

Master's Thesis Dissertation in Bioengineering

Modelling niche differentiation among anammox bacteria and partial-nitrification process for mainstream saline wastewater treatment

David Morgado Pereira

FEUP Supervisor: Professor Manuel Simões

KAUST Supervisor: Professor Pascal Saikaly

KAUST Co-Supervisor: Dr. Muhammad Ali

Water Desalination and Reuse Center - KAUST

Integrated Master in Bioengineering - Master Branch of Biological Engineering

September 2020

"The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny...'"

Isaac Asimov

"Extraordinary claims require extraordinary evidence."

Carl Sagan

Acknowledgments

I would like to thank Prof. Pascal Saikaly for accepting my application to develop my thesis under your supervision at KAUST. Thank you for your guidance, expertise and insights throughout this journey. Your ~~door~~ zoom was always open whenever I needed to solve any issue.

I would also like to acknowledge Dr. Muhammed Ali. I am grateful for your help in my only month in the laboratory, for your availability whenever I ran into trouble or had a question about my research and writing and also for your fruitful inputs on my dissertation.

I wish to express my sincere appreciation to Prof. Manuel Simões, for your support not only on the development of this project but also throughout my academic path at FEUP.

I would thank KAUST for providing the opportunity to enroll in the Visiting Student Research Program and for making sure my life in the city was safe and welcoming during these unfortunate times.

I wish to thank all the friends I have made during my time in KAUST; even though COVID-19 made things harder for everyone, we managed to create a great family and support each other. I would like to pay my special regards to Eduardo. He embraced this adventure with me, both at FEUP and KAUST, and was the perfect quarantine roommate I could have asked for (amazing cooking skills!).

For all my friends, that during these years at FEUP, made sure there was always time to not focus on studying: you know who you are, and I am genuinely happy about that, but you owe me! And for those who were always there for me whenever I was struggling, thank you for your help. I owe you!

To Filipa, for all the support, patience and affection, that despite the distance, was always present and helped me in everything I needed! Thank you so much!

To my parents and sister, I must express my deepest gratitude for the unconditional support and continuous encouragement throughout my years of study. Now you can stop asking how much longer it is going to take to finish. It's done! Obrigado por tudo!

Lastly, to SARS-COV2, for attempting to ruin this work: nice try!

Declaration

I declare, under honour, that this work is original and that all non-original contributions were duly referenced with identification of the source.

David Morgado Pereira
28/09/2020

Preface

I joined professor Pascal group in February 2020 to work on an important research topic related to the integration of partial-nitrification and anaerobic ammonium oxidation (anammox) process with granular activated carbon (GAC) for the treatment of ammonium from mainstream wastewater. The initial project was designed for 5 months. It involved the operation of two sequential batch reactors (one with GAC and another without GAC) in evaluating their nitrogen removal efficiency (daily sampling for water quality measurements) and identify differences in the microbial communities (bi-weekly sampling of the granules and GAC) between the reactors.

In the first month at KAUST, I took safety training to work in the lab, had the training to work with specific equipment, learned how to set up the bioreactors, conducted preliminary experiments with GAC, and performed some water quality measurements. However, by the end of March, as a consequence of the COVID-19 pandemic, all the the experimental work at KAUST was inevitably postponed (except critical experiments). During this period, KAUST leadership requested all students to stay at home. Initially, the lockdown was planned until mid-April, but at beginning of May, all the laboratories were requested to stop their activities. Even though a gradual reopening eventually occurred, students were not allowed to return to the laboratory before September.

After the request at the beginning of May and given that the preliminary data that was collected would be insufficient for my thesis, an alternative master thesis topic was discussed between me, prof. Pascal Saikaly and prof. Manuel Simões. This new topic would still focus on partial-nitrification and anammox but as a theoretical approach by developing a mathematical model of the system. Therefore, those are the starting points of the dissertation I am presenting here.

Abstract

Clean water scarcity is recognized as a major threat to humane activity and it is expected to aggravate by 2050. Seawater has been suggested as a suitable alternative to freshwater, with the possibility of being directly utilized for non-potable uses such as toilet flushing. However, the salinity from the generated wastewater could affect the performance of conventional biological nitrogen removal (BNR) processes. The partial-nitrification and anaerobic ammonium oxidation (anammox) (PN/A) process is considered as one of the most promising and efficient processes for BNR and has the potential of treating saline wastewater. However, due to the slow growth of these bacteria, time-consuming experiments have to be performed before one can draw any conclusion.

Therefore, one-dimensional biofilm models were developed using the AQUASIM software to understand the niche differentiation among anammox bacteria and their cooperation and competition in the PN/A process with aerobic ammonia-oxidizing bacteria (AOB) and archaea (AOA), and nitrite-oxidizing bacteria (NOB). The main goal was to determine the microbial community, bioreactor configuration and operational conditions that achieve the highest nitrogen removal efficiency (NRE) for mainstream saline wastewater treatment. Two bioreactor configurations were used: a granular sequencing batch reactor (GSBR) and a membrane-aerated biofilm reactor (MABR). The influent composition was set to simulate mainstream wastewater conditions at 25 °C: pre-treated sewage (no carbon), 1.7% salinity and a nitrogen loading rate (NLR) of $0.3 \text{ mg}_N \text{ L}^{-1} \text{ d}^{-1}$ (influent concentration of $50 \text{ mg}_{\text{NH}_4^+ - \text{N}} \text{ L}^{-1}$).

The fittest anammox bacteria for saline wastewater treatment were “*Candidatus Kuenenia stuttgartiensis*” and “*Candidatus Scalindua* sp.” In the GSBR, the maximum NRE obtained was 92%, at optimal dissolved oxygen (DO) concentration of $0.40 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$, with granules of AOB and anammox bacteria, while suppressing NOB. A sensitivity analysis for $K_{\text{O}_2}^{\text{NOB}} / K_{\text{O}_2}^{\text{AOB}}$ was performed and it has revealed that NOB suppression was always possible under low DO concentrations ($<0.50 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$). Due to the salinity conditions, marine AOA was considered in the system. In this scenario, AOA outcompeted both AOB and anammox bacteria and the NRE was lower than 90%. Cooperation between AOA and anammox bacteria was evaluated through counter-diffusion of oxygen and ammonium using an MABR. The NRE obtained was 90%, using a wider range of oxygen concentrations. AOA did not cooperate with AOB in any simulation.

Keywords – Partial-nitrification anammox (PN/A), Ammonia-Oxidizing Archaea (AOA), Granular Sequencing Batch Reactor (GSBR), Membrane-Aerated Biofilm Reactor (MABR), AQUASIM

Resumo

A escassez de água potável é reconhecida como uma das principais ameaças à actividade humana e é esperado que se agrave até 2050. A água do mar tem vindo a ser sugerida como uma alternativa sustentável à água potável, uma vez que pode ser utilizada diretamente para usos não-potáveis, tais como para descarga em uso sanitário. No entanto, a elevada salinidade das águas residuais provenientes da utilização doméstica pode limitar o desempenho dos processos convencionais de remoção biológica de nitrogénio (RBN). O processo de nitrificação parcial e oxidação anaeróbia de amónia (*anammox*) (NP/A) é considerado um dos mais promissores e eficientes processos de RBN e tem potencial para o tratamento de águas residuais salinas. No entanto, devido ao crescimento lento destas bactérias, quaisquer conclusões em relação à sua eficiência estão dependentes da realização de experiências demoradas.

Consequentemente, foram desenvolvidos modelos de biofilme unidimensional utilizando o software AQUASIM que visou compreender a diferenciação de nicho entre bactérias *anammox* e a sua cooperação e competição no processo PN/A com bactérias aeróbias oxidantes de amoníaco (AOB) e nitrito (NOB), e archaea oxidantes de amoníaco (AOA). O principal objetivo foi determinar a comunidade microbiana, a configuração do biorreator e as condições operacionais que permitem obter a maior eficiência de remoção de nitrogénio (ERN) para o tratamento de águas residuais salinas. Foram utilizadas duas configurações de biorreator: reator batch sequencial de grânulos (RBSG) e um reator de biofilme aerado por membrana (MABR). A composição do influente foi definida para simular as condições de águas residuais principais a 25 °C: esgotos pré tratados (sem carbono), 1.7% de salinidade e uma taxa de carga de nitrogénio de $0.3 \text{ mg}_N \text{ L}^{-1} \text{ d}^{-1}$ (concentração de influente $50 \text{ mg}_{\text{NH}_4^+ - N} \text{ L}^{-1}$).

As bactérias *anammox* mais adequadas para o tratamento de águas residuais salinas foram "*Candidatus Kuenenia stuttgartiensis*" e *Candidatus Scalindua sp.* No RBSG, ERN máxima de 92% foi obtida, a uma concentração ótima de oxigénio dissolvido (OD) de $0.40 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$, com grânulos de AOB e *anammox*, e com supressão das NOB. Uma análise de sensibilidade para $K_{\text{O}_2}^{\text{NOB}} / K_{\text{O}_2}^{\text{AOB}}$ foi realizada e revelou que a supressão de NOB foi sempre possível sob baixas concentrações de OD ($<0.50 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$). Devido às condições de salinidade, AOA marinhas foram consideradas no sistema. Neste caso, as AOA superaram tanto as AOB como as bactérias *anammox*, e a ERN foi inferior a 90%. A cooperação entre AOA e as bactérias *anammox* foi avaliada através de contra-difusão de oxigénio e amónio, utilizando um MABR. A ERN foi de 90%, usando uma gama mais ampla de concentrações de oxigénio. AOA e AOB não cooperaram em nenhuma simulação.

Keywords – Nitrificação Parcial e *anammox* (NP/A), Archaea Oxidantes de Amoníaco (AOA), Reator Batch Sequencial de Grânulos (RBSG), Reator de Biofilme por Membrana Aerada (MABR), AQUASIM

Contents

1	Introduction	1
2	Literature Review	5
2.1	Microbial community	5
2.1.1	Ammonia-Oxidizing Bacteria (AOB)	5
2.1.2	Nitrite-Oxidizing Bacteria (NOB)	6
2.1.3	Ammonia-Oxidizing Archaea (AOA)	6
2.1.4	Anammox Bacteria	7
2.2	Partial-Nitrification Anammox (PN/A)	10
2.2.1	Advantages of partial-nitrification anammox	11
2.2.2	Mainstream anammox	11
2.2.3	Bioreactor configurations	13
2.2.4	Suppression of nitrite-oxidizing bacteria	14
3	Biofilm modelling	17
3.1	AQUASIM	18
3.2	Objectives of this work	19
4	Materials and Methods	21
4.1	Reactor parameters	21
4.2	Niche differentiation among anammox bacteria	21
4.3	PN/A in a granular sequencing batch reactor (GSBR)	23
4.4	PN/A in a membrane-aerated biofilm reactor (MABR)	24
4.5	Biofilm thickness	25
4.5.1	Model Resolution	26
4.6	Kinetic parameters	26
4.7	AQUASIM implementation	27
4.7.1	GSBR	27
4.7.2	MABR	28
5	Results and Discussion	31
5.1	Niche differentiation among anammox bacteria	31
5.1.1	Optimal conditions	31
5.1.2	Saline conditions	33
5.2	PN/A-GSBR	35
5.2.1	Scenario 1: $K_{O_2}^{NOB} > K_{O_2}^{AOB}$	35
5.2.2	Scenario 2: $K_{O_2}^{NOB} < K_{O_2}^{AOB}$	39
5.3	PN/A-GSBR including AOA	42
5.4	PN/A-MABR	45
6	Conclusions	49
6.1	Future work	50
	References	51
	Appendix	61
A1	Free ammonia	61
A2	Model description	62
A3	Model considerations	65
A4	Mass transfer parameters	68
A5	MABR	69
A6	Kinetic adaptation to salinity	69

List of Figures

Figure 1	Decline in urban water availability (adapted from The United Nations World Water Development Report 2020).	1
Figure 2	Schematic representation of the nitrification/denitrification process and partial-nitrification anammox (red dotted line), based on Castro-Barros et al. (2017). Microbial community: anammox bacteria (blue), ammonia oxidizers (green), nitrite oxidizer bacteria (red) and heterotrophic bacteria (grey).	2
Figure 3	Schematic diagram of A-B stage adapted from McCarty (2018).	12
Figure 4	Confocal laser scanning microscope (CLSM) image from Ali et al. (2016) showing the <i>in situ</i> spatial organization of AOB (green), NOB (red) and anammox bacteria (blue) in a PN/A granule; Schematic representation of the interaction between bacteria and substrate inside the PN/A granule.	16
Figure 5	The lab-scale partial-nitrification and anammox bioreactor (working volume of 3.5 L) in the Water Desalination and Reuse Center (WDRC) laboratory at KAUST. Schematic representation of the SBR cycle and of the co-diffusion of oxygen and ammonium in the granular biofilm.	23
Figure 6	Schematic representation of the duration of each stage in the sequencing batch reactor cycle.	23
Figure 7	Schematic representation of a membrane-aerated biofilm reactor.	24
Figure 8	Schematic diagram of the granule-based SBR as implemented in the AQUASIM using the advective approach.	27
Figure 9	Schematic diagram of the granule-based SBR as implemented in the AQUASIM using the diffusive approach.	28
Figure 10	Schematic diagram of the MABR as implemented in the AQUASIM.	29
Figure 11	Steady-state distribution of anammox bacteria competing in a granule at different nitrogen loading rates. The centre of the granule is represented at 0 mm and the substrate (S_{NO_2} and S_{NH_4}) diffuses to the granule at 0.75 mm. At NLR of $0.1 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$, the growth of the biomass was slower and the simulation was stopped when the granule radius reached 0.50 mm.	31
Figure 12	Steady-state distribution of anammox bacteria competing in a granule at different nitrogen loading rates, with a salinity effect implemented in the microbial kinetics (Equations A6 and A7). The centre of the granule is represented at 0 mm and the substrate (S_{NO_2} and S_{NH_4}) diffuses to the granule at 0.75 mm. At NLR of $0.1 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$, the growth of the biomass was slower and the simulation was stopped when the granule radius reached 0.50 mm.	34
Figure 13	Nitrogen fractions in the effluent at different DO concentrations, for $K_{\text{O}_2}^{\text{NOB}} > K_{\text{O}_2}^{\text{AOB}}$. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).	35
Figure 14	Operation of the sequencing batch reactor cycle at $S_{\text{O}_2} = 0.40 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$: a) at 50 days (not steady-state); b) at 1500 days (steady-state). Aeration is switched on at 24 min and switched off at 192 min of the cycle. Nitrogen fractions concentration are read on the left y axis while dissolved oxygen concentration is read on the right y axis.	36
Figure 15	a) Steady-state distribution of biomass and particulate inerts in a granule at the optimal oxygen concentration for which maximum nitrogen removal is achieved ($S_{\text{O}_2} = 0.40 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$); b) Close up (0.60 - 0.75 mm) of the gradient profile of oxygen (dotted line) in the biofilm. The centre of the granule is represented at a granule radius of 0 mm; substrate diffuses in co-diffusion to the granule at 0.75 mm.	37
Figure 16	Comparison of dynamic model behaviour under the influence of bulk oxygen concentrations of 0.20, 1 and $2 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$: a), c) and e) Steady-state distribution of biomass and particulate inerts in the granule; the centre of the granule is represented at a granule radius of 0 mm; substrate diffuses in co-diffusion to the granule at 0.75 mm. b), d) and f) Evolution of effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas. Nitrogen gas fraction represents the NRE. At $S_{\text{O}_2} = 0.20 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$, steady-state was not reached even after 2000 days of operation. At the other oxygen concentrations, steady-state was reached after 1000 days of operation.	38

Figure 17	Nitrogen fractions in the effluent at different DO concentrations, for $K_{O_2}^{NOB} < K_{O_2}^{AOB}$. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).	39
Figure 18	Evolution of bulk concentrations of ammonium, nitrite, nitrate and nitrogen gas between the two scenarios (different $K_{O_2}^{NOB}$) at different bulk oxygen concentrations. Nitrogen gas fraction represents the NRE.	40
Figure 19	Nitrogen fractions in the effluent at different DO concentrations, considering AOA in the model. $S_{NH_4in} = 50 \text{ mg}_{N-NH_4^+} \text{ L}^{-1}$. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).	42
Figure 20	Comparison of dynamic model behaviour under the influence of bulk oxygen concentrations between 0.05 and 0.20 $\text{mg}_{O_2} \text{ L}^{-1}$: a), c), e) and g) Steady-state distribution of biomass (with AOA, AOB, NOB and anammox bacteria) and particulate inerts; the centre of the granule is represented at a granule radius of 0 mm; substrate diffuses in co-diffusion to the granule at 0.75 mm. b), d), f) and h) Evolution of effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas. Nitrogen gas fraction represents the nitrogen removal efficiency. Steady-state was reached after 2000 days of operation.	43
Figure 21	Nitrogen fractions in the effluent of a membrane-aerated biofilm reactor, at different $J_{O_2}/J_{NH_4^+}$ ratios. $S_{NH_4in} = 50 \text{ mg}_{N-NH_4^+} \text{ L}^{-1}$. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).	45
Figure 22	Optimal nitrogen removal for a $J_{O_2}/J_{NH_4^+}$ ratio of 1.85: a) Distribution of biomass (with AOA and anammox bacteria) and particulate inerts in the membrane biofilm; AOB and NOB were outcompeted. b) Evolution of effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas. c) Gradient profiles of substrate concentration in the counter-diffusion biofilm. Oxygen is supplied at a distance from the membrane of 0 mm and ammonium diffuses in counter-diffusion at 0.75 mm. d) Close up (0-0.15 mm) of the substrate gradient profiles and AOA biomass fraction in the biofilm.	46
Figure 23	Nitrate accumulation due to the presence of NOB for a $J_{O_2}/J_{NH_4^+} = 4$: a) Distribution of biomass (with AOA, NOB and anammox bacteria) and particulate inerts in the membrane biofilm; AOB was outcompeted. Oxygen is supplied at a distance from the membrane of 0 mm and ammonium diffuses in counter-diffusion at 0.75 mm. b) Evolution of effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas.	47
Figure 24	Membrane-aerated biofilm reactor not including AOA. Nitrogen fractions in the effluent at different $J_{O_2}/J_{NH_4^+}$ ratios. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).	47
Figure 25	MABR not considering AOA. Distribution of biomass (with AOB, NOB and anammox bacteria) and particulate inerts on the membrane biofilm: a) for an optimal $J_{O_2}/J_{NH_4^+}$ ratio of 1.85 and b) for a $J_{O_2}/J_{NH_4^+}$ ratio of 4. Oxygen is supplied at a distance from the membrane of 0 mm and ammonium diffuses in counter-diffusion at 0.75 mm.	48
Figure A1	The model-fitted relationship between specific ammonium consumption rate and different salinity levels, for “ <i>Ca. Scalindua sp.</i> ” and “ <i>Ca. Kuenenia stuttgartiensis</i> ”, using a Gaussian equation (Wu et al., 2019). Modified Boltzmann equation predicting inhibition of “ <i>Ca. Brocadia sinica</i> ” at different seawater ratios (Li et al., 2018a).	70

List of Tables

Table 1	Anammox bacteria species competing in the model and respective kinetic parameters.	22
Table 2	Interaction between particulate species and dissolved components in the biofilm . .	26
Table 3	$\mu_{\max}$ implemented in the model for a salinity of 1.70%.	33
Table 4	Nitrogen fractions in the effluent for a dissolved oxygen concentration between 0.10 and 0.15 mgO ₂ L ⁻¹ , considering an influent of $S_{\text{NH}_4\text{in}} = 50 \text{ mg}_{\text{NH}_4^+-\text{N}} \text{ L}^{-1}$	44
Table A1	Free ammonia concentration at different pH, for $S_{\text{NH}_4\text{in}} = 50 \text{ mg}_{\text{NH}_4^+-\text{N}} \text{ L}^{-1}$ and T = 25 °C.	61
Table A2	State variables used in the model.	62
Table A3	Process kinetic rate equations.	63
Table A4	Stoichiometric matrix of the model.	64
Table A5	Literature review on the oxygen affinity constant of AOB (<i>Nitrosomonas</i>) and NOB (<i>Nitrospira/Nitrobacter</i>).	66
Table A6	Kinetic and stoichiometric parameters of the model.	67
Table A7	Diffusion coefficients.	68
Table A8	Literature review on partial-nitrification anammox in membrane-aerated biofilms reactors.	69
Table A9	Fitting parameters of Figure A1.	70
Table A10	Literature review on recent studies for wastewater treatment with marine and freshwater anammox bacteria at different salinities.	71

Glossary

List of abbreviations

Term	Definition
AMO	Ammonia Monooxygenase
AMX	Anammox Bacteria
AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
ASM	Activated Sludge Models
BNR	Biological Nitrogen Removal
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred-Tank Reactor
DO	Dissolved Oxygen
GBRMP	Good Biofilm Reactor Modeling Practice
GSBR	Granular Sequencing Batch Reactor
HRT	Hydraulic Retention Time
IFAS	Integrated Fixed-film Activated Sludge
MABR	Membrane-Aerated Biofilm Reactor
MBBR	Membrane Bed Biofilm Reactor
MBR	Membrane Reactor
N/DN	Nitrification and Denitrification
NLR	Nitrogen Loading Rate
NOB	Nitrite Oxidizing Bacteria
NRE	Nitrogen Removal Efficiency
NXR	Nitrate Oxidoreductase enzyme
OMZ	Oxygen Minimum Zone
PN/A	Partial-nitritation anammox
SBR	Sequencing Batch Reactor
SRT	Solid Retention Time
UASB	Upflow Anaerobic Sludge Blanket
VSS	Volatile Suspended Solids
WW	Wastewater
WWTP	Wastewater Treatment Plant

List of symbols

Term	Definition	Dimension
ϵ_l	Liquid biofilm fraction	-
ϵ_s	Solid biofilm fraction	-
$\mu_{\max}$	Maximum growth rate	T^{-1}
b	Decay rate coefficient	T^{-1}
D_i	Diffusion coefficient implemented on AQUASIM	$L^2 T^{-1}$
E_a	Activation energy	$M L^2 T^{-2} N^{-1}$
H_{O_2}	Non-dimensional Henry's law coefficient for oxygen	-
$J_{NH_4^+}$	Ammonium flux	$M_N L^{-2} T^{-1}$
J_{O_2}	Oxygen flux	$M_{O_2} L^{-2} T^{-1}$
K_i	Affinity constant for substrate i	$M L^{-3}$
k_{O_2}	Overall mass transfer coefficient of oxygen	$L T^{-1}$
L_F	Biofilm thickness	L
$L_{F_{max}}$	Steady-state biofilm thickness	L
N_G	Number of granules	-
S_i	Dissolved species	$M L^{-3}$
u_a	Attachment rate	$L T^{-1}$
u_{det}	Detachment rate	$L T^{-1}$
u_F	The advective velocity of biofilm growth	$L T^{-1}$
X_i	Particulate species	$M_{COD} L^{-3}$
Y	Biomass Yield coefficient	$M_{COD} M_N^{-1}$

1 Introduction

Water is regarded as an essential natural resource on Earth. However, freshwater systems are constantly affected by anthropogenic activities as a result of increasing population, economic development and shifting consumption patterns (Boretti and Rosa, 2019). Two-thirds of the global population live under conditions of severe water scarcity for at least one month of the year (Mekonnen and Hoekstra, 2016). The 2018 edition of the United Nations World Water Development Report (UNESCO, 2018) stated that nearly 6 billion people will suffer from clean water scarcity by 2050. Because of the high population density of cities and increasing urbanization, urban water supply is particularly vulnerable. It is estimated that by 2050, 685 million people living in over 570 cities will face an additional decline in freshwater availability of at least 10% due to climate change (Figure 1) (UNESCO, 2020).

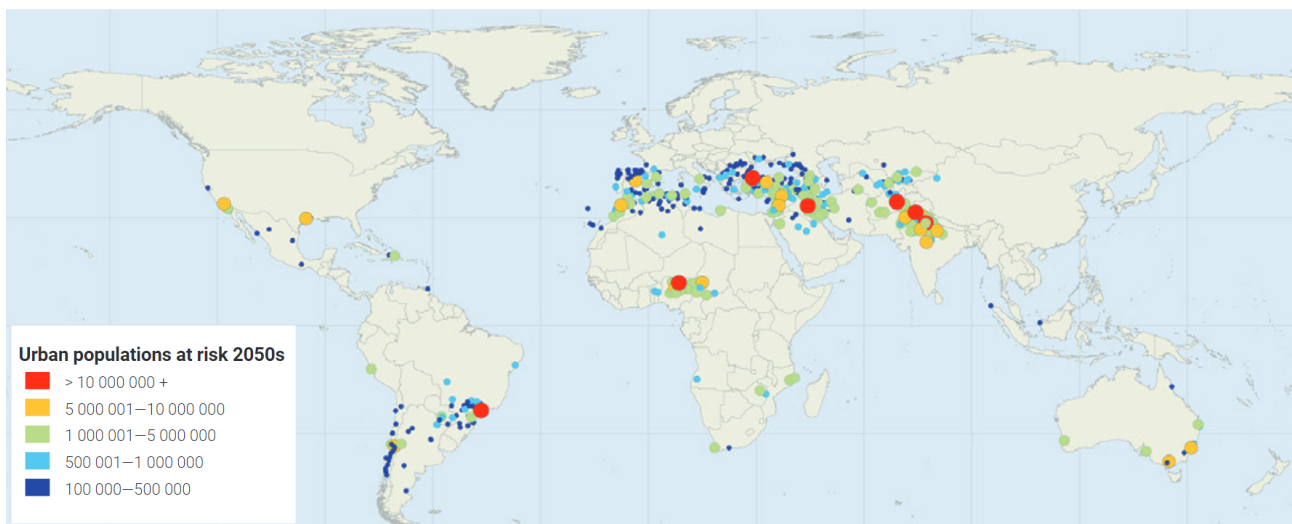


Figure 1: Decline in urban water availability (adapted from The United Nations World Water Development Report 2020).

Water management and improvement in wastewater treatment are critical in order not to jeopardize this essential resource and to achieve sustainable development (Grant et al., 2012). Traditionally, the goal of wastewater treatment was to protect downstream users from health risks. In more recent times, protecting nature by preventing nutrient pollution in surface waters has become an extra goal (Verstraete et al., 2009). There is probably no other elemental cycle where the human impact has been as dramatic as it is the case for nitrogen. Nitrogen pollution in aquatic systems can cause acidification of these water bodies, stimulate eutrophication, or even induce adverse effects on human health and economy (Camargo and Alonso, 2006). Organic nitrogen, ammonium, nitrite and nitrate are present in wastewater discharges from domestic households and industries, and thus nitrogen removal technologies have been implemented into wastewater treatment plants (WWTPs).

Nitrogen removal from wastewater via biological processes has been accounted for a more effective and relatively inexpensive approach in favour of the physicochemical processes (Kehrein et al., 2020). The

conventional approach via nitrification/denitrification (N/DN) (Figure 2) is widely implemented and acts as an effective method to achieve biological nitrogen removal (BNR) in many domestic and industrial wastewater treatment facilities. Although this N/DN pathway has already demonstrated high nitrogen removal efficiencies in many full-scale WWTP worldwide, the process has significant drawbacks that can increase treatment costs and decrease the sustainability of wastewater treatment (Capocelli et al., 2019). Aeration is the largest energy-consumption component and accounts for 55-70% of the total energy demand (Gandiglio et al., 2017). Based on the stoichiometry, the complete oxidation of NH_4^+ requires 4.57 g of oxygen per gram of $\text{NH}_4^+ - \text{N}$ oxidized (Figure 2). Another downside is the demand for an external organic carbon source in denitrification (such as methanol or acetate) (requires 2.86 g of COD per gram of $\text{NO}_3^- - \text{N}$ oxidized), which reduces its potential use for energy recovery via biogas/methane production from anaerobic sludge digestion (McCarty, 2018).

Therefore, new technologies have been developed. Partial-nitrification anammox (PN/A) process (Figure 2 and section 2.2) is considered as an innovative and promising solution to replace conventional nitrogen removal design and convert WWTP from energy-consuming facilities to self-sufficient or even energy-producing systems (Maktabifard et al., 2018).

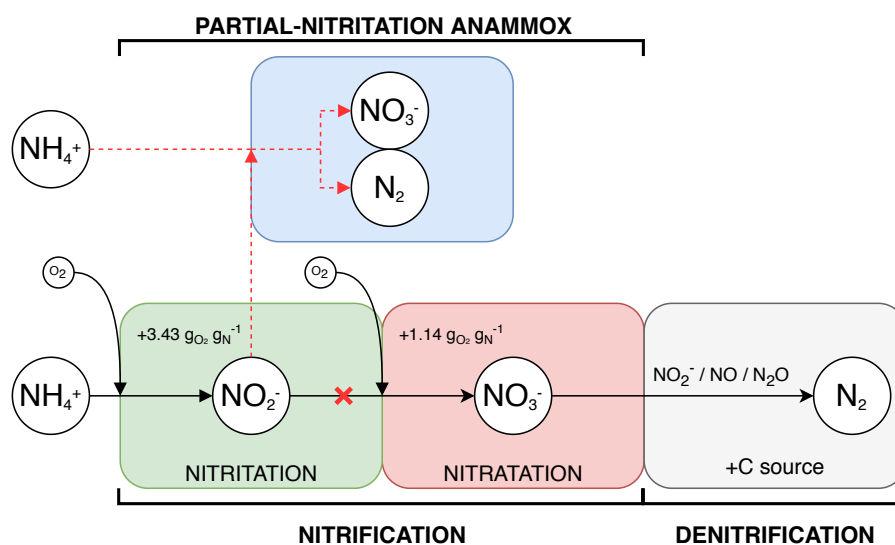


Figure 2: Schematic representation of the nitrification/denitrification process and partial-nitrification anammox (red dotted line), based on Castro-Barros et al. (2017). Microbial community: anammox bacteria (blue), ammonia oxidizers (green), nitrite oxidizer bacteria (red) and heterotrophic bacteria (grey).

Nevertheless, relying exclusively on wastewater treatment is not sufficient to solve the water scarcity issue. Considering that, on the one hand, over 50% of the world's population resides within 60 km of the coastline and, on the other hand, seawater accounts for 97.5% of all water resources on the planet, the use of seawater has been proposed as a good approach to the problem (Shannon et al., 2008).

Desalination is an option to augment freshwater supplies by removing dissolved salts from brackish or saltwater. According to the estimate by Jones et al. (2019), there are 16 thousand operational desalination

plants globally, producing around 95 million m³ d⁻¹ of desalinated water. Nonetheless, desalination is still hindered by high costs and high energy consumption (Park et al., 2020).

Meanwhile, seawater can be directly utilized for non-potable uses in some industrial applications (Lefebvre and Moletta, 2006) as well as for toilet flushing, which represents 30% of the total domestic water demand (Liu et al., 2016a). Several coastal cities and nations have already implemented dual water supply systems (fresh and seawater) to alleviate the water shortage (Tang et al., 2007). Hong Kong has adopted this system since the 1950s for 80% of its 7 million inhabitants, reducing its annual amount of freshwater demand by at least 22% (Chen et al., 2012; Leung et al., 2012). In a life-cycle assessment on seawater for toilet flushing, Liu et al. (2016a) reported that this is a promising water supply alternative for modern cities, and particularly for those that are located within 30 km of the seashore and with a population density higher than 3000 people per square kilometer.

The resultant saline sewage is composed of 33-50% of seawater, indicating that the salinity of the generated wastewater ranges from 1 to 1.7% (Yang et al., 2015). This wastewater could limit the performance of conventional N/DN processes, as salinity is considered a common stress factor to the microbial community in the WWTPs (Wang et al., 2017b). On the other hand, partial-nitrification with marine anammox bacteria has the potential to be utilized for nitrogen removal from saline wastewater as it is naturally adapted for those conditions (Ali et al., 2020).

However, due to the slow growth of these bacteria (Kuenen, 2008), lengthy and time-consuming experiments have to be carried out before one can speak of a steady-state system. Therefore, a computational model could provide significant insight into the whole process while saving time and money. Mathematical modelling is a widely acknowledged tool for fundamental understanding, design and optimization of wastewater treatment processes (Henze et al., 2008). It is essential to demonstrate a model of PN/A process for saline wastewater treatment that could be further validated by experimental work. Experiments with partial-nitrification and marine anammox are currently being implemented in the Water Desalination and Reuse Center (WDRC) at KAUST, envisioning the treatment of saline wastewater for toilet flushing applications.

Therefore, a model-based study will be developed to determine the setup that achieves the highest nitrogen removal efficiency for mainstream saline wastewater treatment. This model will help to understand possible bottlenecks and optimizations to the current system in the laboratory at WDRC.

The next chapter will focus firstly on the microbial community that is present in BNR processes and how these microorganisms adapt or tolerate salinity; then, the state of the art for partial-nitrification anammox, its implementation in the mainstream application, and the current challenges and bottlenecks of this technology will be detailed. Lastly, recent approaches for biofilm modelling and, in particular, the implementation on AQUASIM will be explained.

2 Literature Review

2.1 Microbial community

The central dogma of nitrification has stated that this is a two-step process, where metabolic labour is strictly divided between two distinct groups of microorganisms: ammonia is first oxidized to nitrite by ammonia-oxidizing bacteria (AOB), and the nitrite is subsequently oxidized to nitrate by the nitrite-oxidizing bacteria (NOB) (Daims et al., 2016).

First, the discovery of ammonia oxidation in an entirely different domain of life, with the cultivation of the first ammonia-oxidizing archaea (AOA) *Nitrosopumilus maritimus* (Könneke et al., 2005), prompted a significant re-assessment of the paradigm for ammonia oxidation. Therefore, AOA will be one of the ammonia-oxidizers considered in this study.

Then, Daims et al. (2015) and Van Kessel et al. (2015) identified microorganisms performing complete nitrification on their own (COMplete AMMonia OXidation - commamox) challenging the strict division between the two nitrifying groups, and thus caused another paradigm shift in our understanding of nitrification. Although a commamox species, "*Candidatus Nitrospira inopinata*" has been isolated in pure culture, facilitating the study of its growth kinetics (Kits et al., 2017), little is known about the ecology of this bacteria in different environments. Hence, commamox was not considered in this work. However, it should be noted that the discovery of this microorganism could change the approach to wastewater treatment in the future (Mehrani et al., 2020).

2.1.1 Ammonia-Oxidizing Bacteria (AOB)

AOB are the primary players in the ammonia oxidation in wastewater treatment. All members of the AOB group belong to the Proteobacteria phylum. The majority of species belong to the Betaproteobacteria class (*Nitrosomonas*, including *Nitrosococcus mobilis*; *Nitrosospira*; *Nitrosovibrio* and *Nitrosolobus*) and a few AOB, mostly found in marine environments, are classified as Gammaproteobacteria (*Nitrosococcus* and *Nitrosococcus oceanii*) (Purkhold et al. 2000). Almost all AOB genera found in different types of wastewater treatment environments are *Nitrosomonas* and *Nitrosospira*, and *Nitrosomonas europaea* has been considered the model ammonia-oxidizing bacteria in research until today (Lehtovirta-Morley, 2018).

Although the basic metabolism is more or less uniform for all AOB, different physiological requirements exist among the different strains (Koops and Pommerening-Ro, 2001). The substrate affinity and salt tolerance differ significantly among ammonia-oxidizers. As for salt resistance, which is the environmental condition studied in this work, not only the marine genera but some species of *Nitrosomonas*, namely *N. europaea*, has been described as robust under salt stress (up to $30 \text{ g}_{\text{NaCl}} \text{ L}^{-1}$) (Cui et al., 2016; Liu et al., 2008; Moussa et al., 2006; Navarro et al., 2015; Wang et al., 2017a). Hence, *N. europaea* will be the chosen AOB in this

work.

It is important to note that a wider microbial diversity, even inside the same functional group, is always preferable in wastewater treatment bioreactors. Enriching different species with the ability to remove ammonia under different environmental conditions enables functional resistance of an ecosystem to environmental perturbations. In this way, the loss of one species due to perturbation will be substituted by another species in the community (Louca et al., 2018; Saikaly and Oerther, 2011). However, for modelling purposes, this was not considered and the whole bacteria group is represented by the single species *Nitrosomonas europaea*.

2.1.2 Nitrite-Oxidizing Bacteria (NOB)

NOB is the functional group that contains all the bacteria with the ability to gain energy from the oxidation of nitrite to nitrate (with exception of commamox). Currently, there are seven known NOB genera affiliated with four bacterial phyla, including Proteobacteria (*Nitrobacter*, *Nitrotoga*, *Nitrococcus*), *Nitrospirae* (*Nitrospira*, “*Candidatus Nitromaritima*”), *Chloroflex* (*Nitrolancea*) and *Nitrospirae* (*Nitrospira*) (Feng et al., 2017).

Nitrospira and *Nitrobacter* genera are the most common NOBs in WWTPs (Mehrani et al., 2020). The prediction of their dominance has commonly been based on the *K/r* hypothesis (Nogueira and Melo, 2006): *Nitrobacter* is an *r*-strategist (high maximum specific growth and substrate utilization rates at high substrate concentrations), and *Nitrospira* is a *K*-strategy (high substrate affinity at low substrate concentrations). These affinity differences have been reported in many different species from each genus (Nowka et al., 2015): lower $K_{\text{NO}_2^-}$ values of *Nitrospira* (9-27 μM) compared to *Nitrobacter* (49-544 μM).

Both genera have been reported as resistant to salt stress (up to 16 $\text{g}_{\text{NaCl}} \text{L}^{-1}$), but there are still inconclusive answers about which one is the dominant. Chen et al. (2003) and Moussa et al. (2006) reported higher salinity tolerance of *Nitrobacter* over *Nitrospira*, where Bassin et al. (2012) and Wang et al. (2017a) have described the opposite. Wang et al. (2017a) observed an overgrowth of *Nitrospira* at even higher salinity (20 g L^{-1}), under low nitrite and low oxygen concentrations.

For marine species, values between 19 and 120 μM for $K_{\text{NO}_2^-}^{\text{NOB}}$ were reported by Jacob et al. (2017). As mentioned by these authors, higher substrate affinities were expected because marine nitrite concentrations are usually low. However, non-marine *Nitrospira* exhibits even higher nitrite affinity than the marine counterpart and therefore, $K_{\text{NO}_2^-}^{\text{NOB}}$ of 9 μM will be used in the model.

2.1.3 Ammonia-Oxidizing Archaea (AOA)

The first and rate-limiting step of nitrification, the aerobic oxidation of ammonia to nitrite, has been, for a long time, assumed to be performed only by bacteria (AOB). As aforementioned, this misconception was refuted by the isolation of the AOA *Nitrosopumilus maritimus*, which also contains putative genes for

ammonia monooxygenase (AMO) (Francis et al., 2005), the key enzyme responsible for ammonia oxidation. AOA can be found in many diverse environments: marine and freshwater (Hatzenpichler, 2012), soils (Leininger et al., 2006), and wastewater treatment systems (Li et al., 2016; Park et al., 2006; Sauder et al., 2017). It is assumed that AOA and AOB share the same stoichiometric reaction, using ammonia as a substrate for generating energy. However, they differ in the proposed pathways for oxidizing ammonia (Könneke et al., 2014) and have distinct kinetic features (Prosser and Nicol, 2012). Within the competition for ammonia between aerobic-ammonia oxidizers, AOA have shown an extremely high affinity towards ammonium ($K_{\text{NH}_4^+}^{\text{AOA}} = 133 \text{ nM}$ vs $K_{\text{NH}_4^+}^{\text{AOB}} = 100 \mu\text{M}$) (Kits et al., 2017; Martens-Habbena et al., 2009) and higher oxygen affinity constant when compared to their bacteria counterpart ($K_{\text{O}_2}^{\text{AOA}} = 0.128 \text{ mg L}^{-1}$ vs $K_{\text{O}_2}^{\text{AOB}} = 0.40 \text{ mg L}^{-1}$) (Stahl and De La Torre, 2012).

Compared with many natural environments, WWTPs contain relatively high levels of ammonium, which should favor AOB over AOA (more NH_4^+ available, favours higher maximum growth rate over higher substrate affinity). Many studies reported the dominance of AOB in municipal and industrial WWTPs (Gao et al., 2013; Wells et al., 2009). Nonetheless, in WWTPs with lower influent levels of ammonium (hence, substrate affinity plays a significant role), AOA tended to be numerically dominant (Roy et al., 2017; Sauder et al., 2012), although the contributions of AOA and AOB to the total ammonium oxidation still need to be elucidated.

However, the saline wastewater could be an important differential factor in the competition among ammonia-oxidizers. AOA have been reported to coexist with anammox bacteria (detailed in section 2.1.4) in marine oxygen minimum zones (OMZs) of the Black Sea (Kuypers et al., 2003), as in these low-oxygen environments, AOA can provide nitrite to anammox bacteria (Lam et al., 2007). OMZs cooperation between marine anammox bacteria and AOA has been demonstrated in a lab-scale model by Yan et al. (2012). Recently, Val del Rio et al. (2018) applied PN/A process to saline wastewater and, even though nitrification occurred, no AOB was detected among the abundant bacteria. The authors stated that one plausible explanation could be the presence of *Nitrosopumilus*, which has also been found in the archaea fraction of other anammox reactors.

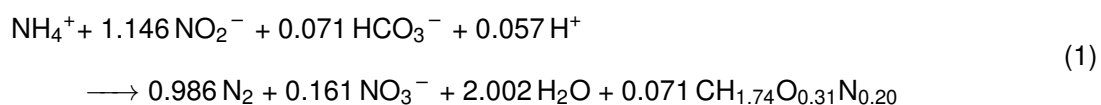
Considering this trend of increased reports of AOA in many engineered systems and give that the treatment of saline wastewater is the scope of this study, the marine AOA *Nitrosopumilus maritimus* will be implemented in the model to assess the competition and cooperation among AOB and anammox bacteria.

2.1.4 Anammox Bacteria

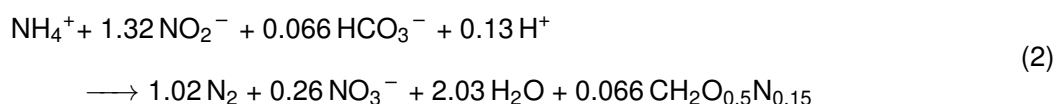
The anaerobic ammonium oxidation (anammox) process, which had been predicted by thermodynamic considerations (Broda, 1977), was discovered by Mulder (1992) in a denitrifying bioreactor operated in the Netherlands. During the anammox process, ammonium (electron donor) and nitrite (electron acceptor) react to form nitrogen gas and nitrate under anoxic conditions (Van De Graaf et al., 1995). Their anoxic and

autotrophic nature means that this process needs neither oxygen nor organic carbon to oxidize ammonium. All anammox species possess a unique organelle-like cell compartment (anammoxosome), which contains the essential enzymes to perform the anammox reaction (van Niftrik et al., 2008). The anammoxosome membrane is rich in ladderane-lipids, which have only been found in association with anammox bacteria and can be used to positively identify these organisms in their natural or man-made environment (Kuenen, 2008). A visual indication of the presence of anammox bacteria in bioreactors is the red colour of the biomass due to the heme *c* group of the protein cytochrome *c* in their metabolism (Kartal et al., 2013).

The metabolic reaction equation of the anammox process is still subjected to debate. Due to the difficulties in cultivating a pure culture, stoichiometric equations are intrinsically not completely precise. Nevertheless, various enrichment cultures of anammox species in membrane bioreactors (MBRs) (>90% purity) have been conducted, which allowed studying their ecophysiology and biochemistry (Ali et al., 2015; Awata et al., 2013; Oshiki et al., 2013; Van Der Star et al., 2008). The updated stoichiometric reaction obtained via enriched culture is formulated in equation 1 (Lotti et al., 2014):



However, the first stoichiometric reaction (equation 2), which was derived via mass balance of substrates/products in a sequencing batch reactor (Strous et al., 1998), is still widely used for reactor design and operation purposes. Thus, it will be the one implemented in the stoichiometric matrix in the model (Table A4):



So far, more than 30 species under 6 *Candidatus* genera within the Planctomycetes phylum have been reported among natural and engineered ecosystems. The anammox bacteria can be classified as freshwater anammox: “*Candidatus* Brocadia” (Strous et al., 1998), “*Ca. Kuenenia*” (Schmid et al., 2000), “*Ca. Anammoxoglobus*” (Kartal et al., 2007), “*Ca. Jettenia*” (Quan et al., 2008) and “*Ca. Anammoximicrobium*” (Khramenkov et al., 2013) and marine anammox “*Ca. Scalindua*” (Kuypers et al., 2003).

The most abundant genera are “*Ca. Brocadia*” and “*Ca. Kuenenia*” who are commonly retrieved from natural and engineered freshwater ecosystems. “*Ca. Jettenia*” is the lowest abundant genus among all anammox bacterial genera, and the majority of them were retrieved from wastewater treatment plants and bioreactors (Sonthiphand et al., 2014). However, population shifts of anammox bacteria have been frequently reported:

- from “*Ca. B. anammoxidans*” to “*Ca. Anammoxoglobus propionicus*”, due to the presence of acetate and propionate (Kartal et al., 2007);

- from “*Ca. Brocadia* sp.” to “*Ca. K. stuttgartiensis*” due to substrate limitation (Van Der Star et al., 2008);
- from “*Ca. B. fulgida*” to “*Ca. Brocadia* sp.40” (Park et al., 2010) and similarly from “*Ca. B. fulgida*” to “*Ca. K. stuttgartiensis*” (Park et al., 2015);
- from “*Ca. Kuenenia*” to “*Ca. B. anammoxidans*”, just after a temperature shock (Isanta et al., 2015a);
- from “*Ca. B. fulgida*” at low salinity ($0\text{--}3\text{ g}_{\text{NaCl}}\text{ L}^{-1}$) to “*Ca. K. stuttgartiensis*” at high salinity ($30\text{ g}_{\text{NaCl}}\text{ L}^{-1}$) (Gonzalez-Silva et al., 2017);
- from “*Ca. Brocadia*” to “*Ca. Kuenenia*” in an SBR at lower temperature ($10\text{ }^{\circ}\text{C}$) (De Cocker et al., 2018);
- from “*Ca. Kuenenia*” to “*Ca. Brocadia*” (Wang et al., 2019).

These population shifts are not a systematic competition and these phenomena are not replicable or well interpreted due to various factors affecting the results (Zhang and Okabe, 2020). Nevertheless, this suggests that genus or species-specific niche differentiation and competition exist. Different anammox bacterial species compete for the same resources (limiting substrate or living space), which consequently leads to the exclusion of an inferior species, or to a decline in its population. There are several aspects that can shape the community composition:

- Different kinetic and physiological properties of anammox bacteria (maximum specific growth rate, affinity to limiting substrate, optimal pH and temperature, oxygen tolerance) (Oshiki et al., 2016; Zhang and Okabe, 2020);
- Susceptibility to various compounds or utilization of fatty acids (Kartal et al., 2008);
- Aggregation capability (Hou et al., 2015; Ali et al., 2018): some anammox bacteria exhibit a higher tendency to form aggregates in which different micro-environments are developed due to substrate diffusion limitation and higher microbial activity.

Another critical factor is the salinity tolerance. As previously noted, the global distribution pattern of anammox bacterial communities is controlled primarily by salinity (Sonthiphand et al., 2014). Even though “*Ca. Scalindua*” is the only marine anammox genus, other freshwater anammox bacteria have been reported to be salt-tolerant and to be suitable for saline wastewater treatment (Li et al., 2018b):

- “*Ca. K. stuttgartiensis*” gradually adapted to salt concentrations as high as $30\text{ g}_{\text{NaCl}}\text{ L}^{-1}$ (Gonzalez-Silva et al., 2017; Kartal et al., 2006);
- “*Ca. Jettenia*” is very sensitive to salinity with a half-maximal inhibitory concentration (IC_{50}) of only $4\text{ g}_{\text{NaCl}}\text{ L}^{-1}$ (Ali et al., 2015); Meng et al. (2018) reported that “*Ca. Jettenia*” was largely inhibited by

the increased salinity ($20 \text{ g}_{\text{NaCl}} \text{ L}^{-1}$), whereas “*Ca. Kuenenia*” were well adapted to the same salt concentration;

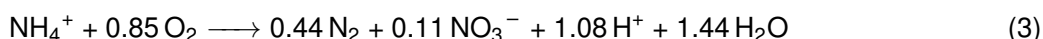
- “*Ca. Brocadia fulgida*” can endure salinity up to $15 \text{ g}_{\text{NaCl}} \text{ L}^{-1}$ (Malovanyy et al., 2015a);
- “*Ca. Brocadia sinica*” has shown high tolerance to salinity, and the activity remained at 58% in 100% seawater (3.5% salinity) (Li et al., 2018a).

These reported salt adaptation and other studies performed with anammox and saline wastewater (see Table A10) demonstrate the potential solution of using different anammox bacteria species for saline wastewater treatment.

2.2 Partial-Nitritation Anammox (PN/A)

With the discovery of anammox bacteria, new processes have been developed to replace N/DN. Usually, nitrogen is present in wastewater only as ammonium. However, anammox metabolism requires both ammonium and nitrite as substrates and thus, the anammox process has to be combined with another process to generate the required nitrite.

In the partial-nitritation, half of the ammonium is converted to nitrite by ammonia-oxidizers (AOA/AOB) (Figure 2), and subsequently, anammox bacteria can convert the other half of ammonium and the resultant nitrite to nitrogen gas (equation 2). It is crucial to suspend the nitrification reaction at the nitritation step, where the nitrite is produced and accumulated rather than oxidized further to nitrate (Figure 2). If NOB coexists with anammox bacteria in the system, there will be a competition for nitrite, and the anammox bacteria growth will be limited (section 2.2.4). The overall stoichiometry of the PN/A process is represented in equation 3.



In the early implementations of PN/A, since the partial-nitritation process requires oxic conditions whereas anammox requires anoxic conditions, nitrogen removal was initially developed in a two-stage anammox reactor configuration: SHARON-Anammox (Single reactor system for High activity Ammonium Removal Over Nitrite) (van Dongen et al., 2001; Hellings et al., 1998).

Although the two-stage system is easy to optimize because aerobic ammonia-oxidizers and anammox bacteria have different growth requirements, single-stage reactor configuration became the current mainstream due to lower capital investment, operational cost and footprint (Jeanningros et al., 2010). Some of the single-stage systems that have been developed since then are:

- CANON (Completely Autotrophic Nitrogen removal Over Nitrite) (Third et al., 2001);

- OLAND (Oxygen Limited Autotrophic Nitrification-Denitrification) (Kuai and Verstraete, 1998);
- SNAP (Single-stage nitrogen removal using Anammox and Partial-nitrification) (Furukawa et al., 2006);
- DEMON (DEammNONification) (Wett, 2006);
- ANITAMOX (Veuillet et al., 2013).

Over the past decade, full-scale installation of anammox systems has become increasingly popular for treating ammonium-rich sidestream at mesophilic temperatures ($>500 \text{ mg L}^{-1}$, 30°C). There are presently more than 110 full-scale anammox-based treatment plants worldwide, and approximately 90% of the plants are being operated as single-stage reactor configurations (Ali and Okabe, 2015; Bowden et al., 2015; Lackner et al., 2014).

2.2.1 Advantages of partial-nitrification anammox

As highlighted in the first chapter, determining the best biological processes for nitrogen removal in municipal wastewater treatment is vital for a sustainable future development. The main limitations in the conventional N/DN process are the large amount of oxygen required for nitrification, the production of a substantial amount of sludge and the emission of greenhouse gases such NO and N_2O . Partial-nitrification anammox offers a sustainable alternative for the same purpose. Its merits are:

- 90% less of the excess sludge production reducing the management cost: the treatment of the generated sludge represents up to 50-60% of the WWTP budge and the fact that less sludge is produced due to the slow growth of the microorganisms involved allows to save money (Cao et al., 2017);
- Elimination of 100% of organic carbon and the need to supply an external carbon source for the reaction due to the autotrophic behavior of both nitrifying and anammox bacteria, which could otherwise be converted to bioenergy (Kartal et al., 2010; McCarty, 2018);
- Fux and Siegrist (2004) estimated that PN/A could achieve an overall saving of up to 40%, including capital, operational and utilities costs, and Siegrist et al. (2008) reported a 63% energy reduction for aeration;
- Less production of greenhouse effect gases such as N_2O and NO (about 1% of a full-scale system) (Kampschreur et al., 2009a) as most of the N_2O production is generally attributed to nitrifiers (Kampschreur et al., 2009b).

2.2.2 Mainstream anammox

The advantages of PN/A process and its success for sidestream treatment encourages exploring its application in mainstream domestic water. By far, the best process examined in this context is the A-B

configuration (Figure 3) with the anaerobic treatment of organic carbon (A-stage) followed by mainstream partial-nitrification anammox treatment (B-stage). It far exceeds all the others in net energy production while significantly reducing biosolids handling needs (McCarty, 2018).

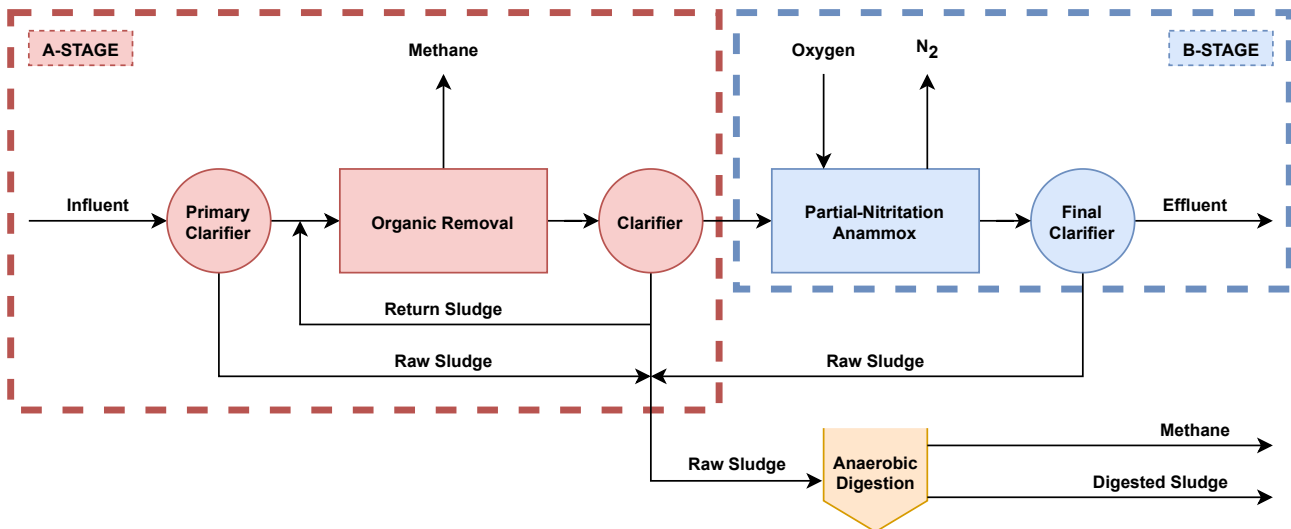


Figure 3: Schematic diagram of A-B stage adapted from McCarty (2018).

A recent model-based study demonstrated that a high rate activated sludge (HRAS)-PN/A system could achieve energy-positive status with a net energy production of 5000 kWh d⁻¹, satisfactory effluent quality and a 107% lower operational cost than conventional activated sludge systems (Jia et al., 2020).

Although the research targeting the direct application of PN/A to mainstream is progressing at a fast pace, in both lab and pilot-scale applications (De Clippeleir et al., 2013; Gilbert et al., 2015; Isanta et al., 2015b; Hoekstra et al., 2019; Laureni et al., 2019; Lotti et al., 2015b), achieving a practical application is still a big challenge, and no full-scale installations have been reported yet.

Several of the main challenges that must be addressed are:

- High COD/N ratio of domestic wastewater may lead to heterotrophic bacteria outcompeting anammox bacteria. This could be solved using the A-B configuration from Figure 3: with the consumption of organic carbon diverted via a pre-removal step, the C/N ratio decreases, and mainstream PN/A can be implemented as the BNR process, allowing the WWTP to shift from an energy consumer to a net energy producer (McCarty, 2018; Kartal et al., 2010);
- Effective retention of anammox bacteria (section 2.2.3) due to the mainstream low-strength nitrogen condition: typical values of 20-75 mg_{NH₄⁺-N} L⁻¹ (Henze and Comeau, 2008);
- A robust nitrification process is challenging to maintain via selective retention of AOB against NOB (section 2.2.4) due to low temperatures;
- A stable system that can treat saline wastewater (i.e. for toilet flushing applications), without compromising the microbial community.

2.2.3 Bioreactor configurations

One of the bottlenecks in all anammox based applications is the slow growth of these bacteria. To prevent biomass washout, the choice of a suitable biomass retention mechanism is an essential aspect of the design of the reactor. An ideal reactor configuration for PN/A should facilitate oxygen-mass transfer and have effective biomass retention (biofilm or granular-based process) (Kartal et al., 2010).

The main advantage of biofilm-based bioreactors over conventional suspended growth systems (activated sludge) is based on their ability to decouple two fundamental process parameters in wastewater treatment: solids retention time (SRT) and hydraulic retention time (HRT) (Henze et al., 2008). The combination of both characteristics allows biofilm reactors to achieve high biomass concentrations (and hence high treatment efficiencies) and high biomass retention times in relatively small reactor volumes. On the other hand, biofilms are very efficient nutrient scavengers and, with proper control of the oxygen flux, they allow stratification and the establishment of redox conditions within their structure (Stewart and Franklin, 2008): aerobic ammonia-oxidizers grow in the aerobic biofilm regions while anaerobic ammonium oxidizers populate the remaining anoxic portion of the biofilm.

There are three major implemented configurations that integrate the mentioned advantages (Li et al., 2018c): sequencing batch reactor (SBR) (floc/granule, operating convenience), moving bed biofilm reactor (MBBR) (biofilm, large surface area), and membrane-aerated biofilm reactor (MABR) (counter-diffusion gradient).

The SBR is one of the most common processes used in partial-nitrification anammox (Lackner et al., 2014). The patterns of feeding, aeration, and settling in the SBR can be flexible, maintain targeted dissolved oxygen concentrations, retain desirable organisms, and achieve stable performance. The intermittent aeration provides desired alternating aerobic/anoxic operational conditions for enriching different types of organisms. Moreover, the cycle pattern in an SBR can be used to promote granular sludge, where biomass grows in compact aggregates (granules) without any carrier material (Winkler et al., 2011).

Granulation in an SBR, well described in the literature by Beun et al. (1999), Fernández et al. (2008), Vlaeminck et al. (2012) and Winkler et al. (2013), is mainly achieved by controlling:

- Hydraulic retention time: short HRTs enhance the washout of suspended biomass, improving the granulation in the system;
- Sludge settling time: by reducing this parameter, more compact granules are formed and sludge with worst settling properties is washed out;
- Hydraulic shear stress: During the aeration period, adequate selective pressure by the upflow velocity of the liquid creates shear forces imposed on the biomass. The higher the superficial gas velocity, the higher the stress forces on the granules, resulting in more dense and compact granules and improved sludge settling velocity.

- Feeding regime: timing of feeding (pulse versus continuous), and the volume exchanged per cycle will determine the concentration range of substrates 'experienced' by the sludge, which can affect the granule formation.

The MBBRs are another technology where partial-nitrification anammox has been implemented (i.e. ANITAMOX (Veuillet et al., 2013)). The reactor can operate in an SBR mode and employs the use of special plastic carriers, such as Kaldnes rings (K1®, K3®, etc.), to provide a large surface for the formation of biofilm. Carriers are mixed in the tank by aeration, which facilitates contact between substrate in the influent and bacterial groups distributed in the different layers of the biofilm in the carrier. Recently, they have been used as hybrid systems, where biofilms and flocs coexist (also referred to as integrated fixed-film activated sludge or IFAS). Faster-growing aerobic nitrifiers tend to enrich in the floc fraction, with direct access to dissolved substrates, while anammox serves as a NO_2^- sink in an anoxic biofilm. As a result, it is possible to control the retention times of the two biomass fractions, which might have potential advantages for PN/A applications (Laureni et al., 2019; Pedrouso et al., 2019; Trojanowicz et al., 2019).

The MABRs are another emerging wastewater treatment technology that have shown a huge potential to reduce the environmental and economic impacts of wastewater treatment plants (Castrillo et al., 2019). It became of great interest for precise control of oxygen supply for the single-stage nitrification-anammox process (Nerenberg, 2016). The MABR allows independent control of electron donors and acceptors because of the counter-diffusion delivery, providing a higher gas transfer efficiency and substrate utilization than convention bubble diffusers used in granular systems and MBBRs (Syron and Casey, 2013). The intramembrane oxygen pressure and the membrane mass transfer coefficient determine the rate of oxygen transfer to the biofilm. If anaerobic/anoxic degradation is required, then the oxygen pressure must be carefully balanced to maintain the anoxic zone at the biofilm–liquid. Since oxygen and wastewater are supplied from opposite sides of the biofilm, a unique profile of oxygen and nutrients develops in the biofilm, creating an oxygen concentration gradient that could favor the co-growth of ammonia-oxidizers and anammox bacteria (Pellicer-Nàcher and Smets, 2014).

2.2.4 Suppression of nitrite-oxidizing bacteria

The main bottleneck in order to achieve higher efficiencies of BNR is the suppression of NOB in the PN/A process (Cao et al., 2017). The previous optimized engineered strategies are required to favour the growth of AOB and retain the slower-growing anammox bacteria, while outcompeting the undesired nitrifiers. Several operational strategies are already implemented in sidestream applications, but they are not feasible under mainstream conditions:

- inhibition of NOB due to high concentrations of free ammonia (NH_3) or free nitrous acid (HNO_2) (Anthonisen et al., 1976) cannot occur due to the low ammonium concentrations in the influent (Table A1);

- at temperatures $> 25^{\circ}\text{C}$, $\mu_{\max}^{AOB} > \mu_{\max}^{NOB}$ which results in selective NOB washout at low SRT. By controlling the SRT to a value that is lower than the minimum required for NOB but greater than the minimum required for AOB, NOB are physically unable to reproduce quickly enough in order to remain in the system. On the contrary, at mainstream temperatures, these differences in growth rates are minimal (Hellings et al., 1998; Jubany et al., 2008).

For that reason, other methods have been proposed: the maintenance of a residual ammonium concentration level in the bioreactor, and the use of transient anoxia with intermittent aeration.

Isanta et al. (2015b), Poot et al. (2016) and Soler-Jofra et al. (2019) described that a residual ammonium concentration directly influences the ammonium oxidation rate from AOB and thus, it should be maintained in order to keep the growth rate of AOB higher than that of NOB.

In transient anoxia, the intermittent aeration with short anoxic periods followed by aerobic periods creates a lag phase in the response of NOB activity at the beginning of the aeration phase, which does not happen with AOB. This method has been reported to successfully outcompete NOB in PN/A systems, even at low temperatures (Gilbert et al., 2014; Ma et al., 2015; Wett et al., 2013).

Nevertheless, dissolved oxygen (DO) is the essential factor in the successful start-up and operation on partial-nitrification anammox (Lackner et al., 2014). Both DO and microbial distribution of the biomass in the biofilm have an impact on the nitrogen conversion and removal. A general criterion is that optimum DO should facilitate AOB growth, suppress NOB, and not inhibit anammox activity. The theoretical nitrate production accounts for 11% of the total nitrogen supply in the PN/A process (equation 3). Therefore, an increase in nitrate production in the effluent might indicate the overgrowth of NOB in the microbial community.

In the biofilm system, AOA/AOB, NOB and anammox compete for living space. Under aerated conditions, AOB grows on the outer surface layer, while NOB is slightly deeper (several μm) and anammox are located inside the anoxic interior, as verified by molecular microbial observation (Figure 4) (Ali et al., 2016; Vlaeminck et al., 2010; Winkler et al., 2011). As such, the substrate gradients, mass transfer and kinetics parameters determine the dominance, coexistence, or suppression of the microbial community. The layered structure of the biofilm is needed in order to outcompete NOB (Picioreanu et al., 2016; Poot et al., 2016).

However, the use of limiting DO concentrations to maintain stable conversion of ammonium to nitrite, based on differences between oxygen affinity constants (K_{O_2}) of AOB and NOB, is still a controversial matter. Previously, the typical strategy was to operate at low DO concentration to accumulate nitrite during nitrification. This strategy is based on a well-accepted assumption that AOB have a higher DO affinity for oxygen than NOB ($K_{O_2}^{NOB} > K_{O_2}^{AOB}$) (Guisasola et al., 2005; Jubany et al., 2008; Rittmann and McCarty, 2001; Wiesmann, 1994).

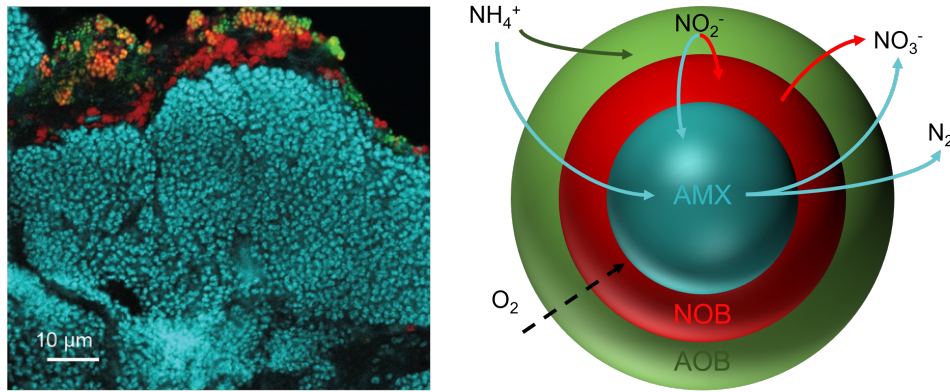


Figure 4: Confocal laser scanning microscope (CLSM) image from Ali et al. (2016) showing the *in situ* spatial organization of AOB (green), NOB (red) and anammox bacteria (blue) in a PN/A granule; Schematic representation of the interaction between bacteria and substrate inside the PN/A granule.

The repression of the nitrite oxidation in biofilm reactors through this concept was identified both in experiments and in modelling approaches (Brockmann and Morgenroth, 2010; Corbalá-Robles et al., 2016; Pérez et al., 2009; Volcke et al., 2010). Nonetheless, several studies contradict this common process understanding. It has been reported that operation at high DO concentrations can also lead to nitrite accumulation with reported lower K_{O_2} for NOB when compared to AOB (De Clippeleir et al., 2013; Malovanyy et al., 2015b; Wett et al., 2013; Manser et al., 2005; Regmi et al., 2014).

In fact, there is a wide range of oxygen affinity constants reported in the literature (Table A5). In order to account for these inconsistencies in published data, several explanations are commonly provided:

- The “strategist explanation” as previously detailed in (section 2.1.2) about the K/r hypothesis of *Nitrospira* and *Nitrobacter* ($K_{O_2}^{Nitrobacter} > K_{O_2}^{Nitrospira}$);
- The “diffusional explanation” where values that are obtained do not consider diffusion limitation within flocs, biomass granules, or biofilms. Several studies have focused on measuring/calibrating experimentally “half-saturation constants” for different substrates in the context of different biological processes and, the kinetic parameters differ from study to study (Arnaldos et al., 2015).

For example, Wu et al. (2017) have reported a higher affinity for AOB ($K_{O_2}^{AOB} < K_{O_2}^{NOB}$) and explained that the enrichment of the biomass strongly impacts the $K_{O_2}^{AOB}$, and thus, the conflicting reports in the literature. On the other hand, Val del Rio et al. (2019) presented a mathematical methodology to determine the intrinsic parameters in granular sludge, based on data obtained by respirometric assays and concluded a higher intrinsic affinity for NOB.

Therefore, to approach this problem, both scenarios will be evaluated in the model: $K_{O_2}^{NOB} > K_{O_2}^{AOB}$ and $K_{O_2}^{NOB} < K_{O_2}^{AOB}$. To ease the interpretation of this sensitivity analysis, $K_{O_2}^{AOB}$ was maintained constant ($0.40 \text{ mg}_{O_2} \text{ L}^{-1}$) and the $K_{O_2}^{NOB}/K_{O_2}^{AOB}$ ratio was varied between 2 (typical kinetics) and 0.5 (representing the K/r hypothesis), by changing $K_{O_2}^{NOB}$.

3 Biofilm modelling

A mathematical model attempts to incorporate science, engineering and conceptual understanding of a real-world application into an executable tool. While the results of a good model should reproduce the relevant phenomena of a system, model failures identify areas needing further research. In this way, the consistent implementation of these models improves our understanding of how a system works and behaves (Wanner et al., 2006).

The first models to be developed on wastewater treatment were the activated sludge models (ASM) (Henze, 2000). They became the accepted approach to design and assist the operation of WWTPs due to their low complexity while still providing the necessary detail of processes and components to address real-world applications. However, with the developments on wastewater treatment, granular sludge technology started to be established across industrial and municipal wastewater treatment plants. As pointed out in Section 2.2.3, pollutant removal in these reactors relies on microorganisms that grow in spherical aggregates that can freely move within the reactor. This provides a high biofilm specific surface area and promotes mass transfer of substrate towards the microorganisms (Boltz et al., 2010), leading to micro-scale substrate gradients and different biomass spatial distribution inside the granular biofilm. Therefore, applying ASMs to biofilm processes is not possible due to the importance of mass-transport processes. In ASM, the diffusion of the substrate in the flocs is not considered, and apparent half-saturation coefficients are used. On the other hand, in the modelling of biofilm systems, diffusion of substrates and intrinsic half-saturation coefficients have to be explicitly included (Picioreanu et al., 2016).

Contrarily to ASM, a consistent and widely used protocol is not available to guide the use of biofilm models to design and operate biofilm reactors. Nonetheless, Wanner et al. (2006) published a comprehensive review of biofilm models, detailing about their structure and their implementation to different scenarios. Recently, Rittmann et al. (2018) established a framework called "Good Biofilm Reactor Modeling Practice" (GBRMP), with guidance on how to develop and apply a biofilm model to obtain accurate and meaningful results on experimental applications. Baeten et al. (2019) reviewed 167 available models in the scientific literature for anaerobic, aerobic and PN/A granular sludge reactors (similar phenomena: liquid, gas and granule transport) where they assessed the different approaches, goals and complexity of the models.

Based on these reviews, a one-dimensional numerical model was developed in this work. It represents multispecies and multi-substrate biofilms with the primary assumption that the composition of particulate and dissolved compounds is homogeneous in one dimension perpendicular to the substratum (Morgenroth, 2008). This is a valid simplification when vertical gradients are several orders of magnitude higher than those in the directions parallel to the biofilm surface (Wanner and Gujer, 1986). Since this applies to most biofilm systems, this approach is sufficient for the majority of questions that engineers need to address.

3.1 AQUASIM

The model was implemented using the software package AQUASIM v2.1d (Reichert, 1994, 1998). The program allows to develop a 1D multispecies and multi-substrate model, calculating substrate removal in a biofilm reactor for any specified microbial system. It predicts the development of the biofilm thickness, spatial distribution of substrates and microbial species in the biofilm and in the bulk fluid over time.

The equations describing these phenomena and how AQUASIM solves them are briefly described below (Equations 4, 5 and 6). The detailed equations are available in the following publications: Wanner and Reichert (1996), Reichert and Wanner (1997) and Reichert (1998).

The partial differential equation describing molecular diffusion, substrate utilization inside a biofilm and its accumulation is based on the Fick's second law of diffusion. AQUASIM differentiates between a solid fraction (ϵ_s) and a liquid fraction (ϵ_l) within the biofilm. It is then assumed that diffusion takes place only in the liquid fraction, resulting in the following equation:

$$\underbrace{\frac{\partial(\epsilon_l \cdot S_{F,i})}{\partial t}}_{\text{Accumulation}} = \underbrace{(\epsilon_l \cdot D_w) \frac{\partial^2 S_{F,i}}{\partial z^2}}_{\text{Diffusion}} + \underbrace{r_{F,i}}_{\text{Reaction}} \quad (4)$$

$S_{F,i}$ is the concentration of substrate i in the biofilm [M L^{-3}]; z (instead of x) is distance from the substratum to the bulk liquid [L]; t is time [T]; D_w is diffusion coefficient in water, and $\epsilon_l \cdot D_w$ is the effective diffusion coefficient in the biofilm [$\text{L}^2 \text{T}^{-1}$] (Table A7); $r_{F,i}$ is the rate of the substrate i conversion per biofilm volume [$\text{M L}^{-3} \text{T}^{-1}$].

For the simulation of multispecies and multi-substrate, the conversions rates ($r_{F,i}$) are linked to process rates (ρ) (Table A3) and stoichiometric values v (Table A6). The general equation for any compound i and process j is described as:

$$r_{F,i} = \sum_{j=1}^n v_{i,j} \rho_j \quad (5)$$

i is the index of each variable in the model (substrate S or biomass X); $v_{i,j}$ represent the stoichiometric matrix in Table A4.

The biofilm thickness (L_F) is calculated from a balance of growth, decay, attachment, and detachment:

$$\frac{\partial L_F}{\partial t} = u_F(L_F) + u_a - u_{det} \quad (6)$$

$u_F(L_F)$ is the net effect of biofilm expansion as the result of growth and decay processes within the biofilm [L T^{-1}]; u_a is the rate of attachment [L T^{-1}] and u_{det} is the rate of detachment [L T^{-1}]. To simulate biofilms with a constant predefined thickness and no attachment, the biofilm detachment velocity can be set to $u_F(L_F)$.

The workflow of how to implement a biofilm model on AQUASIM is explained in Reichert (1998) and Wanner and Morgenroth (2004). The main inputs that must be defined are:

- The state variables: dissolved components, which exist in the bulk liquid and diffuse to and in the biofilm (substrate), and particulate components (active or inert biomass) which do not diffuse (Table A2);
- The biological process rates (such as growth rate and substrate utilization by Monod kinetics, Table A3) and the parameters that represent the system that is being modelled (kinetic parameters, yield and diffusion coefficients) (Table A6 and Table A7);
- The transformation processes (utilization and diffusion of substrate and synthesis or decay of active biomass) via stoichiometric matrix (Table A4);
- The geometric and hydraulic data such as biofilm area, bulk liquid volume and inflow rate.

To begin a simulation, initial conditions such as the influent and bulk liquid concentrations of all components, and the initial biofilm thickness must be known.

3.2 Objectives of this work

As aforementioned, biofilm modelling on wastewater treatment processes, including partial-nitrification anammox (PN/A), are becoming increasingly popular. However, the different models that have been published (Baeten et al., 2019), only account for freshwater conditions and the kinetics parameters for the microorganisms involved have not been changed or revised according to the recent literature.

Hence, this work aims to: review kinetics parameters from recent literature, mainly on anammox bacteria, and model a PN/A process for saline wastewater treatment which has implications on the microbial kinetics implemented in the model.

The model will be implemented using the AQUASIM software and different scenarios will be simulated to:

- Understand the niche differentiation among anammox bacteria based on microbial kinetics;
- Evaluate nitrogen removal performance by PN/A in a granular sequencing batch reactor (GSBR) considering anammox bacteria, ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) and nitrite-oxidizing bacteria (NOB) (marine or salinity adapted species);
- Compare different kinetic oxygen affinities for AOB and NOB;
- Assess the competition for oxygen, ammonium and nitrite between AOA, AOB, NOB and anammox bacteria;
- Evaluate nitrogen removal performance by PN/A in a membrane-aerated biofilm reactor.

The main goal of this work is to determine the microbial community, bioreactor configuration and operational conditions that achieve the highest nitrogen removal efficiency for mainstream saline wastewater treatment.

4 Materials and Methods

Three one-dimensional biofilm models were developed with the AQUASIM software to simulate the following scenarios:

- Niche differentiation among anammox bacteria (granular biofilm);
- Partial-nitrification anammox in a granular sequencing batch reactor (PN/A-GSBR) (granular biofilm);
- Partial-nitrification anammox in a membrane-aerated biofilm reactor (PN/A-MABR) (planar biofilm in counter-diffusion).

4.1 Reactor parameters

The bulk and biomass volume chosen for this work are based on the lab-scale bioreactor in Figure 5. Thus, the total reactor volume (bulk liquid and biofilm) was assumed to be 3.5 L. The bulk liquid is assumed to be homogeneous and well-mixed, and external mass transfer limitation to the biofilm has been neglected (no boundary layer was implemented). The reactor temperature is assumed constant ($T = 25\text{ }^{\circ}\text{C}$; the model did not explicitly account for pH). The biofilm porosity is assumed constant (75%). Its value is determined by the initial fractions of the particulate components. There was no diffusive mass transport of solids in the biofilm and the only movement of solids was caused by biomass growth or decay (to inert fraction). The density of the biomass was set to $80\text{ kg}_{\text{COD}}\text{ m}^{-3}$ (typical values used by Corbalá-Robles et al. (2016) and Val del Rio et al. (2018), considering $0.75\text{ g}_{\text{VSS}}\text{ g}_{\text{COD}}^{-1}$ (Henze, 2000)).

4.2 Niche differentiation among anammox bacteria

Marine and freshwater-adapted anammox bioreactors could be utilized for nitrogen removal in the B-stage of saline mainstream wastewater treatment (Ali et al., 2020; Li et al., 2018b). Therefore, this model was developed to evaluate niche differentiation among anammox bacteria to assess the most suitable anammox species for the subsequent simulations of this work.

The species used in the AQUASIM model were chosen based on the availability of their microbial growth kinetics and salinity tolerance (Oshiki et al., 2016; Zhang and Okabe, 2020) (Table 1).

Recent work by Lotti et al. (2015a) and Zhang et al. (2017b) postulated that much lower μ_{max} obtained in anammox bacteria may result from the beyond-optimum condition for anammox enrichment and the maximum growth rate of “*Candidatus Jettenia caeni*” and “*Candidatus Brocadia sinica*” has been revised and implemented in this study.

On the other hand, from previous modelling approaches on PN/A (Corbalá-Robles et al., 2016; Hao et al., 2001; Hubaux et al., 2015; Van Hulle, 2005; Laurenzi et al., 2019; Li et al., 2019; Pérez et al., 2014; Volcke

et al., 2010; Vázquez-Padín et al., 2009), the kinetic parameters from “*Candidatus Brocadia anammoxidans*” have been considered “the standard anammox parameters” to evaluate bioreactor performance. Therefore, that species was also included in this work despite not showing any salt tolerance (Table 1).

Table 1: Anammox bacteria species competing in the model and respective kinetic parameters.

	“ <i>Ca. Brocadia</i> ⁽¹⁾ anammoxidans”	“ <i>Ca. Brocadia</i> ⁽²⁾ sinica”	“ <i>Ca.</i> ⁽³⁾ Scalindua sp.”	“ <i>Ca. Jettenia</i> ⁽⁴⁾ caeni”	“ <i>Ca. Kuenenia</i> ⁽⁵⁾ stuttgartiensis”
Optimal T (°C)	33	37	25	37	37
$\mu_{\max}^{(*)}$ (d ⁻¹)	0.033	0.138	0.048	0.076	0.071
E_a (kJ mol ⁻¹)	70	56	81.4	55.4	70
Y (g _{COD} g _N ⁻¹)	0.182	0.164	0.078	0.146	0.182
$K_{\text{NH}_4^+}$ (μM)	5	28	3	17.1	3 (**)
$K_{\text{NO}_2^-}$ (μM)	5	34	0.45	35.6	3
Salinity tolerance (g _{NaCl} /L)	0	15-35	Marine	4	30

⁽¹⁾ (Strous et al., 1998); ⁽²⁾ (Oshiki et al., 2016; Zhang et al., 2017b); ⁽³⁾ (Awata et al., 2013; Van De Vossenberg et al., 2008);

⁽⁴⁾ (Ali et al., 2015; Zhang et al., 2017b); ⁽⁵⁾ (Van Der Star et al., 2008).

^(*) $\mu_{\max}$ was corrected to T = 25 °C using the relationship expressed by the modified Arrhenius equation (equation A3).

^(**) $K_{\text{NH}_4^+}$ was never estimated for “*Ca. K. stuttgartiensis*”. The availability of nitrite is more relevant to its niche differentiation among anammox bacteria, as ammonium is usually more abundant in both natural and man-made ecosystems. Therefore, it was assumed that $K_{\text{NH}_4^+}$ is at least equal to $K_{\text{NO}_2^-}$.

The initial fraction of each anammox bacteria was the same ($\epsilon = 0.05$). The reactor behaviour was simulated for an influent containing ammonium and nitrite in a $\text{NH}_4^+:\text{NO}_2^- = 1$ rate, which means that based on the anammox stoichiometry, nitrite is the limiting substrate. For PN/A process, nitrite is produced by AOB/AOA, and it is not immediately available from the influent.

The competition among anammox bacteria was studied using four different nitrogen loading rates in order to simulate mainstream and sidestream wastewater treatment: 0.1 and 0.3 g_N L⁻¹ d⁻¹ (mainstream) and 0.5 and 1 g_N L⁻¹ d⁻¹ (sidestream).

4.3 PN/A in a granular sequencing batch reactor (GSBR)

The operational conditions that were used in this model are based on the experimental lab-scale SBR in Figure 5.

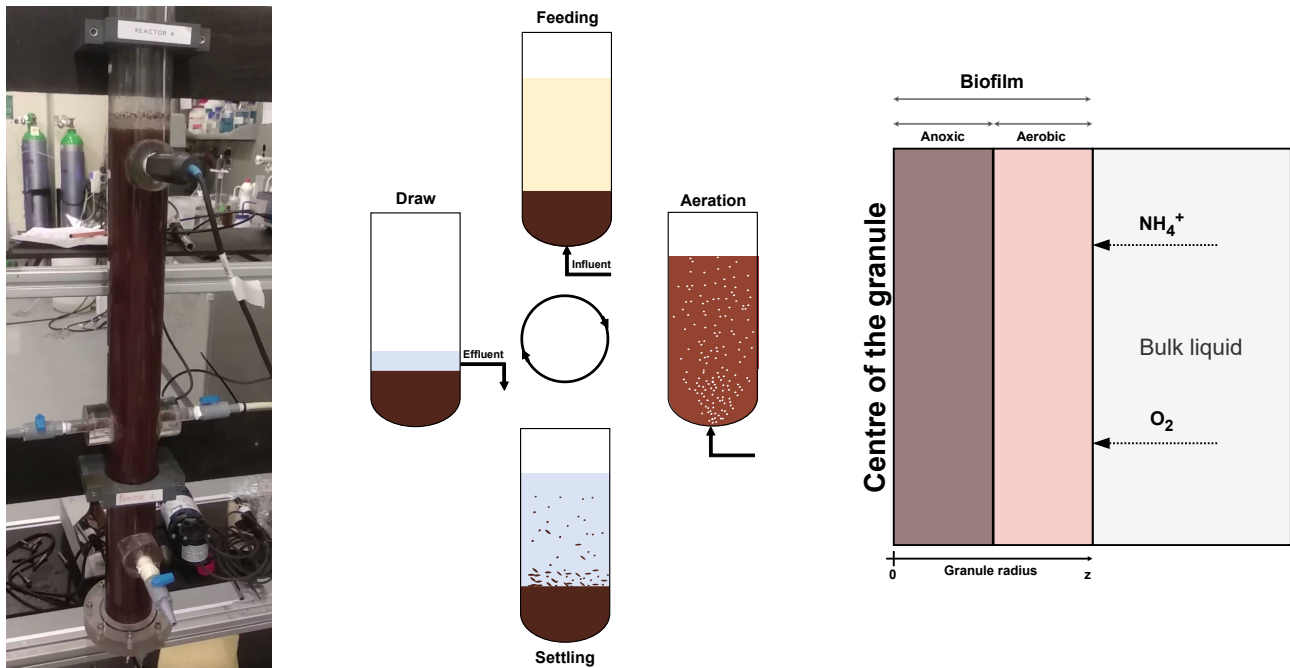


Figure 5: The lab-scale partial-nitrification and anammox bioreactor (working volume of 3.5 L) in the Water Desalination and Reuse Center (WDRC) laboratory at KAUST. Schematic representation of the SBR cycle and of the co-diffusion of oxygen and ammonium in the granular biofilm.

The cycle of the sequencing batch reactor is represented in Figure 6: feeding - 24 min; aeration - 168 min; settling phase - 24 min and effluent discharge - 24 min. Each cycle has a duration of 4 h which results in an HRT of 0.1667 d and an NLR of $0.3 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$, for a typical mainstream influent concentration of $50 \text{ mg}_{\text{NH}_4^+ - \text{N}} \text{ L}^{-1}$.

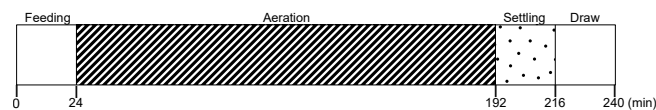


Figure 6: Schematic representation of the duration of each stage in the sequencing batch reactor cycle.

Two sets of simulations were carried out in this bioreactor configuration: with and without ammonia-oxidizing archaea (AOA). As aforementioned in Section 2.1.3, the detection of AOA in WWTPs suggested the potential application of AOA to oxidize ammonium under even lower DO levels, with longer retention time and consequently lower energy consumption. Since the growth kinetics of AOA are significantly different from AOB (Table A6), AOA is likely a better partner for anammox bacteria than AOB when treating mainstream wastewater.

A sensitivity analysis on oxygen affinity constant (K_{O_2}) for AOB and NOB was also simulated (see section 2.2.4):

- Scenario 1: $K_{O_2}^{NOB} > K_{O_2}^{AOB}$;
- Scenario 2: $K_{O_2}^{NOB} < K_{O_2}^{AOB}$.

The inflow composition was set to represent pre-treated sewage (from A-stage, Figure 3), without soluble nor particulate COD and with an ammonium concentration of $50 \text{ mg}_{\text{NH}_4^+-\text{N}} \text{ L}^{-1}$. The initial fraction of the biomass was: $\epsilon_{\text{AMX}} = 0.1$, $\epsilon_{\text{AOA}+\text{AOB}} = 0.1$ and $\epsilon_{\text{NOB}} = 0.05$.

The nitrogen removal efficiency (NRE), at steady-state, was calculated from the nitrogen gas (S_{N_2}) produced by anammox, divided by the ammonium concentration in the influent ($S_{\text{NH}_4, \text{in}}$). The effluent concentration was obtained from the last minute of the settling phase of the SBR. The NRE of the system was evaluated at different dissolved oxygen concentrations during the aeration phase: from 0.20 up to 2 mg L^{-1} .

4.4 PN/A in a membrane-aerated biofilm reactor (MABR)

The MABR was modelled with the reactor parameters described in section 4.1. Biological nitrogen removal in membrane-aerated biofilms has been previously modelled and experimentally developed (Table A8).

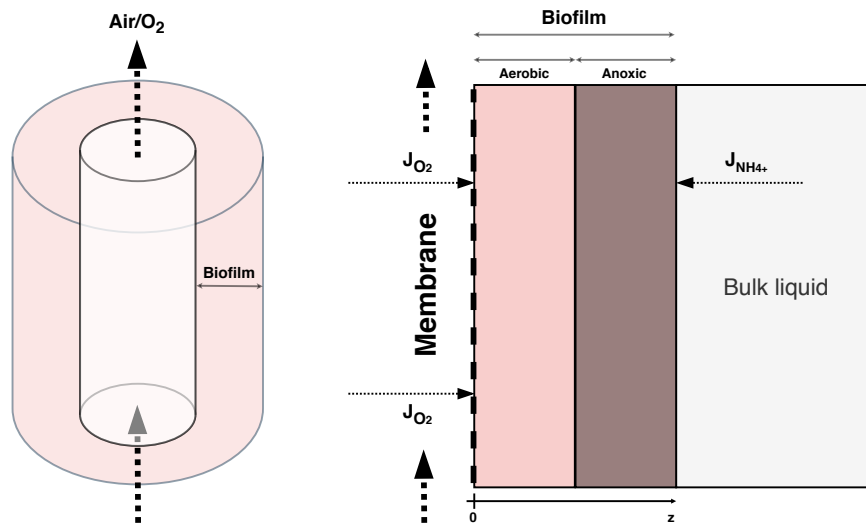


Figure 7: Schematic representation of a membrane-aerated biofilm reactor.

Oxygen is supplied through a gas-permeable membrane that also serves as a biofilm (Figure 7). The NRE was evaluated by changing the oxygen flux (J_{O_2}) (equation 9) supplied from the membrane to the biofilm, in order to compare different $J_{O_2}/J_{\text{NH}_4^+}$ ratios in the bioreactor (same parameter used by others on Table A8).

The surface to volume ratio was chosen based on previous modelling experiments by Ni and Yuan (2013) and Liu et al. (2016b) ($245 \text{ m}^2 \text{ m}^{-3}$). The specific loading rate of NH_4^+ was maintained constant ($J_{\text{NH}_4^+} = 1.225 \text{ g}_\text{N} \text{ m}^{-2} \text{ d}^{-1}$, considering the same operational parameters from the GSBR (HRT = 0.1667 d and NLR

$= 0.3 \text{ g}_N \text{ L}^{-1} \text{ d}^{-1}$). The inflow composition and initial biomass fraction of the biomass were also the same as in the PN/A-GSBR.

4.5 Biofilm thickness

For the niche differentiation and PN/A-GSBR, the biofilm grows in granules. The number of granules, in combination with the granule diameter, can affect the outcome of the simulation because this influences the overall liquid/granule interfacial area. However, the use of a system with a granule size distribution was avoided in this model. It is possible to include several biofilm compartments with different granule diameters in AQUASIM, in order to simulate a granule size distribution (Volcke et al., 2012), but it would significantly increase the complexity of the numerical computations, and it is not expected to contribute to a better understanding of the system. Therefore, the granules are grown from an initial radius of 0.01 mm to a predefined steady state granule radius of 0.75 mm, which is representative values for anammox (An et al., 2013) and implemented in other PN/A models (Hubaux et al., 2015; Jia et al., 2020; Li et al., 2019; Pérez et al., 2014; Volcke et al., 2010).

Granules were considered to be symmetrical spherical biofilms in which the concentration of all the compounds was constant for any given radius. Thus, a radius-dependent biofilm area was set in AQUASIM to correctly represent the spherical symmetry of the granules (equation 7) (Reichert, 1998).

$$A = 4\pi \cdot N_G (L_F + z)^2 \quad (7)$$

A is the area of the biofilm; N_G is the number of granules in the bioreactor; L_F : current granule radius; z is the distance from the substratum to the biofilm.

The number of granules (N_G) was calculated such that, the reactor eventually contained 1 L of particulate material, comprising both active biomass as well as inert matter generated from biomass decay ($V_{biofilm} = 1 \text{ L}$, then $N_G = 5.66 \times 10^5$).

For the MABR, biofilm thicknesses ranging from 0.20 to 0.60 mm were observed over long operation periods. It is known that different levels of shear stress, transmembrane gas pressure and oxygen loading during MABR operation also affected the biofilm parameters (Pellicer-Nàcher et al., 2014). However, in this case, the biofilm was assumed to have the same thickness ($L_{Fmax} = 0.75 \text{ mm}$) as in the GSBR simulation, to allow some degree of comparison between both scenarios.

The biofilm thickness at steady-state, in both bioreactor configurations, is predefined by defining the detachment rate (u_{det}) as the following:

$$\text{if } u_F > 0, u_{det} = u_F \left(\frac{L_F}{L_{Fmax}} \right)^4 \text{ else, } u_{det} = 0 \quad (8)$$

L_F : current biofilm thickness; $L_{F_{max}}$: the steady-state biofilm thickness; u_F : the advective velocity of biofilm growth at the biofilm surface.

4.5.1 Model Resolution

The grid number in the biofilm compartment on AQUASIM was set to 30 (Hubaux et al., 2015). For this model resolution, simulations were typically run for up to 2000 days to reach steady-state conditions. Steady-state was assumed when:

- the biofilm thickness was equal to 0.75 mm;
- there were no differences in biomass distribution in the biofilm;
- there were no differences in the effluent concentration of the different nitrogen fractions.

4.6 Kinetic parameters

The one-dimensional models describe the relationships among five dissolved components (Table A2): ammonium-nitrogen (S_{NH_4}), nitrite-nitrogen (S_{NO_2}), nitrate-nitrogen (S_{NO_3}), dinitrogen gas (S_{N_2}) and dissolved oxygen (S_{O_2}); and five particulate species that are competing for substrate and space in the biofilm (Table 2).

Table 2: Interaction between particulate species and dissolved components in the biofilm

	X_{AOA}	X_{AOB}	X_{NOB}	X_{AMX}
S_{O_2}	+	+	+	–
S_{NH_4}	+	+	–	+
S_{NO_2}	–	–	+	+

+ competitive; – non-competitive

Inert biomass (X_I) was produced by biomass decay using the death-regeneration approach (Van Loosdrecht and Henze, 1999). Living cells are turned partly into inert material. Ammonium is consumed for biomass growth and energy production, and is also produced during biomass decay. The wastewater is assumed to contain no COD, and heterotrophic growth was neglected. This is a simplification since it is known that heterotrophs can be present in autotrophic reactors even when no external COD source is supplied (Fux et al., 2002; Van Hulle, 2005; Wyffels et al., 2004).

The process kinetic rate equations are described in Table A3. The stoichiometric matrix is described in Table A4. The parameters implemented in the stoichiometric matrix and in the kinetic rate equations are described in Table A6. Diffusion coefficients characterizing mass transfer within the biofilm are discretized in Table A7. Other model considerations are detailed in Section A3.

4.7 AQUASIM implementation

4.7.1 GSBR

In the laboratory, influent is fed from the bottom of the reactor through the settled bed of granules. At the end of each cycle, the effluent is discharged from a port at half of the reactor height. The AQUASIM software does not allow the volume of the bulk liquid in the biofilm compartment to vary in time.

As a way of circumventing this issue, Beun et al. (2001) has developed a new approach to represent the mass transport, the fill, conversion and discharge processes in the time-dependent SBR. This approach was later replicated by other authors (Corbalá-Robles et al., 2016; Kampschreur et al., 2007; De Kreuk et al., 2007; Vázquez-Padín et al., 2009; Volcke et al., 2012; Winkler et al., 2015; Ni et al., 2008), and it can be described in the following way (Figure 8):

- The biofilm compartment (that represents the granules) is connected to a completely mixed compartment with an advective link;
- A high recirculation flow rate is incorporated from the biofilm compartment to the completely mixed one to ensure the same concentrations on both liquid bulk volumes;
- The influent and effluent draw are simulated by using switch functions on the flowrates of the completely mixed compartment;
- Aeration is simulated by introducing a gas compartment (completely mixed compartment) connected with the biofilm one with a diffusive link.

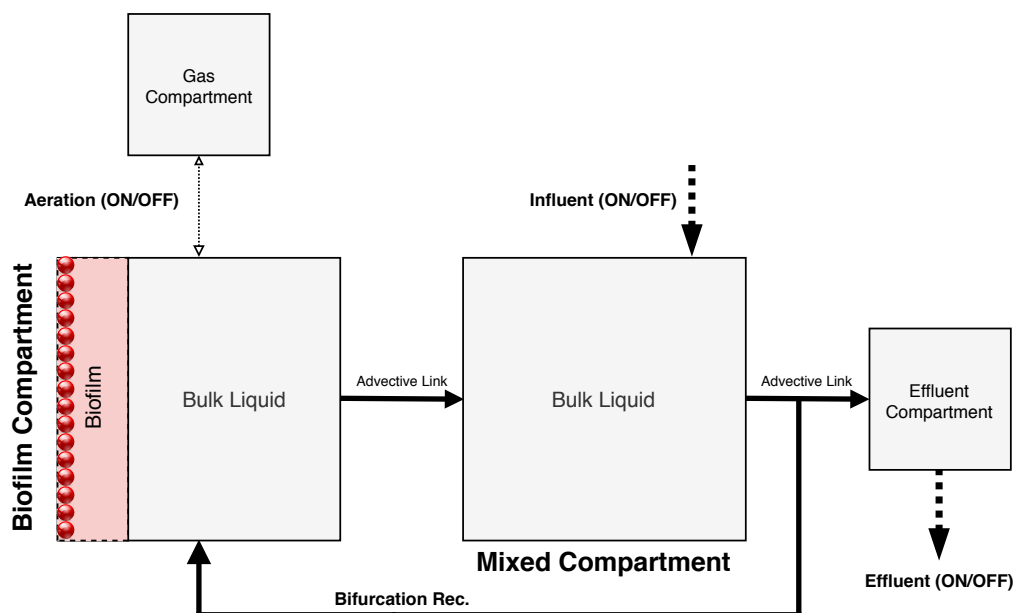


Figure 8: Schematic diagram of the granule-based SBR as implemented in the AQUASIM using the advective approach.

Baeten et al. (2017) showed that caution should be exercised with this popular approach because numerical errors occur when overly high artificial advective flows are used. These errors could stay unnoticed if mass-balances of the simulation results are not checked. As a more reliable solution to this problem, they proposed a new method, using the same switch functions and completely mixed and biofilm compartments, but based on diffusive links between compartments.

In the present model, although the same results were obtained using both approaches, the diffusive way was easier to implement, did result in faster simulations and, therefore, was the one implement for all the simulations. The implemented diffusive approach configuration is illustrated in Figure 9:

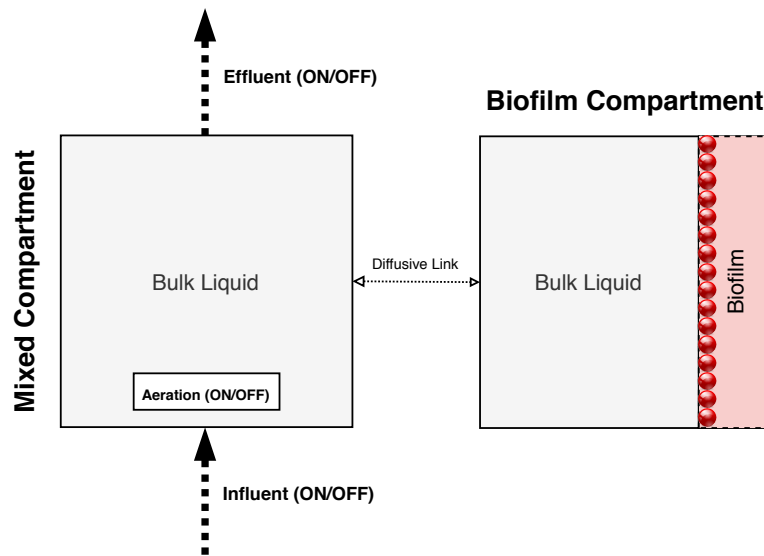


Figure 9: Schematic diagram of the granule-based SBR as implemented in the AQUASIM using the diffusive approach.

4.7.2 MABR

The MABR model was based on the one developed by Ni and Yuan (2013). The biofilm reactor is modelled integrating two different compartments: a completely mixed gas compartment (representing the membrane lumen operated as a flow-through) and a biofilm compartment, containing biofilm and bulk liquid (Figure 10). A physical diffusion link connects the gas compartment to the base of the biofilm. Oxygen concentration in the membrane is determined by the applied gas pressure and the gas flow rate. Thus, the oxygen flux from the gas to the biofilm matrix ($\text{g}_{\text{O}_2} \text{ m}^{-2} \text{ d}^{-1}$) is defined as (Casey et al., 1999):

$$J_{\text{O}_2} = k_{\text{O}_2} \left(\frac{S_{\text{O}_2,g}}{H_{\text{O}_2}} - S_{\text{O}_2} \right) \quad (9)$$

$S_{\text{O}_2,g}$ and S_{O_2} are the concentrations of oxygen in the gas and biofilm matrix compartments (mg L^{-1}), k_{O_2} is the overall mass transfer coefficient of oxygen (1.5 m d^{-1}) (Casey et al., 1999) and H_{O_2} is the non-dimensional Henry's Law coefficient for oxygen (32.7 at 25 °C).

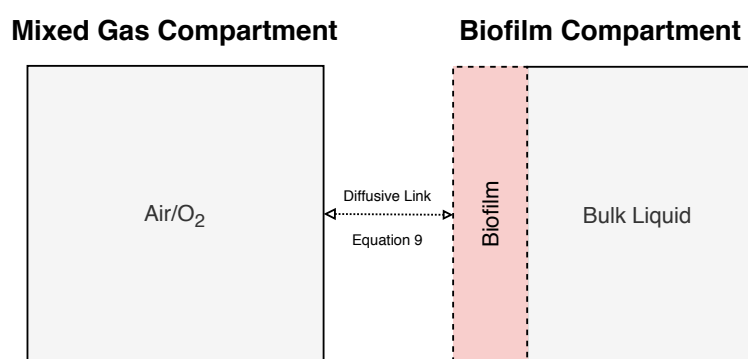


Figure 10: Schematic diagram of the MABR as implemented in the AQUASIM.

5 Results and Discussion

5.1 Niche differentiation among anammox bacteria

5.1.1 Optimal conditions

In this first simulation, the salinity was not evaluated and there were no restrictions in the microbial kinetics of each species from Table 1.

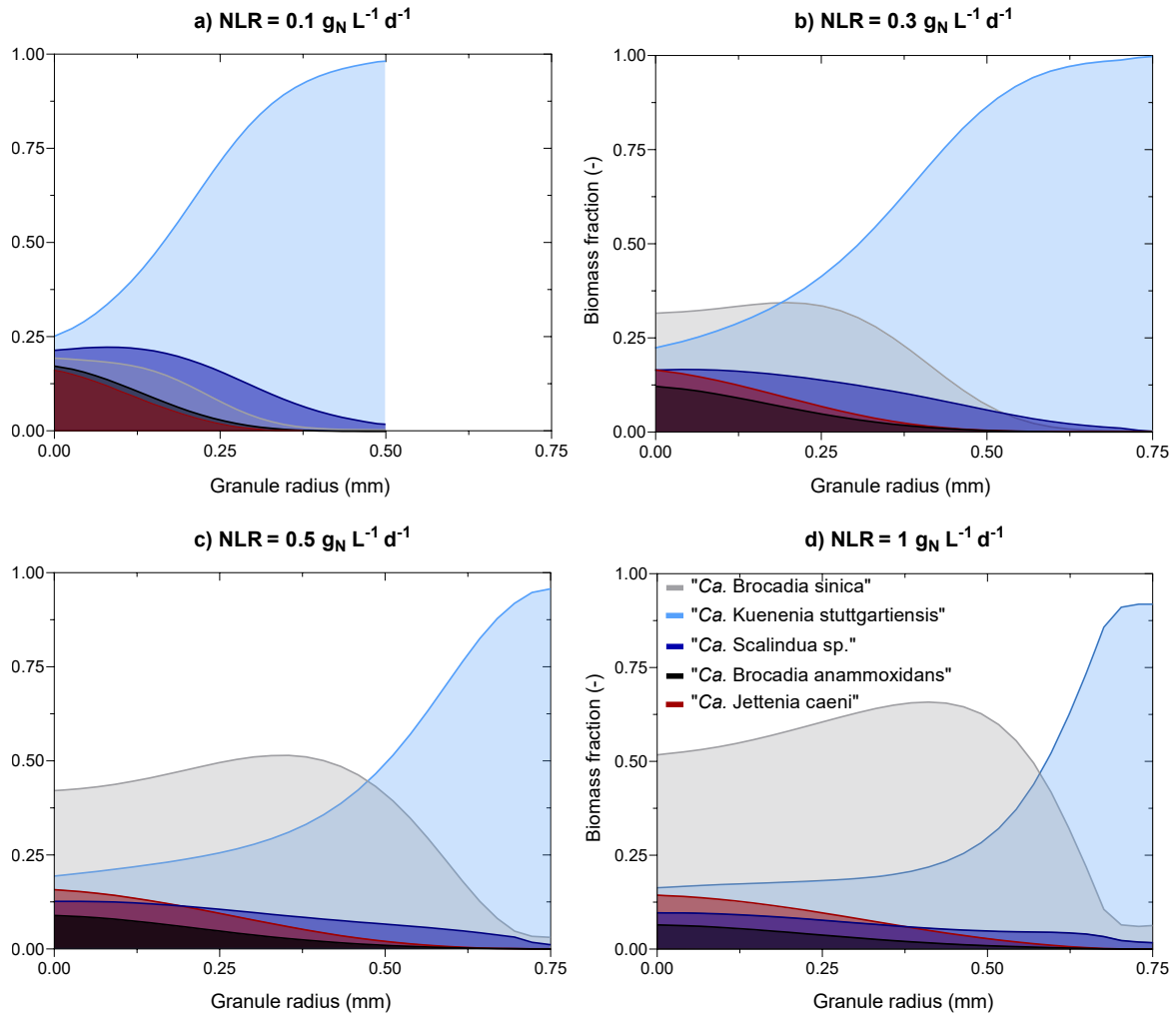


Figure 11: Steady-state distribution of anammox bacteria competing in a granule at different nitrogen loading rates. The centre of the granule is represented at 0 mm and the substrate (S_{NO_2} and S_{NH_4}) diffuses to the granule at 0.75 mm. At NLR of $0.1 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$, the growth of the biomass was slower and the simulation was stopped when the granule radius reached 0.50 mm.

At sidestream values (0.5 and $1 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$), the competition among anammox bacteria resulted in “*Ca. Brocadia sinica*” and “*Ca. Kuenenia stuttgartiensis*” occupying relatively the same volume in the biofilm (Figure 11c and Figure 11d).

“*Ca. B. sinica*” is considered an *r*-strategist that displays a higher growth rate ($\mu_{\text{max}} = 0.138 \text{ d}^{-1}$) than their competitors in niches with high substrate availability. On the other hand, “*Ca. Scalindua sp.*” is regarded as

K-strategist, growing faster than its competitors when the substrate is available at lower concentrations. The higher NLR translates into a higher availability of substrate, which means that the growth rate is more relevant than the *K*-affinity for the spatial distribution of anammox bacteria. Thus, the niche differentiation in Figure 11d: “*Ca. B. sinica*” is the most representative inside the granules (up to 0.50 mm) and “*Ca. Kuenenia stuttgartiensis*” occupies the rest of the volume until the surface. In fact, “*Ca. Brocadia sinica*” is the dominant species in granular-based full-scale wastewater treatment systems (Speth et al., 2016), which usually operate at higher nitrogen concentrations.

At mainstream conditions (0.1 and $0.3 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$), nutrient limitation due to competition and diffusion in the biofilm changed the bacteria's spatial distribution. “*Ca. Kuenenia stuttgartiensis*” was still the most dominant species in the granule. However, at $0.3 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$ (Figure 11b) even though “*Ca. B. sinica*” still appears in the centre of the granule, due to the ecology behavior of “*Ca. Scalindua sp.*”, this species is distributed from the surface of the granule until the centre of the granule, and becomes the second most abundant species for an NLR of $0.1 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$ (Figure 11a).

Concerning “*Ca. B. anammoxidans*” and “*Ca. J. caeni*”, these two species were always outcompeted by a better anammox species within the same ecology: in terms of *K*-affinity, “*Ca. Scalindua sp.*” has a higher substrate affinity than “*Ca. B. anammoxidans*”; and regarding maximum growth rate, “*Ca. Brocadia sinica*” has a higher μ_{max} than “*Ca. J. caeni*”. Therefore, these species could never proliferate in the granule and only represented a small biomass fraction in the centre region. At $0.1 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$, “*Ca. J. caeni*” (*r*-strategist) and “*Ca. B. anammoxidans*” (*K*-strategist), occupied the same biomass fraction and exhibited the same behavior inside the biofilm, despite the opposite microbial kinetics.

It is important to notice that, “*Ca. B. anammoxidans*” is the “standard species” used in almost all biofilm modeling approaches involving anammox bacteria (Baeten et al., 2019). However, these results illustrate that nitrogen removal and the associated growth of anammox bacteria are underestimated when the microbial kinetics of “*Ca. B. anammoxidans*” are implemented to represent the whole bacterial group.

On the other hand, successful competition of “*Ca. J. caeni*” at low NLR ($<0.1 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$) over “*Ca. B. sinica*” and “*Ca. K. stuttgartiensis*” has been reported by Zhang et al. (2017a). Based on the results of this simulation, the kinetic model could not appropriately explain the outcomes of the experimental competition, which means there could be inaccuracies on $K_{\text{NO}_2^-}$ for “*Ca. J. caeni*”. The authors also propose that under a low growth rate, the microbial maintenance rate significantly impacts the microbial competition. Therefore, information about this parameter and an experimental re-evaluation of this kinetics should be implemented in the future.

Surprisingly, the three best salinity tolerant species were the most abundant in the biofilm. “*Ca. K. stuttgartiensis*” was the most abundant species in all nitrogen loading rates tested and can tolerate salinities up to $30 \text{ g}_{\text{NaCl}} \text{ L}^{-1}$ (Gonzalez-Silva et al., 2017; Kartal et al., 2006). The second most abundant species was

“*Ca. Brocadia sinica*” which has been reported to tolerate seawater (3.5% salinity) (Li et al., 2018a). The third most abundant species was “*Ca. Scalindua sp.*”, which belongs to the only marine anammox genus (Sonthiphand et al., 2014).

5.1.2 Saline conditions

To better describe the niche differentiation among “*Ca. Kuenenia stuttgartiensis*”, “*Ca. Scalindua sp.*” and “*Ca. Brocadia sinica*”, a salinity effect was incorporated in the maximum growth rate of the kinetic model (Table 3). Even though there is not a general way to implement this concept in a mathematical model, recent studies have been published trying to approach the problem (Wu et al., 2019; Li et al., 2018a). Therefore, based on the work from these authors (see section A6), the effect of 1.7% salinity was simulated (i.e. for toilet flushing application - see section 1).

From the previous simulation, “*Ca. B. sinica*”, “*Ca. K. stuttgartiensis*” and “*Ca. Scalindua sp.*” are the anammox bacteria that will most likely be present in a biofilm for mainstream saline wastewater treatment. Moreover, “*Ca. J. caeni*” and “*Ca. B. anammoxidans*” have been reported to have lower or no tolerance to saline conditions (Table 1) and their growth would be inhibited.

Table 3: $\mu_{\max}$ implemented in the model for a salinity of 1.70%.

	“ <i>Ca. Scalindua sp.</i> ”	“ <i>Ca. K. stuttgartiensis</i> ”	“ <i>Ca. B. sinica</i> ”
Optimal Salinity (%)	2.80	1.25	0
Inhibition Rate (%)	22	7	36
$\mu_{\max}$ adapted (d ⁻¹)	0.037	0.066	0.088

There are several differences between the results with the salinity effect (Figure 12) and the previous results (Figure 11):

- Considering that there was only a 7% inhibition at 1.7% salinity for “*Ca. Kuenenia stuttgartiensis*” (Table 3), it was expected that this species would be even more abundant and to dominate the biofilm;
- The salinity inhibition played a role in the spatial distribution of “*Ca. Brocadia sinica*”. Lower growth rate translated in a decrease in the biomass fraction of this species at all nitrogen loading rates;
- The lower fraction of “*Ca. B. sinica*” shifted into a higher representativity of “*Ca. Scalindua sp.*” in the biofilm, mainly at mainstream conditions. “*Ca. Scalindua sp.*” was clearly the second most abundant species in the granule at low NLR (Figure 12a,b).

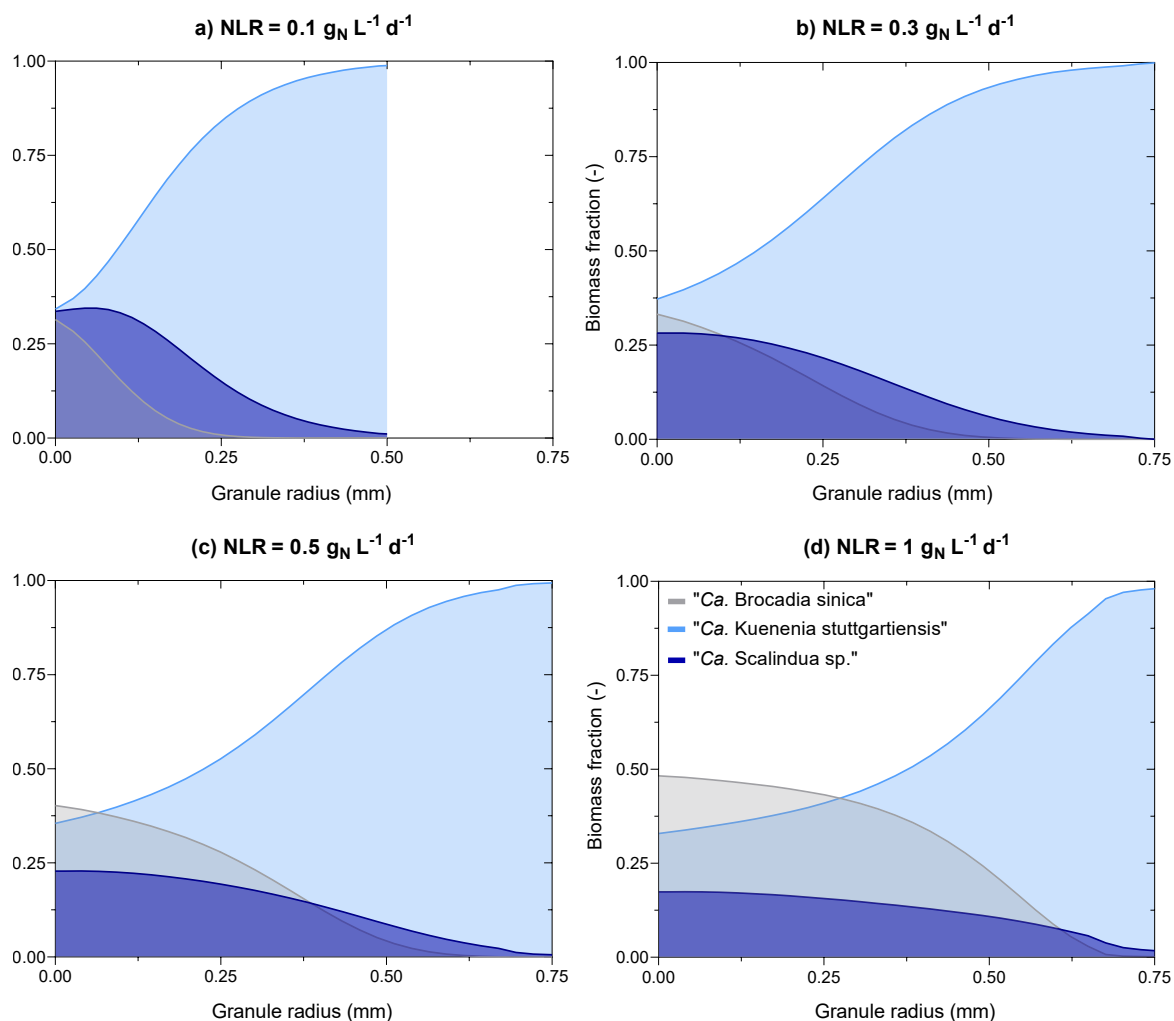


Figure 12: Steady-state distribution of anammox bacteria competing in a granule at different nitrogen loading rates, with a salinity effect implemented in the microbial kinetics (Equations A6 and A7). The centre of the granule is represented at 0 mm and the substrate (S_{NO_2} and S_{NH_4}) diffuses to the granule at 0.75 mm. At NLR of $0.1 \text{ g}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$, the growth of the biomass was slower and the simulation was stopped when the granule radius reached 0.50 mm.

These results indicate that “*Ca. Kuenenia*” and “*Ca. Scalindua*” are the most suitable species for saline wastewater treatment and agree with what has been reported in experimental studies (Table A10). It is important to consider that all the kinetic parameters reported here were adapted for 25 °C using the Arrhenius equation (Equation A3), but only “*Ca. Scalindua sp.*” has optimal growth at room temperature (Van De Vossenberg et al., 2008). This means that an over or underestimation of the kinetic parameters of other anammox bacteria might have occurred.

Although “*Ca. Scalindua sp.*” is a marine anammox, the dual system evaluated in this simulation (freshwater and seawater resulting in 1.7% salinity) affects the growth rate and performance of this marine species (22% inhibition Table 3). This is one of the reasons why salinity-adapted freshwater anammox have been proposed as a better option for saline wastewater treatment (Gonzalez-Silva et al., 2017; Li et al., 2018b). On the other hand, in several studies where adaptation of anammox bacteria to salinity is reported, artificial seawater instead of real seawater is used (Table A10). This has implications on the interpretation of the results since it is known that certain elements in the seawater medium might be the differentiation factor

between the growth of “*Ca. Scalindua* sp.” over other anammox bacteria (Wei et al., 2016).

Therefore, in order to improve the salinity effect in a mathematical model, there is the need of developing more correlations such as the ones used in this model and validating them with more experimental data, within the same operational conditions (such as temperature and the use of real seawater), and also extending this approach to other species.

Lastly, another critical aspect that was not studied was the aggregation ability of anammox bacteria. Based purely on kinetic parameters, there is a niche advantage by other anammox bacteria over “*Ca. B. sinica*” at low NLR. However, it has been reported that “*Ca. B. sinica*” has significant advantages in the efficient and rapid start-up of anammox reactors due to its aggregation capability (Ali et al., 2018).

5.2 PN/A-GSBR

5.2.1 Scenario 1: $K_{O_2}^{NOB} > K_{O_2}^{AOB}$

In this scenario, the common approach of considering higher affinity for oxygen by AOB was simulated ($K_{O_2}^{NOB} = 0.80 \text{ mg}_{O_2} \text{ L}^{-1} > K_{O_2}^{AOB} = 0.40 \text{ mg}_{O_2} \text{ L}^{-1}$).

In Figure 13, the effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas are represented at different DO concentrations.

In PN/A process, there is an optimal bulk oxygen concentration at which the nitrogen gas production reaches a maximum. The maximum nitrogen removal efficiency was 92%, and it was achieved at a DO concentration of $0.40 \text{ mg}_{O_2} \text{ L}^{-1}$ (Figure 13, 91% efficiency at $0.50 \text{ mg}_{O_2} \text{ L}^{-1}$). Similar efficiencies have been accomplished by other models (Corbalá-Robles et al., 2016; Mozumder et al., 2014).

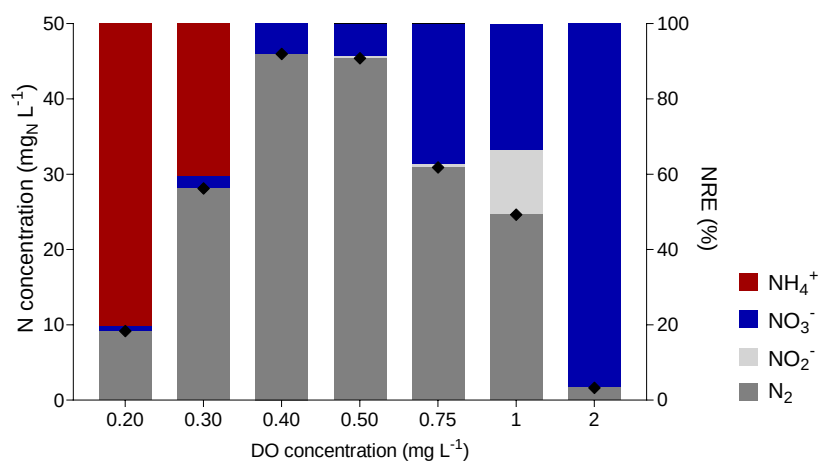


Figure 13: Nitrogen fractions in the effluent at different DO concentrations, for $K_{O_2}^{NOB} > K_{O_2}^{AOB}$. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).

The operation of the SBR during the maximal nitrogen removal is described in Figure 14. The influent ($S_{NH_4in} = 50 \text{ mg}_{NH_4^+-N} \text{ L}^{-1}$) enters the reactor during the first 24 min of the cycle. After that, aeration is

switched on during the next 168 min. At this time, ammonium starts being oxidized by AOB, with oxygen as the electron acceptor, producing nitrite. Anammox bacteria convert ammonium and the now available nitrite to nitrogen gas and nitrate.

When the aeration is switched off, a settling phase (24 minutes) is simulated. Nitrogen gas and nitrate are still produced during this settling phase and until the effluent is discharged in the last 24 min of the cycle. These phenomena are easily observed before steady-state (Figure 14a): there is a more abrupt consumption of nitrite followed by nitrogen gas production, immediately after aeration is switched off. At steady-state, the maximum nitrogen removal is achieved as the oxygen is switched off, which could mean that a lower HRT and resultant lower aeration periods could be implemented in the system (Figure 14b).

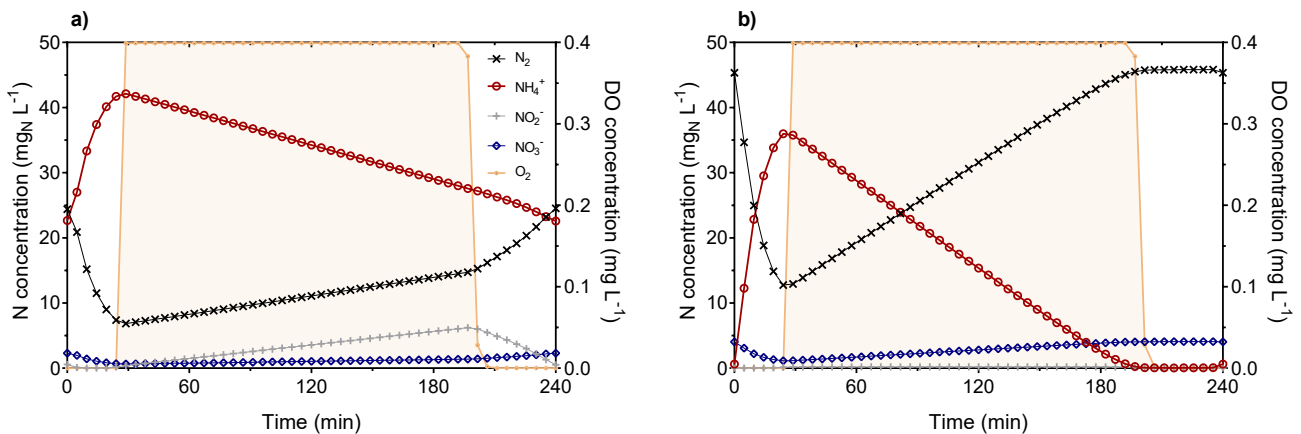


Figure 14: Operation of the sequencing batch reactor cycle at $S_{O_2} = 0.40 \text{ mg}_{O_2} \text{ L}^{-1}$: **a)** at 50 days (not steady-state); **b)** at 1500 days (steady-state). Aeration is switched on at 24 min and switched off at 192 min of the cycle. Nitrogen fractions concentration are read on the left y axis while dissolved oxygen concentration is read on the right y axis.

The typical steady-state biomass distribution profile of the microbial community is represented in Figure 15a: AOB at the outside of the granule close to the bulk liquid to consume the oxygen and the ammonium that are available. AOB is present in the biofilm until DO concentration reaches $0 \text{ mg}_{O_2} \text{ L}^{-1}$ (0.69 - 0.75 mm, Figure 15b). Both anammox bacteria are represented in the biofilm, situated just after AOB, in the inner and anoxic part of the granule, consuming ammonium and nitrite that is produced by AOB and needs to diffuse to the biofilm. Due to the oxygen gradient that is created inside the granule, AOB shields anammox bacteria from the oxygen.

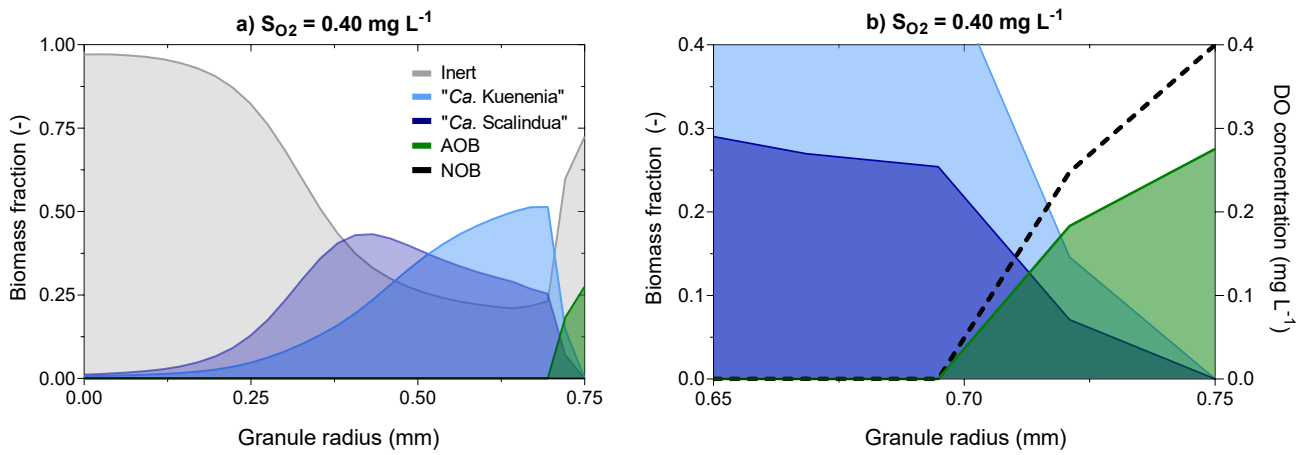


Figure 15: **a)** Steady-state distribution of biomass and particulate inerts in a granule at the optimal oxygen concentration for which maximum nitrogen removal is achieved ($S_{O_2} = 0.40 \text{ mg O}_2 \text{ L}^{-1}$); **b)** Close up (0.60 - 0.75 mm) of the gradient profile of oxygen (dotted line) in the biofilm. The centre of the granule is represented at a granule radius of 0 mm; substrate diffuses in co-diffusion to the granule at 0.75 mm.

Anammox bacteria and AOB completely outcompeted NOB. As described in the niche differentiation simulation, "*Ca. Scalindua* sp." and "*Ca. K. stuttgartiensis*" cohabit the biofilm. Inert particulate originated from biomass decay accumulates in the centre of the granule. This behaviour is under the experimental findings of Vlaeminck et al. (2010) and Winkler et al. (2011).

At very low oxygen concentrations (Figure 16a, $S_{O_2} = 0.20 \text{ mg O}_2 \text{ L}^{-1}$), part of the ammonium remains unconverted due to the oxygen limitation of ammonia-oxidizers. AOB cannot convert ammonium to nitrite and, in turn, anammox bacteria does not have nitrite to convert to N_2 . "*Ca. Scalindua* sp." was the only anammox bacteria in the biofilm, which confirms that the availability of nitrite was low (higher NO_2^- affinity). Overall, the biofilm grows very slowly - the granule radius only reached 0.50 mm, even after 2000 days of operation (Figure 16b). As mentioned in Section 2.1.2, the performance of biofilm reactors for autotrophic nitrogen removal is strongly governed by NOB control. In this scenario, the NOB washout was mainly driven by the competition for oxygen with AOB, with the latter exhibiting higher oxygen affinity ($K_{O_2}^{NOB} = 2 \times K_{O_2}^{AOB}$).

However, at a DO concentration of $1 \text{ mg O}_2 \text{ L}^{-1}$, NOB was not outcompeted by anammox bacteria (Figure 16a). With the increase of DO concentration, anammox tend to situate deeper in the granule. Due to the required diffusive transport of nitrite from the aerobic to the anoxic region, nitrite oxidizers will observe somewhat higher nitrite concentrations than anammox cells. Even though this competition is compensated by the higher affinity of the anammox bacteria, at this DO concentration, the kinetic differences were not enough. Both growth of NOB and anammox bacteria was suboptimal, and the competition between the two resulted in nitrate accumulation (due to higher NOB activity) and nitrite accumulation (AOB still outcompetes NOB for oxygen) (Figure 16b). At $S_{O_2} = 2 \text{ mg O}_2 \text{ L}^{-1}$, anammox bacteria are washed out from the bioreactor by NOB (Figure 16c) and since there is enough oxygen for both nitrifiers, all the ammonium is converted to nitrate (Figure 16d).

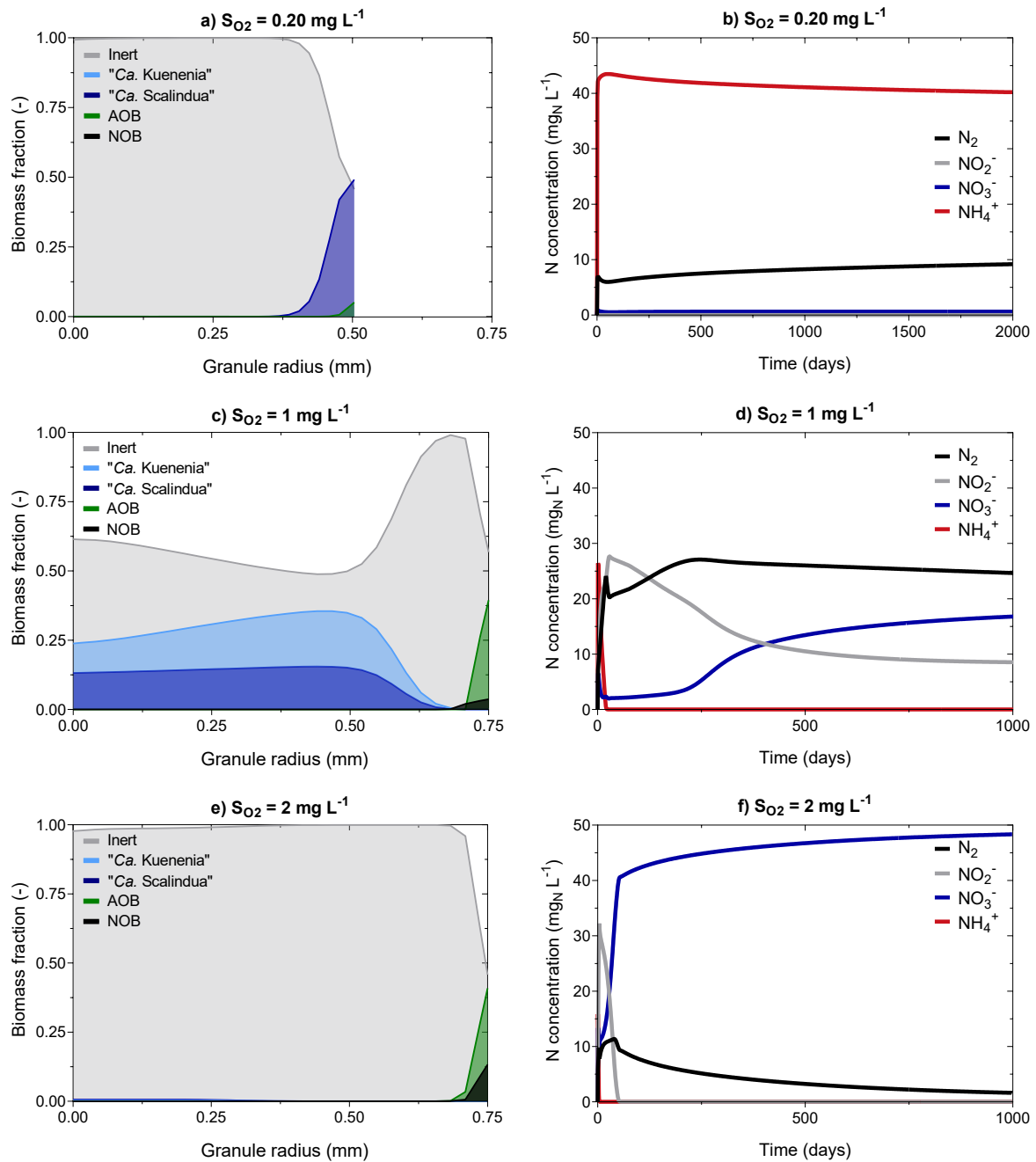


Figure 16: Comparison of dynamic model behaviour under the influence of bulk oxygen concentrations of 0.20, 1 and 2 $\text{mg O}_2 \text{ L}^{-1}$: **a), c) and e)** Steady-state distribution of biomass and particulate inerts in the granule; the centre of the granule is represented at a granule radius of 0 mm; substrate diffuses in co-diffusion to the granule at 0.75 mm. **b), d) and f)** Evolution of effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas. Nitrogen gas fraction represents the NRE. At $S_{\text{O}_2} = 0.20 \text{ mg O}_2 \text{ L}^{-1}$, steady-state was not reached even after 2000 days of operation. At the other oxygen concentrations, steady-state was reached after 1000 days of operation.

5.2.2 Scenario 2: $K_{O_2}^{NOB} < K_{O_2}^{AOB}$

In this scenario, the main objective was to evaluate the differences in nitrogen removal efficiency under the same DO concentrations as the previous scenario, considering higher affinity for oxygen by NOB as reported on Table A5 ($K_{O_2}^{NOB} = 0.20 \text{ mg}_{O_2} \text{ L}^{-1} < K_{O_2}^{AOB} = 0.40 \text{ mg}_{O_2} \text{ L}^{-1}$).

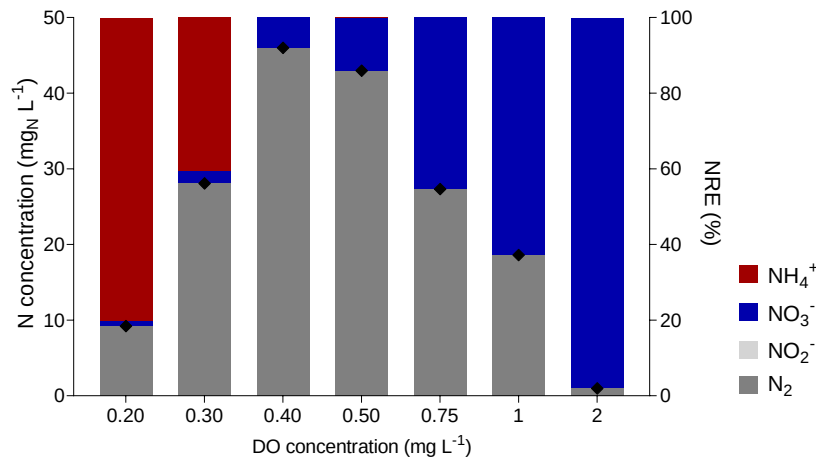


Figure 17: Nitrogen fractions in the effluent at different DO concentrations, for $K_{O_2}^{NOB} < K_{O_2}^{AOB}$. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).

The results obtained with these kinetics (Figure 17) were similar to the previous simulation. The same maximum NRE was achieved for the same DO concentration ($S_{O_2} = 0.40 \text{ mg}_{O_2} \text{ L}^{-1}$).

Although NOB has higher affinity for oxygen, anammox bacteria still outcompeted the latter for nitrite ($K_{NO_2^-}^{AMX} = 0.45/3 \mu\text{M} < K_{NO_2^-}^{NOB} = 9 \mu\text{M}$). The higher $K_{O_2}^{NOB}$ resulted in higher nitrate production at $S_{O_2} > 0.40 \text{ mg}_{O_2} \text{ L}^{-1}$, and in the complete consumption of nitrite in the bioreactor (no unconverted nitrite in the effluent when compared with Figure 13).

Moreover, since NOB is outcompeting AOB for oxygen, less nitrite is produced per day. Consequently, the same effluent concentration were achieved at steady-state (at $S_{O_2} < 0.50 \text{ mg}_{O_2} \text{ L}^{-1}$), but the whole process was slower (Figure 18).

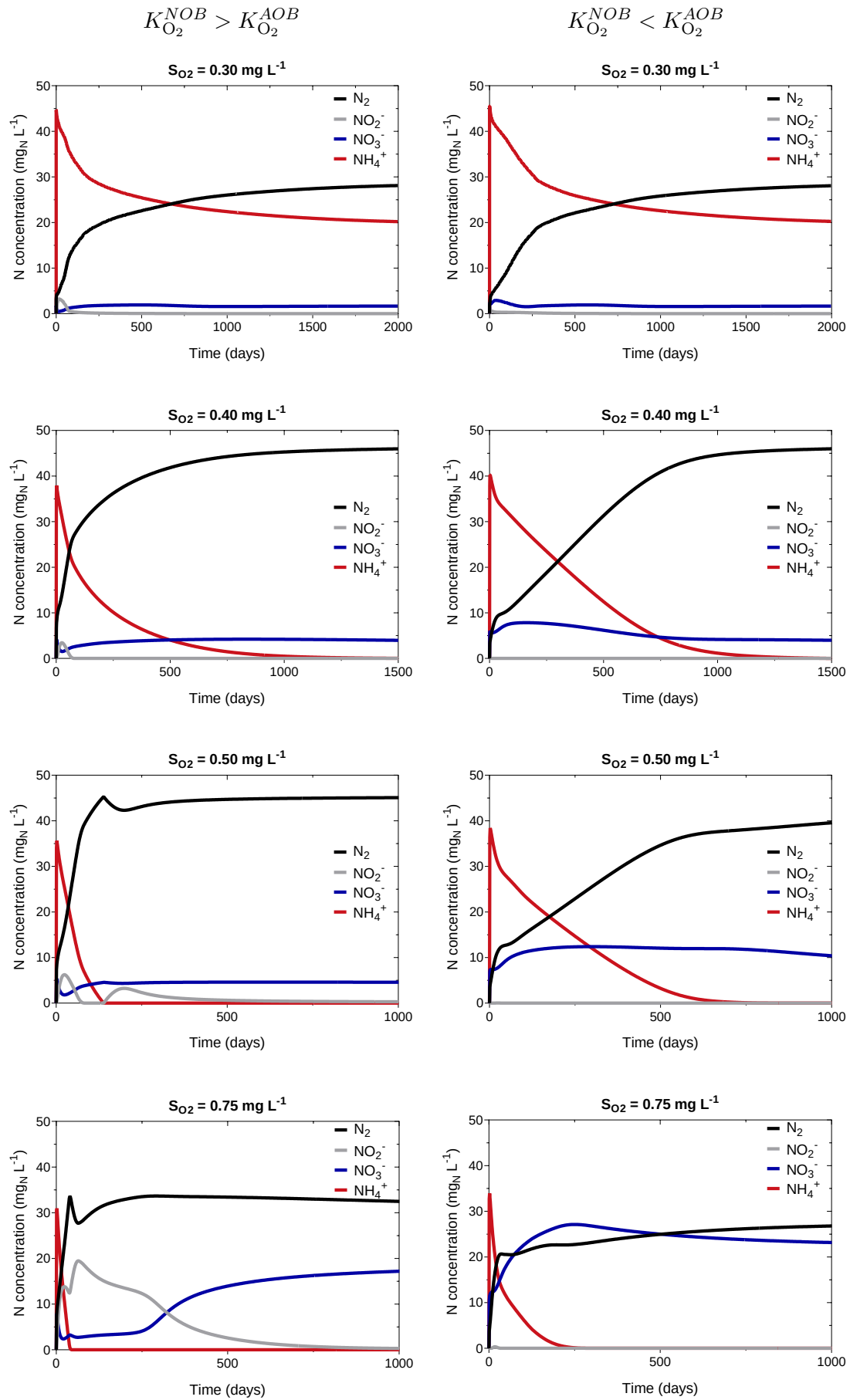


Figure 18: Evolution of bulk concentrations of ammonium, nitrite, nitrate and nitrogen gas between the two scenarios (different $K_{\text{O}_2}^{\text{NOB}}$) at different bulk oxygen concentrations. Nitrogen gas fraction represents the NRE.

Nevertheless, NOB repression and higher AOB/NOB activity have been reported using a strategy of operating at higher DO level (i.e. $>1.5 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$), with low reported values for $K_{\text{NO}_2^-}^{\text{NOB}}$. However, these studies also used other strategies to control NOB: transient anoxia with intermittent aeration (Malovanyy et al., 2015b; Wett et al., 2013); and AVN (Ammonium vs Nitrite) controller, which adjusts the DO to the desired set-point during the aeration period based on online measurements of NH_4^+ (Regmi et al., 2014). For our simulations, only based on the microbial kinetics and with a constant nitrogen loading rate, increasing the DO did not result in the washout of NOB and even undermined the nitrogen removal efficiency (Figure 17 and Figure 18).

These results are in agreement with what has been reported by other modelling approaches. In solely biofilm PN/A systems, the ratio of the oxygen affinity constants, $K_{\text{O}_2}^{\text{NOB}}/K_{\text{O}_2}^{\text{AOB}}$ and the ratio of the nitrite affinity constants, $K_{\text{NO}_2^-}^{\text{NOB}}/K_{\text{NO}_2^-}^{\text{AMX}}$ are reported to be the main parameters controlling microbial competition (Brockmann and Morgenroth, 2010).

Hao et al. (2001) predicted by mathematical modelling that even for $K_{\text{O}_2}^{\text{NOB}} = 0.2 \times K_{\text{O}_2}^{\text{AOB}}$, if the following ratio $K_{\text{NO}_2^-}^{\text{NOB}}/K_{\text{NO}_2^-}^{\text{AMX}} > 3$ is verified, NOB lose the competition for nitrite against anammox bacteria and PN/A process is maintained in the biofilm.

Pérez et al. (2014) and Corbalá-Robles et al. (2016) also reported the above conclusion and further demonstrated that there is a minimum NH_4^+ concentration, $(S_{\text{NH}_4})_{\text{min}}$, where nitrate is only produced by anammox, and NOB are either washed out from the granular sludge or present in very low numbers. Corbalá-Robles et al. (2016) stated that, for example, a change in the ratio $K_{\text{O}_2}^{\text{NOB}}/K_{\text{O}_2}^{\text{AOB}}$ from 5.88 to 0.14 would still repress NOB if the ammonium concentration would also increase from 0.31 to $220 \text{ mg}_{\text{NH}_4^+ - \text{N}} \text{ L}^{-1}$. On the other hand, when the reactor is operated in such away that ammonium concentration in the bulk is lower than $(S_{\text{NH}_4})_{\text{min}}$, NOB are able to proliferate in the granular sludge and no efficient repression of the nitrite oxidation is attained. Additionally, Pérez et al. (2014) described that if $K_{\text{O}_2}^{\text{NOB}} < 0.1 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$, there is an asymptotic behaviour from $(S_{\text{NH}_4})_{\text{min}}$ and NOB suppression cannot take place at any DO and NH_4^+ concentration.

In another example, although in a different bioreactor configuration (hybrid MBBR-IFAS), NOB repression was achieved at low and high DO (0.15 and $1.5 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$) for values of $K_{\text{O}_2}^{\text{NOB}}/K_{\text{O}_2}^{\text{AOB}} < 1$ (Laureni et al., 2019). In this case, instead of $(S_{\text{NH}_4})_{\text{min}}$, a maximum volumetric activity of anammox in the granule was the dominant mechanism controlling NOB washout.

Given that no differences were observed between the two scenarios, the common approach of $K_{\text{O}_2}^{\text{AOB}} < K_{\text{O}_2}^{\text{NOB}}$ was used for the subsequent simulations.

5.3 PN/A-GSBR including AOA

In Figure 19, the effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas are represented at different DO concentrations (0.05 to 0.20 mg_{O₂} L⁻¹).

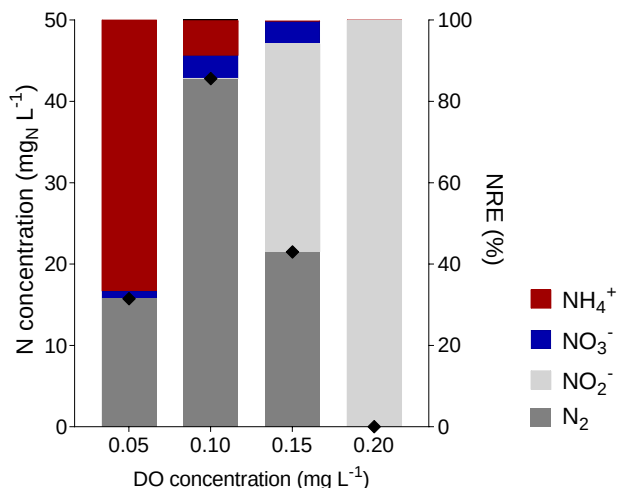


Figure 19: Nitrogen fractions in the effluent at different DO concentrations, considering AOA in the model. $S_{\text{NH}_4\text{in}} = 50 \text{ mg}_{\text{N-NH}_4^+} \text{ L}^{-1}$. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).

At all DO concentrations, AOB was always outcompeted by AOA. At very low oxygen concentrations (Figure 20b, $S_{\text{O}_2} = 0.05 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$), ammonium remained unconverted, even considering the high affinity of AOA for oxygen ($K_{\text{O}_2}^{\text{AOA}} = 0.128 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$). For an oxygen concentration of $0.10 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$, the NRE was 86%, but autotrophic removal of ammonium was not complete, as 9% of NH_4^+ was still present in the effluent (Figure 20d). “*Ca. Scalindua sp.*” was the only anammox bacteria that subsisted in the biofilm (Figure 20a,c).

As some ammonium remained unconverted due to oxygen limitations to ammonia-oxidizing archaea, it was expected that with the increase of dissolved oxygen in the reactor, nitrogen removal would also increase. This could have also been achieved by increasing the aeration time in the cycle.

However, at $S_{\text{O}_2} = 0.15 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$, AOA not only outcompeted AOB for oxygen but also both anammox species for ammonium (Figure 19). The higher affinity of AOA ($K_{\text{NH}_4^+}^{\text{AOA}} = 133 \text{ nM}$) when compared to anammox ($K_{\text{NH}_4^+}^{\text{AOA}} = 3 \mu\text{M}$ for both species, Table 1) led to the accumulation of nitrite in the reactor, while no ammonium was available for the anaerobic conversion to N_2 (Figure 20f). The abundance of “*Ca. K. stuttgartiensis*” over “*Ca. Scalindua sp.*” next to AOA in the biofilm further reinforces this statement (Figure 20e).

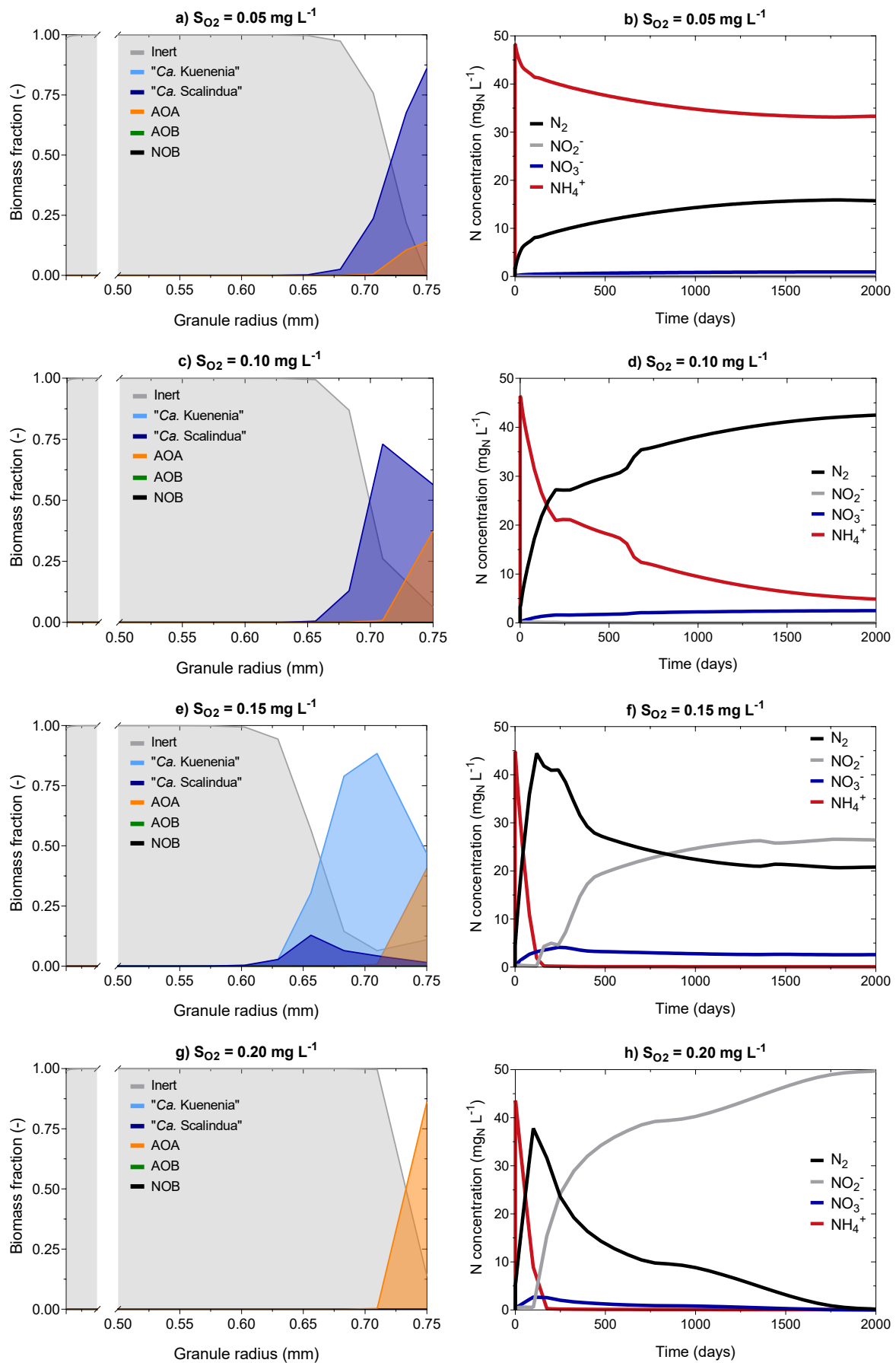


Figure 20: Comparison of dynamic model behaviour under the influence of bulk oxygen concentrations between 0.05 and 0.20 mg O₂ L⁻¹: **a), c), e) and g)** Steady-state distribution of biomass (with AOA, AOB, NOB and anammox bacteria) and particulate inerts; the centre of the granule is represented at a granule radius of 0 mm; substrate diffuses in co-diffusion to the granule at 0.75 mm. **b), d), f) and h)** Evolution of effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas. Nitrogen gas fraction represents the nitrogen removal efficiency. Steady-state was reached after 2000 days of operation.

To evaluate if there was a peak in the nitrogen removal efficiency, smaller steps in DO concentration were tested, from the range of 0.10 to 0.15 mg_{O₂} L⁻¹ (Table 4).

Table 4: Nitrogen fractions in the effluent for a dissolved oxygen concentration between 0.10 and 0.15 mg_{O₂} L⁻¹, considering an influent of $S_{\text{NH}_4^+-\text{N}} = 50 \text{ mg}_{\text{NH}_4^+-\text{N}} \text{ L}^{-1}$

DO	N ₂	NO ₂ ⁻	NO ₃ ⁻	NH ₄ ⁺	NRE
	mg L ⁻¹				%
0.10	42.77	0.07	2.79	4.31	86%
0.11	44.96	0.09	4.87	0.05	90%
0.12	42.95	2.33	4.61	0.06	86%
0.15	21.50	25.61	2.73	0.05	43%

At $S_{\text{O}_2} = 0.11 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$ (Table 4), there is a peak in the nitrogen gas production resulting in 90% of NRE while all the ammonium was converted. Nonetheless, as it can be detected at $S_{\text{O}_2} = 0.12 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$, an increase of 0.01 mg_{O₂} L⁻¹ already resulted in nitrite accumulation inside the bioreactor. The pattern of nitrite accumulation continues until $S_{\text{O}_2} = 0.15 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$ and, at $S_{\text{O}_2} = 0.20 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$ (Figure 20g), only AOA existed in the biofilm, while ammonium was fully converted to nitrite.

Although a 90% nitrogen removal efficiency was obtained (PN/A with only AOB: 92%), current control strategies rely on a range of DO concentrations to allow a response time from the system. The limited optimal DO concentration of 0.11 mg_{O₂} L⁻¹ may be challenging to maintain due to the impact of feed disturbances, biomass microbial activity, and high dependence on the accuracy of the measurement sensors (Saxena et al., 2019). Thereby, the restricted DO concentration, where nitrogen removal is maximum, is not desired.

Regarding modelling approaches with ammonia-oxidizing archaea, Pan et al. (2016) were one of the firsts to include this nitrifier in a PN/A model. They stated that there was cooperation between AOA and AOB, and AOA would only outcompete AOB under low ammonium concentrations (1.3 mg_{NH₄⁺-N} L⁻¹). As a result, AOA would be a better partner to anammox bacteria when compared to AOB for the treatment of low strength nitrogen sewage. However, in their study, the affinity for ammonium of both AOA ($K_{\text{NH}_4^+}^{\text{AOA}} = 0.06 \text{ mg L}^{-1}$) and anammox bacteria ($K_{\text{NH}_4^+}^{\text{AMX}} = 0.05 \text{ mg L}^{-1}$) was quite similar. Therefore, no competition could have been captured.

In the present model, even for 50 mg_{NH₄⁺-N} L⁻¹, AOA outcompeted both AOB and anammox bacteria. These results agree with the recent study published by Straka et al. (2019). They claimed that the higher affinity of AOA for ammonium when compared to anammox bacteria suggests that AOA/anammox cooperation is only possible where AOA do not outcompete anammox for the substrate. Using a biofilm model, they postulated that environments with counter-diffusion of ammonium and oxygen favour this cooperation. This statement will be evaluated in the next section.

5.4 PN/A-MABR

From the mentioned results, if AOA is present in the microbial community, it will outcompete both AOB and anammox bacteria in a co-diffusion gradient. Nitrogen removal with AOA was not optimal when compared with PN/A process with only AOB. Therefore, an engineering solution that allows AOA and anammox cooperation in a counter-diffusion gradient is the membrane-aerated biofilm reactor (MABR): oxygen is provided through a membrane to the base of the biofilm, whereas ammonium is supplied from the bulk liquid.

The steady-state behaviour in terms of nitrogen removal at different $J_{O_2}/J_{NH_4^+}$ ratios is summarized in Figure 21.

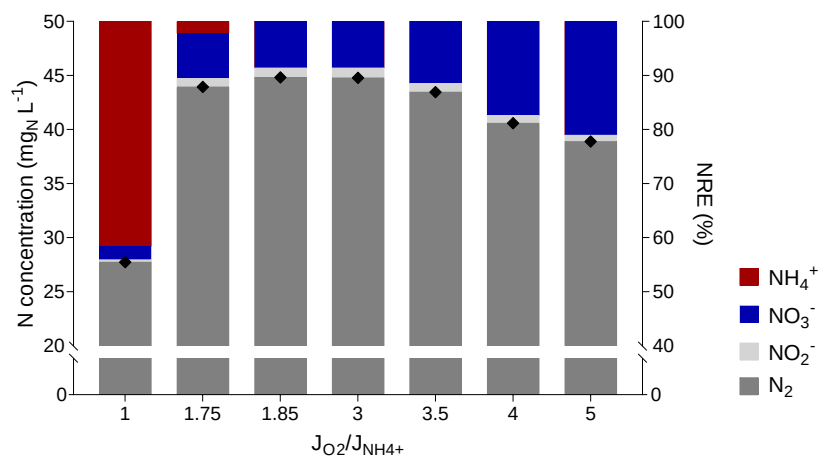


Figure 21: Nitrogen fractions in the effluent of a membrane-aerated biofilm reactor, at different $J_{O_2}/J_{NH_4^+}$ ratios. $S_{NH_4in} = 50 \text{ mg}_{N-NH_4^+} \text{ L}^{-1}$. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).

The maximum nitrogen removal efficiency of 90% was observed for a ratio of $J_{O_2}/J_{NH_4^+}$ from 1.85 to 3. This wider range of acceptable ratios where NRE is optimal was also reported by Terada et al. (2007) and Liu et al. (2016b).

Consistently with the results from the GSBR, AOA outcompeted AOB. The steady-state microbial community distribution and the resulting substrate profiles along the biofilm are shown in Figure 22a and Figure 22b. AOA were dominant in the inner layer (0-0.06 mm) of the biofilm while “*Ca. K. stuttgartiensis*” dominated over “*Ca. Scalindua sp.*” in the rest of the biofilm (more than 75% of the biomass fraction from 0.06-0.75 mm). These observations were coincident with the corresponding substrate profiles along the biofilm: DO decreased from the base through the inner layer of the biofilm where AOA subsisted (Figure 22c), and NH_4^+ showed the inverse trend, resulting in the abundance of anammox bacteria near the outer layer of the biofilm. Notably, in the region where AOA was active, consumption of oxygen and NH_4^+ and consequent production of NO_2^- can be observed (Figure 22d).

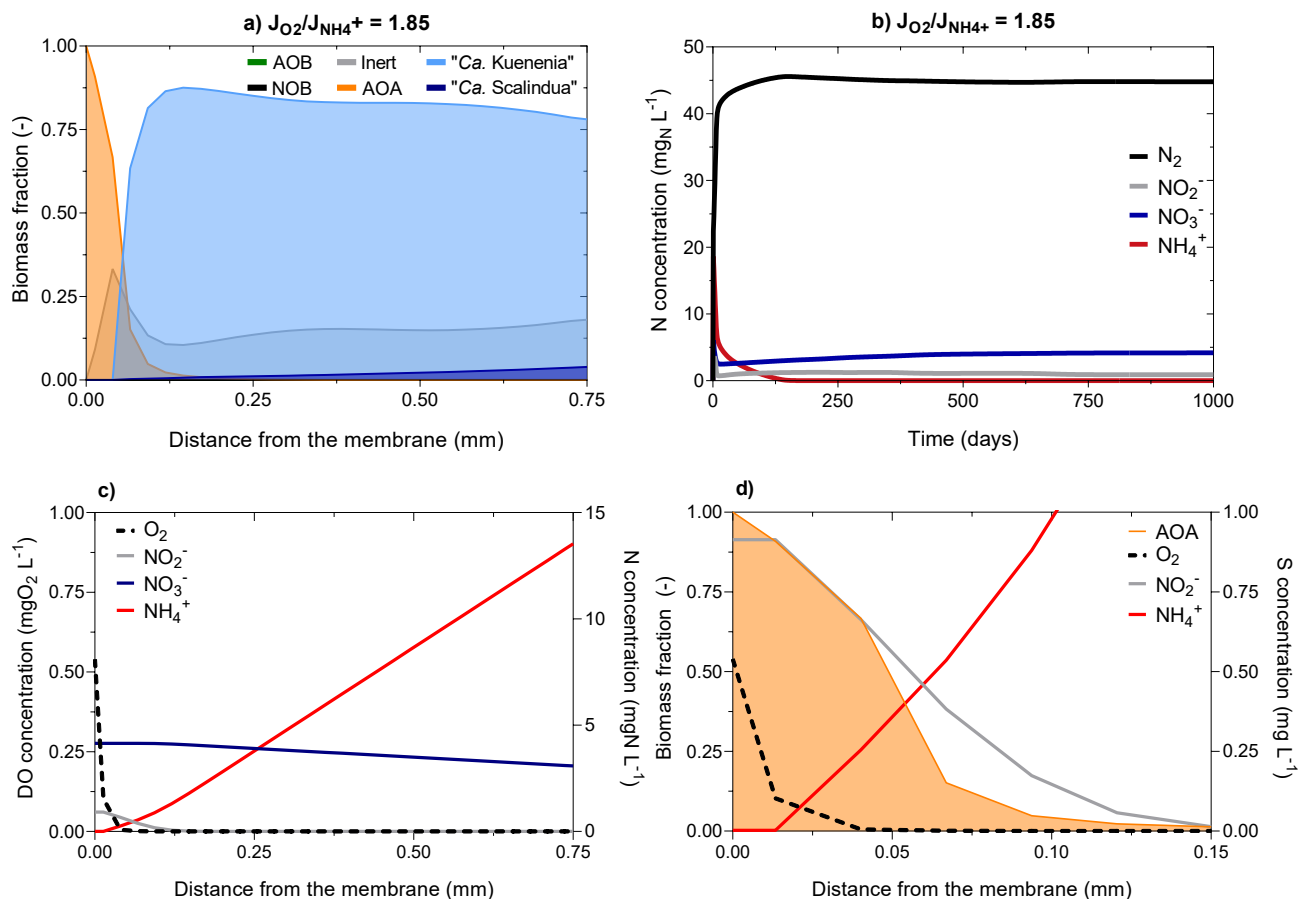


Figure 22: Optimal nitrogen removal for a $J_{O_2}/J_{NH_4^+}$ ratio of 1.85: **a)** Distribution of biomass (with AOA and anammox bacteria) and particulate inerts in the membrane biofilm; AOB and NOB were outcompeted. **b)** Evolution of effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas. **c)** Gradient profiles of substrate concentration in the counter-diffusion biofilm. Oxygen is supplied at a distance from the membrane of 0 mm and ammonium diffuses in counter-diffusion at 0.75 mm. **d)** Close up (0-0.15 mm) of the substrate gradient profiles and AOA biomass fraction in the biofilm.

Oxygen loading can be controlled by adjustment of gas flow rate and applied pressure. Therefore, control of J_{O_2} against the fluctuation of $J_{NH_4^+}$ could be most easily attained with membrane aeration (Syron et al., 2015). However, the inherent system geometry of membrane-aerated biofilm complicates the task of inhibiting and controlling NOB to ensure sufficient and stable nitrification. It has been reported that even if the $J_{O_2}/J_{NH_4^+}$ is maintained, high J_{O_2} can still promote NOB growth close to the membrane (Ma et al., 2017; Terada et al., 2007; Wang et al., 2009).

At a ratio of $J_{O_2}/J_{NH_4^+}$ equals 1, ammonium remained unconverted in the reactor due to oxygen limitations similar to the low oxygen concentrations in the previous simulations (Figure 21). For $J_{O_2}/J_{NH_4^+} = 4$, NOB started to grow in the biofilm, occupying a small region (0-0.035 mm) close to the biofilm base (Figure 23a), and NRE decreased at the expense of nitrate accumulation in the reactor (Figure 23b).

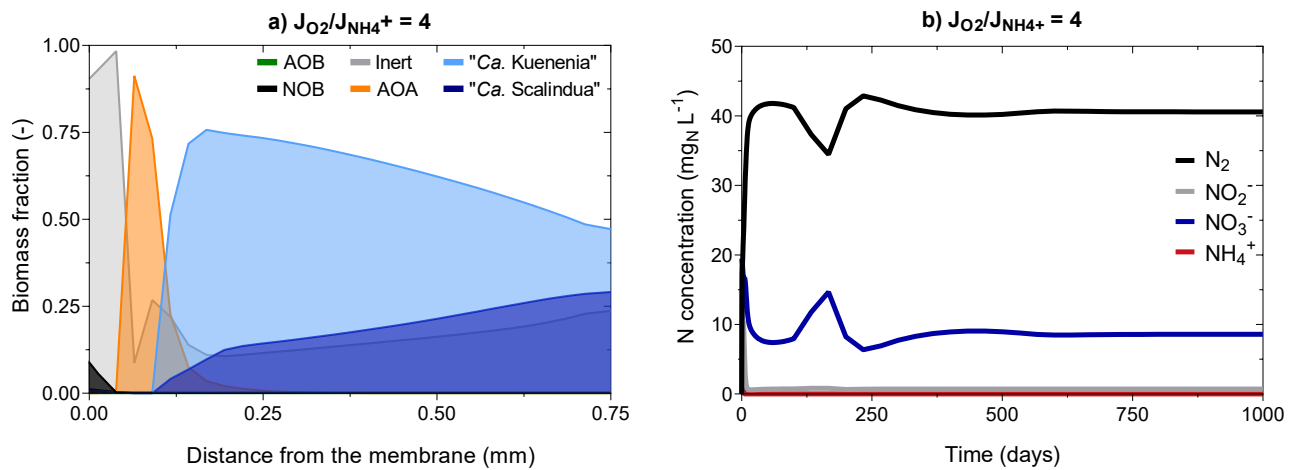


Figure 23: Nitrate accumulation due to the presence of NOB for a $J_{O_2}/J_{NH_4^+} = 4$: **a)** Distribution of biomass (with AOA, NOB and anammox bacteria) and particulate inerts in the membrane biofilm; AOB was outcompeted. Oxygen is supplied at a distance from the membrane of 0 mm and ammonium diffuses in counter-diffusion at 0.75 mm. **b)** Evolution of effluent concentrations of ammonium, nitrite, nitrate and nitrogen gas.

Due to the outcompetition of AOA over AOB, a simulation with only the latter was performed. The steady-state behaviour in terms of nitrogen removal at different $J_{O_2}/J_{NH_4^+}$ ratios for this scenario is summarized in Figure 24.

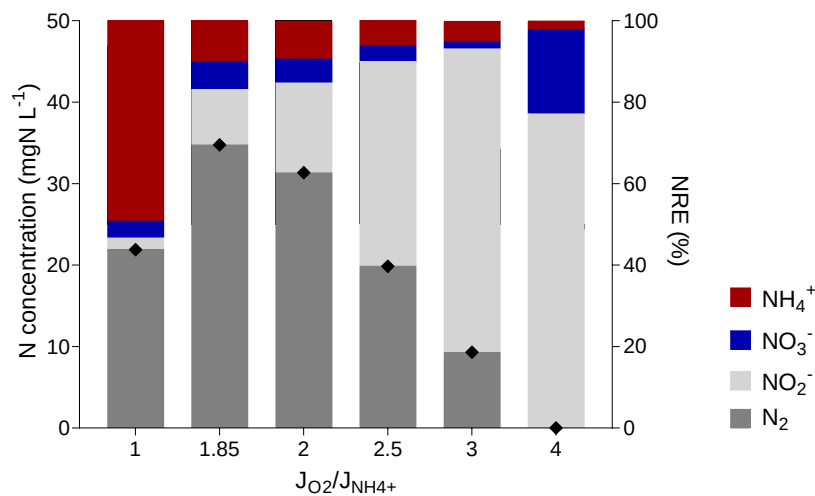


Figure 24: Membrane-aerated biofilm reactor not including AOA. Nitrogen fractions in the effluent at different $J_{O_2}/J_{NH_4^+}$ ratios. Nitrogen removal efficiency (NRE) is indicated by the diamonds (black).

Contrary to what was expected, a lower maximum nitrogen removal efficiency (70%) was obtained when compared with all the other models described in Table A8.

By observing the biofilm in Figure 25a, AOB is the dominant species in the membrane of the reactor (0-0.06 mm), while anammox bacteria are at the outer layer, close to the bulk liquid. There is a small region in the biofilm (0.06-0.20 mm), where the biofilm consists of 100% of inert biomass. Both bacteria guilds moved away from this region and did not interact directly with each other.

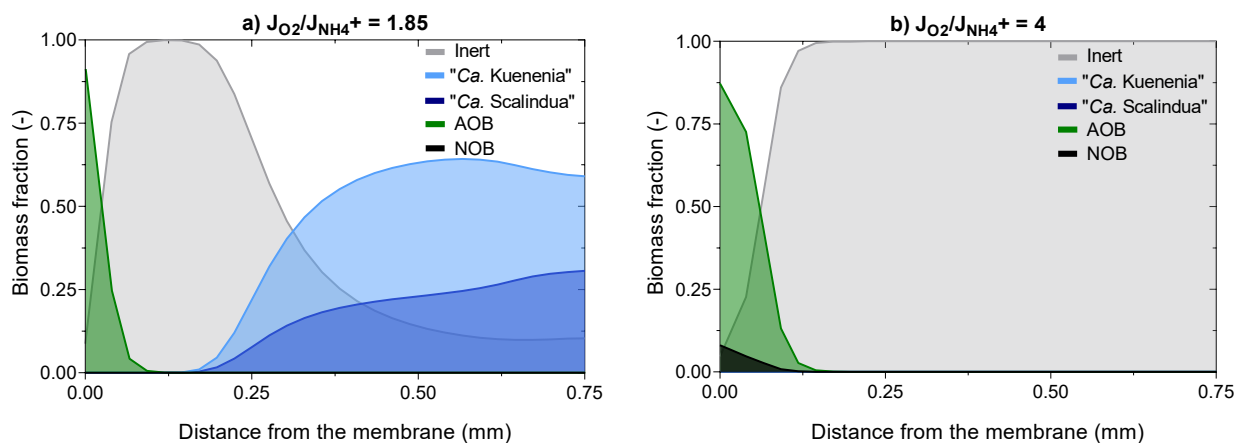


Figure 25: MABR not considering AOA. Distribution of biomass (with AOB, NOB and anammox bacteria) and particulate inerts on the membrane biofilm: **a)** for an optimal $J_{O_2}/J_{NH_4^+}$ ratio of 1.85 and **b)** for a $J_{O_2}/J_{NH_4^+}$ ratio of 4. Oxygen is supplied at a distance from the membrane of 0 mm and ammonium diffuses in counter-diffusion at 0.75 mm.

Therefore, it is suggested that this is causing a diffusion limitation of nitrite from AOB to anammox bacteria, and nitrite was accumulated in the MABR with the increase of $J_{O_2}/J_{NH_4^+}$ ratio (Figure 24).

One plausible explanation for this problem might be the different kinetic parameters used in this study, mainly the growth and decay rate parameters for AOB (μ_{max}^{AOB} and b_{AOB}) when compared to other studies (Table A8). A higher biomass decay resulted in the accumulation of inert biomass, which affected the substrate diffusion. Eventually, for a higher oxygen flux, NOB reappeared in the biofilm, consumed the accumulated nitrite and converted it to nitrate (Figure 24 - $J_{O_2}/J_{NH_4^+} = 4$), while anammox bacteria was ultimately washed out from the MABR (Figure 25b).

This stratification of the biofilm can also be observed in Figure 23a and in a model-based study by Liu et al. (2016b). In Figure 23a, at steady-state, NOB appears in the biofilm while there is a protective layer of inert biomass close to the membrane. This inert fraction resulted from the AOB and NOB decay at the beginning of the experiment: a higher oxygen availability promoted their initial growth which is corroborated by the nitrate peak around 150 days of simulation in Figure 23b.

6 Conclusions

The aim of this work was to understand how salinity impacts the niche differentiation among anammox bacteria and their cooperation and competition in the PN/A process with AOB, AOA and NOB. A salinity effect was considered in the microbial kinetics, as salinity-adapted and marine anammox bacteria could be utilized for nitrogen removal from saline wastewater. Two bioreactor configurations were used: GSBP and MABR. To this end, several models using AQUASIM software were developed.

- Without considering salinity in the model, “*Ca. K. stuttgartiensis*” and “*Ca. Brocadia sinica*” outcompeted “*Ca. J. caeni*” and “*Ca. B. anammoxidans*” at all nitrogen loading rates (NLR). “*Ca. Scalindua sp.*” is also represented in the biofilm, mainly at mainstream conditions ($<0.3 \text{ mg}_\text{N} \text{ L}^{-1} \text{ d}^{-1}$).
- The previous three anammox bacteria were compared in another simulation, considering 1.7% salinity in the influent. In this case, “*Ca. K. stuttgartiensis*” (fittest salinity-adapted freshwater anammox) and “*Ca. Scalindua sp.*” (marine anammox) were the most representative species, especially at mainstream nitrogen loading rates, which were the conditions used in the PN/A process.
- In a GSBP, PN/A process with granules of “*Ca. K. stuttgartiensis*” and “*Ca. Scalindua sp.*”, AOB and NOB, achieved 92% of nitrogen removal efficiency under optimal oxygen concentrations of $0.40 - 0.50 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$.
- A sensitivity analysis of the oxygen affinity constants of AOB and NOB was performed. In both scenarios ($K_{\text{O}_2}^{\text{NOB}} < K_{\text{O}_2}^{\text{AOB}}$ and $K_{\text{O}_2}^{\text{NOB}} > K_{\text{O}_2}^{\text{AOB}}$), under a low DO concentration, NOB was always washed out from the system due to the competition for nitrite with anammox bacteria. The same effluent substrate concentrations were reached at steady-state, but the whole process was slower for $K_{\text{O}_2}^{\text{NOB}} < K_{\text{O}_2}^{\text{AOB}}$ due to the competition for oxygen among nitrifiers.
- With the addition of AOA to the previous simulation, the maximum nitrogen removal efficiency (90%) was obtained at a specific DO concentration of $0.11 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$. Smaller deviations in DO ($0.01 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$) did result in nitrite accumulation in the bioreactor. AOA outcompeted both AOB and anammox bacteria. To assess this problem, counter-diffusion of oxygen and ammonium was implemented using an MABR.
- In a MABR, AOA still outcompeted AOB but not anammox bacteria. This resulted in a nitrogen removal efficiency of 90%, under a broader range of oxygen concentrations (represented as a ratio of oxygen and ammonium fluxes, $J_{\text{O}_2}/J_{\text{NH}_4^+}$). However, in an MABR without AOA, the nitrogen removal efficiency was only 70% due to diffusion limitations in the biofilm.

This model demonstrated that the preferred anammox bacteria to use in PN/A for saline wastewater are “*Ca. K. stuttgartiensis*” and “*Ca. Scalindua sp.*” The GSBP with a microbial community of AOB and

anammox bacteria with NOB control, under a DO concentration of $0.40 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$, was the most efficient (92%) approach for removing nitrogen from saline wastewater. Due to the salinity conditions, AOA could potentially grow in the system and PN/A is only possible in an MABR (90% NRE). This reactor has the operational advantages of working in a broader range of oxygen concentrations, allowing the control of the air flow rate with influent fluctuations.

6.1 Future work

As recommendations for future work, it is essential to consider improvements that can be included in the simulations performed. Two main concerns related to the kinetic parameters should be taken into account:

- as noticed by Zhang et al. (2017b), experimental work on “*Ca. J. caeni*” could not be replicated by the microbial kinetics available in the literature. Inaccuracies on parameters for different anammox species, as well as non-implementation and lack of information on others such as maintenance rate could eventually compromise the modelling results obtained. Therefore, a re-evaluation of these discrepancies is critical.
- although a salinity effect was incorporated in the kinetics of anammox bacteria, this approach was based on a few studies (Wu et al., 2019; Li et al., 2018a), in which the representativity of the whole species is far from being validated. Moreover, this approach is not available and it was not implemented for AOA, AOB and NOB. More experimental studies that could provide empirical values, correlations, salt-dependent functions, or even other kinetic parameters at different salt concentrations are necessary to improve the modelling of this environmental/stress condition.

Despite the fact that AOA have been detected in several WWTPs, and produced interesting results in this model, their contribution to nitrification still needs to be elucidated with more experimental approaches. Another microorganism that could be implemented in a future model is commamox *Nitrospira*. Since their discovery, high abundances of these microorganisms have been detected in some drinking water systems and wastewater treatment plants (Lawson and Lücker, 2018). Therefore, understanding the ecology and physiological parameters regulating these microorganisms, such as substrate availability, salinity tolerance, and cooperation and competition between other microbial communities, might improve and create novel strategies for wastewater treatment in the future (Koch et al., 2019).

Lastly, as pinpointed by Rittmann et al. (2018) in their framework for GBRMP, the fifth and sixth steps of biofilm modelling are to calibrate and validate the developed model. Therefore, an experimental approach of this work should be performed to validate the results obtained.

References

- Ali, M. and Okabe, S. (2015). Anammox-based technologies for nitrogen removal: Advances in process start-up and remaining issues. *Chemosphere*, 141:144–153.
- Ali, M., Oshiki, M., Awata, T., Isobe, K., Kimura, Z., Yoshikawa, H., Hira, D., Kindaichi, T., Satoh, H., Fujii, T., and Okabe, S. (2015). Physiological characterization of anaerobic ammonium oxidizing bacterium 'Candidatus Jettenia caeni'. *Environmental Microbiology*, 17(6):2172–2189.
- Ali, M., Rathnayake, R. M., Zhang, L., Ishii, S., Kindaichi, T., Satoh, H., Toyoda, S., Yoshida, N., and Okabe, S. (2016). Source identification of nitrous oxide emission pathways from a single-stage nitrification-anammox granular reactor. *Water Research*, 102:147–157.
- Ali, M., Shaw, D. R., and Saikaly, P. E. (2020). Application of an enrichment culture of the marine anammox bacterium "Ca. Scalindua sp. AMX11" for nitrogen removal under moderate salinity and in the presence of organic carbon. *Water Research*, 170.
- Ali, M., Shaw, D. R., Zhang, L., Haroon, M. F., Narita, Y., Emwas, A. H., Saikaly, P. E., and Okabe, S. (2018). Aggregation ability of three phylogenetically distant anammox bacterial species. *Water Research*, 143:10–18.
- An, P., Xu, X., Yang, F., and Li, Z. (2013). Comparison of the characteristics of anammox granules of different sizes. *Biotechnology and Bioengineering*, 118(3):446–454.
- Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S., and Srinath, E. G. (1976). Inhibition of nitrification by ammonia and nitrous acid. *Water Pollution Control Federation*, 48(5):835–852.
- Arnaldos, M., Amerlinck, Y., Rehman, U., Maere, T., Van Hoey, S., Naessens, W., and Nopens, I. (2015). From the affinity constant to the half-saturation index: Understanding conventional modeling concepts in novel wastewater treatment processes. *Water Research*, 70:458–470.
- Augusto, M. R., Camiloti, P. R., and de Souza, T. S. O. (2018). Fast start-up of the single-stage nitrogen removal using anammox and partial nitrification (SNAP) from conventional activated sludge in a membrane-aerated biofilm reactor. *Bioresource Technology*, 266:151–157.
- Awata, T., Oshiki, M., Kindaichi, T., Ozaki, N., Ohashi, A., and Okabe, S. (2013). Physiological characterization of an anaerobic ammonium-oxidizing bacterium belonging to the "Candidatus scalindua" group. *Applied and Environmental Microbiology*, 79(13):4145–4148.
- Baeten, J. E., Batstone, D. J., Schraa, O. J., van Loosdrecht, M. C., and Volcke, E. I. (2019). Modelling anaerobic, aerobic and partial nitrification-anammox granular sludge reactors - A review. *Water Research*, 149:322–341.
- Baeten, J. E., Van Loosdrecht, M. C. M., and Volcke, E. I. P. (2017). Improving the Accuracy of Granular Sludge and Biofilm Reactor Simulations in Aquasim Through Artificial Diffusion. *Biotechnol. Bioeng.*, 114:2131–2136.
- Bassin, J. P., Kleerebezem, R., Muyzer, G., Rosado, A. S., Van Loosdrecht, M. C., and Dezotti, M. (2012). Effect of different salt adaptation strategies on the microbial diversity, activity, and settling of nitrifying sludge in sequencing batch reactors. *Applied Microbiology and Biotechnology*, 93(3):1281–1294.
- Beun, J. J., Heijnen, J. J., and van Loosdrecht, M. C. M. (2001). N-Removal in a granular sludge sequencing batch airlift reactor. *Biotechnology and Bioengineering*, 75(1):82–92.
- Beun, J. J., Hendriks, A., Van Loosdrecht, M. C. M., Morgenroth, E., Wilderer, P. A., and Heijnen, J. J. (1999). Aerobic granulation in a sequencing batch reactor. *Water Research*, 33:2283–2290.
- Blackburne, R., Vadivelu, V. M., Yuan, Z., and Keller, J. (2007). Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter. *Water Research*, 41(14):3033–3042.
- Blackburne, R., Yuan, Z., and Keller, J. (2008). Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. *Biodegradation*, 19(2):303–312.
- Boltz, J. P., Morgenroth, E., and Sen, D. (2010). Mathematical modelling of biofilms and biofilm reactors for engineering design. *Water Science and Technology*, 62(8):1821–1836.
- Boretti, A. and Rosa, L. (2019). Reassessing the projections of the World Water Development Report. *npj Clean Water*, 2(1).
- Bowden, G., Stensel, D., and Tsuchihashi, R. (2015). Technologies for Sidestream Nitrogen Removal. *Water Environment Research Foundation*, page 110.
- Brockmann, D. and Morgenroth, E. (2010). Evaluating operating conditions for outcompeting nitrite oxidizers and maintaining partial nitrification in biofilm systems using biofilm modeling and Monte Carlo filtering. *Water Research*, 44(6):1995–2009.
- Broda, E. (1977). Two kinds of lithotrophs missing in nature. *Zeitschrift für allgemeine Mikrobiologie*, 17(6):491–493.
- Camargo, J. A. and Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*, 32(6):831–849.
- Cao, Y., van Loosdrecht, M. C., and Daigger, G. T. (2017). Mainstream partial nitrification-anammox in municipal wastewater treatment: status, bottlenecks, and further studies. *Applied Microbiology and Biotechnology*, 101(4):1365–1383.
- Capocelli, M., Prisciandaro, M., Piemonte, V., and Barba, D. (2019). A technical-economical approach to promote the water treatment & reuse processes. *Journal of Cleaner Production*, 207:85–96.
- Casey, E., Glennon, B., and Hamer, G. (1999). Oxygen Mass Transfer Characteristics in a Membrane-Aerated Biofilm Reactor. *Biotechnol Bioeng.*, 62:183–192.

- Castrillo, M., Díez-Montero, R., Esteban-García, A. L., and Tejero, I. (2019). Mass transfer enhancement and improved nitrification in MABR through specific membrane configuration. *Water Research*, 152:1–11.
- Castro-Barros, C. M., Jia, M., van Loosdrecht, M. C., Volcke, E. I., and Winkler, M. K. (2017). Evaluating the potential for dissimilatory nitrate reduction by anammox bacteria for municipal wastewater treatment. *Bioresource Technology*, 233:363–372.
- Chen, G. H., Chui, H. K., Wong, C. L., Tang, D. T. W., Lu, H., Jiang, F., and Van Loosdrecht, M. C. M. (2012). An Innovative Triple Water Supply System and a Novel SANI® Process to Alleviate Water Shortage and Pollution Problem for Water-scarce Coastal Areas in China. *Journal of Water Sustainability*, 2(2):121–129.
- Chen, G. H., Wong, M. T., Okabe, S., and Watanabe, Y. (2003). Dynamic response of nitrifying activated sludge batch culture to increased chloride concentration. *Water Research*, 37(13):3125–3135.
- Corbalá-Robles, L., Picioreanu, C., Van Loosdrecht, M. C., and Pérez, J. (2016). Analysing the effects of the aeration pattern and residual ammonium concentration in a partial nitrification-anammox process. *Environmental Technology*, 37(6):694–702.
- Cui, Y. W., Zhang, H. Y., Ding, J. R., and Peng, Y. Z. (2016). The effects of salinity on nitrification using halophilic nitrifiers in a Sequencing Batch Reactor treating hypersaline wastewater. *Scientific Reports*, 6.
- Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R. H., Von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P. H., and Wagner, M. (2015). Complete nitrification by *Nitrospira* bacteria. *Nature*, 528(7583):504–509.
- Daims, H., Lückner, S., and Wagner, M. (2016). A New Perspective on Microbes Formerly Known as Nitrite-Oxidizing Bacteria. *Trends in Microbiology*, 24(9):699–712.
- De Clippeleir, H., Vlaeminck, S. E., De Wilde, F., Daeninck, K., Mosquera, M., Boeckx, P., Verstraete, W., and Boon, N. (2013). One-stage partial nitrification/anammox at 15 °C on pretreated sewage: Feasibility demonstration at lab-scale. *Applied Microbiology and Biotechnology*, 97(23):10199–10210.
- De Cocker, P., Bessière, Y., Hernandez-Raquet, G., Dubos, S., Mozo, I., Gaval, G., Caligaris, M., Barillon, B., Vlaeminck, S. E., and Sperandio, M. (2018). Enrichment and adaptation yield high anammox conversion rates under low temperatures. *Bioresource Technology*, 250:505–512.
- De Kreuk, M. K., Picioreanu, C., Hosseini, M., Xavier, J. B., and Van Loosdrecht, M. C. (2007). Kinetic model of a granular sludge SBR: Influences on nutrient removal. *Biotechnology and Bioengineering*, 97(4):801–815.
- Downing, L. S. and Nerenberg, R. (2008). Effect of oxygen gradients on the activity and microbial community structure of a nitrifying, membrane-aerated biofilm. *Biotechnology and Bioengineering*, 101(6):1193–1204.
- Feng, Y., Lu, X., Al-Hazmi, H., and Mäkinen, J. (2017). An overview of the strategies for the deammonification process start-up and recovery after accidental operational failures.
- Fernández, I., Vázquez-Padín, J. R., Mosquera-Corral, A., Campos, J. L., and Méndez, R. (2008). Biofilm and granular systems to improve Anammox biomass retention. *Biochemical Engineering Journal*, 42(3):308–313.
- Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., and Oakley, B. B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, 102(41):14683–14688.
- Furukawa, K., Lieu, P. K., Tokitoh, H., and Fujii, T. (2006). Development of single-stage nitrogen removal using anammox and partial nitrification (SNAP) and its treatment performances. *Water Science and Technology*, 53(6):83–90.
- Fux, C., Bohler, M., Huber, P., Brunner, I., and Siegrist, H. (2002). Biological treatment of ammonium-rich wastewater by partial nitrification and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant. *Journal of Biotechnology*, 99(3):295–306.
- Fux, C. and Siegrist, H. (2004). Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitrification/anammox: environmental and economical considerations. *Water Science and Technology*, 50(10):19–26.
- Gandiglio, M., Lanzini, A., Soto, A., Leone, P., and Santarelli, M. (2017). Enhancing the energy efficiency of wastewater treatment plants through co-digestion and fuel cell systems.
- Gao, J. F., Luo, X., Wu, G. X., Li, T., and Peng, Y. Z. (2013). Quantitative analyses of the composition and abundance of ammonia-oxidizing archaea and ammonia-oxidizing bacteria in eight full-scale biological wastewater treatment plants. *Bioresource Technology*, 138:285–296.
- Gilbert, E. M., Agrawal, S., Brunner, F., Schwartz, T., Horn, H., and Lackner, S. (2014). Response of different *Nitrospira* Species to anoxic periods depends on operational DO. *Environmental Science and Technology*, 48(5):2934–2941.
- Gilbert, E. M., Agrawal, S., Schwartz, T., Horn, H., and Lackner, S. (2015). Comparing different reactor configurations for Partial Nitrification/Anammox at low temperatures. *Water Research*, 81:92–100.
- Gilmore, K. R., Terada, A., Smets, B. F., Love, N. G., and Garland, J. L. (2013). Autotrophic nitrogen removal in a membrane-aerated biofilm reactor under continuous aeration: A demonstration. *Environmental Engineering Science*, 30(1):38–45.
- González-Cabaleiro, R., Curtis, T. P., and Ofiteiru, I. D. (2019). Bioenergetics analysis of ammonia-oxidizing bacteria and the estimation of their maximum growth yield. *Water Research*, 154:238–245.
- Gonzalez-Silva, B. M., Rønning, A. J., Andreassen, I. K., Bakke, I., Cervantes, F. J., Østgaard, K., and Vadstein, O. (2017). Changes in the microbial community of an anammox consortium during adaptation to marine conditions revealed by 454 pyrosequencing. *Applied Microbiology and Biotechnology*, 101(12):5149–5162.
- Grant, S. B., Saphores, J.-D., Feldman, D. L., Hamilton, A. J., Fletcher, T. D., Cook, P. L. M., Stewardson, M., Sanders, B. F., Levin, L. A., Ambrose, R. F., Deletic, A., Brown, R., Jiang, S. C., Rosso, D., Cooper, W. J., and Marusic, I. (2012). Taking the "Waste" Out of "Wastewater" for Human Water Security and Ecosystem Sustainability. *Science*, 337(6095):681–685.

- Guisasola, A., Jubany, I., Baeza, J. A., Carrera, J., and Lafuente, J. (2005). Respirometric estimation of the oxygen affinity constants for biological ammonium and nitrite oxidation. *Journal of Chemical Technology and Biotechnology*, 80(4):388–396.
- Guo, Y., Sugano, T., Song, Y., Xie, C., Chen, Y., Xue, Y., and Li, Y. Y. (2020). The performance of freshwater one-stage partial nitrification/anammox process with the increase of salinity up to 3.0%. *Bioresource Technology*, 311(May):123489.
- Hao, X., Heijnen, J. J., and Van Loosdrecht, M. C. (2001). 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., Heijnen, J. J., and Van Loosdrecht, M. C. (2002). Model-based evaluation of temperature and inflow variations on a partial nitrification-ANAMMOX biofilm process. *Water Research*, 36(19):4839–4849.
- Hatzenpichler, R. (2012). Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. *Applied and Environmental Microbiology*, 78(21):7501–7510.
- Hellinga, C., Schellen, A. A., Mulder, J. W., Van Loosdrecht, M. C., and Heijnen, J. J. (1998). The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water. *Water Science and Technology*, 37(9):135–142.
- Henze, M. (2000). *Activated sludge models ASM1, ASM2, ASM2d and ASM3*. IWA Publishing.
- Henze, M. and Comeau, Y. (2008). Wastewater Characterization. In *Biological Wastewater Treatment: Principles, Modelling and Design*, chapter 3, pages 33–53. IWA Publishing.
- Henze, M., van Loosdrecht, M. C., Ekama, G. A., and Brdjanovic, D. (2008). *Biological Wastewater Treatment: Principles, Modelling and Design*. IWA Publishing.
- Hoekstra, M., Geilvoet, S. P., Hendrickx, T. L., van Erp Taalman Kip, C. S., Kleerebezem, R., and van Loosdrecht, M. C. (2019). Towards mainstream anammox: lessons learned from pilot-scale research at WWTP Dokhaven. *Environmental Technology*, 40(13):1721–1733.
- Hou, X., Liu, S., and Zhang, Z. (2015). Role of extracellular polymeric substance in determining the high aggregation ability of anammox sludge. *Water Research*, 75:51–62.
- Huang, X., Mi, W., Ito, H., and Kawagoshi, Y. (2021). Probing the dynamics of three freshwater Anammox genera at different salinity levels in a partial nitrification and Anammox sequencing batch reactor treating landfill leachate. *Bioresource Technology*, 319(September 2020):124112.
- Hubaux, N., Wells, G., and Morgenroth, E. (2015). Impact of coexistence of flocs and biofilm on performance of combined nitrification-anammox granular sludge reactors. *Water Research*, 68:127–139.
- Isanta, E., Bezerra, T., Fernández, I., Suárez-Ojeda, M. E., Pérez, J., and Carrera, J. (2015a). Microbial community shifts on an anammox reactor after a temperature shock using 454-pyrosequencing analysis. *Bioresource Technology*, 181:207–213.
- Isanta, E., Reino, C., Carrera, J., and Pérez, J. (2015b). Stable partial nitrification for low-strength wastewater at low temperature in an aerobic granular reactor. *Water Research*, 80:149–158.
- Jacob, J., Nowka, B., Merten, V., Sanders, T., Spieck, E., and Dähnke, K. (2017). Oxidation kinetics and inverse isotope effect of marine nitrite-oxidizing isolates. *Aquatic Microbial Ecology*, 80(3):289–300.
- Jeanningros, Y., Vlaeminck, S. E., Kaldate, A., Verstraete, W., and Graveleau, L. (2010). Fast start-up of a pilot-scale deammonification sequencing batch reactor from an activated sludge inoculum. *Water Science and Technology*, 61(6):1393–1400.
- Jetten, M. S., Strous, M., van de Pas-Schoonen, K. T., Schalk, J., van Dongen, U. G., van de Graaf, A. A., Logemann, S., Muyzer, G., van Loosdrecht, M. C., and Kuenen, J. G. (1998). The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews*, 22(5):421–437.
- Jia, M., Solon, K., Vandeplassche, D., Venugopal, H., and Volcke, E. I. (2020). Model-based evaluation of an integrated high-rate activated sludge and mainstream anammox system. *Chemical Engineering Journal*, 382.
- Jones, E., Qadir, M., van Vliet, M. T., Smakhtin, V., and mu Kang, S. (2019). The state of desalination and brine production: A global outlook. *Science of the Total Environment*, 657:1343–1356.
- Jubany, I., Carrera, J., Lafuente, J., and Baeza, J. A. (2008). Start-up of a nitrification system with automatic control to treat highly concentrated ammonium wastewater: Experimental results and modeling. *Chemical Engineering Journal*, 144(3):407–419.
- Kampschreur, M. J., Picoreanu, C., Tan, N., Kleerebezem, R., Jetten, M. S. M., and van Loosdrecht, M. C. M. (2007). Unraveling the Source of Nitric Oxide Emission During Nitrification. *Water Environment Research*, 79(13):2499–2509.
- Kampschreur, M. J., Poldermans, R., Kleerebezem, R., Van Der Star, W. R., Haarhuis, R., Abma, W. R., Jetten, M. S., and Van Loosdrecht, M. C. (2009a). Emission of nitrous oxide and nitric oxide from a full-scale single-stage nitrification-anammox reactor. *Water Science and Technology*, 60(12):3211–3217.
- Kampschreur, M. J., Temmink, H., Kleerebezem, R., Jetten, M. S., and van Loosdrecht, M. C. (2009b). Nitrous oxide emission during wastewater treatment. *Water Research*, 43(17):4093–4103.
- Kartal, B., De Almeida, N. M., Maalcke, W. J., Op den Camp, H. J., Jetten, M. S., and Keltjens, J. T. (2013). How to make a living from anaerobic ammonium oxidation. *FEMS Microbiology Reviews*, 37(3):428–461.
- Kartal, B., Koleva, M., Arsov, R., van der Star, W., Jetten, M. S., and Strous, M. (2006). Adaptation of a freshwater anammox population to high salinity wastewater. *Journal of Biotechnology*, 126(4):546–553.
- Kartal, B., Kuenen, J. G., and Van Loosdrecht, M. C. (2010). Sewage treatment with anammox. *Science*, 328(5979):702–703.

- Kartal, B., Rattray, J., van Niftrik, L. A., van de Vossenberg, J., Schmid, M. C., Webb, R. I., Schouten, S., Fuerst, J. A., Damsté, J. S., Jetten, M. S., and Strous, M. (2007). Candidatus "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. *Systematic and Applied Microbiology*, 30(1):39–49.
- Kartal, B., Van Niftrik, L., Rattray, J., Van De Vossenberg, J. L., Schmid, M. C., Sinninghe Damsté, J., Jetten, M. S., and Strous, M. (2008). Candidatus 'Brocadia fulgida': An autofluorescent anaerobic ammonium oxidizing bacterium. *FEMS Microbiology Ecology*, 63(1):46–55.
- Kehrein, P., Van Loosdrecht, M., Osseweijer, P., Garfi, M., Dewulf, J., and Posada, J. (2020). A critical review of resource recovery from municipal wastewater treatment plants-market supply potentials, technologies and bottlenecks. *Environmental Science: Water Research and Technology*, 6(4):877–910.
- Khramenkov, S. V., Kozlov, M. N., Kevbrina, M. V., Dorofeev, A. G., Kazakova, E. A., Grachev, V. A., Kuznetsov, B. B., Polyakov, D. Y., and Nikolaev, Y. A. (2013). A novel bacterium carrying out anaerobic ammonium oxidation in a reactor for biological treatment of the filtrate of wastewater fermented sludge. *Microbiology (Russian Federation)*, 82(5):628–636.
- Kits, D. K., Sedlacek, C. J., Lebedeva, E. V., Han, P., Bulaev, A., Pjevac, P., Daebeler, A., Romano, S., Albertsen, M., Stein, L. Y., Daims, H., and Wagner, M. (2017). Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature*, 549(7671):269–272.
- Koch, H., van Kessel, M. A., and Lückner, S. (2019). Complete nitrification: insights into the ecophysiology of comammox Nitrospira. *Applied Microbiology and Biotechnology*, 103(1):177–189.
- Könneke, M., Bernhard, A. E., De La Torre, J. R., Walker, C. B., Waterbury, J. B., and Stahl, D. A. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature*, 437(7058):543–546.
- Könneke, M., Schubert, D. M., Brown, P. C., Hügler, M., Standfest, S., Schwander, T., Schada Von Borzyskowski, L., Erb, T. J., Stahl, D. A., and Berg, I. A. (2014). Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO₂ fixation. *Proceedings of the National Academy of Sciences of the United States of America*, 111(22):8239–8244.
- Koops, H.-P. and Pommerening-Ro, A. (2001). Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. *FEMS Microbiology Ecology*, 37:1–9.
- Kuai, L. and Verstraete, W. (1998). Ammonium Removal by the Oxygen-Limited Autotrophic Nitrification-Denitrification System. *Applied and Environmental Microbiology*, 64(11):4500–4506.
- Kuenen, J. G. (2008). Anammox bacteria: from discovery to application. *Nature Reviews Microbiology*, 6(4):320–326.
- Kuypers, M. M., Silekers, A. O., Lavik, G., Schmid, M., Jørgensen, B. B., Kuenen, J. G., Sinninghe Damsté, J. S., Strous, M., and Jetten, M. S. (2003). Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. *Nature*, 422(6932):608–611.
- Lackner, S., Gilbert, E. M., Vlaeminck, S. E., Joss, A., Horn, H., and van Loosdrecht, M. C. (2014). Full-scale partial nitrification/anammox experiences - An application survey. *Water Research*, 55:292–303.
- Lackner, S., Terada, A., and Smets, B. F. (2008). Heterotrophic activity compromises autotrophic nitrogen removal in membrane-aerated biofilms: Results of a modeling study. *Water Research*, 42(4-5):1102–1112.
- Lam, P., Jensen, M. M., Lavik, G., McGinnis, D. F., Schubert, C. J., Amann, R., Thamdrup, B., and Kuypers, M. M. M. (2007). Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. *Proceedings of the National Academy of Sciences of the United States of America*, 104(17):7104–7109.
- Laureni, M., Weissbrodt, D. G., Villez, K., Robin, O., de Jonge, N., Rosenthal, A., Wells, G., Nielsen, J. L., Morgenroth, E., and Joss, A. (2019). Biomass segregation between biofilm and flocs improves the control of nitrite-oxidizing bacteria in mainstream partial nitrification and anammox processes. *Water Research*, 154:104–116.
- Lawson, C. E. and Lückner, S. (2018). Complete ammonia oxidation: an important control on nitrification in engineered ecosystems? *Current Opinion in Biotechnology*, 50:158–165.
- Lefebvre, O. and Moletta, R. (2006). Treatment of organic pollution in industrial saline wastewater: A literature review. *Water Research*, 40(20):3671–3682.
- Lehtovirta-Morley, L. E. (2018). Ammonia oxidation: Ecology, physiology, biochemistry and why they must all come together.
- Leininger, S., Urich, T., Schlöter, M., Schwark, L., Qi, J., Nicol, G. W., Prosser, J. I., Schuster, S. C., and Schleper, C. (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, 442(7104):806–809.
- Leung, R. W. K., Li, D. C. H., Yu, W. K., Chui, H. K., Lee, T. O., van Loosdrecht, M. C. M., and Chen, G. H. (2012). Integration of seawater and grey water reuse to maximize alternative water resource for coastal areas: the case of the Hong Kong International Airport. *Water Science and Technology*, 65(3):410–417.
- Li, J., Qi, P., Li, R., Wang, J., and Wang, D. (2018a). Carbon and nitrogen removal through "Candidatus Brocadia sinica"-dominated simultaneous anammox and denitrification (SAD) process treating saline wastewater. *Biochemical Engineering Journal*, 140:72–76.
- Li, J., Qi, P., Qiang, Z., Dong, H., Gao, D., and Wang, D. (2018b). Is anammox a promising treatment process for nitrogen removal from nitrogen-rich saline wastewater? *Bioresource Technology*, 270(July):722–731.
- Li, X., Klaus, S., Bott, C., and He, Z. (2018c). Status, Challenges, and Perspectives of Mainstream Nitrification-Anammox for Wastewater Treatment. *Water Environment Research*, 90(7):634–649.
- Li, X., Sun, Y., Wang, Z. W., and He, Z. (2019). Theoretical understanding of the optimum conditions for a mainstream granular nitrification-anammox reactor coupled with anaerobic pretreatment. *Science of the Total Environment*, 669:683–691.
- Li, Y., Ding, K., Wen, X., Zhang, B., Shen, B., and Yang, Y. (2016). A novel ammonia-oxidizing archaeon from wastewater treatment plant: Its enrichment, physiological and genomic characteristics. *Scientific Reports*, 6(1):23747.

- Liu, S., Yang, F., Gong, Z., and Su, Z. (2008). Assessment of the positive effect of salinity on the nitrogen removal performance and microbial composition during the start-up of CANON process. *Applied Microbiology and Biotechnology*, 80(2):339–348.
- Liu, X., Dai, J., Wu, D., Jiang, F., Chen, G., Chui, H. K., and van Loosdrecht, M. C. (2016a). Sustainable Application of a Novel Water Cycle Using Seawater for Toilet Flushing. *Engineering*, 2(4):460–469.
- Liu, X., Kim, M., and Nakhla, G. (2017). Operational conditions for successful partial nitrification in a sequencing batch reactor (SBR) based on process kinetics. *Environmental Technology (United Kingdom)*, 38(6):694–704.
- Liu, Y., Ngo, H. H., Guo, W., Peng, L., Pan, Y., Guo, J., Chen, X., and Ni, B. J. (2016b). Autotrophic nitrogen removal in membrane-aerated biofilms: Archaeal ammonia oxidation versus bacterial ammonia oxidation. *Chemical Engineering Journal*, 302:535–544.
- Lotti, T., Kleerebezem, R., Abelleira-Pereira, J. M., Abbas, B., and van Loosdrecht, M. C. (2015a). Faster through training: The anammox case. *Water Research*, 81:261–268.
- Lotti, T., Kleerebezem, R., Lubello, C., and van Loosdrecht, M. C. (2014). Physiological and kinetic characterization of a suspended cell anammox culture. *Water Research*, 60:1–14.
- Lotti, T., Kleerebezem, R., and Van Loosdrecht, M. C. M. (2015b). Effect of Temperature Change on Anammox Activity. *Biotechnology and Bioengineering*, 112(1).
- Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., O'Connor, M. I., Ackermann, M., Hahn, A. S., Srivastava, D. S., Crowe, S. A., Doebeli, M., and Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. *Nature Ecology & Evolution*, 2(6):936–943.
- Ma, B., Bao, P., Wei, Y., Zhu, G., Yuan, Z., and Peng, Y. (2015). Suppressing nitrite-oxidizing bacteria growth to achieve nitrogen removal from domestic wastewater via anammox using intermittent aeration with low dissolved oxygen. *Scientific Reports*, 5(July):1–9.
- Ma, Y., Domingo-Félez, C., Plósz, B. G., and Smets, B. F. (2017). Intermittent Aeration Suppresses Nitrite-Oxidizing Bacteria in Membrane-Aerated Biofilms: A Model-Based Explanation. *Environmental Science and Technology*, 51(11):6146–6155.
- Maktabifard, M., Zaborowska, E., and Makinia, J. (2018). Achieving energy neutrality in wastewater treatment plants through energy savings and enhancing renewable energy production. *Reviews in Environmental Science and Biotechnology*, 17(4):655–689.
- Malovanyy, A., Plaza, E., Trela, J., and Malovanyy, M. (2015a). Ammonium removal by partial nitrification and Anammox processes from wastewater with increased salinity. *Environmental Technology*, 36(5):595–604.
- Malovanyy, A., Trela, J., and Plaza, E. (2015b). Mainstream wastewater treatment in integrated fixed film activated sludge (IFAS) reactor by partial nitrification/anammox process. *Bioresource Technology*, 198:478–487.
- Manser, R., Gujer, W., and Siegrist, H. (2005). Consequences of mass transfer effects on the kinetics of nitrifiers. *Water Research*, 39(19):4633–4642.
- Martens-Habbena, W., Berube, P. M., Urakawa, H., De La Torre, J. R., and Stahl, D. A. (2009). Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature*, 461(7266):976–979.
- McCarty, P. L. (2018). What is the Best Biological Process for Nitrogen Removal: When and Why? *Environmental Science and Technology*, 52(7):3835–3841.
- Mehrani, M. J., Sobotka, D., Kowal, P., Ciesielski, S., and Makinia, J. (2020). The occurrence and role of Nitrospira in nitrogen removal systems. *Bioresource Technology*, 303.
- Mekonnen, M. M. and Hoekstra, A. Y. (2016). Sustainability: Four billion people facing severe water scarcity. *Science Advances*, 2(2):1–7.
- Meng, Y., Yin, C., Zhou, Z., and Meng, F. (2018). Increased salinity triggers significant changes in the functional proteins of ANAMMOX bacteria within a biofilm community. *Chemosphere*, 207:655–664.
- Morgenroth, E. (2008). Biofilm modelling. In *Biological Wastewater Treatment: Principles, Modelling and Design*, chapter 17, pages 457–492. IWA Publishing.
- Moussa, M. S., Sumanasekera, D. U., Ibrahim, S. H., Lubberding, H. J., Hooijmans, C. M., Gijzen, H. J., and Van Loosdrecht, M. C. (2006). Long term effects of salt on activity, population structure and floc characteristics in enriched bacterial cultures of nitrifiers. *Water Research*, 40(7):1377–1388.
- Mozumder, M. S. I., Picioreanu, C., Van Loosdrecht, M. C., and Volcke, E. I. (2014). Effect of heterotrophic growth on autotrophic nitrogen removal in a granular sludge reactor. *Environmental Technology (United Kingdom)*, 35(8):1027–1037.
- Mulder, A. (1992). Anoxic Ammonia Oxidation.
- Navarro, R. R., Aoyagi, T., Kimura, M., Itoh, H., Sato, Y., Kikuchi, Y., Ogata, A., and Hori, T. (2015). High-Resolution Dynamics of Microbial Communities during Dissimilatory Selenate Reduction in Anoxic Soil. *Environmental Science and Technology*, 49(13):7684–7691.
- Nerenberg, R. (2016). The membrane-biofilm reactor (MBfR) as a counter-diffusional biofilm process. *Current Opinion in Biotechnology*, 38:131–136.
- Ni, B. J., Yu, H. Q., and Sun, Y. J. (2008). Modeling simultaneous autotrophic and heterotrophic growth in aerobic granules. *Water Research*, 42(6-7):1583–1594.
- Ni, B. J. and Yuan, Z. (2013). A model-based assessment of nitric oxide and nitrous oxide production in membrane-aerated autotrophic nitrogen removal biofilm systems. *Journal of Membrane Science*, 428:163–171.

- Nogueira, R. and Melo, L. F. (2006). Competition between *Nitrospira* spp. and *Nitrobacter* spp. in nitrite-oxidizing bioreactors. *Biotechnology and Bioengineering*, 95(1):169–175.
- Nowka, B., Daims, H., and Spieck, E. (2015). Comparison of oxidation kinetics of nitrite-oxidizing bacteria: Nitrite availability as a key factor in niche differentiation. *Applied and Environmental Microbiology*, 81(2):745–753.
- Oshiki, M., Awata, T., Kindaichi, T., Satoh, H., and Okabe, S. (2013). Cultivation of planktonic anaerobic ammonium oxidation (anammox) bacteria using membrane bioreactor. *Microbes and Environments*, 28(4):436–443.
- Oshiki, M., Satoh, H., and Okabe, S. (2016). Ecology and physiology of anaerobic ammonium oxidizing bacteria.
- Pan, Y., Ni, B. J., Liu, Y., and Guo, J. (2016). Modeling of the interaction among aerobic ammonium-oxidizing archaea/bacteria and anaerobic ammonium-oxidizing bacteria. *Chemical Engineering Science*, 150:35–40.
- Park, H., Rosenthal, A., Jezek, R., Ramalingam, K., Fillos, J., and Chandran, K. (2010). Impact of inocula and growth mode on the molecular microbial ecology of anaerobic ammonia oxidation (anammox) bioreactor communities. *Water Research*, 44(17):5005–5013.
- Park, H., Sundar, S., Ma, Y., and Chandran, K. (2015). Differentiation in the Microbial Ecology and Activity of Suspended and Attached Bacteria in a Nitrification-Anammox Process. *Biotechnol. Bioeng*, 112:272–279.
- Park, H. D. and Noguera, D. R. (2007). Characterization of two ammonia-oxidizing bacteria isolated from reactors operated with low dissolved oxygen concentrations. *Journal of Applied Microbiology*, 102(5):1401–1417.
- Park, H. D., Wells, G. F., Bae, H., Griddle, C. S., and Francis, C. A. (2006). Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. *Applied and Environmental Microbiology*, 72(8):5643–5647.
- Park, K., Kim, J., Yang, D. R., and Hong, S. (2020). Towards a low-energy seawater reverse osmosis desalination plant: A review and theoretical analysis for future directions. *Journal of Membrane Science*, 595(October 2019):117607.
- Park, M. R., Park, H., and Chandran, K. (2017). Molecular and Kinetic Characterization of Planktonic *Nitrospira* spp. Selectively Enriched from Activated Sludge. *Environmental Science and Technology*, 51(5):2720–2728.
- Pedrouso, A., Trela, J., Val del Rio, A., Mosquera-Corral, A., and Plaza, E. (2019). Performance of partial nitrification-anammox processes at mainstream conditions in an IFAS system. *Journal of Environmental Management*, 250(August):109538.
- Pellicer-Nàcher, C., Franck, S., Gülay, A., Rusalleda, M., Terada, A., Al-Soud, W. A., Hansen, M. A., Sørensen, S. J., and Smets, B. F. (2014). Sequentially aerated membrane biofilm reactors for autotrophic nitrogen removal: Microbial community composition and dynamics. *Microbial Biotechnology*, 7(1):32–43.
- Pellicer-Nàcher, C. and Smets, B. F. (2014). Structure, composition, and strength of nitrifying membrane-aerated biofilms. *Water Research*, 57:151–161.
- Pellicer-Nàcher, C., Sun, S., Lackner, S., Terada, A., Schreiber, F., Zhou, Q., and Smets, B. F. (2010). Sequential aeration of membrane-aerated biofilm reactors for high-rate autotrophic nitrogen removal: Experimental demonstration. *Environmental Science and Technology*, 44(19):7628–7634.
- Pérez, J., Costa, E., and Kreft, J. U. (2009). Conditions for partial nitrification in biofilm reactors and a kinetic explanation. *Biotechnology and Bioengineering*, 103(2):282–295.
- Pérez, J., Lotti, T., Kleerebezem, R., Picioreanu, C., and van Loosdrecht, M. C. (2014). Outcompeting nitrite-oxidizing bacteria in single-stage nitrogen removal in sewage treatment plants: A model-based study. *Water Research*, 66:208–218.
- Picioreanu, C., Pérez, J., and van Loosdrecht, M. C. (2016). Impact of cell cluster size on apparent half-saturation coefficients for oxygen in nitrifying sludge and biofilms. *Water Research*, 106:371–382.
- Picioreanu, C., van Loosdrecht, M. C. M., and Heijnen, J. J. (1997). Modelling the effect of oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. *Water Science and Technology*, 36(1):147–156.
- Poot, V., Hoekstra, M., Geleijnse, M. A., van Loosdrecht, M. C., and Pérez, J. (2016). Effects of the residual ammonium concentration on NOB repression during partial nitrification with granular sludge. *Water Research*, 106:518–530.
- Prosser, J. I. and Nicol, G. W. (2012). Archaeal and bacterial ammonia-oxidisers in soil: The quest for niche specialisation and differentiation. *Trends in Microbiology*, 20(11):523–531.
- Quan, Z. X., Rhee, S. K., Zuo, J. E., Yang, Y., Bae, J. W., Park, J. R., Lee, S. T., and Park, Y. H. (2008). Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor. *Environmental Microbiology*, 10(11):3130–3139.
- Regmi, P., Miller, M. W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., and Bott, C. B. (2014). Control of aeration, aerobic SRT and COD input for mainstream nitrification/denitrification. *Water Research*, 57:162–171.
- Reichert, P. (1994). AQUASIM – A tool for simulation and data analysis of aquatic systems. *Water Science and Technology*, 30(2):21–30.
- Reichert, P. (1998). AQUASIM 2.0 - User Manual: Computer Program for the Identification and Simulation of Aquatic Systems. Technical report, Swiss Federal Institute for Environmental Science and Technology (EAWAG).
- Reichert, P. and Wanner, O. (1997). Movement of solids in biofilms: Significance of liquid phase transport. *Water Science and Technology*, 36(1):321–328.
- Rittmann, B. E., Boltz, J. P., Brockmann, D., Daigger, G. T., Morgenroth, E., Sørensen, K. H., Takács, I., Van Loosdrecht, M., and Vanrolleghem, P. A. (2018). A framework for good biofilm reactor modeling practice (GBRMP). *Water Science and Technology*, 77(5):1149–1164.
- Rittmann, B. E. and McCarty, P. L. (2001). *Environmental Biotechnology: Principles and Applications*. McGraw Hill.

- Rongsayamanont, C., Limpiyakorn, T., Law, B., and Khan, E. (2010). Relationship between respirometric activity and community of entrapped nitrifying bacteria: Implications for partial nitrification. *Enzyme and Microbial Technology*, 46(3-4):229–236.
- Roy, D., McEvoy, J., Blonigen, M., Amundson, M., and Khan, E. (2017). Seasonal variation and ex-situ nitrification activity of ammonia oxidizing archaea in biofilm based wastewater treatment processes. *Bioresource Technology*, 244:850–859.
- Saikaly, P. E. and Oerther, D. B. (2011). Diversity of Dominant Bacterial Taxa in Activated Sludge Promotes Functional Resistance following Toxic Shock Loading. *Microbial Ecology*, 61(3):557–567.
- Sauder, L. A., Albertsen, M., Engel, K., Schwarz, J., Nielsen, P. H., Wagner, M., and Neufeld, J. D. (2017). Cultivation and characterization of *Candidatus Nitrosocosmicus exaquare*, an ammonia-oxidizing archaeon from a municipal wastewater treatment system. *ISME Journal*, 11(5):1142–1157.
- Sauder, L. A., Peterse, F., Schouten, S., and Neufeld, J. D. (2012). Low-ammonia niche of ammonia-oxidizing archaea in rotating biological contactors of a municipal wastewater treatment plant. *Environmental Microbiology*, 14(9):2589–2600.
- Saxena, N., Nawaz, A., and Lee, M. (2019). Comprehensive Review of Control and Operational Strategies for Partial Nitrification/ANAMMOX System. *Industrial and Engineering Chemistry Research*, 58:10635–10651.
- Schmid, M., Twachtmann, U., Klein, M., Strous, M., Juretschko, S., Jetten, M., Metzger, J. W., Schleifer, K.-H., and Wagner, M. (2000). Molecular Evidence for Genus Level Diversity of Bacteria Capable of Catalyzing Anaerobic Ammonium Oxidation. *Systematic and Applied Microbiology*, 23:93–106.
- Seuntjens, D., Carvajal-Arroyo, J. M., Ruopp, M., Bunse, P., De Mulder, C. P., Lochmatter, S., Agrawal, S., Boon, N., Lackner, S., and Vlaeminck, S. E. (2018). High-resolution mapping and modeling of anammox recovery from recurrent oxygen exposure. *Water Research*, 144:522–531.
- Shannon, M. A., Bohn, P. W., Elimelech, M., Georgiadis, J. G., Mariñas, B. J., and Mayes, A. M. (2008). Science and technology for water purification in the coming decades. *Nature*, 452(7185):301–310.
- Siegrist, H., Salzgeber, D., Eugster, J., and Joss, A. (2008). Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal. *Water Science and Technology*, 57(3):383–388.
- Soler-Jofra, A., Wang, R., Kleerebezem, R., van Loosdrecht, M. C., and Pérez, J. (2019). Stratification of nitrifier guilds in granular sludge in relation to nitrification. *Water Research*, 148:479–491.
- Sonthiphand, P., Hall, M. W., and Neufeld, J. D. (2014). Biogeography of anaerobic ammonia-oxidizing (anammox) bacteria. *Frontiers in Microbiology*, 5(AUG).
- Speth, D. R., In'T Zandt, M. H., Guerrero-Cruz, S., Dutilh, B. E., and Jetten, M. S. (2016). Genome-based microbial ecology of anammox granules in a full-scale wastewater treatment system. *Nature Communications*, 7.
- Stahl, D. A. and De La Torre, J. R. (2012). Physiology and diversity of ammonia-oxidizing archaea. *Annual Review of Microbiology*, 66:83–101.
- Stewart, P. S. (2003). Diffusion in biofilms. *Journal of Bacteriology*, 185(5):1485–1491.
- Stewart, P. S. and Franklin, M. J. (2008). Physiological heterogeneity in biofilms. *Nature Reviews Microbiology*, 6(3):199–210.
- Straka, L. L., Meinhardt, K. A., Bollmann, A., Stahl, D. A., and Winkler, M. K. (2019). Affinity informs environmental cooperation between ammonia-oxidizing archaea (AOA) and anaerobic ammonia-oxidizing (Anammox) bacteria. *ISME Journal*, 13(8):1997–2004.
- Strous, M., Heijnen, J. J., Kuenen, J. G., and Jetten, M. S. M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology*, 50:589–596.
- Strous, M., Van Gerven, E., Gijs Kuenen, J., and Jetten, M. (1997). Effects of Aerobic and Microaerobic Conditions on Anaerobic Ammonium-Oxidizing (Anammox) Sludge. *Applied and Environmental Microbiology*, 63(6):2446–2448.
- Syron, E. and Casey, E. (2013). Membrane Aerated Biofilm Reactors. In Hoek, E. M. and Tarabara, V. V., editors, *Encyclopedia of Membrane Science and Technology*. John Wiley & Sons, Inc.
- Syron, E., Semmens, M. J., and Casey, E. (2015). Performance analysis of a pilot-scale membrane aerated biofilm reactor for the treatment of landfill leachate. *Chemical Engineering Journal*, 273:120–129.
- Tang, S. L., Yue, D. P. T., and Ku, D. C. C. (2007). *Engineering and Costs of Dual Water Supply Systems*. IWA Publishing.
- Terada, A., Lackner, S., Tsuneda, S., and Smets, B. F. (2007). Redox-stratification controlled biofilm (ReSCoBi) for completely autotrophic nitrogen removal: The effect of co- versus counter-diffusion on reactor performance. *Biotechnology and Bioengineering*, 97(1):40–51.
- Third, K. A., Sliemers, A. O., Kuenen, J. G., and Jetten, M. S. (2001). The CANON system (completely autotrophic nitrogen-removal over nitrite) under ammonium limitation: Interaction and competition between three groups of bacteria. *Systematic and Applied Microbiology*, 24(4):588–596.
- Trojanowicz, K., Trela, J., and Plaza, E. (2019). Possible mechanism of efficient mainstream partial nitrification/anammox (PN/A) in hybrid bioreactors (IFAS). *Environmental Technology (United Kingdom)*, 0(0):1–15.
- UNESCO (2018). The United Nations world water development report 2018: Nature-Based Solutions for Water. Technical report.
- UNESCO (2020). The United Nations World Water Development Report 2020: Water and Climate Change. Technical report.
- Val del Rio, A., Campos, J. L., Da Silva, C., Pedrouso, A., and Mosquera-Corral, A. (2019). Determination of the intrinsic kinetic parameters of ammonia-oxidizing and nitrite-oxidizing bacteria in granular and flocculent sludge. *Separation and Purification Technology*, 213:571–577.

- Val del Rio, A., Pichel, A., Fernandez-Gonzalez, N., Pedrouso, A., Fra-Vázquez, A., Morales, N., Mendez, R., Campos, J. L., and Mosquera-Corral, A. (2018). Performance and microbial features of the partial nitrification-anammox process treating fish canning wastewater with variable salt concentrations. *Journal of Environmental Management*, 208:112–121.
- Van De Graaf, A., Mulder, A., De Bruijn, P., Jetten, M., Robertson, A., and Kuenen, J. (1995). Anaerobic Oxidation of Ammonium Is a Biologically Mediated Process. *Applied and Environmental Microbiology*, 61(4):1246–1251.
- Van De Vossenberg, J., Rattray, J. E., Geerts, W., Kartal, B., Van Niftrik, L., Van Donselaar, E. G., Sinninghe Damsté, J. S., Strous, M., and Jetten, M. S. (2008). Enrichment and characterization of marine anammox bacteria associated with global nitrogen gas production. *Environmental Microbiology*, 10(11):3120–3129.
- Van Der Star, W. R., Miclea, A. I., Van Dongen, U. G., Muyzer, G., Picioreanu, C., and Van Loosdrecht, M. C. (2008). The membrane bioreactor: A novel tool to grow anammox bacteria as free cells. *Biotechnology and Bioengineering*, 101(2):286–294.
- van Dongen, U., Jetten, M., and van Loosdrecht, M. (2001). The SHARON®-Anammox® process for treatment of ammonium rich wastewater. *Water Science and Technology*, 44(1):153–160.
- Van Hulle, S. (2005). *Modelling, simulation and optimization of autotrophic nitrogen removal processes*. PhD thesis, Ghent University.
- Van Kessel, M. A., Speth, D. R., Albertsen, M., Nielsen, P. H., Op Den Camp, H. J., Kartal, B., Jetten, M. S., and Lücker, S. (2015). Complete nitrification by a single microorganism. *Nature*, 528(7583):555–559.
- Van Loosdrecht, M. C. M. and Henze, M. (1999). Maintenance, endogeneous respiration, lysis, decay and predation. *Water Science and Technology*, 39(1):107–117.
- van Niftrik, L., Geerts, W. J., van Donselaar, E. G., Humbel, B. M., Yakushevskaya, A., Verkleij, A. J., Jetten, M. S., and Strous, M. (2008). Combined structural and chemical analysis of the anammoxosome: A membrane-bounded intracytoplasmic compartment in anammox bacteria. *Journal of Structural Biology*, 161(3):401–410.
- Vandekerckhove, T. G., Bodé, S., De Mulder, C., Vlaeminck, S. E., and Boon, N. (2019). ¹³C incorporation as a tool to estimate biomass yields in thermophilic and mesophilic nitrifying communities. *Frontiers in Microbiology*, 10(FEB).
- Vannecke, T. P. W. and Volcke, E. I. P. (2015). Modelling microbial competition in nitrifying biofilm reactors; Modelling microbial competition in nitrifying biofilm reactors. *Biotechnol. Bioeng.*, 112:2550–2561.
- Vázquez-Padín, J. R., Pozo, M. J., Jarpa, M., Figueroa, M., Franco, A., Mosquera-Corral, A., Campos, J. L., and Méndez, R. (2009). Treatment of anaerobic sludge digester effluents by the CANON process in an air pulsing SBR. *Journal of Hazardous Materials*, 166(1):336–341.
- Verstraete, W., Van de Caveye, P., and Diamantis, V. (2009). Maximum use of resources present in domestic "used water". *Bioresource Technology*, 100(23):5537–5545.
- Veuliet, F., Lacroix, S., Bausseron, A., Gonidec, E., Ochoa, J., Christensson, M., and Lemaire, R. (2013). Integrated fixed-film activated sludge ANITA™Mox process – a new perspective for advanced nitrogen removal. *Water Science and Technology*, 69(5):915–922.
- Vlaeminck, S. E., De Clippeleir, H., and Verstraete, W. (2012). Microbial resource management of one-stage partial nitrification/anammox. *Microbial Biotechnology*, 5(3):433–448.
- Vlaeminck, S. E., Terada, A., Smets, B. F., De Clippeleir, H., Schaubroeck, T., Bolea, S., Demeestere, L., Mast, J., Boon, N., Carballa, M., and Verstraete, W. (2010). Aggregate size and architecture determine microbial activity balance for one-stage partial nitrification and anammox. *Applied and Environmental Microbiology*, 76(3):900–909.
- Volcke, E. I., Picioreanu, C., De Baets, B., and Van Loosdrecht, M. C. (2010). Effect of granule size on autotrophic nitrogen removal in a granular sludge reactor. *Environmental Technology*, 31(11):1271–1280.
- Volcke, E. I., Picioreanu, C., De Baets, B., and van Loosdrecht, M. C. (2012). The granule size distribution in an anammox-based granular sludge reactor affects the conversion-Implications for modeling. *Biotechnology and Bioengineering*, 109(7):1629–1636.
- Wang, R., Terada, A., Lackner, S., Smets, B. F., Henze, M., Xia, S., and Zhao, J. (2009). Nitrification performance and biofilm development of co- and counter-diffusion biofilm reactors: Modeling and experimental comparison. *Water Research*, 43(10):2699–2709.
- Wang, Y., Chen, J., Zhou, S., Wang, X., Chen, Y., Lin, X., Yan, Y., Ma, X., Wu, M., and Han, H. (2017a). 16S rRNA gene high-throughput sequencing reveals shift in nitrogen conversion related microorganisms in a CANON system in response to salt stress. *Chemical Engineering Journal*, 317:512–521.
- Wang, Y., Niu, Q., Zhang, X., Liu, L., Wang, Y., Chen, Y., Negi, M., Figeys, D., Li, Y. Y., and Zhang, T. (2019). Exploring the effects of operational mode and microbial interactions on bacterial community assembly in a one-stage partial-nitrification anammox reactor using integrated multi-omics. *Microbiome*, 7(1).
- Wang, Z., van Loosdrecht, M. C., and Saikaly, P. E. (2017b). Gradual adaptation to salt and dissolved oxygen: Strategies to minimize adverse effect of salinity on aerobic granular sludge. *Water Research*, 124:702–712.
- Wanner, O., Eberl, H., Morgenroth, E., Noguera, D., Picioreanu, C., Rittmann, B., and van Loosdrecht, M. (2006). *Mathematical modeling of biofilms*. IWA Pub., Hove.
- Wanner, O. and Gujer, W. (1986). A multispecies biofilm model. *Biotechnology and Bioengineering*, 28(3):314–328.
- Wanner, O. and Morgenroth, E. (2004). Biofilm modeling with AQUASIM. *Water Science and Technology*, 49(11-12):137–144.
- Wanner, O. and Reichert, P. (1996). Mathematical modeling of mixed-culture biofilms. *Biotechnology and Bioengineering*, 49(2):172–184.

- Wei, Q., Kawagoshi, Y., Huang, X., Hong, N., Van Duc, L., Yamashita, Y., and Hama, T. (2016). Nitrogen removal properties in a continuous marine anammox bacteria reactor under rapid and extensive salinity changes. *Chemosphere*, 148:444–451.
- Wells, G. F., Park, H. D., Yeung, C. H., Eggleston, B., Francis, C. A., and Criddle, C. S. (2009). Ammonia-oxidizing communities in a highly aerated full-scale activated sludge bioreactor: Betaproteobacterial dynamics and low relative abundance of Crenarchaea. *Environmental Microbiology*, 11(9):2310–2328.
- Wett, B. (2006). Solved upscaling problems for implementing deammonification of rejection water. *Water Science and Technology*, 53(12):121–128.
- Wett, B., Omari, A., Podmirseg, S. M., Han, M., Akintayo, O., Gómez Brandón, M., Murthy, S., Bott, C., Hell, M., Takács, I., Nyhuis, G., and O'Shaughnessy, M. (2013). Going for mainstream deammonification from bench to full scale for maximized resource efficiency. *Water Science and Technology*, 68(2):283–289.
- Wiesmann, U. (1994). Biological Nitrogen Removal from Wastewater. In *Advances in Biochemical Engineering/Biotechnology*, volume 51. Springer Berlin Heidelberg.
- Winkler, M. K., Kleerebezem, R., Kuenen, J. G., Yang, J., and Van Loosdrecht, M. C. (2011). Segregation of biomass in cyclic anaerobic/aerobic granular sludge allows the enrichment of anaerobic ammonium oxidizing bacteria at low temperatures. *Environmental Science and Technology*, 45(17):7330–7337.
- Winkler, M. K., Kleerebezem, R., Strous, M., Chandran, K., and Van Loosdrecht, M. C. (2013). Factors influencing the density of aerobic granular sludge. *Applied Microbiology and Biotechnology*, 97(16):7459–7468.
- Winkler, M. K., Le, Q. H., and Volcke, E. I. (2015). Influence of Partial Denitrification and Mixotrophic Growth of NOB on Microbial Distribution in Aerobic Granular Sludge. *Environmental Science and Technology*, 49(18):11003–11010.
- Wu, J., Zhang, Y., Zhang, M., and Li, Y. (2017). Effect of nitrifiers enrichment and diffusion on their oxygen half-saturation value measurements. *Biochemical Engineering Journal*, 123:110–116.
- Wu, Z., Meng, H., Huang, X., Wang, Q., Chen, W. H., Gu, J. D., and Lee, P. H. (2019). Salinity-driven heterogeneity toward anammox distribution and growth kinetics. *Applied Microbiology and Biotechnology*, 103(4):1953–1960.
- Wyffels, S., Van Hulle, S. W., Boeckx, P., Volcke, E. I., Van Cleemput, O., Vanrolleghem, P. A., and Verstraete, W. (2004). Modeling and simulation of oxygen-limited partial nitrification in a membrane-assisted bioreactor (MBR). *Biotechnology and Bioengineering*, 86(5):531–542.
- Yan, J., Haaijer, S. C., Op den Camp, H. J., van Niftrik, L., Stahl, D. A., Könneke, M., Rush, D., Sinninghe Damsté, J. S., Hu, Y. Y., and Jetten, M. S. (2012). Mimicking the oxygen minimum zones: Stimulating interaction of aerobic archaeal and anaerobic bacterial ammonia oxidizers in a laboratory-scale model system. *Environmental Microbiology*, 14(12):3146–3158.
- Yang, M., Liu, J., Zhang, X., and Richardson, S. D. (2015). Comparative Toxicity of Chlorinated Saline and Freshwater Wastewater Effluents to Marine Organisms. *Environmental Science and Technology*, 49(24):14475–14483.
- Yokota, N., Watanabe, Y., Tokutomi, T., Kiyokawa, T., Hori, T., Ikeda, D., Song, K., Hosomi, M., and Terada, A. (2018). High-rate nitrogen removal from waste brine by marine anammox bacteria in a pilot-scale UASB reactor. *Applied Microbiology and Biotechnology*, 102(3):1501–1512.
- Yu, H., Li, J., Dong, H., and Qiang, Z. (2019). Nitrogen removal performance of marine anammox bacteria treating nitrogen-rich saline wastewater under different inorganic carbon doses: High inorganic carbon tolerance and carbonate crystal formation. *Bioresource Technology*, 288(May):121565.
- Zhang, L., Narita, Y., Gao, L., Ali, M., Oshiki, M., Ishii, S., and Okabe, S. (2017a). Microbial competition among anammox bacteria in nitrite-limited bioreactors. *Water Research*, 125:249–258.
- Zhang, L., Narita, Y., Gao, L., Ali, M., Oshiki, M., and Okabe, S. (2017b). Maximum specific growth rate of anammox bacteria revisited. *Water Research*, 116:296–303.
- Zhang, L. and Okabe, S. (2020). Ecological niche differentiation among anammox bacteria. *Water Research*, 171.

Appendix

A1 Free ammonia

Ammonia is dissolved in wastewater as the ammonium ion (NH_4^+) and un-ionized ammonia (NH_3). These are in an equilibrium affected by the temperature and pH. Therefore, the free ammonia concentration (FA) ($\text{mg}_{\text{NH}_3} \text{L}^{-1}$) can be calculated as a function of the total ammonia concentration as nitrogen ($\text{mg}_\text{N} \text{L}^{-1}$), pH and temperature (T in $^\circ\text{C}$) of the bulk liquid (Anthonisen et al., 1976):

$$\text{NH}_3 \text{ (mg}_{\text{NH}_3} \text{ L}^{-1}) = \frac{17}{14} \times \frac{S_{\text{NH}_4\text{in}}}{K_a \cdot 10^{-\text{pH}} + 1} \quad (\text{A1})$$

$$K_a = \frac{[\text{NH}_4^+]}{[\text{NH}_3][\text{H}^+]} = \exp\left(\frac{6344}{273 + T}\right) \quad (\text{A2})$$

Table A1: Free ammonia concentration at different pH, for $S_{\text{NH}_4\text{in}} = 50 \text{ mg}_{\text{NH}_4^+-\text{N}} \text{L}^{-1}$ and $T = 25^\circ\text{C}$.

pH	6	7	7.5	8	9
FA ($\text{mg}_{\text{NH}_3} \text{L}^{-1}$)	0.028	0.34	1.07	3.26	18.12

Considering a low ammonium concentration in the effluent and that the usual pH in wastewater treatment is around 7-8, the concentration of FA in the water can be neglected.

A2 Model description

The state variables are listed in Table A2. The process kinetic rate equations are described in Table A3. The stoichiometric matrix is presented in Table A4. The parameters implemented in the stoichiometric matrix and in the kinetic rate equations are described in Table A6.

Table A2: State variables used in the model.

Component	Definition	Unit
Model dissolved components		
S_{NH_4}	Ammonium nitrogen	$\text{mg}_\text{N L}^{-1}$
S_{NO_2}	Nitrite nitrogen	$\text{mg}_\text{N L}^{-1}$
S_{NO_3}	Nitrate nitrogen	$\text{mg}_\text{N L}^{-1}$
S_{O_2}	Dissolved oxygen	$\text{mg}_{\text{O}_2} \text{L}^{-1}$
S_{N_2}	Dinitrogen gas	$\text{mg}_\text{N L}^{-1}$
Model particulate components		
X_{AOA}	Ammonia-oxidizing archaea	$\text{mg}_{\text{COD}} \text{L}^{-1}$
X_{AOB}	Ammonia-oxidizing bacteria	$\text{mg}_{\text{COD}} \text{L}^{-1}$
X_{AMX}	Anammox bacteria	$\text{mg}_{\text{COD}} \text{L}^{-1}$
X_{NOB}	Nitrite-oxidizing bacteria	$\text{mg}_{\text{COD}} \text{L}^{-1}$
X_I	Inert, non-biodegradable organics	$\text{mg}_{\text{COD}} \text{L}^{-1}$

Table A3: Process kinetic rate equations.

Process	Kinetics rates expressions
1. Growth of AOA	$\mu_{max}^{AOA} \frac{S_{O_2}}{K_{O_2}^{AOA} + S_{O_2}} \frac{S_{NH_4}}{K_{NH_4^+}^{AOA} + S_{NH_4}} X_{AOA}$
2. Decay of AOA	$b_{AOA} X_{AOA}$
3. Growth of AOB	$\mu_{max}^{AOB} \frac{S_{O_2}}{K_{O_2}^{AOB} + S_{O_2}} \frac{S_{NH_4}}{K_{NH_4^+}^{AOB} + S_{NH_4}} X_{AOB}$
4. Decay of AOB	$b_{AOB} X_{AOB}$
5. Growth of NOB	$\mu_{max}^{NOB} \frac{S_{O_2}}{K_{O_2}^{NOB} + S_{O_2}} \frac{S_{NO_2}}{K_{NO_2^-}^{NOB} + S_{NO_2}} X_{NOB}$
6. Decay of NOB	$b_{NOB} X_{NOB}$
7. Growth of Anammox	$\mu_{max}^{AMX} \frac{K_{O_2}^{AMX}}{K_{O_2}^{AMX} + S_{O_2}} \frac{S_{NH_4}}{K_{NH_4^+}^{AMX} + S_{NH_4}} \frac{S_{NO_2}}{K_{NO_2^-}^{AMX} + S_{NO_2}} X_{AMX}$
8. Decay of Anammox	$b_{AMX} X_{AMX}$

Table A4: Stoichiometric matrix of the model.

Variable $i \rightarrow$	S_{NH_4}	S_{O_2}	S_{NO_2}	S_{NO_3}	S_{N_2}	X_{AOA}	X_{AOB}	X_{NOB}	X_{AMX}	X_I
Process $j \downarrow$	N	O ₂	N	N	N	COD	COD	COD	COD	COD
1	$-i_{\text{NXB}} - \frac{1}{Y_{\text{AOA}}}$	$1 - \frac{3.43}{Y_{\text{AOA}}}$	$\frac{1}{Y_{\text{AOA}}}$			1				
2	$i_{\text{NXB}} - i_{\text{NXI}} \cdot f_I$					-1				f_I
3	$-i_{\text{NXB}} - \frac{1}{Y_{\text{AOB}}}$	$1 - \frac{3.43}{Y_{\text{AOB}}}$	$\frac{1}{Y_{\text{AOB}}}$				1			
4	$i_{\text{NXB}} - i_{\text{NXI}} \cdot f_I$						-1			f_I
5	$-i_{\text{NXB}}$	$1 - \frac{1.14}{Y_{\text{NOB}}}$	$-\frac{1}{Y_{\text{NOB}}}$	$\frac{1}{Y_{\text{NOB}}}$				1		
6	$i_{\text{NXB}} - i_{\text{NXI}} \cdot f_I$							-1		f_I
7	$-i_{\text{NXB}_{\text{AMX}}} - \frac{1}{Y_{\text{AMX}}}$		$-\frac{1}{Y_{\text{AMX}}} - 1/1.14$	$1/1.14$	$\frac{2}{Y_{\text{AMX}}}$				1	
8	$i_{\text{NXB}_{\text{AMX}}} - i_{\text{NXI}} \cdot f_I$								-1	f_I

A3 Model considerations

The microbial kinetic parameters chosen for this model consider the use of saline wastewater and temperature of 25 °C.

Anammox bacteria are completely inhibited by oxygen until micro-aerobic conditions ($< 0.02 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$) are reached again (Strous et al., 1997; Seuntjens et al., 2018). Therefore, due to this reversible condition, anammox bacteria growth is modeled with two Monod terms for ammonium and nitrite and one inhibition term for oxygen (Table A3).

Maximum growth rate were converted to 25 °C using the relationship express expressed by the modified Arrhenius equation (Hao et al., 2002):

$$\mu_{max}^{AOB}(T) = \mu_{max}^{AOB}(T_{ref}) \cdot \exp\left(\frac{E_a^{AOB} \cdot (T - T_{ref})}{R \cdot T \cdot T_{ref}}\right) \quad (\text{A3})$$

The partial nitrification process is catalysed by the AMO enzyme of ammonia-oxidizing microorganisms. Hence the activation energy (E_a) of AOA was analogous to that of AOB (68 kJ mol^{-1}) (Jetten et al., 1998). E_a value of NOB for the nitrite oxidation process was 44 kJ mol^{-1} (Jetten et al., 1998). E_a values for each anammox bacteria species are characterised in Table 1.

Analogous to growth rate, the decay rate coefficient (b) for AOB and NOB were calculated at 25 °C based on the equations from Jubany et al. (2008):

$$b_{AOB} = 1.651 \times 10^{11} \cdot \exp\left(\frac{-8183}{273 - T}\right) \quad (\text{A4})$$

$$b_{NOB} = 8.626 \times 10^6 \cdot \exp\left(\frac{-5295}{273 - T}\right) \quad (\text{A5})$$

The decay rate coefficient for AOA and all anammox bacteria was assumed to be $0.14^* \mu_{max}$ (Straka et al., 2019) as this information is mostly missing for anammox bacteria (Zhang and Okabe, 2020).

Regarding the biomass yield coefficients, Könneke et al. (2014) compared the biomass yield of marine AOA *Nitrosopumilus maritimus* and the marine AOB *N. oceanii*, and a 1.5 times higher AOA yield was observed. It was found that AOA uses the energy gained by ammonia oxidation for biomass formation in a much more efficient way than its bacterial counterpart (Könneke et al., 2014; Vandekerckhove et al., 2019). However, as mentioned before, *N. europaea* is the chosen AOB for this model as it can adapt to saline environments. The yield of *N. europaea* varies between 0.17-0.25. Recently, González-Cabaleiro et al. (2019) predicted the highest possible true yield of $0.184 \text{ g}_{\text{COD}} \text{ g}_{\text{N}}^{-1}$ for AOB (After unit conversion, using a typical biomass composition of $\text{C}_5\text{H}_7\text{O}_2\text{N}_1$, corresponding with $1.42 \text{ g}_{\text{COD}} \text{ g}_{\text{biomass}}^{-1}$).

Therefore, in the model, the yield of AOA was set to $0.1337 \text{ g}_{\text{COD}} \text{ g}_{\text{N}}^{-1}$ (Könneke et al., 2014) (assuming

1.44 g_{COD} g_{VSS}⁻¹ (Henze, 2000) and the yield of AOB was set between 0.093 and 0.184 g_{COD} g_N⁻¹ (Straka et al., 2019). However, the different values for the yield of AOB did not affect the results and thus, that data was not shown or discussed, and the value of 0.184 g_{COD} g_N⁻¹ was used in all simulations.

For nitrite oxidizers, the yield of *Nitrospira* and *Nitrobacter* varies between 0.057 to 0.14 g_{COD} g_N⁻¹ in the literature (Blackburne et al., 2007; Mehrani et al., 2020; Park and Noguera, 2007; Park et al., 2017; Wiesmann, 1994). However, Vannecke and Volcke (2015) compared 60 species of NOB and observed a mean yield value of 0.08 g_{COD} g_N⁻¹, which also has been reported by other studies (Jubany et al., 2008; Laurenzi et al., 2019; Pérez et al., 2014). Hence, it is the value assumed for this work.

For the affinity kinetic constants, two scenarios were tested: $K_{O_2}^{NOB} = 2 \times K_{O_2}^{AOB}$ (typical kinetics) and $K_{O_2}^{NOB} = 0.5 \times K_{O_2}^{AOB}$ (representing the K/r hypothesis). The value of $K_{O_2}^{AOB}$ was fixed at 0.40 mg L⁻¹, also based on the value reported by Vannecke and Volcke (2015). Similar values are reported for *Nitrosomonas* on Table A5. $K_{O_2}^{NOB}$ varied between 0.80 and 0.20 mg L⁻¹.

Table A5: Literature review on the oxygen affinity constant of AOB (*Nitrosomonas*) and NOB (*Nitrospira*/*Nitrobacter*).

Experimental conditions	$K_{O_2}^{AOB}$	$K_{O_2}^{NOB}$	T (°C)	References
Respirometric assay/lab-scale	0.30	1.10 ⁽¹⁾	20	(Wiesmann, 1994)
Literature/Reference Value	0.50	0.68 ⁽²⁾	5-30	(Rittmann and McCarty, 2001)
Respirometric/lab-scale	0.75	1.75	25	(Guisasola et al., 2005)
MBR lab-scale	0.18	0.13 ⁽¹⁾	15	(Manser et al., 2005)
MABR lab-scale	0.24	0.40 ⁽¹⁾	22	(Downing and Nerenberg, 2008)
Enriched culture lab-scale SBR	0.03	0.43 ⁽²⁾	19-23	(Blackburne et al., 2008)
Respirometric assay/lab	0.74	1.75 ⁽²⁾	25	(Jubany et al., 2008)
Respirometric assay/WWTP	0.33	0.97 ⁽²⁾	23	(Rongsayamanont et al., 2010)
PN/A WWTP	0.37	0.16	20	(Wett et al., 2013)
N/DN Pilot scale	1.16	0.16 ⁽¹⁾	25	(Regmi et al., 2014)
Respirometric assay/lab-scale	0.19	0.26 ⁽¹⁾	25	(Wu et al., 2017)
PN/A lab-scale SBR	0.36	0.50	35	(Liu et al., 2017)
Mathematical approach	0.14	0.09 ⁽¹⁾	20	(Val del Rio et al., 2019)
Co-culture CSTR	0.24	-	30	(Park and Noguera, 2007)
Enriched culture lab-scale SBR	-	0.33 ⁽¹⁾	22	(Park et al., 2017)
Literature review	0.40	0.97	30	(Vannecke and Volcke, 2015)

⁽¹⁾ *Nitrospira*

⁽²⁾ *Nitrobacter*

Table A6: Kinetic and stoichiometric parameters of the model.

Parameter	Definition	Values	Unit	Reference
Ammonia-Oxidizing Archaea (AOA)				
Y_{AOA}	Yield coefficient for AOA	0.1337	$\text{g}_{\text{COD}} \text{g}_{\text{N}}^{-1}$	(Könneke et al., 2014)
μ_{max}^{AOA}	Maximum growth rate of AOA	0.50	d^{-1}	(Könneke et al., 2014)
b_{AOA}	Decay rate coefficient of AOA	0.07	d^{-1}	(Straka et al., 2019)
$K_{\text{O}_2}^{AOA}$	S_{O_2} affinity constant for AOA	0.128	$\text{mg}_{\text{O}_2} \text{L}^{-1}$	(Stahl and De La Torre, 2012)
$K_{\text{NH}_4^+}^{AOA}$	S_{NH_4} affinity constant for AOA	0.0019	$\text{mg}_{\text{N}} \text{L}^{-1}$	(Martens-Habbena et al., 2009)
Ammonia-Oxidizing Bacteria (AOB)				
Y_{AOB}	Yield coefficient for AOB	0.184	$\text{g}_{\text{COD}} \text{g}_{\text{N}}^{-1}$	(González-Cabaleiro et al., 2019)
μ_{max}^{AOB}	Maximum growth rate of AOB	1.30	d^{-1}	(Wiesmann, 1994)
b_{AOB}	Decay rate coefficient of AOB	0.20	d^{-1}	(Jubany et al., 2008)
$K_{\text{O}_2}^{AOB}$	S_{O_2} affinity constant for AOB	0.40	$\text{mg}_{\text{O}_2} \text{L}^{-1}$	(Vannecke and Volcke, 2015)
$K_{\text{NH}_4^+}^{AOB}$	S_{NH_4} affinity constant for AOB	1.40	$\text{mg}_{\text{N}} \text{L}^{-1}$	(Zhang and Okabe, 2020)
Nitrite-Oxidizing Bacteria (NOB)				
Y_{NOB}	Yield coefficient for NOB	0.08	$\text{g}_{\text{COD}} \text{g}_{\text{N}}^{-1}$	(Vannecke and Volcke, 2015)
μ_{max}^{NOB}	Maximum growth rate of NOB	0.85	d^{-1}	(Park et al., 2017)
b_{NOB}	Decay rate coefficient of NOB	0.17	d^{-1}	(Jubany et al., 2008)
$K_{\text{O}_2}^{NOB}$	S_{O_2} affinity constant for NOB	0.80/0.20	$\text{mg}_{\text{O}_2} \text{L}^{-1}$	Scenario 1 and 2
$K_{\text{NO}_2^-}^{NOB}$	S_{NO_2} affinity constant for NOB	0.126	$\text{mg}_{\text{N}} \text{L}^{-1}$	(Jacob et al., 2017) (Nowka et al., 2015)
Anammox Bacteria (see Table 1)				
b_{AMX}	Decay rate coefficient of anammox	$0.14 \mu_{\text{max}}$	d^{-1}	(Straka et al., 2019)
$K_{\text{O}_2}^{AMX}$	S_{O_2} inhibiting coefficient for anammox	0.02	$\text{mg}_{\text{DO}} \text{L}^{-1}$	(Seuntjens et al., 2018)

Other stoichiometric parameters				
i_{NXB}	Nitrogen content of biomass	0.083	$g_N g_{COD}^{-1}$	$CH_{1.8}O_{0.5}N_{0.2}$ ⁽¹⁾
$i_{NXB_{AMX}}$	Nitrogen content of biomass in anammox	0.058	$g_N g_{COD}^{-1}$	$CH_2O_{0.5}N_{0.15}$ ⁽²⁾
i_{NXI}	Nitrogen content of X_I	0.06	$g_N g_{COD}^{-1}$	ASM1 (Henze, 2000)
f_I	Fraction of X_I in biomass decay	0.08	$g_{COD} g_{COD}^{-1}$	ASM1 (Henze, 2000)

⁽¹⁾ Using a typical biomass composition of $CH_{1.8}O_{0.5}N_{0.2}$, corresponding to $1.3659 g_{COD} g_{biomass}^{-1}$

⁽²⁾ Using an anammox biomass composition of $CH_2O_{0.5}N_{0.15}$ (see equation 2), corresponding to $1.51 g_{COD} g_{biomass}^{-1}$

A4 Mass transfer parameters

Diffusion coefficients in water (D_w) were first converted into effective diffusion coefficient in the biofilm (D_e) by using the average relative effective diffusion coefficient (D_r) over different types of biofilms (Stewart, 2003). The calculated effective diffusion coefficients ($D_e = D_r \cdot D_w$) were then divided by the porosity ($\epsilon_l = 0.75$). This was necessary because AQUASIM multiplies the porosity with the input values of the diffusion coefficient as a default estimate of the effective diffusion coefficient Equation (4).

Table A7: Diffusion coefficients.

	D_w 25 °C ($10^{-4} m^2 d^{-1}$)	D_r	D_e ($10^{-4} m^2 d^{-1}$)	AQUASIM ($10^{-4} m^2 d^{-1}$)	Reference
D_{NH_4}	1.70	0.78	1.33	1.77	(Stewart, 2003)
$D_{NO_2^-}$	1.40	0.66	0.92	1.23	(Picioreanu et al., 1997)
$D_{NO_3^-}$	1.40	0.66	0.92	1.23	(Picioreanu et al., 1997)
D_{O_2}	1.62	0.57	0.92	1.23	(Stewart, 2003)
D_{N_2}	1.73	0.57	0.99	1.31	(Stewart, 2003)

A5 MABR

Table A8: Literature review on partial-nitrification anammox in membrane-aerated biofilms reactors.

Approach	NH ₄ ⁺ in (mg L ⁻¹)	NLR (g _N L ⁻¹ d ⁻¹)	L _F (mm)	J _{O₂} /J _{NH₄⁺}	NRE	Reference
Experimental	500-780	1.08	0.50	1.73	72%	(Pellicer-Nàcher et al., 2010)
Experimental	-	0.185	-	2.18	75%	(Gilmore et al., 2013)
Experimental	50	0.05	-	(0.5 mg _{O₂} L ⁻¹)	84%	(Augusto et al., 2018)
Model AOB	250	variable	0.75	1.58	90%	(Terada et al., 2007)
Model AOB	250	variable	0.75	1.70	93%	(Lackner et al., 2008)
Model AOB	250	variable	0.75	2.00	75%	(Ni and Yuan, 2013)
Model AOB	30	variable	0.20	1.85	86%	(Liu et al., 2016b)
Model AOA	30	variable	0.20	2.40	92%	(Liu et al., 2016b)

A6 Kinetic adaptation to salinity

To better describe the growth behaviour with salinity for *Ca. Kuenenia stuttgartiensis*, “*Ca. Scalindua* sp.” and “*Ca. Brocadia sinica*”, a salinity effect was incorporated in the Monod equation in the kinetic model.

The first approach was developed by Wu et al. (2019), which implemented a salinity-induced growth equation of anammox for niche estimation between “*Ca. Scalindua* sp.” and “*Ca. K. stuttgartiensis*”. A Gaussian equation (Equation A6) was fitted with the data from Kartal et al. (2006) and Awata et al. (2013), evaluating the salinity effect on substrate consumption rate for both anammox bacteria species (Figure A1). This way, it is possible to calculate how much the performance of anammox is being inhibited by salinity and implement that parameter on the maximum growth rate (Table 3).

$$r_{\text{NH}_4^+} = r_{\text{NH}_4^+,0} + \left(\frac{A}{w\sqrt{\frac{\pi}{2}}} \right) \cdot \exp \left[-2 \left(\frac{S_{\text{salt}} - S_{\text{salt},c}}{w} \right)^2 \right] \quad (\text{A6})$$

$r_{\text{NH}_4^+}$: ammonium consumption rate ($\mu\text{mol}_{\text{NH}_4^+} \text{mg}_{\text{VSS}}^{-1} \text{d}^{-1}$); $r_{\text{NH}_4^+,0}$: ammonium consumption rate without salt addition; A and w : numerical values with no physical meaning obtained by fitting the data to Gauss equation; S_{salt} : salt concentration; $S_{\text{salt},c}$: salt concentration where maximum ammonium consumption was obtained.

The second approach was developed by Li et al. (2018a), which used a modified Boltzmann model (Equation A7) that indicates how much the performance of “*Ca. B. sinica*” is being inhibited by a percentage of seawater content in the influent (100% seawater = 3.5% salinity) (Figure A1). This parameter was also

implement on the maximum growth rate (Table 3).

$$I = I_{max} + \frac{I_{min} - I_{max}}{1 + \exp\left(\frac{s - s_c}{s_d}\right)} \quad (A7)$$

I : Inhibition rate (%); I_{min} : Minimum inhibition rate; I_{max} : Maximum inhibition rate; s : seawater content (%); s_c : inflection point from Boltzmann equation; s_d : constant value of seawater content (%).

Table A9: Fitting parameters of Figure A1.

	"Ca. Kuenenia"	"Ca. Scalindua"	"Ca. Brocadia"	
$r_{\text{NH}_4^+,0}$	1.53	− 39.34	I_{min}	0%
w	2.20	4.15	I_{max}	46.02%
A	58.5	507.64	s_c	36.30%
$S_{salt,c}$	1.25	2.80	s_d	10.32%

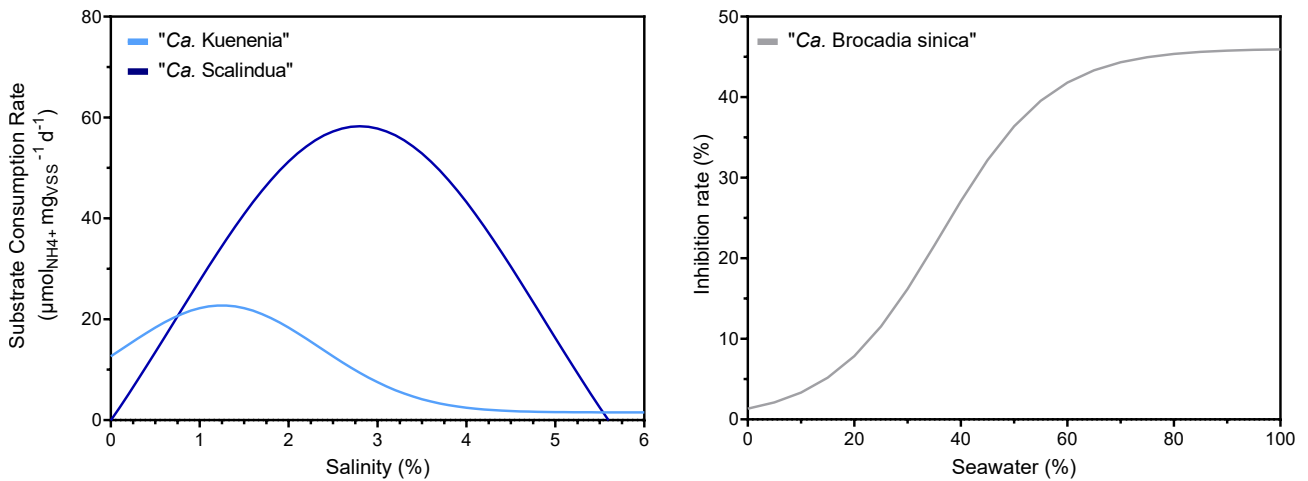


Figure A1: The model-fitted relationship between specific ammonium consumption rate and different salinity levels, for "Ca. Scalindua sp." and "Ca. Kuenenia stuttgartiensis", using a Gaussian equation (Wu et al., 2019). Modified Boltzmann equation predicting inhibition of "Ca. Brocadia sinica" at different seawater ratios (Li et al., 2018a).

Table A10: Literature review on recent studies for wastewater treatment with marine and freshwater anammox bacteria at different salinities.

Bioreactor	T (°C)	Salinity (%)	NLR (g _N L ⁻¹ d ⁻¹)	NRE (%)	Microbial Community	Reference
PN/A SBR	33	Synthetic WW (1-2%)	0.32	73	<i>Nitrospira</i> (NOB) <i>N. europaea</i> (AOB) “ <i>Ca. Kuenenia</i> ”	(Wang et al., 2017a)
Anammox SBR	30	Real seawater (3%)	0.89	80	“ <i>Ca. Brocadia</i> ”	(Li et al., 2018a)
Anammox SBR	30	Synthetic WW (0.7-0.9%)	0.25	80	<i>Nitrospira</i> (NOB) <i>Nitrosopumilus</i> (AOA) “ <i>Ca. Brocadia</i> ” “ <i>Ca. Scalindua</i> ”	(Val del Rio et al., 2018)
Anammox UASB	29	Waste brine (2.7%)	14.2	75	“ <i>Ca. Scalindua wagneri</i> ”	(Yokota et al., 2018)
Anammox SBR	20	Real Seawater (3.5%)	3.78	90	“ <i>Ca. Scalindua</i> sp.”	(Yu et al., 2019)
Anammox MBR	25	Real Seawater (3.5%)	0.40	90	“ <i>Ca. Scalindua</i> AMX11”	(Ali et al., 2020)
PN/A Airlift reactor	25	Synthetic WW (2.5%)	1.00	74	<i>Nitrosomonas</i> (AOB) “ <i>Ca. Kuenenia</i> ”	(Guo et al., 2020)
PN/A SBR	23	Landfill leachate (3%)	1.22	86	<i>Nitrospira</i> (NOB) <i>Nitrosomonas</i> (AOB) “ <i>Ca. Kuenenia</i> ”	(Huang et al., 2021)