SUPPLEMENTARY INFORMATION

Impact of cell cluster size on apparent half-saturation coefficients for oxygen in nitrifying sludge and biofilms

Cristian Picioreanu, Julio Pérez*, Mark C.M. van Loosdrecht

Department of Biotechnology, Faculty of Applied Sciences, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands. (C.Picioreanu@tudelft.nl, J.O.PerezCanestro@tudelft.nl, M.C.M.vanLoosdrecht@tudelft.nl)

* Present address: Department of Chemical Engineering, School of Engineering, Universitat Autònoma de Barcelona, Barcelona 08193, Spain

Corresponding author: Cristian Picioreanu

Address: Department of Biotechnology, Faculty of Applied Sciences, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands.

Email: C.Picioreanu@tudelft.nl

CONTENT

Table S1. Number of colonies of each size in simulations with distributed colony size.

Colony size (μm)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AOB-MBR	31	66	60	45	31	21	14	10	7	5	3	2	2	1	1
NOB-MBR	234	190	99	49	25	13	7	4	2	1	1	1	0	0	0
AOB-CAS	12	31	34	29	24	18	14	11	8	6	5	4	3	2	2
NOB-CAS	974	479	187	76	33	15	7	4	2	1	1	0	0	0	0

Table S2. Literature review of oxygen half-saturation coefficients and growth yields of AOB and NOB

$K_{O,AOB}$ (mgO_2/L)	Genus/Species	Conditions	Reference
0.30	*Nitrosomonas*	20 °C, pH 7.9	(Loveless and Painter, 1968)
0.25	*Nitrosomonas europaea*		(Peeters et al., 1969)
0.5	*Nitrosomonas*		(Laudelout et al., 1974)
0.32	Ammonium oxidizers	25 °C, pH 7.3-7.8	(Hanaki et al., 1990)
0.04 (high μ) 0.48 (low μ)	*Nitrosomonas europaea* ATCC (axenic)	25 °C, pH 7.5	(Laanbroek and Gerards, 1993)
0.27	*Nitrosomonas europaea*	25 °C, pH 7.5	(Laanbroek et al., 1994)
0.16	*Nitrosomonas* ATCC(axenic)	30 °C	(Hunik et al., 1994)
0.18	*Nitrosomonas oligotropha*	20 °C, pH 7.5	(Manser et al., 2005)
0.07		30 °C, pH 7.8	(Sliekers et al., 2005)
0.24	*Nitrosomonas europaea*		(Park and Noguera, 2007)
1.22	*Nitrosomonas oligotropha*		(Park and Noguera, 2007)
0.033	*Nitrosomonas*	20 °C	(Blackburne et al., 2008)
0.1	*Nitrosospira* enrichment	30 °C	(Terada et al., 2013)
$K_{O,NOB}$ (mgO_2/L)	**Species**	**Conditions**	**Reference**
1.84	*Nitrobacter winogradskyi*		(Peeters et al., 1969)
2	*Nitrobacter*		(Laudelout et al., 1974)
0.7	*Nitrobacter winogradskyi*	25 °C, pH 7.5	(Laanbroek and Gerards, 1993)
0.8	*Nitrobacter hamburgensis*	25 °C, pH 7.5	(Laanbroek et al., 1994)
0.54	*Nitrobacter agilis*	30 °C	(Hunik et al., 1994)
0.13	*Nitrospira*	20 °C, pH 7.5	(Manser et al., 2005)
0.04		30 °C, pH 7.8	(Sliekers et al., 2005)
0.54	*Nitrospira*	20 °C ?	(Blackburne et al., 2007b)
0.43	*Nitrobacter*	20 °C ?	(Blackburne et al., 2008)
Y_{AOB} (gCOD/gN-NH_4)	**Species**	**Conditions**	**Reference**
0.071	*Nitrosomonas*		(Downing et al., 1964; Knoles et al., 1965)
0.284	*Nitrosomonas*		(Beccari et al., 1979)
0.20	*Nitrosomonas* ATCC		(Belser and Schmidt, 1980)
0.24	*Nitrosospira* AV2		(Belser and Schmidt, 1980)
0.10	*Nitrosolobus* AV3		(Belser and Schmidt, 1980)
0.241	*Nitrosomonas europaea*		(Keen and Prosser, 1987)
0.213	*Nitrosomonas*	Literature and measurements	(Wiesmann, 1994)
0.288	*Nitrosomonas*	“True” yield	(Chandran and Smets, 2001)
0.198	*Nitrosomonas*		(Blackburne et al., 2007a)
0.18	Ammonium oxidizers		(Jubany et al., 2008)
0.15	*Nitrosomonas*	Observed	(Ahn et al., 2008)
Y_{NOB} (gCOD/gN-NO_2)	**Species**	**Conditions**	**Reference**
0.03	*Nitrobacter*		(Boon and Landelout, 1962)
0.03	NOB in act. sludge		(Knoles et al., 1965)
0.12	*Nitrobacter*		(Stratton and McCarty, 1967)
0.03			(Alleman, 1985; Beccari et al., 1979)
0.198	*Nitrobacter*		(Keen and Prosser, 1987)
0.06	Average nitrifiers		(Wiesmann, 1994)
0.114			(Chandran and Smets, 2001)
0.102	*Nitrobacter*		(Blackburne et al., 2007a)
0.213	*Nitrospira*		(Blackburne et al., 2007b)
0.04	*Nitrobacter*	Observed	(Ahn et al., 2008)
0.08	*Nitrobacter*		(Jubany et al., 2008)
0.064		yield per cell	(Nowka et al., 2015)

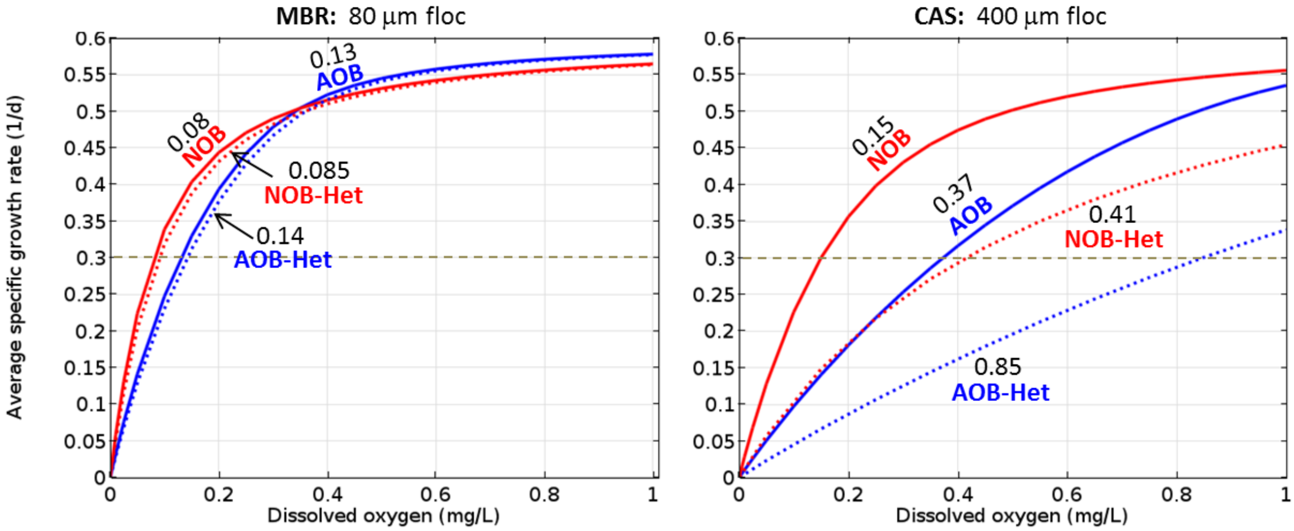

Figure S1. Effect of heterotrophs on the apparent oxygen half-saturation coefficients.

Average specific growth rates are presented function of dissolved oxygen concentration for a small (80 µm) and for a large (400 µm) floc, as resulted from the 3-D heterogeneous model with uniform size of AOB and NOB colonies. *Blue curves*: AOB; *Red curves*: NOB. *Thick continuous lines*: only AOB and NOB microcolonies; *Dotted lines*: uniformly distributed heterotrophs besides AOB and NOB microcolonies.

The simulations include the same model parameters as for the results presented in Figure 2, with the additional $C_{x,H}$=20 g/L (local concentration of heterotrophs in the floc), Y_H=0.67 gCOD/gCOD (yield biomass produced from substrate), $K_{O,H}$=0.2 mgO_2/L (half-saturation coefficient for heterotrophs) and $\mu_{\max,H}$=5 1/d (maximum specific growth rate of heterotrophs). Numbers on the graphs represent the calculated half-saturation coefficients, $K_{O,AOB,3d}$ and $K_{O,NOB,3d}$, in mg O_2/L, for each case with or without heterotrophs.

Although the background oxygen consumption by heterotrophs affected oxygen uptake rates of both AOB and NOB leading to increased values of $K_{O,AOB,3d}$ and $K_{O,NOB,3d}$, the difference between these values would not be much impacted by the presence of heterotrophs. The inversion of apparent oxygen half-saturation coefficients ($K_{O,AOB,3d}$ > $K_{O,NOB,3d}$) compared with the intrinsic ones ($K_{O,AOB}$ < $K_{O,NOB}$) is exactly the same.

REFERENCES

Ahn, J.H., Yu, R., Chandran, K., 2008. Distinctive microbial ecology and biokinetics of autotrophic ammonia and nitrite oxidation in a partial nitrification bioreactor. Biotechnol. Bioeng. 100, 1078–1087. doi:10.1002/bit.21863

Alleman, J.E., 1985. Elevated Nitrite Occurrence in Treatment Systems. Water Sci. Technol. 17, 409–419.

Beccari, M., Marani, D., Ramadori, R., 1979. A critical analysis of nitrification alternatives. Water Res. 13, 185–192. doi:10.1016/0043-1354(79)90091-5

Belser, L.W., Schmidt, E.X., 1980. Growth and Oxidation Kinetics of Three Genera of Ammonia Oxidizing Nitrifiers. FEMS Microbiol. Lett. 7, 213–216. doi:10.1111/j.1574-6941.1980.tb01628.x

Blackburne, R., Vadivelu, V.M., Yuan, Z., Keller, J., 2007b. Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter. Water Res. 41, 3033–3042. doi:10.1016/j.watres.2007.01.043

Blackburne, R., Vadivelu, V.M., Yuan, Z., Keller, J., 2007a. Determination of growth rate and yield of nitrifying bacteria by measuring carbon dioxide uptake rate. Water Environ. Res. 79, 2437–2445. doi:10.2175/106143007X212139

Blackburne, R., Yuan, Z., Keller, J., 2008. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation 19, 303–312. doi:10.1007/s10532-007-9136-4

Boon, B., Landelout, H., 1962. Kinetics of the nitrite oxidation by Nitrobacter winogradskyi. Biochem. J. 85, 440–447. doi:10.1042/bj0850440

Chandran, K., Smets, B.F., 2001. Estimating biomass yield coefficient for autotrophic ammonia and nitrite oxidation from batch experiments. Water Res. 35, 3153–3156.

Downing, A.L., Painter, H.A., Knoles, G., 1964. Nitrification in the activated sludge process. J. Inst. Sew. Purif. 2, 130–158.

Hanaki, K., Wantawint, C., Ohgaki, S., 1990. Effects of the activity of heterotrophs on nitrification in a suspended growth reactor.pdf 24, 289–296.

Hunik, J.H., Bos, C.G., Van der Hoogen, M.P., De Gooijer, C.D., Tramper, J., 1994. Co-lmmobilized Nitrosomonas europaea and Nitrobacter agilis Cells: Validation of a Dynamic Model for Simultaneous Substrate Conversion and Growth in K-Carrageenan Gel Beads. Biotechnol. Bioeng. 43, 1153–1163.

Jubany, I., Carrera, J., Lafuente, J., Baeza, J.A., 2008. Start-up of a nitrification system with automatic control to treat highly concentrated ammonium wastewater: Experimental results and modeling. Chem. Eng. J. 144, 407–419. doi:10.1016/j.cej.2008.02.010

Keen, G.A., Prosser, J.I., 1987. Steady state and transient growth of autotrophic nitrifying bacteria. Arch. Microbiol. 147, 73–79. doi:10.1007/BF00492908

Knoles, G., Downing, A.L., Barrett, M.J., 1965. Determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of an electronic computer. J. Gen. Microbiol. 38, 263–278.

Laanbroek, H.J., Bodelier, P.L.E., Gerards, S., 1994. Oxygen consumption kinetics of Nitrosomonas europaea and Nitrobacter hamburgensis grown in mixed continuous cultures at different oxygen concentrations. Arch. Microbiol. 161, 156–162. doi:10.1007/s002030050036

Laanbroek, H.J., Gerards, S., 1993. Competition for limiting amounts of oxygen between Nitrosomonas europaea and Nitrobacter winogradskyi grown in mixed continuous cultures. Arch. Microbiol. 159, 453–459. doi:10.1007/BF00288593

Laudelout, H., Lambert, R., Fripiat, J.L., Pham, M.L., 1974. Effect of temperature in the velocity of oxidation of ammonia to nitrate in mixed nitrifier culture. Ann. Microbiol . 125B, 75–84.

Loveless, J., Painter, H., 1968. The influence of metal ion concentrations and pH value on the growth of a Nitrosomonas strain isolated from activated sludge. J. Gen. Microbiol. 52, 1–14.

Manser, R., Gujer, W., Siegrist, H., 2005. Consequences of mass transfer effects on the kinetics of nitrifiers. Water Res. 39, 4633–4642. doi:10.1016/j.watres.2005.09.020

Nowka, B., Daims, H., Spieck, E., 2015. Comparison of oxidation kinetics of nitrite-oxidizing bacteria: Nitrite availability as a key factor in niche differentiation. Appl. Environ. Microbiol. 81, 745–753. doi:10.1128/AEM.02734-14

Park, H.D., Noguera, D.R., 2007. Characterization of two ammonia-oxidizing bacteria isolated from reactors operated with low dissolved oxygen concentrations. J. Appl. Microbiol. 102, 1401–1417. doi:10.1111/j.1365-2672.2006.03176.x

Peeters, T.L., Van Goal, A.D., Landelout, H., 1969. Kinetics study of oxygen limited respiration in nitrifying bacteria. Bact. Proc. 58, 141.

Sliekers, A.O., Haaijer, S.C.M., Stafsnes, M.H., Kuenen, J.G., Jetten, M.S.M., 2005. Competition and coexistence of aerobic ammonium- and nitrite-oxidizing bacteria at low oxygen concentrations. Appl. Microbiol. Biotechnol. 68, 808–817. doi:10.1007/s00253-005-1974-6

Stratton, F.E., McCarty, P.L., 1967. Prediction of Nitrification Effects on the Dissolved Oxygen Balance of Streams. Environ. Sci. Technol. 1, 405–410. doi:10.1021/es60005a003

Terada, A., Sugawara, S., Yamamoto, T., Zhou, S., Koba, K., Hosomi, M., 2013. Physiological characteristics of predominant ammonia-oxidizing bacteria enriched from bioreactors with different influent supply regimes. Biochem. Eng. J. 79, 153–161. doi:10.1016/j.bej.2013.07.012

Wiesmann, U., 1994. Biological Nitrogen Removal from Wastewater. Adv. Biochem. Eng. Biotechnol. 51, 113–154. doi:10.1007/BFb0008736