Supporting Information

Glycerol-driven Denitratation: Process Kinetics, Microbial Ecology, and Operational Controls

AUTHOR NAMES: *Matthew P. Baideme,*[a,#] *Chenghua Long,*[a] *Luke T. Plante,*[a,b] *Jeffrey A. Starke,*[b,1] *Michael A. Butkus,*[b] *and Kartik Chandran*[a,*]

AUTHOR AFFILIATIONS:

[a] Department of Earth and Environmental Engineering, Columbia University, New York, NY 10027, U.S.A.

[b] Department of Geography and Environmental Engineering, United States Military Academy, West Point, NY 10996, U.S.A.

[1] Present Address: Opus College of Engineering, Marquette University, Milwaukee, WI 53233, U.S.A.

[#] Co-corresponding author: Department of Earth and Environmental Engineering, Columbia University, 500 W. 120th St., New York, NY 10027. Email: mpb2177@columbia.edu.

[*] Corresponding Author: Department of Earth and Environmental Engineering, Columbia University, New York, NY 10027, U.S.A. Phone: 212.854.9027. Fax: 212.854.7081. E-mail: kc2288@columbia.edu.

Calculations, Derivations, Methods

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Calculations and Derivations

Electron acceptor, organic electron donor, and cell synthesis half-reactions and Gibb's free energy:[1]

$$R_c:\ (0.20)CO_2 + (0.05)HCO_3^- + (0.05)NH_4^+ + H^+ + e^- = (0.05)C_5H_7O_2N + (0.45)H_2O$$

$$R_d:\ (0.21)CO_2 + H^+ + e^- = (0.07)C_3H_8O_3 + (0.21)H_2O$$

$$\Delta G_a^{0'} = 38.88\frac{kJ}{eeq}$$

$$R_{a,\ NO_3^-\rightarrow N_2}:\ (0.20)NO_3^- + (1.20)H^+ + e^- = (0.10)N_2 + (0.60)H_2O$$

$$\Delta G_a^{0'} = -72.20\frac{kJ}{eeq}$$

$$R_{a,\ NO_3^-\rightarrow NO_2^-}:\ (0.50)NO_3^- + H^+ + e^- = (0.50)NO_2^- + (0.50)H_2O$$

$$\Delta G_a^{0'} = -41.65\frac{kJ}{eeq}$$

$$R_{a,\ NO_2^-\rightarrow N_2}:\ (0.33)NO_2^- + (1.33)H^+ + e^- = (0.17)N_2 + (0.67)H_2O$$

$$\Delta G_a^{0'} = -92.56\frac{kJ}{eeq}$$

$$R_{in,\ acetylCoA}:\ (0.22)CO_2 + H^+ + e^- = (0.11)C_2H_3O + (0.33)H_2O$$[1–3]

$$\Delta G_{in}^{0'} = 30.90\frac{kJ}{eeq}$$

$$R = f_e(R_a - R_d) + f_s(R_c - R_d) = f_eR_a + f_sR_c - R_d$$

$$1 = f_e + f_s$$

$$R_{NO_3^-\rightarrow N_2}:\ f_e\left[(0.20)NO_3^- + (0.07)C_3H_8O_3 + (0.20)H^+\right] + f_s\left[(0.05)NH_4^+ + (0.05)HCO_3^- + (0.07)C_3H_8O_3\right] = f_e\left[(0.10)N_2 + (0.39)H_2O + (0.21)CO_2\right] + f_s\left[(0.05)C_5H_7O_2N + (0.24)H_2O + (0.01)CO_2\right]$$

$$R_{NO_3^-\rightarrow NO_2^-}:\ f_e\left[(0.50)NO_3^- + (0.07)C_3H_8O_3\right] + f_s\left[(0.05)NH_4^+ + (0.05)HCO_3^- + (0.07)C_3H_8O_3\right] = f_e\left[(0.50)NO_2^- + (0.29)H_2O + (0.21)CO_2\right] + f_s\left[(0.05)C_5H_7O_2N + (0.24)H_2O + (0.01)CO_2\right]$$

$$R_{NO_2^-\rightarrow N_2}:\ f_e\left[(0.33)NO_2^- + (0.07)C_3H_8O_3 + (0.33)H^+\right] + f_s\left[(0.05)NH_4^+ + (0.05)HCO_3^- + (0.07)C_3H_8O_3\right] = f_e\left[(0.17)N_2 + (0.45)H_2O + (0.21)CO_2\right] + f_s\left[(0.05)C_5H_7O_2N + (0.24)H_2O + (0.01)CO_2\right]$$

$R_{ic,\ glycerol \rightarrow acetylCoA}$: $(0.07)C_3H_8O_3 + (0.01)CO_2 = (0.11)acetylCoA + (0.12)H_2O$

1. Thermodynamic derivation of COD requirements for glycerol-driven denitrification using the Reaction Energetics Method for predicting bacterial yield.

A combination of TEEM1[1] and the modifications incorporated into the TEEM2[2] thermodynamic models was employed to determine stoichiometric coefficients for glycerol-driven chemoorganoheterotrophic denitrification.

$$\Delta G_{ic}^{0'} = \Delta G_{in}^{0'} - \Delta G_{d}^{0'} = 30.90\frac{kJ}{eeq} - 38.88\frac{kJ}{eeq} = -7.98\frac{kJ}{eeq}$$

$\Delta G_{in}^{0'} = 30.90\frac{kJ}{e^-\ eq}$ to represent the energy required to convert the cell carbon source to an intermediate compound (acetyl-CoA) prior to full oxidation.[2]

$\Delta G_{d}^{0'} = 38.88\frac{kJ}{e^-\ eq}$ for the heterotrophic reaction.[1]

$$\Delta G_{ic}^{0} = \Delta G_{ic}^{0'} - RTv_{H^+}ln[10^{-7}] = -7.98\frac{kJ}{eeq} - \left(0.008314\frac{kJ}{mol \cdot K}\right)(273.15 + 23K)(0)ln[10^{-7}] = = -7.98\frac{kJ}{eeq}$$

$$\Delta G_{ic} = \Delta G_{ic}^{0} + RTlnQ = \Delta G_{ic}^{0} + RTln\left(\frac{[C_2H_3O]^{0.11}[H_2O]^{0.12}}{[CO_2]^{0.008}[C_3H_8O_3]^{0.07}}\right)$$

Assume average atmospheric $CO_{2(g)}$ concentration, $P_{CO_2} = 409ppm = 4.09 \cdot 10^{-4}atm$,

$$[CO_{2(g,headspace)}] = [CO_{2(aq)}] = \frac{P_{N_2}}{K_H} = \frac{4.09 \cdot 10^{-4}atm}{29.41\frac{atm}{M}} = 1.39 \cdot 10^{-5}M$$

therefore,

Initial $[C_3H_8O_3] = \left(300\frac{mgCOD}{L}\right)\left(\frac{1gCOD}{1000mgCOD}\right)\left(\frac{1molCOD}{32gCOD}\right)\left(\frac{1molC_3H_8O_3}{3.5molCOD}\right) = 2.68 \cdot 10^{-3}M$ per cycle.

Assume all glycerol is converted to acetyl-CoA, $[C_3H_8O_3] = [acetylCoA]$.

System was operated at room temperature (23°C) and buffered at $pH = 7.5$, or $[H^+] = 10^{-7.5} M$.

$$\Delta G_{ic} = -7.98\frac{kJ}{eeq} + \left(0.008314\frac{kJ}{mol \cdot K}\right)(273.15 + 23K)ln\left(\frac{[2.68 \cdot 10^{-3}M]^{0.11}[1M]^{0.12}}{[1.39 \cdot 10^{-5}M]^{0.008}[2.68 \cdot 10^{-3}M]^{0.07}}\right) = -8.34\frac{kJ}{eeq}$$

$$\Delta G_r^{0'} = \Delta G_a^{0'} - \Delta G_d^{0'} = -72.20\frac{kJ}{eeq} - 38.88\frac{kJ}{eeq} = -111.08\frac{kJ}{eeq}$$

$$\Delta G_r^0 = \Delta G_r^{0'} - RTv_{H^+} ln[10^{-7}] = -111.08\frac{kJ}{eeq} - \left(0.008314\frac{kJ}{mol \cdot K}\right)(273.15 + 23K$$
$$\frac{kJ}{eeq}$$

$$\Delta G_r = \Delta G_r^0 + RTlnQ = \Delta G_r^0 + RTln\left(\frac{[N_2]^{0.10}[CO_2]^{0.21}[H_2O]^{0.39}}{[NO_3^-]^{0.20}[C_3H_8O_3]^{0.07}[H^+]^{0.20}}\right)$$

Assume completely anoxic reactor with headspace saturated with $N_{2(g)}$, therefore,

$$[N_{2(g,headspace)}] = [N_{2(aq)}] = \frac{P_{N_2}}{K_H} = \frac{1atm}{1639.34\frac{atm}{M}} = 6.10 \cdot 10^{-4} M$$

Initial $[NO_3^-] = \left(100\frac{mgN}{L}\right)\left(\frac{1gN}{1000mgN}\right)\left(\frac{1molN}{14gN}\right) = 7.14 \cdot 10^{-3} M$ per cycle.

$$\Delta G_r = -119.02\frac{kJ}{eeq} + \left(0.008314\frac{kJ}{mol \cdot K}\right)(273.15 + 23K)ln\left(\frac{[6.10 \cdot 10^{-4}M]^{0.10}}{[7.14 \cdot 10^{-3}M]^{0.20}[2.}\right.$$
$$= = -114.67\frac{kJ}{eeq}$$

$A\varepsilon\Delta G_r + \Delta G_s = 0$, at steady-state, assuming that the energy transfer efficiency from the oxidation of electron donor to capture by the electron carrier is equal to that of the electron carrier to electrons captured for cell synthesis.[1]

$$A = -\frac{\Delta G_s}{\varepsilon\Delta G_r}$$

88 $\Delta G_s = \frac{\Delta G_{fa} - \Delta G_d}{\varepsilon^m} + \frac{\Delta G_{in} - \Delta G_{fa}}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}$, where $\Delta G_{pc} = 18.8\frac{kJ}{eeq}$ with NH_4^+ as the nitrogen

89 source for cell synthesis and $C_5H_7O_2N$ is assumed as the cell relative composition.[2] Sufficient

90 NH_4^+ was included in the feed stock for theoretical growth requirements and significant NH_4^+

91 was always remaining in the effluent indicating that additional nitrogen sources (NO_3^- or NO_2^-)

92 were not used for synthesis purposes as they are less energy efficient for the cell.

93 Since glycerol and acetyl-CoA are not C1 compounds, $\Delta G_{fa} = 0$ and $m = n$.[2]

94 $n = -1$ as $\Delta G_p < 0$.

95 $\varepsilon = 0.40$ was assumed based upon experimental data[2] and reported operational influent

96 COD:NO_3^--N ratios.[4–6]

97 $$\Delta G_s = \frac{\Delta G_{in} - \Delta G_d}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon} = \frac{\Delta G_{ic}}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}$$

98 $$A = -\frac{\Delta G_s}{\varepsilon \Delta G_r} = -\frac{\frac{\Delta G_{ic}}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon \Delta G_r} = -\left(\frac{\left(\frac{-8.34\frac{kJ}{eeq}}{0.40^{-1}}\right) + \left(\frac{18.8\frac{kJ}{eeq}}{0.40}\right)}{(0.40)\left(-114.67\frac{kJ}{eeq}\right)}\right) = 0.952$$

99 $$f_s = \frac{1}{1+A} = \frac{1}{1+0.952} = 0.512$$

100 $$f_e = 1 - f_s = 1 - 0.513 = 0.488$$

101 $$R = f_e R_a + f_s R_c - R_d = (0.488)R_a + (0.512)R_c - R_d$$

102 $$R_{NO_3^- \rightarrow N_2}: \; NO_3^- + (0.73)C_3H_8O_3 + (0.26)NH_4^+ + (0.26)HCO_3^- + H^+ = (0.50)N_2 + (1.15)CO_2 + (0.26)C_5H_7O_2N + (3.17)H_2O$$

103 $$COD = \left(0.73\ mol\ C_3H_8O_3\right)\left(\frac{3.5\ mol\ O_2}{1\ mol\ C_3H_8O_3}\right)\left(\frac{32\ g\ O_2}{1\ mol\ O_2}\right) = 82.1\ g\ O_2 = 82.1\ g\ COD$$

104 $$NO_3^- - N = \left(1\ mol\ NO_3^-\right)\left(\frac{1\ mol\ NO_3^- - N}{1\ mol\ NO_3^-}\right)\left(\frac{14\ g\ NO_3^- - N}{1\ mol\ NO_3^- - N}\right) = 14\ g\ NO_3^- - N$$

$COD{:}NO_3^- - N = 82.1\ g\ COD{:}\ 14\ g\ NO_3^- - N = 5.9{:}1$

Using this same process, assumptions of other energy-transfer efficiencies yield the following results:

ε	0.30	0.40	0.50	0.60	0.70	0.80
A	1.809	0.951	0.583	0.383	0.262	0.184
f_s	0.356	0.513	0.632	0.723	0.792	0.845
f_e	0.644	0.487	0.368	0.277	0.208	0.155

As can be seen, the assumption of an energy-transfer efficiency has a drastic effect and, therefore, must be confirmed.

2. Confirmation of thermodynamic assumptions using the Dissipation Method for predicting bacterial yield.

The Dissipation Method for predicting bacterial yield[7–9] was employed to confirm assumptions used in the thermodynamic Reaction Energetics Method determination of COD requirements to support glycerol-driven denitrification.

$\frac{D_s^{0'}}{r_{Ax}} = 200 + 18 \cdot (6 - C)^{1.8} + e^{\left[\left\{(3.8 - \gamma_D)^2\right\}^{0.16} \cdot (3.6 + 0.4C)\right]}$, which describes the heat (Gibbs free energy) dissipated during growth or production of 1 C-mole of biomass.

$C = 3$, which represents the number of carbon atoms in a mole of glycerol.

$\gamma_D = 4.667$, degree of reductance of the carbon in glycerol as the electron donor.[7]

$$\frac{D_s^{0'}}{r_{Ax}} = 200 + 18 \cdot (6-3)^{1.8} + e^{\left[\{(3.8-4.667)^2\}^{0.16} \cdot \{3.6 + (0.4)(3)\}\right]} = 428.06 \frac{kJ}{c\,mol}$$

$$Y_{DX} = \frac{\gamma_D}{\gamma_X} \frac{\Delta G_{eD}^{0'} - \Delta G_{eA}^{0'}}{\left(\Delta G_{eD}^{0'} - \Delta G_{eA}^{0'}\right) + \left[\left(\frac{D_s^{0'}}{r_{Ax}} \cdot \frac{1}{\gamma_X}\right) + \left(\Delta G_{eX}^{0'} - \Delta G_{eD}^{0'}\right)\right]}$$, which represents the bacterial cell yield on the electron donor.

$\Delta G_{eD}^{0'} = 38.88 \frac{kJ}{eeq}$, Gibbs standard free energy for glycerol as the electron donor.[1]

$\Delta G_{eA}^{0'} = -72.20 \frac{kJ}{eeq}$, Gibbs standard free energy for NO_3^- as the electron acceptor.[1]

$\Delta G_{eX}^{0'} = 38.80 \frac{kJ}{eeq}$, assuming $\Delta G_{fX}^{0'} = -67 \frac{kJ}{c-mol}$.[10]

$\gamma_X = 4.2$, degree of reductance of the carbon in biomass.[7]

$$Y_{DX} = \left(\frac{4.667}{4.2}\right)\left[\frac{38.88\frac{kJ}{eeq} - \left(-72.20\frac{kJ}{eeq}\right)}{\left\{38.88\frac{kJ}{eeq} - \left(-72.20\frac{kJ}{eeq}\right)\right\} + \left\{\left(428.06\frac{kJ}{c\,mol} \cdot \frac{1}{4.2}\right) + \left(38.80\frac{kJ}{eeq} - 38.88\frac{kJ}{eeq}\right)\right\}}\right] = 0.580 \frac{c\,mol_X}{c\,mol_D}$$

$$Y_{DX} = 0.522 \frac{eeq_X}{eeq_D}$$

In terms of eeq, $Y_{DX} = f_s^0$, therefore, $f_s^0 = 0.522 \frac{eeq_X}{eeq_D}$.

$f_e^0 = 1 - f_s^0 = 1 - 0.522 = 0.487$

Comparison of f_s^0 calculated using the Dissipation Method with f_s calculated using the Reaction Energetics Method indicates that the energy-transfer efficiency, ε, inherent in the Dissipation Method calculations is $\varepsilon = 0.406$. This confirms the validity of the assumption of $\varepsilon = 0.40$ in the Reaction Energetics Method calculations.

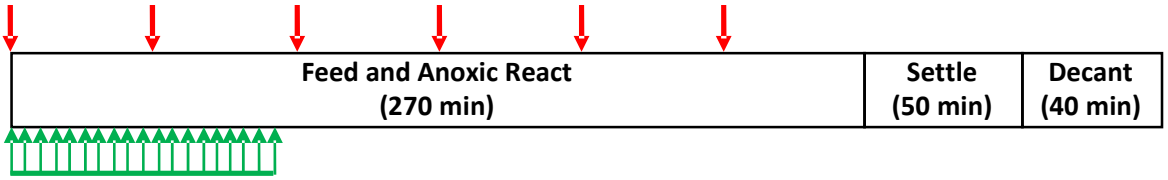
While these calculations are at standard state, it has been shown that there is little difference between predictions at standard state and non-standard state in certain instances provided system pH is close to neutral, substrate concentrations are low, and $\Delta G_{eD}^{0'} - \Delta G_{eA}^{0'} > 20 \frac{kJ}{eeq}$.[2,9] Additionally, as they are simply being used to confirm assumptions made using the reaction energetics method, calculations were not made to convert to non-standard state conditions.

3. Initial reactor buffering methodology prior to pH optimization batch assays.

For pH optimization batch assays, the medium was initially buffered to approximately pH 9.0 but left unbuffered for the remainder of each experiment during which the pH ranged from 7.2 to 9.0.

Table S1. *Components of SBR feed including trace nutrients.* **Trace nutrients were dissolved in deionized water.*

SBR Feed (mg per 100 L SBR feed)	
NO_3^--N	10,000.0
NH_4^+-N	2,500.0
$MgSO_4 \cdot 7H_2O$	20,000.0
KH_2PO_4	8,700.0
$CaCl_2 \cdot 2H_2O$	2,000.0
NaOH	for pH adjustment
Trace Nutrients* (mg per 100 L SBR feed)	
$EDTA \cdot Na_2$	2,010.1
$FeSO_4 \cdot 7H_2O$	500.4
$MnCl_2 \cdot 4H_2O$	172.2
$ZnSO_4 \cdot 7H_2O$	43.1
$CuSO_4 \cdot 5H_2O$	25.0
$CoCl_2 \cdot 6H_2O$	23.8
$Na_2MoO_4 \cdot 2H_2O$	10.0
$NiSO_4 \cdot 6H_2O$	2.1
H_3BO_3	1.1



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155 ***Figure S1.*** *Two feeding strategies, semi-continuous (green arrows; 75-min NO_3^- feed with*

156 *concurrent 72-min glycerol feed, influent COD:NO_3^--N=2.4:1) and pulse (red arrows; each*

157 *pulse contained 4-min NO_3^- feed with concurrent 1-min glycerol feed, influent COD:NO_3^--*

158 *N=2.4:1), were investigated to determine their impact on NO_2^- accumulation.*

159

Table S2. *Effluent sCOD and biomass concentrations supported at each respective influent COD:NO_3^--N ratio.*

	Inf. COD:NO_3^--N				
	2.5:1 (n=6)	2.8:1 (n=11)	3.0:1 (n=10)	4.0:1 (n=7)	5.0:1 (n=6)
Avg $X_{reactor}$ [mg/L COD]	345.4±50.4	423.4±35.4	448.2±60.9	493.4±39.3	692.4±25.6
Avg $sCOD_{eff}$ [mg/L COD]	6.9±6.0	5.3±3.1	9.6±9.5	2.2±4.3	18.7±5.6
$sCOD_{eff}/sCOD_{inf}$ [%]	2.8	1.9	0.3	0.6	3.7

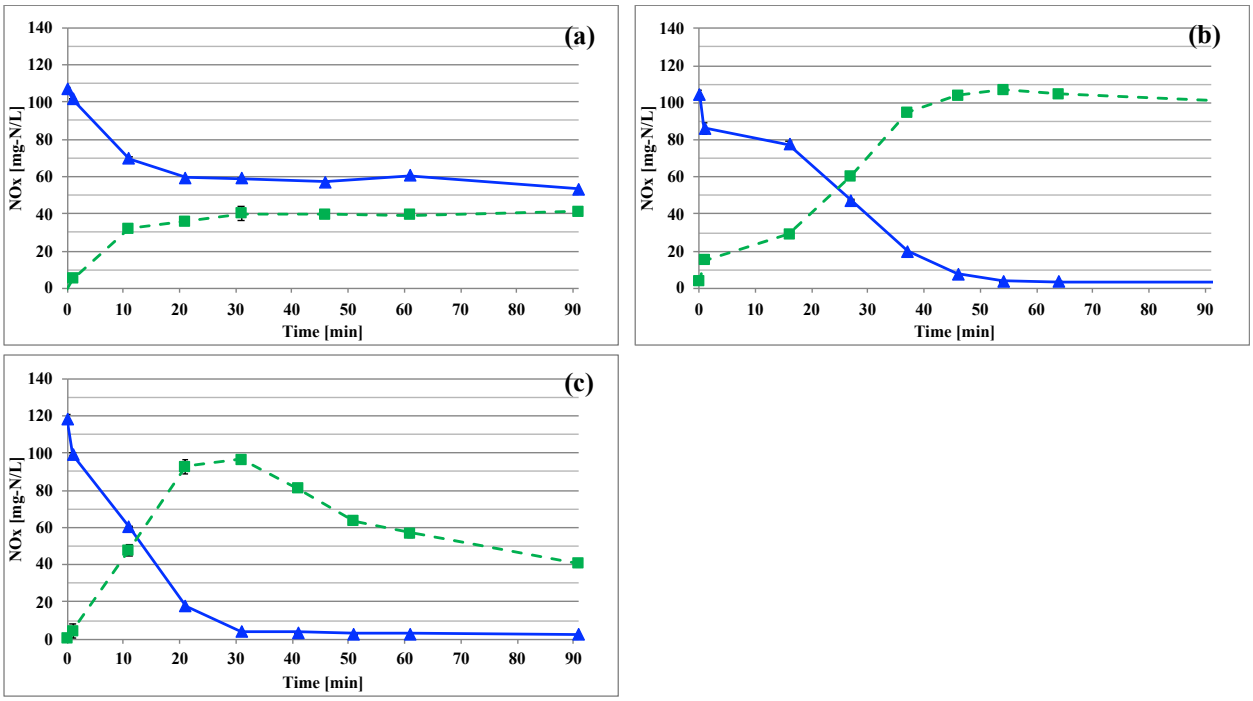
Table S3. *Results of Holm-Sidak post hoc multiple comparison analysis to determine between which NARs a significant difference exists (statistical significance exists at $p<0.05$ and is demarcated using bold font).*

Inf. COD:NO_3^--N	2.5 ($\bar{x}$=0.65)	2.8 ($\bar{x}$=0.69)	3.0 ($\bar{x}$=0.62)	4.0 ($\bar{x}$=0.57)	5.0 ($\bar{x}$=0.11)
2.5		0.496	0.755	0.319	**0.000**
2.8			0.147	**0.006**	**0.000**
3.0				0.329	**0.000**
4.0					**0.000**

Table S4. *Denitratation performance under continuous and pulse operational feeding strategies.*

Influent COD:NO_3^--N	SRT [d]	Operational Feeding Strategy	Avg $NO_{3,eff}^-$ [mg/L NO_3^--N]	Avg $NO_{2,eff}^-$ [mg/L NO_2^--N]
2.4	3	Pulse NO_3^- Pulse COD	11.3 ± 3.3	86.4 ± 7.5
		Continuous NO_3^- Continuous COD	16.0 ± 5.5	70.1 ± 8.4

Contrary to the continuous operational feeding strategy, the pulse operational feeding strategy reduced nearly 90% of the influent NO_3^- despite the limited reaction time for late occurring pulses of NO_3^- and glycerol indicating that influent NO_3^- underwent rapid reduction upon entering the system. This observation was consistent with other studies which reported that specific denitrification rates are higher for pulse-type feeding strategies as compared to continuous feeding strategies resulting in a faster reduction of influent NO_3^-.[11,12] Martins et al.[11] determined that maximum specific denitrification rates were considerably lower for SBR systems with long feeding periods that mimicked continuously-fed, completely mixed systems, than in plug flow-type systems. Similarly, Ryu et al.[12] found that denitrification rates were fastest during slug feeding followed in order by intermittent and continuous feeding strategies during their evaluation of fermented food waste as an external carbon source for nutrient removal in an SBR.



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182 ***Figure S2.*** *Representative* ex situ *NO_3^--N (▲, solid line) and NO_2^--N (○, dotted line) profiles at*

183 *influent COD:NO_3^--N ratios (a) 2.5, (b) 3.0, (c) 5.0.* Ex situ *batch assays were performed using*

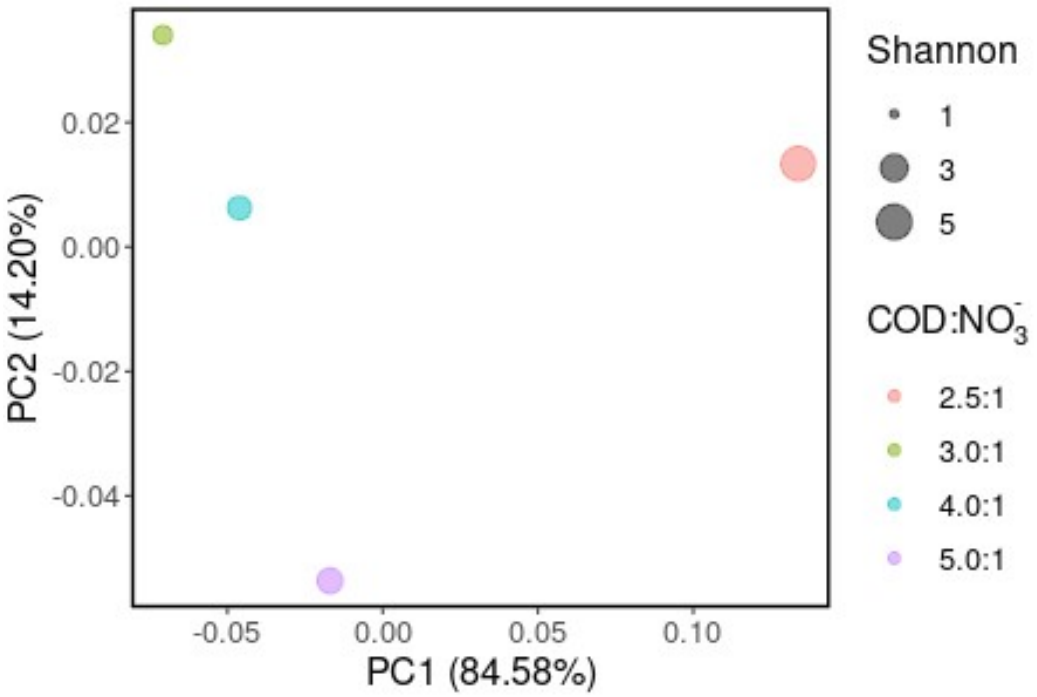
184 *biomass acclimated at each influent COD:NO_3^--N ratio in the parent reactor for at least four*

185 *SRTs.*

186

Table S5. *Estimations of richness and diversity in the microbial communities at influent COD:NO_3^--N ratios.*

Inf. COD:NO_3^--N	Shannon Index	Chao-1 Estimator
2.5:1	4.79	174
3.0:1	1.61	64
4.0:1	2.24	96
5.0:1	2.62	92



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191 ***Figure S3.*** *Principal Coordinates Analysis (PCoA) of weighted Unifrac distances analyzes and*

192 *compares the beta diversity of microbial communities selected for at influent COD:NO_3^--N*

193 *ratios.*

References

1 B. E. Rittmann and P. L. McCarty, *Environmental Biotechnology: Principles and Applications*, McGraw-Hill, Boston, 2001.

2 P. L. McCarty, Thermodynamic electron equivalents model for bacterial yield prediction: Modifications and comparative evaluations, *Biotechnol. Bioeng.*, 2007, **97**, 377–388.

3 R. K. Thauer, K. Jungermann and K. Decker, Energy conservation in chemotrophic anaerobic bacteria, *Bacteriol. Rev.*, 1977, **41**, 100–180.

4 J. Hinojosa, R. Riffat, S. Fink, S. Murthy, K. Selock, C. Bott, I. Takacs, P. Dold and R. Wimmer, in *Proceedings of the 81st Annual Water Environment Federation Technical Exposition and Conference*, Chicago, 2008, pp. 274–288.

5 H. Lu and K. Chandran, Diagnosis and quantification of glycerol assimilating denitrifying bacteria in an integrated fixed-film activated sludge reactor via ^{13}C DNA stable-isotope probing, *Environ. Sci. Technol.*, 2010, **44**, 8943–8949.

6 J. C. Akunna, C. Bizeau and R. Moletta, Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: Glucose, glycerol, acetic acid, lactic acid and methanol, *Water Res.*, 1993, **27**, 1303–1312.

7 J. M. VanBriesen, Evaluation of methods to predict bacterial yield using thermodynamics, *Biodegradation*, 2002, **13**, 171–190.

8 J. J. Heijnen and J. P. van Dijken, In search of a thermodynamic description of biomass yields for the chemotrophic growth of microorganisms, *Biotechnol. Bioeng.*, 1992, **39**, 833–858.

9 J. J. Heijnen, M. C. M. van Loosdrecht and L. Tijhuis, A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs energy dissipation, *Biotechnol. Bioeng.*, 1992, **40**, 1139–1154.

10 J. A. Roels, Application of macroscopic principles to microbial metabolism, *Biotechnol. Bioeng.*, 1980, **22**, 2457–2514.

11 A. M. P. Martins, J. J. Heijnen and M. C. M. van Loosdrecht, Bulking sludge in biological nutrient removal systems, *Biotechnol. Bioeng.*, 2004, **86**, 125–135.

12 H.-D. Ryu, S.-I. Lee, K.-Y. Chung and K.-Y. Kim, Effects of feeding methods on denitrification and phosphorus release, *Environ. Eng. Sci.*, 2007, **24**, 1467–1474.