subset of *Nitrospira* OTUs, not the whole community.

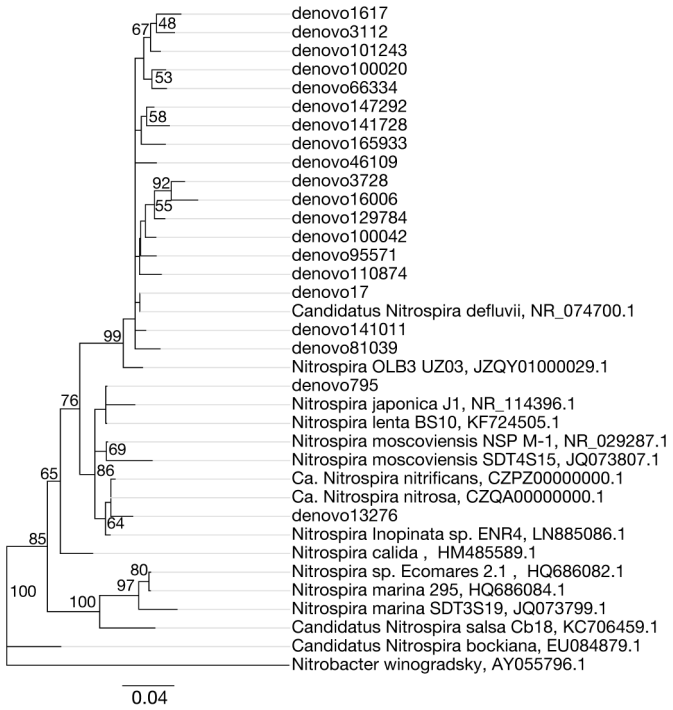

Figure S4. Neighbor-joining consensus tree generated from an alignment of published 16S rRNA sequences and sequences retrieved in this study. *Nitrobacter winogradskyi* was used to root the tree. Bootstrap values, shown at the nodes where the value was greater than 50, are based on 10,000 trials. The scale bar indicates a 4% sequence difference. Accession numbers are presented after the sequence names.

3. Methods

3.1 Floc and microcolony sizes

To compare flocs from the full-scale plant operated with high DO and the pilot plant after stable low DO operation, grab samples were collected from each system, the biomass embedded in an agarose solution, and visualized via microscopy. Initially, an agarose solution was prepared by dissolving 1.5 grams of Multi-Purpose Agarose (Thermo Fisher Scientific, Waltham, MA) in 100mL of deionized water, and heated in a microwave on high until clear, approximately one minute. The heated solution was immediately filtered through a 0.45-µm membrane filter (Nitrocellulose Membrane Filters, EMD Millipore Corp., Darmstadt, Germany) to remove impurities, and the filtrate stored at 50º C during sample preparation. The biomass samples were stained with NucBlue® Live ReadyProbes® Reagent following the manufacturer's protocol (Thermo Fisher Scientific, Waltham, MA). Two to three drops of activated sludge were carefully transferred to a petri dish using a wide mouth pipet and approximately 5 mL of the agarose solution was added. The petri dish was gently rotated to ensure an even distribution of floc and the agarose allowed to solidify. The petri dishes were immediately visualized with a Zeiss Microscope Axio Imager.Z2 and AxioCam MRM camera (Zeiss, Oberkochen, Germany) and images were captured with the AxioVision Rel. 4.8 Software for Image Acquisition and Management for Light Microscopy (Zeiss, Oberkochen, Germany). The surface area of individual floc was measured using the threshold, trace, and analyze features within the ImageJ software, which accounted for the entire irregular shape (Schneider et al. 2012). An equivalent diameter was calculated from the surface area of 356 and 373 individual flocs originating from the full-scale and pilot plant, respectively.

Fluorescence *in situ* hybridization (FISH) was used to analyze AOB microcolonies from the full-scale and pilot-scale plants. Biomass samples were collected from both plants during the third operational phase. The biomass was fixed using a 4% paraformaldehyde solution and stored at -20°C in a 1:1 volume of phosphate-buffered saline and absolute ethanol (Amann and Fuchs 2008). Fixed cells were placed on gelatin-coated glass slides and hybridized with a mixture of hybridization buffer and FISH probes. Oligonucleotide probe Nso1225 (Mobarry et al. 1996) was used to detect AOB. Microbial cells were counterstained with a 1 μg/ml of 4'6-diamidino-2-phenylindol (DAPI). Controls were performed to examine autofluorescence. The slides were visualized with a Zeiss Microscope Axio Imager.Z2 and AxioCam MRM camera (Zeiss, Oberkochen, Germany). Images were captured and analyzed with the AxioVision Rel. 4.8 Software for Image Acquisition and Management for Light Microscopy (Zeiss, Oberkochen, Germany). A positive signal for the Nso1225 oligonucleotide probe was used to identify 96 and 145 AOB microcolonies originating from the full-scale and pilot plant, respectively. An equivalent diameter for each microcolony was calculated from the maximum dimensions in the horizontal and vertical planes.

3.2 Calculations of Oxygen Requirement and Energy Savings

To estimate the energy savings associated with low-DO operation for the full-scale Nine Springs WWTP, oxygen transfer and oxygen requirement calculations were performed. All calculations assumed one treatment train with a three-pass aerobic zone. The first, second, and third aeration basins are 1/7, 3/7, and 3/7 of the total aerated volume, respectively.

Initially, the oxygen requirement for the biodegradation of carbonaceous material plus the oxygen requirement for oxidation of ammoniacal nitrogen were calculated according to Equations S.1 and S.2 (Tchobanoglous 2003).

$$R_O = Q(S_O - S) - 1.42P_{X,bio} + 4.33Q(TKN) \quad \text{(S.1)}$$

Where:

R_O = total oxygen required, grams per day (g/d)

Q = influent flow, meters^3/day (m^3/d), assumed 40 MGD (151,416 m^3/d)

S_O = influent substrate concentration (BOD), (mg/L), assumed 150 mg BOD/L

S = effluent substrate concentration (BOD), (mg/L), assumed 0 mg BOD/L

$P_{X,bio}$ = biomass as VSS wasted, from Equation S.2 (g/d)

TKN = influent nitrogen that is oxidized, (mg TKN/L), assumed 35 mg TKN/L

$$P_{X,bio} = \frac{QY(S_O - S)\left(\frac{1kg}{10^3 g}\right)}{1+(k_d)SRT} + \frac{(f_d)(k_d)QY(S_O - S)SRT\left(\frac{1kg}{10^3 g}\right)}{1+(k_d)SRT} + \frac{QY_n(TKN)\left(\frac{1kg}{10^3 g}\right)}{1+(k_{dn})SRT} \quad \text{(S.2)}$$

Where:

Y = biomass yield, g of VSS per g of BOD consumed, assumed 0.5g VSS/g BOD

Y_n = biomass yield for nitrogen, g VSS per g of NH_4-N, assumed 0.12g VSS/g NH_4-N

k_d = endogenous decay coefficient for heterotrophic organisms, days^{-1}, assumed 0.12 d^{-1}

k_{dn} = endogenous decay coefficient for nitrifying organisms, days^{-1}, assumed 0.08 d^{-1}

f_d = fraction of cell mass remaining as cell debris, unitless, assumed 0.15

SRT = solids retention time, days (d), assumed 10 d

Subsequently, the total biological oxygen requirement was converted to pounds of oxygen per day (lb O_2/d) by multiplying R_O by 0.0022 lb/g. The total biological oxygen requirement was

distributed to each basin as 22%, 55%, and 23%, respectively, based on past observations at the Nine Springs WWTP.

The additional oxygen requirement to maintain a specific level of DO in activated sludge was calculated using a mass balance approach for three aerobic basins with three separate DO concentrations. This mass balance is demonstrated in Equation S.3 (Tchobanoglous 2003). Equation S.4 determined the total actual oxygen requirement for each of the three aerobic basins.

$$AOTR_{LIQUID} = \left[(Q_{IN} - Q_{RAS}{\times}DO) + \left(Q_{prev}(DO - DO_0)\right)\right] \times \frac{3785412 L/MG}{453592\ mg/lb} \quad \text{(S.3)}$$

$$AOTR_{TOTAL} = AOTR_{LIQUID} + R_O \quad \text{(S.4)}$$

Where:

$AOTR_{LIQUID}$ = actual oxygen transfer rate to maintain DO concentration in the liquid, (lb O_2/d)

Q_{IN} = influent flow rate with DO = 0 mg/L, million gallons per day (MGD), assumed 40 MGD

Q_{RAS} = return activated flow rate, (MGD), assumed 0.5 Q_{IN}

Q_{prev} = flow rate from the previous basin, (MGD)

DO = concentration of DO in basin, (mg O_2/L)

DO_0 = concentration of DO in the first basin, (mg O_2/L)

$AOTR_{TOTAL}$ = total actual oxygen requirement for maintaining a specific concentration in the activated sludge and for the biological requirement (lb O_2/d)

The field oxygen transfer efficiency was calculated in Equation S.5 with standard oxygen transfer efficiencies determined specifically for Nine Springs WWTP aeration basins from a previous study. Standard oxygen transfer efficiencies vary with the type of aeration device, basin geometry,

degree of mixing, and the wastewater characteristics (Tchobanoglous 2003). The standard oxygen transfer efficiencies in wastewater (αSOTE) determined for Nine Springs WWTP aeration basins were 0.10, 0.18, and 0.18 for the three aerobic zone passes, respectively. The field oxygen transfer efficiency assumed a water temperature of 20°C and saturated DO concentration of 10.6 mg O_2/L.

$$OTE_{20} = \propto_{SOTE} \times \frac{C_{SAT} - DO}{C_{SAT}} \quad \text{(S.5)}$$

Where:

OTE_{20} = field oxygen transfer efficiency at 20°C, unitless

αSOTE = standard oxygen transfer efficiency for wastewater, unitless

C_{SAT} = concentration of DO at saturation, (mg O_2/L), assumed 10.6 mg O_2/L

DO = concentration of DO in the aeration basin, (mg O_2/L)

The field oxygen transfer efficiency was applied to the $AOTR_{TOTAL}$ for each aeration basin in Equation S.6. In addition, the resulting adjusted oxygen transfer rate was converted to an air flow rate in cubic feet per minute (CFM). The CFM for each aeration basin was summed to determine the total CFM required for the entire aerobic zone.

$$CFM = \frac{AOTR_{TOTAL}}{OTE_{20}} \times \frac{1}{60min \times \rho_{air} \times O_2} \quad \text{(S.6)}$$

Where:

CFM = required airflow rate, cubic feet per minute (ft^3/min)

ρ_{air} = air density, pounds per cubic feet (lb/ft^3), assumed 0.0765 lb/ft^3

O_2 = fraction of oxygen in air, assumed 0.21

The total required airflow rate in CFM was converted to standard CFM (SCFM), using Equation S.7. This equation assumed 25% humidity, saturation pressure of 0.3631 psi, and 20°C air temperature. The SCFM airflow was incorporated into the calculation for the power requirement

of the blowers in the mass flow rate of air (w) within Equation S.8 for U.S. Customary Units (Tchobanoglous 2003).

$$SCFM = \frac{CFM}{[P_{std}/(p_1 - P_{sat}\phi)] \times (T_{act}/T_{std})} \quad \text{(S.7)}$$

$$P_w = \left[\frac{wRT_{act}}{550ne}\left[\left(\frac{p_2}{p_1}\right)^{0.283} - 1\right]\right] \times \frac{0.746kW}{hp} \times \frac{8760h}{yr} \quad \text{(S.8)}$$

Where:

SCFM = standard cubic feet per minute, (SCFM)

P_{std} = standard pressure, pounds per square inch (psi), 14.7psi

P_{sat} = saturation pressure, pounds per square inch (psi), 0.3631psi

p_1 = inlet pressure, (psi), 14.25psi at the Nine Springs 877ft elevation

Φ = blower air humidity, (%), assumed 25/100

T_{act} = actual inlet air temperature, Rankine (°R), assumed 20°C or 527.7 °R

T_{std} = standard temperature, (°R), assumed 520 °R

P_w = power requirement for blowers, kilowatt hours per year (kWh/yr)

w = weight of flow of air, pounds per second (lb/s), SCFM × 0.0765lb/ft^3 × 1min/60s

R = engineering gas constant for air, 53.3 ft·lb/lb air·°R

n = 0.283 for air

e = blower efficiency (usual range is 0.70-0.90), Nine Springs WWTP calculated average is 0.63

p_2 = absolute outlet pressure, (psi), assumed 22.6psia based on 16ft diffuser depth and 1psi pressure loss

4. References

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