Supporting Information

Robust nitritation of anaerobic digester centrate using dual stressors and timed alkali additions

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15 **Table S1.** Strategies for nitritation and nitrogen removal from anaerobic digester centrate.

Short-cut biological nitrogen removal (SBNR) + references	Step 1: Ammonium to nitrite (nitritation)				Step 2: Nitrite to nitrous oxide or to nitrogen gas		Consequence of nitrate production in Step 1
	Target % conversion to nitrite	Selection Strategy for Nitritation			Electron donor & organisms	End product denitrification	
		T	DO	Alkalinity/pH			
Short-cut nitritation/ denitrification to N_2 in a CSTR (SHARON)[15,42]; Short-cut nitritation/ denitrification in a SBR[49]	Complete	35°C	AOB DO: K_{o2} = 1.45 mg NOB DO: K_{o2} = 1.1 mg /L	Set point pH at at 8.1-8.4; alkalinity from denitrification; CO_2 stripping	Methanol or soluble BOD denitrifying heterotrophs	N_2	Increased COD/N is needed for N removal; less biogas production or more methanol needed; increased eutrophication if treated effluent is discharged to environment.
Short-cut nitritation/ denitritation to N_2O by CSTRs in series (CANDO)[1,16–18]	Near complete	32°C	0-1 mg/L	Alkalinity generated by denitrification or added with set-point pH control or timed alkalinity additions (this work)	VFAs or primary effluent as BOD for denitrifying heterotrophs: glycogen-accumulating organisms and polyphosphate-PHB accumulating organisms	N_2O for co-combustion with air of biogas; final combustion products are CO_2, H_2O, N_2, and energy	Influent nitrate production requires additional electron donor (VFAs of soluble BOD).
Anammox-based N removal as N_2: CANON[19]; SHARON[14]; SBR nitritation[2,3]	Partial ~55%			CANON: both alkali and acid are used to set pH; SHARON: centrate alkalinity neutralizes ~50% of the HNO_2. SBR: alkalinity added by pH set-point control	Ammonium plus anaerobic ammonium oxidizing bacteria	N_2	Effluent nitrate must be reduced to nitrite or to N_2; increasing requirement for soluble BOD.

17 **Table S2.** $K_{I,FA}$ coefficients reported for *uncompetitive* (self) inhibition by free ammonia (FA, NH₃)
18 and $K_{I,FNA}$ coefficients reported for *non-competitive* inhibition by free nitrous acid (FNA, HNO₂).
19 For ammonium oxidation by AOB, the combined FA- and FNA-inhibited specific rate of oxidation
20 is given by:

21 $$q_{AOB,\,FA} = \hat{q}_{AOB}\frac{[Total\_Ammonium]}{K_{S,AOB}(1 + [FNA]/K_{I,FNA}) + [Total\_Ammonium](1 + [FNA]/K_{I,FNA} + [FA]/K_{I,FA})}, \quad K_{S,AOB} = 1.1\text{-}4.1$$
22 mM = 15-57 mg NH₄⁺-N/L[43]

23 For nitrite oxidation by NOB, the combined FA- and FNA-inhibited specific rate of oxidation of
24 nitrite is given by:

25 $$q_{NOB,\,FA} = \hat{q}_{NOB}\frac{[Total\_Nitrite]}{K_{S,NOB}(1 + [FNA]/K_{I,FNA}) + [Total\_Nitrite](1 + (1 + [FNA]/K_{I,FNA}) + [FA]/K_{I,FA})}, K_{S,NOB} = 0.6\text{-}2.0$$
26 mM = 8.4-28 mg NO₂⁻-N/L[43]

27 where q = observed specific rate of substrate utilization; $\hat{q}$ = maximum specific rate of substrate
28 utilization.

Inhibition coefficients for FA, $K_{I,FA}$ (mg FA/L)		Inhibition coefficients for FNA, $K_{I,FNA}$ (mg FNA/L)		Temperature (ºC)	Reference
Ammonium oxidation by AOB	Nitrite oxidation by NOB	Ammonium oxidation by AOB	Nitrite oxidation by NOB		
4.93-27.2	0.782	0.564	0.0799-0.320	30	Park and Bae[62]
10	0.75	0.5	0.1	30	Park et al.[28]
55.61	0.805	-	-	35	Magri et al.[63]
62.35	-	-	-	23	Carvallo et al.[64]
7.25	-	-	-	23	Carrera et al.[65]
46-376	0.21-1.31	-	-	30	Torà et al.[61]

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32   **Table S3.** Operational decisions and the rationale for each.

Design Decision	Rationale
Repeatable 1-day cycle duration	To synchronize operation with denitritation reactors operating on a 1-day cycle.
Continuous monitoring with a pH probe to monitor adaptation (phase 2)	Continuous pH monitoring during the adaptation phase provided information needed to set the timing of alkali additions and ensure complete ammonia oxidation to nitrite. This included information on ammonia oxidation rates, as AOB adapted to progressively higher ammonia concentrations. For the DS/SBR, the timing of alkali addition was calibrated to occur when pH approached 6 and FNA inhibition prevented further decrease in pH. Alkali addition relieved FNA inhibition, but re-imposed FA inhibition.
Decision regarding the pH range (SS/SBR:7.30 - 9; DS/SBR: 6 – 9.5)	The operational pH range for each reactor was based upon the calculated equilibrium concentrations of FA and FNA. For the SS/SBR, FA was the sole stressor over the pH range 7.30 – 9. For the DS/SBR, FA and FNA were alternately inhibitory over the pH range 6 – 9.5.
System operation was robust against pH probe failure after a repeating cycle of alkali additions was established. Stable nitrate production was observed in the SS/SBR and stable nitrite production was observed in the DS/SBR	After long-term operation, the rate of ammonia oxidation stabilizes, and the timing of alkali addition becomes fixed.
Three alkali additions were chosen.	The choice of three pH cycles (Initiation, 12-h and 14-h) in this work was a combination of practical operational considerations related to monitoring and stressor dose-contact time. In a follow-up short-term study about 10 days, we have switched to 2 pH cycle (Initiation and 12-h) per SBR cycle by increasing dosage at 12-h and no obvious performance deterioration has been observed

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35   **Table S4.** Algorithm used to calculate the concentration of alkali stock needed for timed alkali

36   addition in the DS/SBR.

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1. Characterize waste
    a. influent alkalinity
    b. influent TKN or TAN (optional: effluent TAN of previous cycle)
2. Design decisions and operating parameters
    a. recycle ratio
    b. working volume
    c. number of alkali inputs per cycle (number of FA exposures per cycle)
    d. volume of alkali to be added per cycle
3. Calculate alkalinity requirement per cycle
4. Calculate alkalinity required per alkali input
5. Calculate the pH after addition of each alkali input
6. Calculate the alkalinity needed to increase the pH in step 5 to the desired upper bound pH (typical range 9-9.5 for FA exposure)
7. Calculate the concentration of alkali stock based on the volume of alkali to be added
8. Check the solubility quotient for NaHCO$_3$ to ensure dissolution of buffer stock(i.e., Q$_c$ < K$_{sp}$)

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Sample calculations for preparation of alkali stock solutions

39   **Step 1: Waste characterization**

40   Influent alkalinity: $ALK_{inf} = 3000 \ mgCaCO_3/L$ (measured)

41   Influent TAN: $TAN_{inf} = 1300 \ mgN/L$ (measured)

42   **Step 2: Design decisions and operating parameters (user choice)**

43   Recycle ratio: $r = 0.25$

44   Working volume: $V = 2 \ L$

45   Number of alkali inputs per cycle: $n = 3$

46 Volume of alkali to be added per cycle: $\Omega = 0.02\ L$

47 Alkalinity at pH 6.3 (end-of-cycle): $ALK_{pH=6.3} = 100\ mgCaCO_3/L$ (assumption)

48

49 **Step 3: Calculate alkalinity requirement per cycle**

50 Total alkalinity added per cycle to neutralize ammonium oxidized to HNO_2:

$$ALK_{total} = TAN \times (1-r) \times 7.14$$
$$= 1300 \times (1-0.25) \times 7.14$$
$$= 6961\ mgCaCO_3/L$$

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52 Alkalinity requirement (difference between total alkalinity for neutralization of HNO_2 and

53 alkalinity in the influent):

$$ALK_{req} = ALK_{total} - ALK_{inf} \times (1-r)$$
$$= 6961 - 3000 \times (1-0.25)$$
$$= 4711\ mgCaCO_3/L$$

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55 **Step 4: Calculate alkalinity required per alkali input**

56 Alkalinity required per alkali input (assumes negligible change in the working volume):

$$ALK_{req,n} = ALK_{req}\ /\ n$$
$$= 4711/3$$
$$= 1571\ mgCaCO_3/L$$

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58 **Step 5: Calculate pH after addition of each alkali input**

59 Define the following expressions,

60
$$\alpha_0 = \frac{[H^+]^2}{K_1 K_2 + K_1[H^+] + [H^+]^2}$$

61
$$\alpha_1 = \frac{K_1[H^+]}{K_1 K_2 + K_1[H^+] + [H^+]^2}$$

$$\alpha_2 = \frac{K_1 K_2}{K_1 K_2 + K_1[H^+] + [H^+]^2}$$

62   $c_{T,pH\,=\,6.3} =$ Total carbonate (carbonic acid + bicarbonate + carbonate)

63   When $pH = pK_1 = 6.3$, (bicarbonate) = (carbonic acid), so $\alpha_1 = 0.5$.

64
$$\begin{aligned}
c_{T,pH\,=\,6.3} &= ALK_{pH\,=\,6.3} \,/\, \alpha_1 \\
&= 100\,/\,0.5 \\
&= 200\ mgCaCO_3/L)
\end{aligned}$$

65   Total carbonate (carbonic acid + bicarbonate + carbonate) after adding alkali:

66
$$\begin{aligned}
c_{T,new} &= c_{T,pH\,=\,6.3} + ALK_{req,\,n} \\
&= 200\ +\ 1571 \\
&= 1771\ mgCaCO_3/L
\end{aligned}$$

67   Bicarbonate after adding alkali:

68
$$\begin{aligned}
\left[HCO_3^-\right]_{new} &= ALK_{pH\,=\,6.3} + ALK_{req,n} \\
&= 100 + 1571 \\
&= 1671\ mgCaCO_3/L
\end{aligned}$$

69   pH after adding alkali:

70
$$\begin{aligned}
\alpha_1 &= \left[HCO_3^-\right]_{new} \,/\, c_{T,new} \\
&= 1671/1771 \\
&= 0.94
\end{aligned}$$

71   Solving for pH using expression for α_1:

72
$$pH_{int,\alpha_1\,=\,0.94} = 7.5$$

73

74　**Step 6: Calculate alkalinity needed to increase the pH in step 5 to the desired upper bound**

75　**pH (9.5)**

76　Bicarbonate concentration at pH 9.5:

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$$\text{At pH } 9.5, \ \alpha_1 = 0.86.$$

$$
\begin{aligned}
\left[HCO_3^-\right]_{pH=9.5} &= \alpha_{1,pH=9.5} \times c_{T,new} \\
78 &= 0.86 \times 1771 \\
&= 1522 \ mgCaCO_3/L
\end{aligned}
$$

79　Hydroxide to be added:

$$
\begin{aligned}
\left[OH^-\right]_{req} &= \left[HCO_3^-\right]_{new} - \left[HCO_3^-\right]_{pH=9.5} \\
80 &= 1671 - 1522 \\
&= 149 \ mgCaCO_3/L
\end{aligned}
$$

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82　**Step 7: Calculate the concentration of alkali stock based on the volume of alkali to be added**

$$
\begin{aligned}
\left[OH^-\right]_{stock} &= \left[OH^-\right]_{req} \times \frac{V}{n\Omega} \\
83 &= 149 \times \frac{2}{3 \times 0.02} \\
&= 5000 \ mg \ CaCO_3/L \\
&= 100 \ mM
\end{aligned}
$$

$$
\begin{aligned}
\left[HCO_3^-\right]_{stock} &= ALK_{req,n} \times \frac{V}{n\Omega} \\
84 &= 1571 \times \frac{2}{3 \times 0.02} \\
&= 52350 \ mgCaCO_3/L \\
&= 1047 \ mM
\end{aligned}
$$

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Nitrogen Balance

89   **Table S5** Nitrogen Balance of SS/SBR and DS/SBR and at the start and end of Day 100

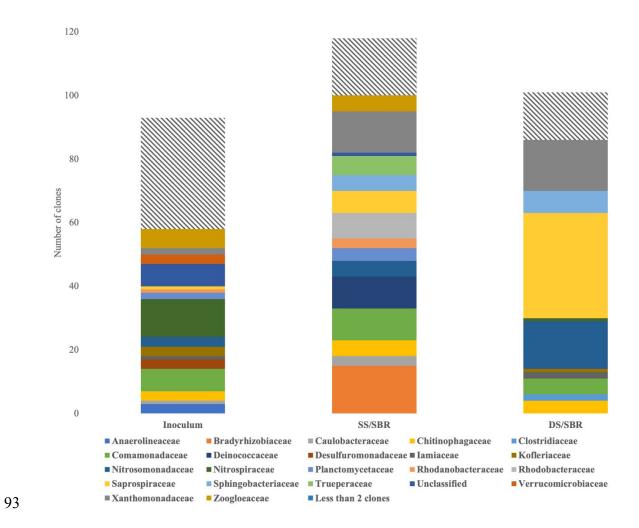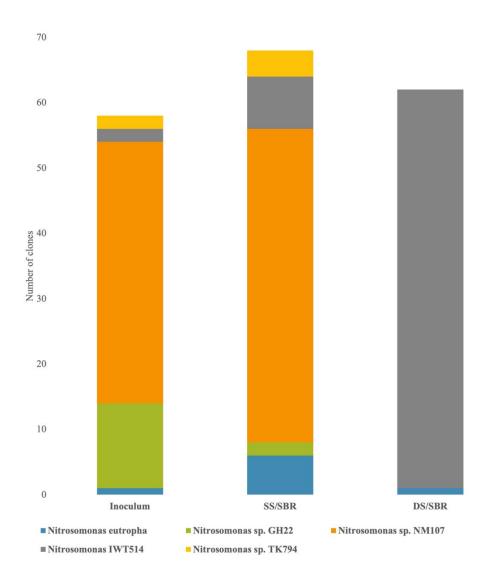|	Initial				Final			
	TKN	NO_2^--N	NO_3^--N	TN	TKN	NO_2^--N	NO_3^--N	TN
SS/SBR	1,217	0	274	1,491	68.0	0	1,408	1,476
DS/SBR	1,082	302	2.3	1,386	69.4	1,300	8.3	1,378

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Figure S1 Relative 16s rRNA gene abundance in clone libraries prepared for the inoculum (Day

95   0) and in samples from SS/SBR and DS/SBR on Day 100.

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50

40
Number of clones

30

20

10

0

| Inoculum | SS/SBR | DS/SBR |

■ **Nitrosomonas eutropha**   ■ **Nitrosomonas sp. GH22**   ■ **Nitrosomonas sp. NM107**

■ **Nitrosomonas IWT514**   ■ **Nitrosomonas sp. TK794**

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97   **Figure S2** Phylogeny of *amo*A genes in clone libraries prepared for the inoculum (Day 0) and in

98   samples from the SS/SBR and the DS/SBR on Day 100.

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