

Supplementary materials

Enhancement of mainstream nitrogen removal via simultaneous partial nitrification, anammox and denitrification by gel entrapment technique

Ming Zeng¹, Junfeng Yang¹, Xiaofang Li¹, Nan Wu^{2*}, Peng Pan¹, Chang Wang¹

¹College of Marine and Environmental Sciences, Tianjin University of Science & Technology, 300457 Tianjin, China

²College of Engineering and Technology, Tianjin Agricultural University, Tianjin 300384, China

*Corresponding author. E-mail address: nwu@tjau.edu.cn (Nan Wu)

The following are included as supporting information for this paper:

Number of pages: 1

Number of tables: 6

Table S1 Wastewater compositions for batch tests of activated sludge, anammox biofilm and BSgel system

Batch test	Wastewater compositions
Activated sludge	50 mg/L $\text{NH}_4\text{Cl-N}$, 0-100 mg/L sucrose, 27 g/L KH_2PO_4 , 500 mg/L NaHCO_3 , 180 mg/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 300 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.
Anammox biofilm	50 $\text{mg} \cdot \text{L}^{-1}$ $\text{NH}_4\text{Cl-N}$, 50 $\text{mg} \cdot \text{L}^{-1}$ $\text{NaNO}_2\text{-N}$, 27 $\text{mg} \cdot \text{L}^{-1}$ KH_2PO_4 , 500 $\text{mg} \cdot \text{L}^{-1}$ NaHCO_3 , 180 $\text{mg} \cdot \text{L}^{-1}$ $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 300 $\text{mg} \cdot \text{L}^{-1}$ $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The Trace elements were added ($1 \text{ mL} \cdot \text{L}^{-1}$) into the synthetic wastewater. The trace element solution contained 625 $\text{mg} \cdot \text{L}^{-1}$ EDTA, 190 $\text{mg} \cdot \text{L}^{-1}$ $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 430 $\text{mg} \cdot \text{L}^{-1}$ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 220 $\text{mg} \cdot \text{L}^{-1}$ $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 240 $\text{mg} \cdot \text{L}^{-1}$ $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 990 $\text{mg} \cdot \text{L}^{-1}$ $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, and 250 $\text{mg} \cdot \text{L}^{-1}$ $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.
BSgel system	50 $\text{mg} \cdot \text{L}^{-1}$ $\text{NH}_4\text{Cl-N}$, 100 mg/L sucrose, 27 $\text{mg} \cdot \text{L}^{-1}$ KH_2PO_4 , 500 $\text{mg} \cdot \text{L}^{-1}$ NaHCO_3 , 180 $\text{mg} \cdot \text{L}^{-1}$ $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 300 $\text{mg} \cdot \text{L}^{-1}$ $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Trace elements were added ($1 \text{ mL} \cdot \text{L}^{-1}$) into the synthetic wastewater. The trace element solution contained 625 $\text{mg} \cdot \text{L}^{-1}$ EDTA, 190 $\text{mg} \cdot \text{L}^{-1}$ $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 430 $\text{mg} \cdot \text{L}^{-1}$ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 220 $\text{mg} \cdot \text{L}^{-1}$ $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 240 $\text{mg} \cdot \text{L}^{-1}$ $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 990 $\text{mg} \cdot \text{L}^{-1}$ $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, and 250 $\text{mg} \cdot \text{L}^{-1}$ $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

Table S2. Definition of components in the model

Number	Component	Definition	Unit
Model dissolved components			
1	S_O	Dissolved oxygen	$\text{g O}_2 \text{ m}^{-3}$
2	S_S	Readily degradable organic substrate	g COD m^{-3}
3	S_{NH_4}	Ammonium nitrogen	g N m^{-3}
4	S_{NO_2}	Nitrite nitrogen	g N m^{-3}
5	S_{NO_3}	Nitrate nitrogen	g N m^{-3}
Model particulate components			
6	X_{AOB}	Aerobic ammonium-oxidizing bacteria	g COD m^{-3}
7	X_{AMX}	Anaerobic ammonium-oxidizing bacteria	g COD m^{-3}
8	X_{NOB}	Nitrite-oxidizing bacteria	g COD m^{-3}
9	X_H	Heterotrophic bacteria	g COD m^{-3}

10	X_I	Inert, non-biodegradable organics	g COD m^{-3}
----	-------	-----------------------------------	-----------------------

Table S3. Process kinetic rate equations for the model

Process	Kinetics rates expressions
1. Growth of AOB	$\mu_{AOB} \frac{S_{O_2}}{K_{O_2}^{AOB} + S_{O_2}} \frac{S_{NH_4}}{K_{NH_4}^{AOB} + S_{NH_4}} X_{AOB}$
2. Decay of AOB	$b_{AOB} X_{AOB}$
3. Growth of NOB	$\mu_{NOB} \frac{S_{O_2}}{K_{O_2}^{NOB} + S_{O_2}} \frac{S_{NO_2}}{K_{NO_2}^{NOB} + S_{NO_2}} X_{NOB}$
4. Decay of NOB	$b_{NOB} X_{NOB}$
5. Growth of Anammox	$\mu_{AMX} \frac{K_{O_2}^{AMX}}{K_{O_2}^{AMX} + S_{O_2}} \frac{S_{NH_4}}{K_{NH_4}^{AMX} + S_{NH_4}} \frac{S_{NO_2}}{K_{NO_2}^{AMX} + S_{NO_2}} X_{AMX}$
6. Decay of Anammox	$b_{AMX} X_{AMX}$
7. Aerobic growth of X_H	$\mu_H \frac{S_{O_2}}{K_{OH_1} + S_{O_2}} \frac{S_S}{K_{S_1} + S_S} X_H$
8. Anoxic growth of X_H with nitrite reduction	$\mu_H \eta_{H1} \frac{K_{OH_2}}{K_{OH_2} + S_{O_2}} \frac{S_{NO_3}}{K_{NO_3}^{HB} + S_S} \frac{S_S}{K_{S_2} + S_S} X_H$
9. Anoxic growth of X_H with nitrate reduction	$\mu_H \eta_{H2} \frac{K_{OH_3}}{K_{OH_3} + S_{O_2}} \frac{S_{NO_2}}{K_{NO_2}^{HB} + S_{NO_2}} \frac{S_S}{K_{S_3} + S_S} X_H$
10. Decay of X_H	$b_H X_H$

Table S4. Kinetic and stoichiometric parameters of the model

Parameter	Definition	Values	Unit	Source
<i>Aerobic ammonium oxidizing bacteria (AOB)</i>				
Y_{AOB}	yield coefficient for AOB	0.15	$\text{g COD g}^{-1} \text{N}$	Wiesmann, 1994
μ_{AOB}	maximum growth rate of AOB	0.0854	h^{-1}	Wiesmann, 1994
b_{AOB}	decay rate coefficient of AOB	0.0054	h^{-1}	Wiesmann, 1994
$K_{O_2}^{AOB}$	S_{O_2} affinity constant for AOB	0.6	g DO m^{-3}	Wiesmann, 1994
$K_{NH_4}^{AOB}$	S_{NH_4} affinity constant for AOB	2.4	g N m^{-3}	Wiesmann, 1994
<i>Nitrite oxidizing bacteria (NOB)</i>				
Y_{NOB}	yield coefficient for NOB	0.041	$\text{g COD g}^{-1} \text{N}$	Wiesmann, 1994
μ_{NOB}	maximum growth rate of NOB	0.0604	h^{-1}	Wiesmann, 1994
b_{NOB}	decay rate coefficient of NOB	0.0025	h^{-1}	Wiesmann, 1994

$K_{O_2}^{NOB}$	S_{O_2} affinity constant for NOB	2.2	g DO m ⁻³	Wiesmann, 1994
$K_{NO_2}^{NOB}$	S_{NO_2} affinity constant for NOB	5.5	g N m ⁻³	Wiesmann, 1994
<i>Anaerobic ammonium oxidizing bacteria (Anammox)</i>				
Y_{AMX}	yield coefficient for Anammox	0.159	g COD g ⁻¹ N	Strous et al., 1998
μ_{AMX}	maximum growth rate of Anammox	0.0030	h ⁻¹	Koch et al. 2000
b_{AMX}	decay rate coefficient of Anammox	0.00013	h ⁻¹	Hao et al., 2002
$K_{O_2}^{AMX}$	S_{O_2} inhibiting coefficient for Anammox	0.01	g DO m ⁻³	Strous et al., 1998
$K_{NH_4}^{AMX}$	S_{NH_4} affinity constant for Anammox	0.07	g N m ⁻³	Strous et al., 1998
$K_{NO_2}^{AMX}$	S_{NO_2} affinity constant for Anammox	0.05	g N m ⁻³	Hao et al., 2002
<i>Heterotrophic bacteria (HB)</i>				
Y_H	yield coefficient for X _H	0.6	g COD g ⁻¹ COD	Henze et al., 2000
μ_H	maximum growth rate of X _H	0.26	h ⁻¹	Koch et al., 2000
b_H	decay rate coefficient of X _H	0.008	h ⁻¹	Wiesmann, 1994
η_{H1}	anoxic growth factor for nitrate reduction	0.28	—	Hiatt et al., 2008
η_{H2}	anoxic growth factor for nitrite reduction	0.16	—	Hiatt et al., 2008
K_{OH1}	S_{O_2} affinity constant for aerobic growth	0.1	g DO m ⁻³	Hiatt et al., 2008
K_{OH2}	S_{O_2} inhibit constant for nitrate reduction	0.1	g DO m ⁻³	Hiatt et al., 2008
K_{OH3}	S_{O_2} inhibit constant for nitrite reduction	0.1	g DO m ⁻³	Hiatt et al., 2008
K_{S1}	S_S affinity constant for aerobic growth	20	g COD m ⁻³	Hiatt et al., 2008
K_{S2}	S_S affinity constant for nitrate reduction	20	g COD m ⁻³	Hiatt et al., 2008
K_{S3}	S_S affinity constant for nitrite reduction	20	g COD m ⁻³	Hiatt et al., 2008
$K_{NO_3}^{HB}$	S_{NO_3} affinity constant for HB	0.2	g N m ⁻³	Hiatt et al., 2008
<i>Other stoichiometric parameters</i>				
i_{NBM}	Nitrogen content of biomass	0.07	g N g ⁻¹ COD	Henze et al.,

				2000
i_{NXI}	Nitrogen content of X_I	0.02	g N g ⁻¹ COD	Henze et al., 2000
i_{NXS}	Nitrogen content of X_S	0.04	g N g ⁻¹ COD	Henze et al., 2000
f_I	Fraction of X_I in biomass decay	0.10	g COD g ⁻¹ COD	Henze et al., 2000

Table S5. Stoichiometric matrix for the model

Variable	S _{O2}	S _S	S _{NH4}	S _{NO2}	S _{NO3}	S _{N2}	X _S	X _H	X _{AOB}	X _{NOB}	X _{AMX}	X _I
Process	O ₂	COD	N	N	N	N	COD	COD	COD	COD	COD	COD
1	$-\frac{3.43 - Y_{AOB}}{Y_{AOB}}$		$-i_{NBM} - \frac{1}{Y_{AOB}}$	$\frac{1}{Y_{AOB}}$					1			
2			$i_{NBM} - i_{NXI}f_I$				$1 - f_I$		-1			f_I
3	$-\frac{1.14 - Y_{NOB}}{Y_{NOB}}$		$-i_{NBM}$	$-\frac{1}{Y_{NOB}}$	$\frac{1}{Y_{NOB}}$					1		
4			$i_{NBM} - i_{NXI}f_I$				$1 - f_I$			-1		f_I
5			$-i_{NBM} - \frac{1}{Y_{AMX}}$	$-\frac{1}{Y_{AMX}} - \frac{1}{1.14}$	$\frac{1}{1.14}$	$\frac{2}{Y_{AMX}}$					1	
6			$i_{NBM} - i_{NXI}f_I$				$1 - f_I$				-1	f_I
7	$-\frac{1 - Y_H}{Y_H}$	$-\frac{1}{Y_H}$	$-i_{NBM}$					1				
8		$-\frac{1}{Y_H}$	$-i_{NBM}$	$-\frac{1 - Y_H}{1.71Y_H}$		$\frac{1 - Y_H}{1.71Y_H}$		1				
9		$-\frac{1}{Y_H}$	$-i_{NBM}$		$-\frac{1 - Y_H}{2.86Y_H}$	$\frac{1 - Y_H}{2.86Y_H}$		1				
10			$i_{NBM} - i_{NXI}f_I$				$1 - f_I$	-1				f_I

Table S6. Bacterial community diversity index of three samples

	SS	Biofilm	BSgel
Sequences	71928	65824	72038
OTUs	492	566	542
Coverage	0.999	0.999	0.999
Chao1	531.48	616.32	605.42
ACE	532.23	613.73	604.78
Shannon	5.08	5.72	5.59
Simpson	0.916	0.954	0.950

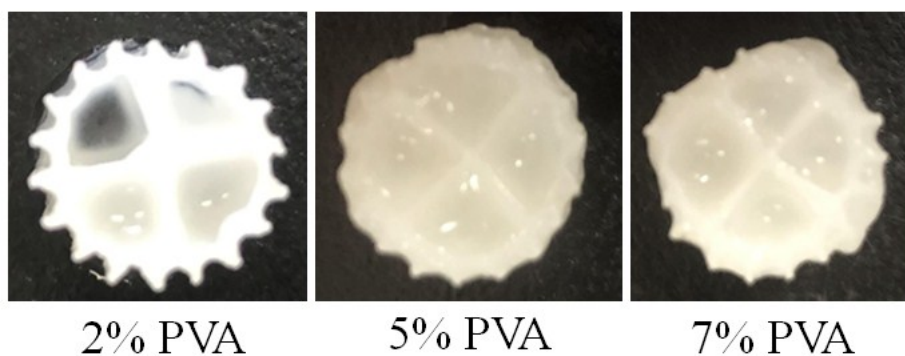


Figure S1 Images of BSgel system prepared by 2%, 5% and 7% PVA concentrations

Additional References

- Hao, X.D., Heijnen, J.J. and van Loosdrecht, M.C.M. (2002) Sensitivity analysis of a biofilm model describing a one-stage completely autotrophic nitrogen removal (CANON) process. *Biotechnology and Bioengineering* 77(3), 266-277.
- Hao, X.D., Heijnen, J.J. and van Loosdrecht, M.C.M. (2002) Model-based evaluation of temperature and inflow variations on a partial nitrification-ANAMMOX biofilm process. *Water Research* 36(19), 4839-4849.
- Henze, M. (2000) Activated sludge models ASM1, ASM2, ASM2d and ASM3, IWA publishing.
- Hiatt, W., and Grady, C. (2008) An updated process model for carbon oxidation, nitrification, and denitrification. *Water Environment Research* 80(11), 2145-2156.
- Koch, G., Egli, K., Van der Meer, J. and Siegrist, H. (2000) Mathematical modeling of autotrophic denitrification in a nitrifying biofilm of a rotating biological contactor. *Water Science and Technology* 41(4-5), 191-198.
- Strous, M., Heijnen, J., Kuenen, J. and Jetten, M. (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology* 50(5), 589-596.
- Wiesmann, U. (1994) Biological nitrogen removal from wastewater. In *Biotechnics/Wastewater*, pp. 113-154, Springer Berlin Heidelberg.