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“Technical implementation and development of control strategies for the integration of the Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO) into wastewater treatment schemes for simultaneous nitrogen removal and energy recovery from nitrogen”

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This work is dedicated to my family and friends, and the people who contribute to a sustainable future.

“There is no passenger on spaceship earth. We are all crew.”

Marshall McLuhan, 1965

Abstract

The **C**oupled **A**erobic-anoxic **N**itrous **D**ecomposition **O**peration (CANDO) is a novel approach for the biological treatment of high strength ammonia wastewater. In this double-stage biological treatment train, aerobic (first stage) and anoxic (second stage) processing conditions are coupled for the generation of nitrite and subsequently nitrous oxide (N_2O). The generated N_2O is harvested for on-site energy recovery. Because of its exothermal decomposition (52 kJ/mol) during combustion, approximately 37% more energy compared to the combustion with oxygen can be generated using biogas as fuel. Additionally, oxygen and carbon demands in the biological stages can be considerably reduced compared to conventional biological nutrient removal by nitrification/denitrification. Furthermore, the process facilitates tight emission control by the intentional combustion of harmful N_2O emissions, which are inherent to other biological nitrogen removal technologies.

High N_2O yields could be achieved earlier under highly controlled lab-scale conditions. Hence, the main objectives of this research were the application and comparison of real feed-stream conditions, the development of process automation and control strategies, as well as N_2O removal processes toward an application of the CANDO at full-scale.

For this purpose, a lab-scale operation of both stages (aerobic/anoxic) was conducted over ten months treating ammonia-rich reject water from a full-scale digester and primary effluent from a local wastewater treatment plant. The investigations were accompanied by a comprehensive measurement campaign to assess long-term stability as well as process dynamics throughout single cycles.

The long-term operation demonstrated the potential for continuous N_2O generation under variable and realistic process conditions considering the feed matrices applied. Eventually, the systems were operated under steady-state conditions for six months without operational failures. The observed process dynamics were similar to previous investigations under controlled conditions and underscored the relevance of endogenous substrate (e.g. polyhydroxybutyrate) respiration as a key for high N_2O yields in biological systems also in technical application. Under time-controlled sequencing-batch-operation, stable nitrogen removal was achieved to average effluent concentrations of 1.8 $mg_{NO_2\text{-}N}$ /L. The application of the oxidation reduction (ORP) potential as surrogate parameter for the abundance of NO_2^- enabled process automation and dynamic control and resulted in highly efficient nitrogen removal, even under varying process conditions, i.e. different COD/N ratios. In addition, it was shown that a COD/N ratio of 4 is required for stable operation considering nitrogen removal and stable mixed liquor volatile suspended sludge (MLVSS) concentrations representing the active biomass in the system. The observed nitrogen removal rates were at the lower end of the range observed for conventional denitritation and denitrification systems, which have a significantly higher demand for both, organic reducing equivalents and aeration energy.

The system performance in terms of N_2O production as ratio of NO_2-N/N_2O-N was comparable to previous lab-scale investigations. On average, 58±8% to 68±8% (depending on the applied removal strategy) N_2O production was observed under steady-state conditions applying either continuous gas stripping or a dedicated stripping phase as part of the sequencing batch cycles.

In addition to conventional gas stripping, a novel process for gas removal i.e., a micro porous hollow-fiber membrane contactor, was tested for N_2O removal. The investigations demonstrated its technical feasibility under controlled conditions and the results are promising regarding process integration. The mass-transfer was stable and comparable to CO_2 removal at similar operating conditions. At continuous operation, 78% of the feed N_2O-N load to the module was extracted. Additionally, mass transfer dynamics and off-gas concentrations were investigated in relation to feed and effluent concentrations as well as operational conditions, i.e., gas and liquid flow rates. The investigations furthermore revealed, that the membrane resistance is negligible, also at low superficial liquid velocities. Compared to conventional N_2O gas stripping (900 ppm), higher off-gas concentrations of approximately 3,700 ppm could be generated in an equal range of dissolved N_2O feed concentrations. In relation to initial NO_2^- loads in the second biological stage, this translates to a removal efficiency of over 98% during continuous operation while maintaining anoxic processing conditions.

Based on these results, future research should be dedicated to the technical implementation of the CANDO process and process integration of biological stages and on-site combustion units for energy recovery in full-scale wastewater treatment processes. The results will potentially facilitate feasibility as well as life-cycle assessments for the determination of the actual performance and environmental impact of the CANDO process in comparison to other state-of-the-art nitrogen removal technologies in technical application.

Key words: Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO); energy recovery; design of experiment (DoE); greenhouse gas emissions; nitrogen removal; nitrous oxide (N_2O); wastewater treatment; sequencing batch reactor (SBR), oxidation reduction potential (ORP); hollow-fiber membrane contactor

Zusammenfassung

Der sogenannte *Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO)* Prozess ist ein vielversprechendes Verfahren zur simultanen Stickstoffelimination und Energierückgewinnung auf kommunalen Kläranlagen; insbesondere aus hoch ammoniumbelasteten Prozesswässern. Das Verfahren ermöglicht außerdem eine Verringerung der Belüftungsenergie wie auch von organischen Substraten durch die gezielte Verkürzung der in konventionellen Verfahren angewendeten autotrophen wie heterotrophen mikrobiologischen Stoffwechselketten. Ferner kann durch die gezielte Produktion und Verbrennung von Lachgas (N_2O) zu molekularem Stickstoff (N_2) neben der Energierückgewinnung auch eine Emissionsminderung des klimaschädlichen Lachgases erreicht und potentiell die CO_2-Bilanz von Kläranlagen verbessert werden. Das Konzept der Integration von Stickstoffelimination, Energierückgewinnung und Emissionsminderung ist bisher einzigartig. Technische Anwendungen existieren bisher jedoch nicht. Vorhergehende Laborstudien haben allerdings bereits das Potenzial der biologischen Lachgasproduktion unter sehr kontrollierten Bedingungen nachgewiesen. Ein Vergleich der Ergebnisse mit etablierten technischen Prozessen ist aufgrund der verwendeten synthetischen Substrate und Gasextraktionsmethoden nicht sinnvoll. Die Prozessdynamik, -führung und -kontrolle unter realen Bedingungen sind bisher weitestgehend unbekannt. Daher bestand das Ziel dieser Arbeit in der Untersuchung möglicher lokal verfügbarer Kohlenstoffquellen in Form von Realabwässern zur Abschätzung und Entwicklung technisch und potentiell wirtschaftlich umsetzbarer Implementationsstrategien in bestehende Verfahren zur Abwasserbehandlung.

Neben der Untersuchung möglicher realer Substrate zur Lachgasproduktion, wurden außerdem Automationsstrategien und Möglichkeiten zur effizienten Lachgasproduktion und -separation untersucht. Dazu wurde ein Betrieb beider Stufen über zehn Monate untersucht, wobei als reale Substrate Prozesswasser und Primärablauf einer lokalen Kläranlage verwendet wurden.

Nach Entwicklung einer Prozessführungsstrategie zur Verwendung von Realsubstraten und der Adaption der Biozönose über etwa drei Monate, wurde der Betrieb für weitere sechs Monate stabil geführt. Während dieser Phase wurden Untersuchungen zur Prozessdynamik durchgeführt, die die mit synthetischen Substraten aufgezeigten Mechanismen und die Relevanz des endogenen katabolen Zellstoffwechsels für die erfolgreiche biologische Lachgasproduktion bestätigten.

Während dieses Betriebs wurden Ablaufwerte im Mittel von 1,8 $mg_{NO_2\text{-}N}$/L gemessen. Die Stickstoffeliminationsraten lagen im unteren Bereich der beschriebenen Denitrifikations- und Denitritationsraten, allerdings bei deutlich geringerem Bedarf an Belüftungsenergie und organischen Reduktionsäquivalenten.

Über die Entwicklung und Anwendung einer Steuerungsstrategie basierend auf dem Redoxpotential wurde die NO_2^- Eliminationsleistung verbessert, sodass keine Nitritkonzentrationen im Ablauf mehr messbar waren. Auch während der Simulation von Betriebsstörungen konnte in dieser Phase eine vollständige NO_2^--Elimination

gewährleistet werden. Die Untersuchungen bestätigten außerdem die Notwendigkeit eines CSB/N Verhältnis von 4 für einen stabilen Betrieb.

Die Umsatzration von NO_2^- zu N_2O in der zweiten Stufe des CANDO-Prozesses lagen bei 58±8% und 68±8% unter Anwendung einer dedizierten Strippphase zum Ende der Batchsequenz bzw. einer kontinuierlichen Gasstrippung während anoxischer Prozessphasen. Die Gesamtumsatzraten über beide Studien lagen bei 53% bzw. 63% im kontinuierlichen Betrieb.

Die Umsatzleistungen unterschieden sich nicht signifikant von den Ergebnissen früherer Studien und der Anwendung synthetischer Substrate, sodass ähnliche Umsatzraten auch unter technischen Bedingungen zu erwarten sind.

Zur potentiellen Prozessintegration der biologischen Stufen zur Lachgasproduktion und der energetischen Nutzung, wurde eine Gasmembran zur Lachgasextraktion untersucht. Unter kontrollierten Bedingungen wurden bis zu 78% des Lachgases aus dem behandelten Massenstrom entfernt. In Relation zu den Betriebskonzentrationen im CANDO Prozess bedeutet dies eine potentielle Extraktionsleistung von etwa 98%. Darüber hinaus lagen die N_2O-Konzentrationen im Abgasstrom des Membrankontaktors während der Simulation einer kontinuierlichen Gasextraktion höher als Werte, die mittels konventioneller Gasstrippung erzeugt wurden. Im Vergleich zu im Mittel 900 ppm bei Gasstripping im Reaktor konnten 3,700 ppm durch Anwendung der Gasmembran erzeugt werden.

Aufgrund dieser Ergebnisse sollten weitere Versuche im technischen Maßstab durchgeführt werden, um Wirtschaftlichkeit und Ökobilanz des Verfahrens besser einschätzen zu können. Die Ergebnisse werden potentiell Vergleiche mit dem aktuellen Stand der Technik ermöglichen, um dadurch das technische Potential des CANDO-Prozesses weiter zu beleuchten.

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Research papers, author contributions and topic related publications

This cumulative doctoral thesis is based on the following peer-reviewed research papers, which are presented in Chapter 5-8:

Paper 1/Chapter 5:

Weißbach, M.; Criddle, C. S.; Drewes, J. E.; Koch, K. (2017). A proposed nomenclature for biological processes that remove nitrogen. In: *Environmental Science: Water Research and Technology.* 3 (1), 10–17.

- Author contributions: Max Weißbach conducted the literature research, developed the terminology and prepared the manuscript. Craig S. Criddle, Jörg E. Drewes and Konrad Koch supervised this study and reviewed the manuscript.

Paper 2/Chapter 6:

Weißbach, M.; Thiel, P.; Drewes, J. E.; Koch, K. (2018). Nitrogen removal and intentional nitrous oxide production from reject water in a coupled nitritation/nitrous denitritation system under real feed-stream conditions. *Bioresource Technology*, 255, 58-66.

- Author contributions: Max Weißbach was responsible for the research plan and designed, assembled and operated the system. Max Weißbach and Paul Thiel conducted lab-measurements and chemical analyses together. Max Weißbach prepared the manuscript. Jörg E. Drewes and Konrad Koch supervised this study and reviewed the manuscript.

Paper 3/Chapter 7:

Weißbach, M.; Gossler, F.; Drewes, J. E.; Koch, K. (2018). Separation of nitrous oxide from aqueous solutions applying a micro porous hollow fiber membrane contactor for energy recovery. *Separation and Purification Technology*, 195, 271-280.

- Author contributions: Max Weißbach designed the research plan, experiments and setup. Experiments including gaseous and dissolved measurements were conducted by Max Weißbach and Fabian Gossler. Max Weißbach was responsible for the data analysis, model development, mass transfer studies and the preparation of this manuscript which was reviewed by Jörg E. Drewes and Konrad Koch.

Paper 4/Chapter 8:

Weißbach, M.; Drewes, J. E.; Koch, K. (2018). Application of the oxidation reduction potential for process control and monitoring nitrite in a Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO). *Chemical Engineering Journal*, 343, 484-491.

- Author contributions: Max Weißbach designed and conducted all experiments including the bioreactor operation, chemical analyses and gaseous measurements. Max Weißbach was responsible for the preparation of this manuscript, which was reviewed by Jörg E. Drewes and Konrad Koch.

Paper 5/in preparation (not included in the present thesis):

Weißbach, M.; Koch, K. Jörg E.; Joss, A.. A comprehensive research reactor design concept for investigating biological wastewater treatment processes. *MethodsX* (in preparation).

Author contributions: Max Weißbach constructed the research plant together with Adriano Joss. Max Weißbach conducted the literature research and developed the theoretical concept as presented in the publication. The manuscript was prepared by Max Weißbach and Adriano Joss, who supervised this study. The manuscript was reviewed by Jörg E. Drewes and Konrad Koch.

Topic related international publications:

The following topic related peer-reviewed papers have also been prepared and published during this PhD study. However, they are not presented as entire manuscripts in this dissertation, but serve as a supportive element in the respective sub-chapters:

Zhu, B., Bradford, L., Huang, S., Szalay, A., Leix, C., **Weissbach, M.**, Tancsics, A., Drewes, J.E.; Lüders, T. (2017). Unexpected diversity and high abundance of putative nitric oxide dismutase (Nod) genes in contaminated aquifers and wastewater treatment systems. *Applied and Environmental Microbiology*, 83 (4).

Horstmeyer, N.; **Weissbach, M.**; Koch, K.; Drewes, J. E. (2018). A novel concept to integrate energy recovery into potable water reuse treatment schemes. *Journal of Water Reuse and Desalination* (in press). DOI: 10.2166/wrd.2017.051

In the following, the most important topic related scientific contributions including German publications, presentations, and posters are listed.

Topic related German publications:

Weißbach M., Wolfram D., Koch K.; Drewes J. E. (2015). *Untersuchung der Umgebungsbedingungen bei der biogenen Lachgasproduktion*. Gesellschaft Deutscher Chemiker, Wasser 2015 – Jahrestagung der Wasserchemischen Gesellschaft (81, 38-42), Frankfurt am Main: Gesellschaft Deutscher Chemiker e.V.

Weißbach, M., Koch, K., N., Drewes, J. (2015). *Lachgas in der biologischen Abwasserbehandlung – eine potentielle Quelle zur nachhaltigen*

Energierückgewinnung. Energieeffiziente Verfahren zur Stickstoffelimination - Berichte aus der Siedlungswasserwirtschaft, Technische Universität München, München.

Koch, K., **Weißbach, M.**, Leix, C., Horstmeyer, N.; Drewes, J. (2015). *Gezielte Erzeugung von Lachgas als alternative Behandlung stickstoffreicher Abwasserteilströme einschließlich einer Energierückgewinnung*. Umwelttechnologie und Energie in Bayern. München, 50–53.

Presentations:

Koch, K., Leix, C., **Weißbach, M**., Drewes, J., Haisch, C., Berger, C., Thaler, K., Nießner, R. (2013). *Enabling Energy Savings and Recovery in Contemporary Wastewater Treatment Facilities through Photoacoustic-Based N_2O Monitoring and Control Strategies*. International Graduate School of Science and Engineering (IGSSE) Kick-off Meeting, 2-3 December 2013, Munich.

Koch, K., Leix, C., **Weißbach, M**., Berger, C., Thaler, K., Haisch, C., Nießner, R., Drewes, J.E. (2014). *Optimization and reduction of N_2O emission in wastewater treatment*. World's Leading Trade Fair for Water, Wastewater and Solid Waste Management (IFAT), 5 May 2014, Munich.

Weißbach, M., Wolfram, D., Leix, C., Koch, K., Drewes, J.E. (2015). *Untersuchung der Umgebungsbedingungen bei der biogenen Lachgasproduktion*. Jahrestagung der Wasserchemischen Gesellschaft - Fachgruppe in der Gesellschaft Deutscher Chemiker; 11-13 May 2015, Schwerin.

Drewes, J.E., Horstmeyer, N., **Weißbach, M.**, Koch, K. (2015) *Potable Water Reuse – Towards an Energy-neutral Treatment Scheme*. TUM-UQ Research Symposium on Water, Environment & Sustainability. 11-12 June 2015, Munich.

Weißbach, M.; Koch, K.; Drewes, J.E. (2015). *Lachgas in der biologischen Abwasserbehandlung - eine potenzielle Quelle zur nachhaltigen Energierückgewinnung*. 43. Abwassertechnischen Seminar, Energieeffiziente Verfahren zur Stickstoffelimination - Innovationen aus der Forschung und Erfahrungen aus der Praxis, 16 July 2015, Garching.

Weißbach, M., Koch K., Drewes, J.E. (2016). *Deammonification, Nitritation/Denitritation and Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO)*. World's Leading Trade Fair for Water, Wastewater and Solid Waste Management (IFAT), 31 May 2016, Munich.

Weißbach, M.; Koch, K.; Drewes, J.E. (2016): *Why your enemy is my friend: Intended N_2O production with the CANDO process*. N_2O Expert Meeting and Workshop, 21-22 September 2016, Bochum.

Posters:

Leix, C., **Weißbach, M.**, Koch, K., Drewes, J., Berger, C., Thaler, K., Haisch, C., Nießner, R. (2015). *N_2O emissions from wastewater treatment processes – Innovative monitoring and minimization/maximization strategies*. 11th IWA Leading Edge Conference on Water and Wastewater Technologies, 26-29 May 2015, Abu Dhabi.

Weißbach, M., Leix, C., Koch, K., Drewes, J., Berger, C., Thaler, K., Haisch, C., Nießner, R (2015). *N_2O emissions from wastewater treatment processes – Innovative monitoring and minimization/maximization strategies. IWA conference, Activated sludge – 100 years and counting*, 12-14 June 2015, Essen.

Weißbach, M., Leix, C., Koch, K., Drewes, J., Berger, C., Thaler, K., Haisch, C., Nießner, R. (2015*). Innovative monitoring and utilization of N_2O emissions in wastewater treatment processes*. The University of Queensland - Technische Universität München - Research Symposium on Water, Environment and Sustainability, 11-12 June 2015, Munich.

Leix, C., **Weißbach, M.**, Koch, K., Drewes, J., Berger, C., Thaler, K., Haisch, C., Nießner, R. (2015). *Innovative Vermeidungs- und Produktionsstrategien für N_2O in der Abwasseraufbereitung*. 44. Abwassertechnisches Seminar (ATS), 14 July 2016, Garching.

Abbreviations

AMO	Ammonium mono-oxygenase
AOB	Ammonium oxidizing bacteria
BNR	Biological nutrient removal
CANDO	Coupled Aerobic-anoxic Nitrous Decomposition Operation
CAS	Conventional activated sludge
cNor	Cytochrome-based nitric oxide reductase
COD	Chemical oxygen demand
D	Deammonification
DN	Denitritation
DO	Dissolved oxygen
GHG	Greenhouse gas
GWP	Global warming potential
HAO	Hydroxylamine oxygenase
MAP	Magnesium-ammonium-phosphate
N	Nitrification
N_2O-N	Nitrous-oxide-nitrogen
NAR	Nitrate reductase
NH_4-N	Ammonia-nitrogen
NirK	Copper-based nitrite reductase
NirS	Cytochrome-base nitrite reductase
NO_2-N	Nitrite-nitrogen
NO_3-N	Nitrate-nitrogen
NOB	Nitrite oxidizing bacteria
NosZ	Nitrous oxide reductase
ORP	Oxidation reduction potential
ORR	Oxidation reduction regime
PHA	Polyhydroxyalcanoate
PHB	Poly-hydroxy-butyrate
PN	Partial nitritation
qNor	Quinol-based nitric oxide reductase
SBR	Sequencing-batch reactor
WWTP	Wastewater treatment plant

Content

Figures

1. Introduction

Natural resources and environmental services have always supported the social, and economic growth and development of societies. However, while the earth's regeneration capacity was sufficient to compensate for the use of natural goods until the 19th century, the exhaustive exploitation initialized by the industrial revolution has continuously led to a turning point on August 13th, 2015, the so-called "world overshoot day". Consequently, the deterioration of environmental systems is becoming increasingly evident and potentially irreversible. Especially regarding mineral cycles and biodiversity within the different environmental spheres as well as the global climate, considerable measures are required to mitigate the anthropogenic impact and maintain the natural balance of the earth support system (Rockström et al., 2009).

The detriments of anthropogenic activity on the environment are manifold. Considering climate change, although controversially discussed, the significant correlation of anthropogenic greenhouse gases (GHG), e.g., carbon dioxide (CO_2), methane (CH_4), or nitrous oxide (N_2O), on the global temperature increase is widely acknowledged (Bernstein, 2008). Common emission sources are industrial processes, wastewater effluents and road-runoff from urban areas. In rural areas, especially agricultural land-use and accompanying fertilizer application need to be accounted for net-positive mass flows of nitrogen (N), phosphorous (P), and carbon (C) into the environment. As a result, these mineral cycles are considerably affected, causing aggravation of terrestrial as well as aquatic systems e.g., by eutrophication (Rockström et al., 2009).

Since these environmental concerns have been related to considerable social and economic risks (Nordhaus, 1994), global decision-makers have framed several resolutions as incentives to maintain thriving social and economic growth in a more sustainable way. In this respect, for instance, the United Nations global development agenda, the "Global Sustainable Development Goals" (Martens and Obenland, 2016), presently lists the protection of the earth's life-support system next to social justice as the highest priority. In this context, among others, specifically, "clean water and sanitation" as well as "affordable and clean energy" are listed to safeguard global environmental integrity (Martens and Obenland, 2016).

For the water and wastewater sector, these demands require considerable changes and have triggered a re-thinking of the conventional treatment i.e., nutrient removal, toward the development of water and resource as well as energy recovery (Batstone et al., 2015; McCarty et al., 2011; Scherson et al., 2013). Discharge standards have already been continuously increased throughout the last decades, which requires the development of more efficient or alternative wastewater treatment schemes (Horstmeyer et al., 2018). In addition, increasing water scarcity due to climate change has resulted in water reuse practices in semi-arid and arid regions. In this respect, especially the increasing energy demand and related operational costs of advanced water treatment processes are presently of concern in addition to technical challenges that need to be overcome. Hence, novel on-site energy saving and recovery measures in addition to established technology, e.g. biogas production, are part of on-going investigations (Horstmeyer et al., 2018) next to the development of advanced water treatment technology (Joss et al., 2008). Among the potential future energy recovery platforms are the utilization of potential and thermal energy as well as more efficient valorization of chemical energy potentials (McCarty et al., 2011).

Considering the utilization of chemical energy in organic matter, at the present state, nitrogen removal is of increasing concern. In this respect, it is noteworthy, that conventional heterotrophic nitrogen removal technologies are typically limiting factors for the energy recovery potential from organic matter contained in wastewater streams and in turn, directly influence the energy balance of operating wastewater treatment plants. Typically, biological nitrogen removal requires organic carbon for nitrogen reduction due to the application of heterotrophic bacteria in treatment processes. Hence, the total biogas potential of organic loads in wastewater is limited by the influent nitrogen loads. Additionally, co-fermentation of external organic substrates potentially imposes additional nitrogen loads to the biological stages depending on its composition. Accordingly, research is conducted toward alternative biological treatment technologies for nitrogen removal including for instance, nitritation/denitritation (Torà et al., 2011) or partial nitritation/deammonification (van Dongen et al., 2001). These processes provide several advantages such as a lower oxygen and organic carbon demand as well as high process intensity. However, different studies have indicated a considerable increase of the carbon footprint compared to conventional nitrification/denitrification due to potential emissions of the potent GHG N_2O (Kampschreur et al., 2008). As a result, the higher feasibility is eventually jeopardized by a considerable climate impact which possibly compromises the energy savings when taking the total CO_2-balance into account (Daelman et al., 2013). Therefore, specific operational strategies for the above mentioned state-of-the-art processes to mitigate N_2O emissions are part of ongoing investigations (Leix et al., 2016).

An alternative approach is based on the recognition of N_2O as a potential regenerative energy source (Scherson et al., 2013). The thermal decomposition of N_2O in high performance gas engines, for instance, increases the energy yield of fuel combustion by the exothermal decomposition of N_2O and is already state-of-the art (Glarborg et al., 1994). Considering wastewater treatment, the ‘Coupled Aerobic-anoxic Nitrous Decomposition Operation’ (CANDO) is the first approach proposing the integration of N_2O as energy source into wastewater treatment schemes (Scherson et al. 2013). The process is based on intentional biological N_2O production from wastewater nitrogen compounds and subsequent energy recovery by combustion with biogas. Presently, the research focus lies on the application for municipal wastewater treatment, but the concept is also potentially applicable in the bioenergy sector in general, for instance, for the treatment of ammonia-rich digester residuals from agricultural biogas plants, as well as for manure or landfill leachates as industrial wastewater treatment.

However, so far only lab-scale experiences with synthetic feed stocks have been published, which are not directly transferable to engineering practice. Hence, further research is necessary to provide a basis for subsequent decision making on scale-up and process integration. In this respect, especially the general viability of real substrates and required operational strategies, but also potential process limitations related to upscaling this process are of interest. The document-in-hand addresses these questions and thereby, contributes to the further development of the CANDO process by extending the previous fundamental research to the development of implementation and control strategies as the next step in transitioning to full-scale implementation. For this purpose, process performance and stability as well as operational strategies to optimize N_2O yields under different treatment conditions were investigated in addition to N_2O harvesting technologies.

The presented results are supporting previous assessments of the CANDO process considering the potential for energy recovery, nitrogen removal and GHG emission mitigation in wastewater treatment. Focusing on real feed-stream conditions and process automation as well as N_2O extraction processes, the findings of this study serve as a comprehensive basis for future process developments for the potential development of the CANDO process and beyond this, present research gaps, which still need to be filled.

2. The role of nitrogen in the water-energy-climate nexus

To illustrate the potential impact and application potential of the CANDO process, the following section provides a detailed reflection on the present role of nitrogen and its applications as well as its emission pathways into the environment. For this purpose, the anthropogenic and natural nitrogen cycles are presented in relation to the present state and development of nitrogen emissions especially focusing on bioenergy generation in the wastewater and agricultural sectors. Subsequently, the potential benefits of intentional N_2O production and combustion will be discussed in relation to environmental protection by simultaneously closing the anthropogenic nitrogen cycle and recovering energy.

2.1 Current state and perspective of anthropogenic nitrogen emissions

Nitrogen is a central element of environmental systems. In terrestrial and aquatic systems as well as in the atmosphere, it plays a key role in mineral and biological cycles and is a basis of organic matter and life (Gruber and Galloway, 2008). In natural balance, nitrogen mass-flows between the different environmental spheres and respective sub-cycles are self-sustaining. The atmospheric inert nitrogen gas (N_2) serves as natural buffer which is activated by biological fixation and transformation into so-called reactive nitrogen (Galloway et al., 2008).

Since the invention of the Haber-Bosch process, anthropogenic nitrogen fixation and subsequent anthropogenic nitrogen emissions of reactive nitrogen have considerably accelerated these natural cycles leading to a considerable imbalance compared to the natural state (Erisman et al., 2008). In the 1990s, for instance, more than 160 tera-grams of N were released into the environment annually, which was more than the amount of biological nitrogen fixation on land (110 tera grams N/year) or by the oceans (140 tera-grams N/year) (Galloway et al., 2008).

In this respect, ammonium (NH_4^+), nitrite (NO_2^-), and NO_3^- as well as N_2O and nitric oxide (NO) are commonly emitted reactive nitrogen species in addition to organically fixed nitrogen. Considering their environmental impacts, the dissolved forms, i.e., NH_4^+, NO_2^- and NO_3^-, are responsible for the eutrophication of water bodies ultimately leading to the depletion of oxygen and potentially fish kill as well as the deterioration of drinking water quality (Vollenweider, 1971).

Anthropogenic gaseous N_2O emissions into the atmosphere are triggering climate change. Presently, anthropogenic emissions account for a third of the total N_2O flux into the atmosphere (Gruber and Galloway, 2008). From the beginning of the industrialization in the late 19th century to the present, the atmospheric concentration of N_2O has significantly increased due to anthropogenic activity. Intensive measurement campaigns have shown, that the present level of 330 ppb has been unprecedented at least during the last 800,000,000 years (IPCC, 2013). Although the atmospheric concentration and the quantity of anthropogenic N_2O emissions is lower by several orders of magnitude (e.g. 0.14 Mt_{N_2O}/a in Germany in 2016) compared to

CO_2 (796 Mt_{CO_2}/a), its climate impact is considerably higher (UBA, 2017b). Its relatively high CO_2-equivalent of 300 leads to the present contribution of approximately 6% to the global GHG index (Ravishankara et al., 2009). Despite considerable mitigation measures in the industry, N_2O concentrations are still increasing (Bernstein, 2008) due to their atmospheric resilience of approximately 124 years (compared to CH_4 of only 12.4 years) (IPCC, 2013). Additionally, N_2O catalyzes the destruction of the stratospheric ozone layer and is suspected to be the main contributor to stratospheric ozone depletion of the last century (Ravishankara et al., 2009).

Despite these detrimental effects, technical nitrogen fixation will likely continue to meet the present and future demands for e.g., food, energy crops and industrial products. Hence, more efficient measures or alternative strategies are necessary to close the urban nitrogen cycle. In this respect, more efficient end-of-pipe treatment targeting the complete conversion of reactive nitrogen into N_2 or recycling within the anthropogenic nitrogen cycle by value-added material chains are viable options.

2.2 Present framework and challenges concerning nitrogen removal and energy recovery from organic matter

For the organic fraction of municipal solid waste (OFMSW) and wastewater, which are rich in NH_4^+ and fixed nitrogen species, typically end-of-pipe treatment is targeted, i.e., mineralization to N_2. In conventional approaches, mineralization is intended by the biological formation of N_2. However, the presently increasing demand for regenerative energy has spurred interest in the energetic valorization of the potential chemical energy contained in waste and wastewater compounds. Considering an average municipal wastewater, for instance, the chemical energy potential of organic compounds is estimated to 1.93 kWh/m³ (McCarty et al., 2011). For energy recovery, the biogas production from OFSMW and wastewater constituents is well established.

Similar to the wastewater treatment sector, the installed biogas production capacity of the agricultural bio energy sector has continuously increased over the last decades as part of the energy revolution. Energetic valorization of organic compounds in waste, wastewater and energy crops is typically based on the anaerobic digestion process. It combines biological conversion of organic substrates into biogas and subsequent electricity and heat generation by combustion (Wellinger et al., 2013). The technology potentially facilitates sustainable electrical base load generation, next to on-demand energy generation, and simultaneous removal of organic carbon from OFSMW and wastewater by the conversion to CO_2 and CH_4. It is hence, a promising technology for urban waste and wastewater treatment and sustainable energy generation (McCarty et al., 2011). In Germany, for instance, approximately 9,000 biogas plants have produced an estimated 32,4 TWh of electrical power in 2016 (UBA, 2017a). In the development toward continuous base-load energy supply, different approaches concerning its application and integration into the electricity grid are presently investigated. For instance, virtual power plants (Saboori et al., 2011) by integration of different biogas plants next to on-site energy recovery and utilization from e.g., industrial and municipal wastes and wastewaters are considered. However, an inherent problem concerns nitrogen loads in organic substrates, as these cannot be removed by anaerobic digestion and therefore, require post-treatment.

Depending on substrate specific and national legislation, the fate of these NH_4^+ loads along with nitrogen emission pathways varies. In contrast to wastewater or waste treatment, the organic substrate quality of agricultural and composting biogas plants, for instance, typically allows for the distribution of digestates as farm land fertilizers.

The present perspective, however, is changing. Due to intensive fertilizer application, considerable groundwater nitrate concentrations (Figure 1) are presently an urgent concern for drinking water quality in Germany. Approximately 28% of German groundwater bodies, for instance, exhibit a bad chemical status due to NO_3^- concentrations greater than 50 mg NO_3-N/L (Keppner et al., 2016). To initiate a trend reversal, German fertilizer regulations have, hence, been tightened in June 2017 (DüMV) and are now limiting fertilizer nitrogen loads, including digestates, to area specific restraints of 50 kg/(ha*a) on a three-year average. Accordingly, the integration of post-treatment measures will likely need to be considered, also in the bioenergy sector.

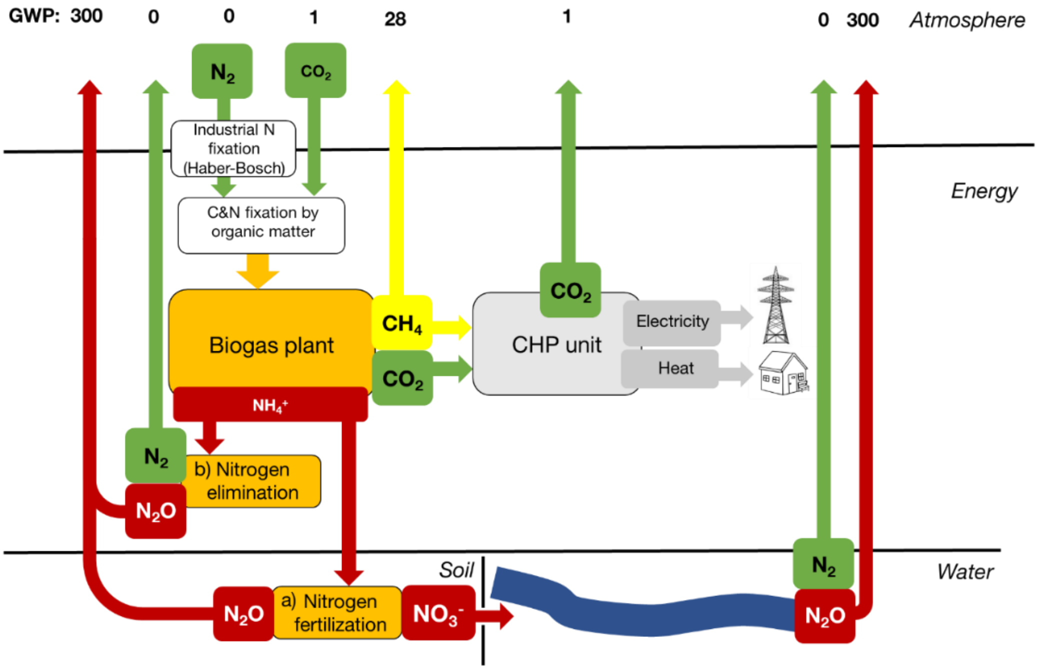


Figure 1. Schematic illustration of the intersections between the anthropogenic and the natural nitrogen cycle focusing on bio-energy generation in a) the agricultural, and b) the wastewater and waste treatment sectors. The global warming potentials (GWP) of each gas is expressed in CO_2-equivalents.

However, in addition to infiltration into groundwater bodies, NH_4^+ is also emitted into the atmosphere after microbial conversion to N_2O or NO or as direct emissions of NH_3. The total quantity of gaseous emissions as well the specific share of each compound is strongly depending on the environmental conditions e.g., soil pH, oxidation reduction regime, but is considered to contribute considerable to global climate change (Mørkved et al., 2007; Wrage et al., 2001). Regarding the GHG emissions from the German agricultural sector, for instance, N_2O contributes approximately 32% to the total CO_2-footprint (UBA, 2017b).

Considering the wastewater and bio waste sector, a different framework needs to be considered than for agricultural biogas production. The application of digestates as fertilizer is typically prohibited due to the risk of hazardous substances e.g., heavy metals (DüMV). Hence, the digestates must not be used as fertilizers in future applications according to present legislation (DüMV) and typically undergo on-site dewatering and subsequent thermal treatment. The ammonia rich reject water from the dewatering process is typically treated on-site to meet the water quality demand of receiving water bodies. Presently, end-of-pipe treatment i.e., technical conversion of NH_4^+ loads into N_2, is the most applied removal strategy. In this respect, either main-stream treatment or side-stream treatment trains are applied. The conventional technology, especially in main-stream treatment, is the biological nitrification/denitrification process. Like in the biological metabolism in soils, N_2O is an inherent intermediate metabolite and is emitted, especially in aerated zones of biological wastewater treatment trains (Kampschreur et al., 2009). The N_2O emission factor ($N_2O\text{-}N_{emitted}/N_{removed}$), however, is highly site-specific. According to a literature study (Weißbach et al., 2015), N_2O emissions from full-scale treatment plants vary from negligible amounts to 6%. On average, about 1.4% of the eliminated nitrogen are emitted as N_2O. Despite this low figure, N_2O emissions can account for up to 80% of the carbon footprint of a wastewater treatment plant (Daelman et al., 2013). Compared to other industries, the wastewater treatment sector contributes approximately 2.8% of all N_2O emissions (IPCC, 2013). The value is comparably low, but considering its adverse impacts, N_2O emissions must be mitigated at every possible level.

In addition to N_2O emissions, nitrification/denitrification bears another disadvantage, which is rooted in the heterotrophic nature of the denitrification stage. The process requires a considerable amount of organic reducing equivalents (measured as the chemical oxygen demand (COD)), which is also the typical resource used for biogas production. For reliable nitrogen elimination approximately 7.6 kg_{COD}/kg_N are necessary (Sobieszuk and Szewczyk, 2006). Hence, considering a complete organic mass-balance for an anaerobic wastewater treatment plant, the energy recovery potential of organic matter is limited by the necessary denitrification capacity. To overcome this issue, presently alternative nitrogen removal processes are being investigated to decouple organic carbon demand and nitrogen elimination.

Accordingly, novel or alternative biological processes as for instance, partial nitritation/deammonification (PN/D) (Joss et al., 2009) or nitritation/denitritation (N/DN) (Wang et al., 2016) have been developed, and successfully penetrated the market. By applying short-cuts in the microbial metabolism, these processes considerably decrease the organic carbon demand or even facilitate complete autotrophic operation. Accordingly, the oxygen demand can be reduced by up to 60% compared to conventional nitrification (Horstmeyer et al., 2018). However, the emission factor of

N_2O is suspected to increase to approximately 3% (Leix et al., 2016) and is hence, considerably higher than in conventional treatment. In this respect, it is noteworthy, that an increase of 1% of the emission factor potentially increases the carbon footprint of a wastewater treatment plant by 30% (Haas and Hartley, 2004).

In addition to biological treatment, physico-chemical nitrogen removal technologies have been investigated comprising value-added material chains. Recycling by e.g., the production of high quality mineral fertilizer (magnesium-ammonium-phosphate (MAP)) or ammonia stripping from digestates are being investigated in this respect. However, presently, especially stripping is not considered economically feasible (Bonmatı and Flotats, 2003) and MAP is presently not accepted on the market.

Hence, it is evident that further research is required to develop efficient strategies for nitrogen removal and simultaneous N_2O emission control for environmental protection in biological systems. From an economic perspective, especially from the agricultural sector, lower carbon and oxygen demand are also desired to maintain competitive energy prices on the energy market in potential future post-treatment requirements. This will be possibly endangered by the necessity of post-processing units for nitrogen removal. However, sustainable development is of utmost importance.

In this respect, the energetic valorization of nitrogen coupled to nitrogen removal (Figure 2) is a potential win-win situation contributing to solving these currently emerging challenges. A promising approach is the intentional biological production of N_2O and on-site energetic utilization in combustion with biogas. Analogous to biomethane combustion yielding CO_2, the combustion of N_2O produces CO_2-neutral N_2.

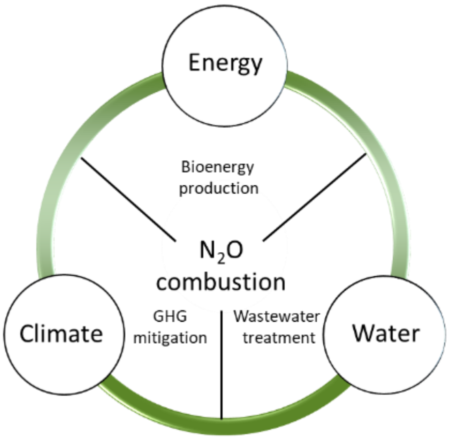


Figure 2. Integration of N_2O combustion into the water-climate-energy nexus serving sustainable energy generation, wastewater treatment and mitigation of GHG emissions.

Additionally, in contrast to oxygen, N_2O decays exothermally, when used as oxidation agent and hence, potentially delivers 37% more energy Eq. (1) compared to oxygen in biomethane combustion (Scherson et al., 2013) Eq. (1):

$$CH_4 + 4\,\mathbf{N_2O} \rightarrow CO_2 + 2\,H_2O + 4\,\mathbf{N_2}, \qquad \Delta H^{\circ}_R = \mathbf{-1{,}219}\ \text{kJ mol}^{-1} \tag{1}$$

$$CH_4 + 2\,O_2 \rightarrow CO_2 + 2\,H_2O, \qquad \Delta H^{\circ}_R = \mathbf{-890}\ \text{kJ mol}^{-1} \tag{2}$$

The final combustion products are N_2 and CO_2. Therefore, the integration of N_2O production and combustion in addition to biomethane utilization is a potential solution toward a more sustainable energy generation from organic matter and nitrogen.

However, a prerequisite for energy recovery from N_2O, is an efficient N_2O production and process integration by biological or chemical processes. As part of this research, the biological N_2O formation pathways and potentials are discussed in the following chapter.

3. Biological N_2O production

3.1 N_2O formation pathways in biological systems

From a biochemical perspective, the microbial nitrogen metabolism is characterized by oxidative and reductive metabolic patterns whose directions strongly depend on the predominant oxidation reduction regime. The complete oxidation pattern ranges from NH_4^+ (oxidation number -III) to NO_3^- (oxidation number +V). In both, aerobic (oxidative) as well as anoxic (reductive) metabolic chains, N_2O is produced as a direct metabolite, but also occurs as a chemical by-product of different biological precursors.

Under aerobic conditions (oxidative regime), when excess oxygen is supplied, the nitrification process i.e., the complete oxidation from NH_4^+ to NO_3^- is the predominant pathway. Complete nitrification can be performed by single organisms e.g., *Nitrospira* (van Kessel et al., 2015). However typically, the oxidation of NH_4^+ to NO_2^- (Nitritation (3)) and subsequent oxidation of NO_2^- to NO_3^- (Nitratation Eq. (4)) are distinct reactions conducted by different specialized microbial guilds i.e., autotrophic ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), respectively. Stoichiometrically, the exergonic catabolic nitrification pathway can be expressed as follows (Schmidt et al., 2003):

Nitritation: $NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O,$ $\left(\Delta G^o = -409 \frac{kJ}{mol}\right)$ (3)

Nitratation: $NO_2^- + 0.5\ O_2 \rightarrow NO_3^-,$ $\left(\Delta G^o = -74 \frac{kJ}{mol}\right)$ (4)

Nitrification: $NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O,$ $\left(\Delta G^o = -483 \frac{kJ}{mol}\right))$ (5)

Nitritation is conducted by the oxidation of NH_4^+ via the intermediate metabolite hydroxylamine (NH_2OH). Firstly, NH_4^+ is oxidized by ammonium mono oxidoreductase (*AMO*) to NH_2OH (Wrage et al., 2001) Eq. (6). According to the present model, hydroxylamine oxidoreductase (*HAO)* further oxidizes NH_2OH to NO (Hooper and Terry, 1979) Eq. (7) or NO_2^- (Schreiber et al., 2012) Eq. (8). In the case of NO, further biological reduction typically yields N_2O Eq. (9). A potentially responsible nitric oxide reductase and further intermediates, however, are still unknown (Cabail et al., 2005; Fernández et al., 2008). Stoichiometrically, the particular reactions can be expressed as follows:

$$NH_4^+ + O_2 + H^+ + 2\ e^- \ \xrightarrow{AMO} \ NH_2OH + H_2O \qquad (6)$$

$$NH_2OH \ \xrightarrow{HAO} \ HNO + 2\ H^+ + 2\ e^- \qquad (7)$$

$$NH_2OH + H_2O \ \xrightarrow{HAO} \ NO_2^- + 5\ H^+ + 4\ e^- \qquad (8)$$

$$NO + H^+ + e^- \ \xrightarrow{?} \ 0.5\ \mathbf{N_2O} + 0.5\ H_2O \qquad (9)$$

Next to the biological pathway of N_2O generation, NH_2OH is a precursor for the chemical disproportionation (Alluisetti et al., 2004) Eq. (10) and oxidation of NH_2OH with oxygen (Anderson, 1964) Eq. (11) as well as nitrous acid (Harper et al., 2015; Terada et al., 2017) Eq. (12) under aerobic conditions:

$$NH_2OH \ \rightarrow \ 0.25\ \mathbf{N_2O} + 0.5\ NH_3 + 0.75\ H_2O \qquad (10)$$

$$NH_2OH + O_2 \ \rightarrow \ 0.5\ \mathbf{N_2O} + 1.5\ H_2O \qquad (11)$$

$$NH_2OH + HNO_2 \ \rightarrow \ \mathbf{N_2O} + 2\ H_2O \qquad (12)$$

Considering thermodynamic properties, the comproportionation between hydroxylamine and nitrous acid Eq. (12) is favored in comparison to hydroxylamine oxidation Eq. (11) (Harper et al., 2015). Additional findings even reported, that it potentially becomes the dominant pathway in aerobic biological systems (Terada et al., 2017) especially under slightly acidic conditions due to the relatively higher availability of free nitrous acid.

A reductive pathway, which occurs under suboxic conditions, where dissolved oxygen is limited, involves the nitrifier-encoded copper containing NO_2^- reductase (*NirK*) and a cytochrome (type-c) based nitric oxide reductase (*cNor*). These enzymes reduce NO_2^- to NO and subsequently N_2O in the aerobic nitrous denitritation pathway (Weißbach et al., 2017; Wrage et al., 2001):

$$NO_2^- + 2\ H^+ + e^- \ \xrightarrow{NirK} \ NO + H_2O \qquad (13)$$

$$NO + H^+ + e^- \ \xrightarrow{cNor} \ 0.5\ \mathbf{N_2O} + 0.5\ H_2O \qquad (14)$$

A few studies reported further N_2O reduction and N_2 formation based on AOB activity under aerobic conditions (aerobic denitrousation), but so far, no enzymatic mechanism has been determined in this respect (Schmidt, 2009).

For both pathways, oxidation of NH_2OH to NO Eq. (7) and NO reduction Eq. (14), oxygen is required for the activation of ammonia (NH_3) by *AMO* to NH_2OH. The latter serves as substrate for *HAO* or electron donor for NO reduction to N_2O, why both pathways can be considered as autotrophic (Schreiber et al., 2012).

In contrast, a second nitrous denitritation pathway is heterotrophic. Instead of oxidizing NH_3, organic reducing equivalents are used during AOB activity (Schmidt, 2009) and the activation of *NirK* and *cNor*. Similar pathways have been also observed for ammonia oxidizing archaea (Santoro et al., 2011).

Under anoxic processing conditions, the dissimilatory heterotrophic reduction of nitrogen compounds is part of the catabolic branch of the microbial metabolism (Schreiber et al., 2012). The denitrification refers to the complete reduction pattern from NO_3^- to N_2 and can be typically conducted by single organisms i.e., heterotrophic denitrifiers (Al-Mamun and Baawain, 2015):

Denitrification: $NO_3^- + 5\ H^+ + 5\ e^- \rightarrow 0.5\ N_2 + 2\ H_2O + OH^-$ (15)

The pattern contains several inherent metabolites in the following order: NO_2^-, NO and N_2O. The reduction is conducted involving different respiratory reductases. The first step involves nitrate reductase (*NAR*), Eq. (16), followed by NO_2^- reduction by either *NirK* (also encoded in nitrifers) or *NirS*, Eq. (17). NO is then further reduced by quinol based (*qNor*) or cytochrome (*cNor*) based reductases to N_2O Eq. (18). Eventually, the copper based nitrous oxide reductase (*NosZ*) reduces N_2O to N_2 (19):

$$NO_3^- + 2\ H^+ + 2\ e^-\ \xrightarrow{NAR}\ NO_2^- + H_2O \quad (16)$$

$$NO_2^- + 2\ H^+ + e^-\ \xrightarrow{NirK/NirS}\ NO + H_2O \quad (17)$$

$$NO + H^+ + e^-\ \xrightarrow{qNor/cNor}\ 0.5\ \mathbf{N_2O} + 0.5\ H_2O \quad (18)$$

$$0.5\ \mathbf{N_2O} + H^+ + e^-\ \xrightarrow{NosZ}\ 0.5\ N_2 + 0.5\ H_2O \quad (19)$$

Considering the reduction to N_2, *Nos* activity is the only known N_2O sink in the microbial metabolism (Schreiber et al., 2012). However, the abundance of dissolved oxygen down to concentrations of 0.09 mg/l were found to significantly inhibit N_2O reduction (Oh and Silverstein, 1999). In the case of subsequent oxygen depletion, N_2O has been found to be reduced which indicated the recovery potential of *NosZ* after oxygen inhibition (Morley et al., 2008).

Within biological nutrient removal, oxidation and reduction patterns potentially occur simultaneously. Concentration differences e.g., in granular activated sludge systems or in transition areas from aerobic to anoxic zones, facilitate simultaneous reduction and oxidation. As a result, the quantification of the particular pathways in a nitrogen mass-balance is challenging and requires further research, especially concerning mechanistic understanding of biological N_2O emissions. A summary of these potentially occurring reactions and interfaces between aerobic and anoxic conditions is given in Weißbach et al. (2017) and supported with a clear terminology to precisely differentiate processes from each other (Figure 3).

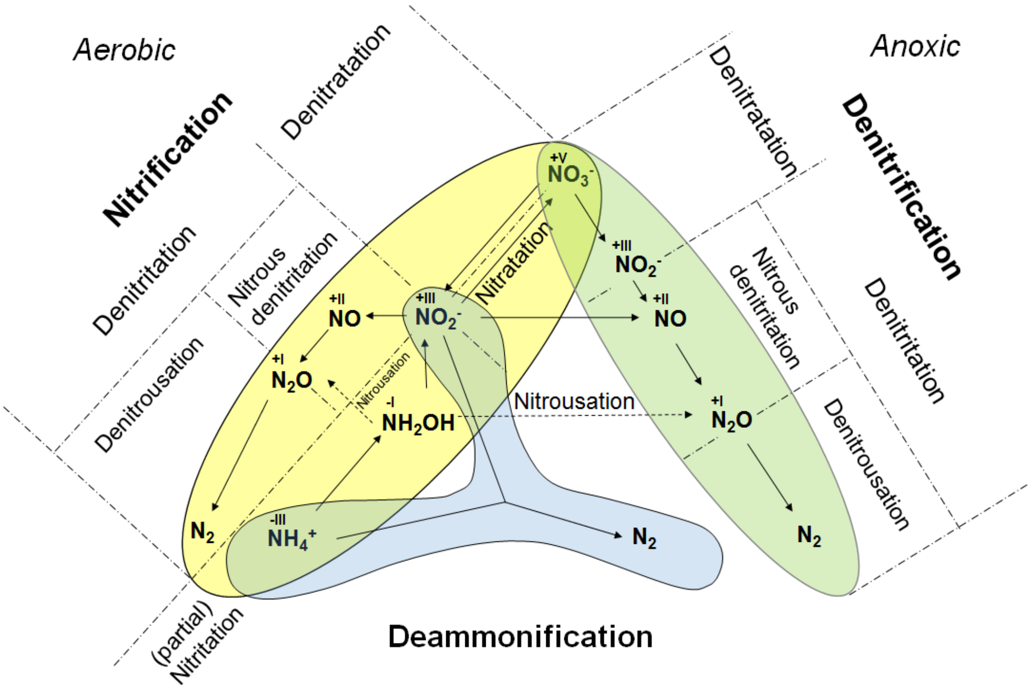


Figure 3. Metabolic pathways potentially occurring in biological nitrogen removal processes (Weißbach et al., 2017)

3.2 Potential processes for intentional microbial N_2O production

Depending on the environmental or technically applied process conditions, the metabolic pathways and respective N_2O yields considerably differ and potentially interfere. Previous studies have assessed N_2O emissions depending on the nitrogen mass-balances of full-scale wastewater treatment plants applying conventional nitrification/denitrification. The results range from negligible amounts (Czepiel et al., 1995) up to 6% (Desloover et al., 2012) in relation to the nitrogen removed. In a literature study by the author, the average emission factor of lab-scale investigations with mixed-culture communities targeting N_2O emissions was assessed to account for approximately 3.3% on average (Weißbach et al., 2015).

Typical factors promoting N_2O production have been reported. Under aerobic conditions, hydroxylamine related pathways are suspected to be dominant (Terada et al., 2017). In this respect, low pH values (Mørkved et al., 2007), high NH_4^+ concentrations (Schreiber et al., 2012) as well as low dissolved oxygen concentrations (Kampschreur et al., 2009; Schreiber et al., 2012) evidently trigger higher N_2O emissions from nitrification systems. Additionally, low COD/N ratios and high NO_2 concentrations have been related to increased emissions, especially in nitrification/denitrification systems (Itokawa et al., 2001; Law et al., 2012).

In novel treatment technologies for biological nitrogen removal e.g., partial nitritation/deammonification or nitritation/denitritation, NO_2^- is the typical precursor for subsequent denitrifying steps. For this purpose, low dissolved oxygen concentrations and low hydraulic retention times are applied in addition to mesophilic temperature conditions to promote AOB growth (Laureni et al., 2016) and ammonia oxidation.

Under these sub-oxic operational conditions, aerobic nitrous denitritation is triggered in addition to N_2O emissions via NH_2OH oxidation. Different studies have assessed the emissions from nitritation processes in full-scale deammonification plants to approximately 3% (Kampschreur et al., 2009; Kampschreur et al., 2008; Leix et al., 2016). Under lab-scale conditions, at pH values below 6.2, up to 54% could be observed. However, the conditions were not sustainable in relation to microbial activity (Mørkved et al., 2007) and hence, cannot be considered for continuous intentional N_2O production.

Under non-limiting denitrifying conditions, N_2O typically plays a minor role, if easily degradable matter is provided and conditions are strictly anoxic. As the rate constant of *NosZ* is considerably higher than e.g., *NirK*, the bottleneck is rather NO_2^- and NO_3^- reduction (Schreiber et al., 2012). However, it can accumulate in the mixed liquor because of its high solubility (1.3 g/L) (Weiss and Price, 1980) and its comparably low Henry constant ($2.4 \cdot 10^{-2}$ mol/atm) (Sander, 1999) under process limiting conditions e.g., low COD/N ratios or supply of hardly degradable organic substrates (Itokawa et al., 2001). In full-scale monitoring studies, off-gas measurements in transient anoxic/aerobic zones often serve an approximation of N_2O emissions, which are related to anoxic production pathways, but due to N_2O production from nitrification activity the results are usually biased (Kampschreur et al., 2009). Additionally, the sensitivity of the dissimilatory nitrous oxide reductase (NosZ) in these transient conditions has an effect on the N_2O yield (Cavigelli and Robertson, 2000). Hence, further research is necessary to quantify the particular effects of each pathway in relation to N_2O production in conventional treatment.

Considerable N_2O production rates occur under alternating anaerobic/anoxic process conditions. Under these conditions, organo-heterotrophic microorganisms which are capable of storing organic carbon intracellularly are selected for (Scherson et al., 2014). The microorganisms build up different endogenous polyhydroxyalcanoates e.g., polyhydroxybutyrate (PHB) or polyhydroxyvalerate, via the consumption of glycogen or polyphosphate in famine phases (Zeng et al., 2003). N_2O production can be observed especially simultaneously to the degradation of these storage compounds, when extracellular organic substrates are depleted. The mechanism is possibly rooted in different metabolic rate constants of endogenous and extracellular substrate respiration and hence, the necessary supply of reducing equivalents. In comparison to denitrification, this potentially leads to an accumulation of the intermediate N_2O (Liu et al., 2015) resulting in a pathway termed anoxic nitrous denitritation (Weißbach et al., 2017).

The comparison of all processes yields a semi-quantitative assessment of processes that could be applied for biological N_2O production according to the present state of knowledge (Figure 3).

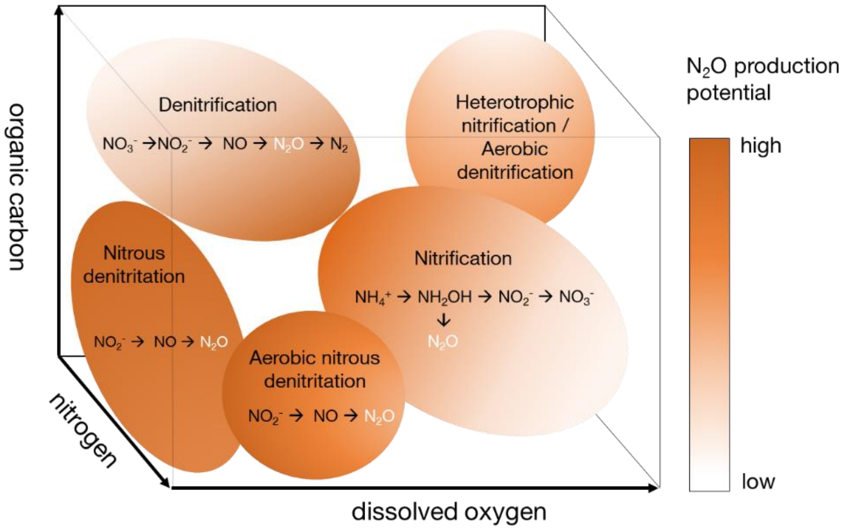


Figure 3. Overview of potentially applicable microbial pathways for N_2O production. The pathways are distinguished by the dissolved oxygen concentration, available organic carbon and nitrogen. Additionally, the particular N_2O production potentials are indicated (adapted from Weißbach et al. (2015)).

It is evident that N_2O generation strongly depends on the oxidation reduction regime (ORR) and the availability of organic carbon or the COD/N ratio, respectively. Generally, alternating ORR and sub-oxic conditions trigger N_2O generation in oxidative reaction chains, while N_2O production under anoxic conditions is dominated by the COD/N ratio. Considering turn-over rates, the anoxic nitrous denitritation pathway is the most promising. Previous studies have indicated conversion ratios of over 70% in relation to NO_3^- (Zeng et al., 2003) and NO_2^- (Scherson et al., 2014) in continuously operated systems providing readily degradable synthetic substrates.

Additional means to potentially optimize N_2O production under anoxic conditions by the inhibition of NosZ have also been previously investigated. Different Nos genes in pure culture experiments, for instance, showed positive (Otte et al., 1996), but also negative (Berks et al., 1993) results regarding the oxygen sensitivity of Nos genes. Considering intentional aerobic N_2O production, a selection toward the abundance of sensitive Nos is promising, but has not been assessed yet. Additionally, low pH values decrease Nos assembly and reaction rates compared to NAR, Nir and Nor (Bergaust et al., 2010; Richardson et al., 2009). As relevant co-factors for the inhibition of Nos, nitrous acid (Zhou et al., 2008), NO (Frunzke and Zumft, 1986) and hydrogen sulfide (Sørensen et al., 1980) as well copper limitation (Granger and Ward, 2003) have also been described.

3.3 The Coupled Aerobic-anoxic Nitrous Decomposition Operation

Presently, the most promising technology for intentional microbial N_2O production, is the Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO) proposed by Scherson et al. (2013) (Figure 5). The process was originally developed for the treatment of ammonia-rich digester liquor produced by anaerobic stabilization at wastewater treatment plants (WWTPs). Its design facilitates energy recovery via intentional generation of N_2O implying tight N_2O emission control and lower carbon and aeration energy demand than necessary for denitritation or denitrification. Additionally, its potential for phosphorous removal has recently been demonstrated (Gao et al., 2017).

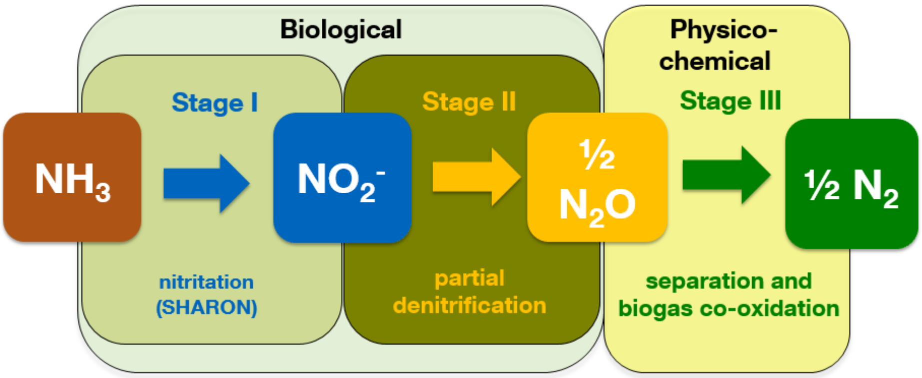


Figure 4. The Concept of CANDO – biological transformation of NH_3 to N_2O in biological stages I-II and physico-chemical treatment of N_2O in stage II for energy recovery

The process combines nitritation (NH_4^+ oxidation to NO_2^-, Eq. (3)) with subsequent anoxic nitrous denitritation (N_2O Eq. (13), Eq. (14)) at low COD/N ratios of approximately three. In contrast to the nitrification/denitrification, oxygen and carbon demand are severely lower. Considering stoichiometries, the oxygen demand can be reduced by 33%, while the demand for readily degradable organic substrates can be reduced by approximately 60% (Scherson et al., 2014; Sobieszuk and Szewczyk, 2006). In an additional step, N_2O is harvested and subsequently combusted with biogas, which enables carbon footprint mitigation, energy recovery and nitrogen removal simultaneously.

Theoretically, 37% more energy can be generated applying the necessary conditions during combustion given in Eq. (1). Additionally, the lower carbon demand increases the energy recovery potential for anaerobic treatment and biogas production. According to a recent study, the energy recovery potential for conventional wastewater can be increased by approximately 0.22 kWh/m³ taking N_2O production and carbon savings into account (Horstmeyer et al., 2018).

Considering operational experience, previous to this study, the biological stages of the CANDO were investigated in two lab-scale setups in long-term operation (Gao et al., 2017; Scherson et al., 2014). The first stage was realized applying the Single High-rate Ammonia Removal Over Nitrite (SHARON) process (Hellinga et al., 1998). In the second stage, N_2O was biologically produced applying the anoxic nitrous denitritation pathway. The pathway was triggered by an alternating operation. In sequencing-batch-operation, the process was primed by an anaerobic feast phase followed by anoxic processing conditions for N_2O production.

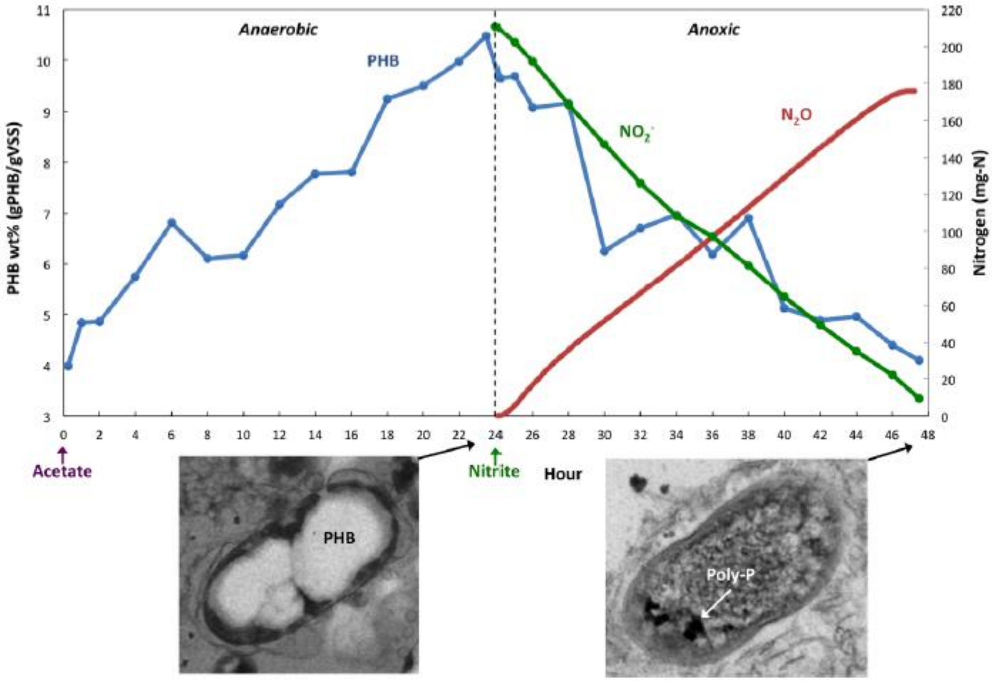


Figure 5. Typical process dynamics as observed for the application of a readily degradable substrate (i.e. acetate) as organic reducing equivalent. (Scherson et al., 2013)

Optimal processing conditions were described for an SBR operation. The published operations (Gao et al., 2017; Scherson et al., 2013) comprised an initial COD pulse in the anaerobic reaction. At the end of the anaerobic reaction phases, extracellular COD has been consumed at steady-state conditions. The anaerobic phase was followed by an anoxic reaction period. At the beginning of this reaction phase acetate (Gao et al., 2017; Scherson et al., 2013) or propionate (Gao et al., 2017) was pulse fed into the reactor. As a result, PHB was produced and extracellular carbon consumed at the end of the anaerobic reaction phase. In the subsequent anoxic reaction phase, NO_2^- was pulsed and metabolized to N_2O by endogenous substrate respiration (Scherson et al., 2013).

Considering process performance, according to the first long-term study (Scherson et al., 2014), an average conversion ratio of over 70% of NO_2-N to N_2O-N can potentially be generated at feed concentrations up to 300 $mg_{NO_2\text{-}N}/L$ per cycle. Additionally, the investigation of process dynamics within single cycles underlined the necessity of an endogenous substrate respiration to obtain high N_2O yields. A following study (Gao et al., 2017) focused on the P-removal potential and achieved phosphorous removal efficiencies of 40.0% and 76.1% providing propionate and acetate, respectively, which illustrates the influence of the composition of organic carbon supplied. Similarly, the N_2O yields were lower (68%) applying propionate compared to acetate (74%).

For the application and feasibility under realistic operational conditions, however, numerous knowledge gaps remain. For instance, the application of synthetic feeds provides the basis for detailed mechanistic studies, but acetate (Scherson et al., 2014) or propionate (Gao et al., 2017) will most likely be not viable in technical application. The obtained process rates and kinetics, however, these substrates serve well for a benchmark considering performance and N_2O yields. To estimate the application potential under realistic processing conditions, however, further research is necessary under real-feed stream conditions as applied in this work. Especially, the composition of organic matter in real wastewater is of concern. Typically, the organic compounds require additional reaction time for intracellular storage as they are not completely readily degradable. The degradation and storage of more complex organic matter under anaerobic conditions has been demonstrated before (Henze and Mladenovski, 1991; Jabari et al., 2016). However, the impact on the intentional N_2O production has not been assessed.

Considering N_2O removal, different strategies and removal technologies have been assessed under lab-scale conditions. Continuous stripping with helium was applied (Scherson et al., 2014), which cannot be considered viable regarding operational expenses in full-scale application and the intended combustion considering off-gas composition. In another study (Gao et al., 2017), stripping toward the end of a batch was conducted for selected cycles with ambient air which could be a viable approach. However, the applied experimental conditions and SBR operation of both studies cannot be compared to each other. Hence, it could not be derived, whether or not the applied stripping strategy (continuous vs. dedicated removal phase) had an impact on the N_2O yield.

Based on the previous findings, this work focuses on operational issues related to process control, stability, feasibility and N_2O extraction methods under real feed-stream conditions to potentially enable scale-up and technology transfer in the future as the main objective.

4. Executive summary

The conducted research was part of the project “Enabling Energy Savings and Recovery in Contemporary Wastewater Treatment Facilities through Photoacoustic-Based N_2O Monitoring and Control Strategies” (PANOWA) carried out at the Chair of Urban Water Systems Engineering (CUWSE) at the Technical University of Munich (TUM) in collaboration with the Chair of Analytical Chemistry (CAC) at TUM, the Criddle Group at Stanford University (Palo Alto, CA, USA), and Eberhard Morgenroth and Adriano Joss at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) (Zurich, CH). The project was funded by the International Graduate School of Science and Engineering at TUM.

The project started in October 2013 and preliminary work consisted of the assembly and optimization of the experimental setup (**Paper #5**, in preparation), a literature study (**Paper #1**) and a first international research visit at the Stanford University (Figure 6). In addition to the literature review, four experimental objectives were proposed focusing on bioprocess optimization and N_2O mass transfer investigations. The objectives and related hypotheses were composed in a research proposal as a basis for the conducted research and briefly summarized as follows:

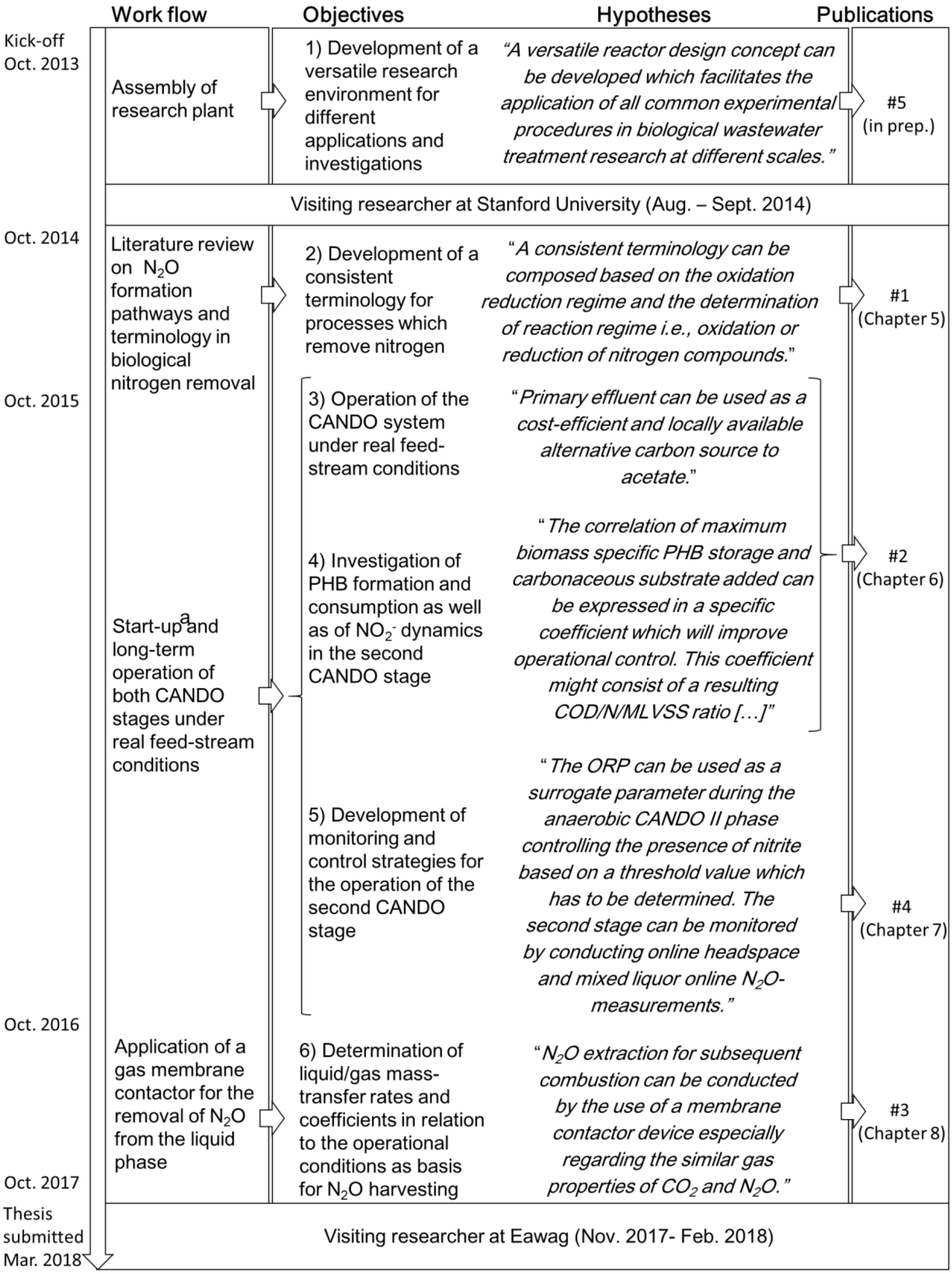
Objective 1 – Design and construction of a versatile laboratory-scale plant for experimental studies on biological wastewater treatment processes

Prerequisite for the experimental work was the design and construction of an automated lab-scale reactor system. For this purpose, a design concept was developed to providing flexibility and scalability to an extend which enables lab-scale investigations and subsequent scale-up without constraining the system to the application of the CANDO process, but for most presently relevant investigations involved in biological wastewater treatment research. Hence, the following hypothesis was formulated:

> *“A versatile reactor design concept can be developed which facilitates the application of all common experimental procedures in biological wastewater treatment research at different scales.”*

This resulting system was successfully applied for monitoring and recording of all relevant process parameters as well as the operation of the CANDO process. Basis for this work was a fruitful collaboration with collaborators at Eawag.

Preparation, assembly and trouble-shooting were finished in June 2015. A common publication (**Paper #5**) about the versatile concept is currently in preparation in collaboration with Dr. Adriano Joss (Eawag). The preparation of this paper was facilitated through the second international visit at Eawag in 2017/18 (Figure 6).



	Work flow	Objectives	Hypotheses	Publications
Kick-off Oct. 2013	Assembly of research plant	1) Development of a versatile research environment for different applications and investigations	*"A versatile reactor design concept can be developed which facilitates the application of all common experimental procedures in biological wastewater treatment research at different scales."*	#5 (in prep.)
	Visiting researcher at Stanford University (Aug. – Sept. 2014)			
Oct. 2014	Literature review on N_2O formation pathways and terminology in biological nitrogen removal	2) Development of a consistent terminology for processes which remove nitrogen	"*A consistent terminology can be composed based on the oxidation reduction regime and the determination of reaction regime i.e., oxidation or reduction of nitrogen compounds.*"	#1 (Chapter 5)
Oct. 2015	Start-up[a] and long-term operation of both CANDO stages under real feed-stream conditions	3) Operation of the CANDO system under real feed-stream conditions	"*Primary effluent can be used as a cost-efficient and locally available alternative carbon source to acetate.*"	#2 (Chapter 6)
		4) Investigation of PHB formation and consumption as well as of NO_2^- dynamics in the second CANDO stage	"*The correlation of maximum biomass specific PHB storage and carbonaceous substrate added can be expressed in a specific coefficient which will improve operational control. This coefficient might consist of a resulting COD/N/MLVSS ratio […]*"	
		5) Development of monitoring and control strategies for the operation of the second CANDO stage	"*The ORP can be used as a surrogate parameter during the anaerobic CANDO II phase controlling the presence of nitrite based on a threshold value which has to be determined. The second stage can be monitored by conducting online headspace and mixed liquor online N_2O-measurements.*"	#4 (Chapter 7)
Oct. 2016	Application of a gas membrane contactor for the removal of N_2O from the liquid phase	6) Determination of liquid/gas mass-transfer rates and coefficients in relation to the operational conditions as basis for N_2O harvesting	"*N_2O extraction for subsequent combustion can be conducted by the use of a membrane contactor device especially regarding the similar gas properties of CO_2 and N_2O.*"	#3 (Chapter 8)
Oct. 2017 Thesis submitted Mar. 2018	Visiting researcher at Eawag (Nov. 2017- Feb. 2018)			

Figure 6. Overview of objectives, conducted research and publications

Objective 2 – Development of a consistent terminology for processes that remove nitrogen

Throughout a preliminary literature study targeting the identification of applicable N_2O formation pathways, some inconsistencies in the applied terminology concerning reaction pathways was noticed. Accordingly, the following hypothesis was formulated:

> "*A consistent terminology can be composed based on the oxidation reduction regime and the determination of reaction regime i.e., oxidation or reduction of nitrogen compounds.*"

Thus, the objective was to conduct a literature review targeting a summary of presently used terminologies to identify ambiguities or inconsistencies and terms, which are outdated. The review was followed by the development of a logical and conservative method to enable the integration of novel terms into historically established terminology (**Paper #1**).

Objective 3 – Operation of the CANDO system under real feed-stream conditions

To support the transition of the CANDO process toward technical implementation, the system was operated using real-feed streams, i.e., digester reject water and primary effluent as substrates. Assuming that the bioavailability of organic carbon contained in primary effluent which was used in the second CANDO stage would not considerably deviate from denitrification, the following hypothesis was proposed:

> "*Primary effluent can be used as a cost-efficient and locally available alternative carbon source to acetate.*"

The respective experimental work started with the inoculation of the nitritation stage. After achieving stable operational conditions, the nitrous denitritation stage was inoculated. Preliminary results indicated that inoculation with conventional activated sludge facilitates a fast start-up. However, after two weeks, the system performance continuously decreased. Eventually, stable operation was achieved after initial operational difficulties and re-inoculations. Throughout final steady-state operation, process performance, stability and different N_2O removal strategies were successfully tested and confirmed the proposed hypothesis. Further details are given in chapter 6 (**Paper #2**).

As part of the related PhD program of the IGSSE, a first international research stay was part of the studies from August to September 2014. The exchange visit served exchange and preparation of a collaboration with the Criddle group who proposed the CANDO process in 2013 for the first time. As a result, the literature study (**Paper #1**) was extended and finalized in collaboration. About the details of this study, the reader is referred to chapter 5.

Objective 4 - Investigation of PHB formation and consumption as well as of NO_2^- dynamics in the second CANDO stage

The COD/N ratio was suspected to differ, considering the biodegradability of real wastewaters, and needed to be adjusted for stable operation under real feed-stream conditions. Additionally, a reliable design parameter for the determination of biomass concentration (MLVSS) specific carbon and nitrogen loads for technical applications and potential scale-up was lacking, but could be derived in addition to the COD/N ratio. Hence the following was hypothesized:

> *"The correlation of maximum biomass specific PHB storage and carbonaceous substrate added can be expressed in a specific coefficient which will improve operational control. This coefficient might consist of a resulting COD/N/MLVSS ratio that could be applied and substitute a given COD/N ratio for continuous operation in order to assure a full conversion of external carbon into intracellular PHB at the beginning of the anoxic period while also preventing N_2O degradation."*

Under real feed-stream conditions, a COD/N ratio of 4 enabled steady-state operation and investigations were conducted concerning the process dynamics of the second CANDO stage. Process rates as well as the relation between the consumption of PHB and N_2O could be confirmed, which elucidates the application potential of complex organic matter as a precursor for endogenous substrate production in the operation of the second CANDO stage. Additionally, a COD/N/MLVSS ratio was successfully determined facilitating the design of future systems and scale-up. The results are presented in Chapter 6 (**Paper #2**).

Objective 5 - Development of monitoring and control strategies for the operation of the second CANDO stage

Previously, ORP had been successfully applied in a study by Lackner et al. (2014) as control parameter for the deammonification process demonstrating that nitrite was depleted at an ORP below -200 mV. Accordingly, it was suspected that the ORP could be used for the second stage of the CANDO according to the following hypothesis:

> *"The ORP can be used as a surrogate parameter during the anaerobic CANDO II phase controlling the presence of nitrite based on a threshold value which has to be determined. The second stage can be monitored by conducting online headspace and mixed liquor online N_2O-measurements."*

Toward the end of the experimental phase, the ORP was investigated in continuous operation as operational variable for process control and automation. The hypothesis was positively tested and the ORP gradient was successfully applied in anaerobic as well as during anoxic processing conditions to monitor NO_2^- abundance. The results are presented in Chapter 7 (**Paper #4**).

Objective 6 - Determination of liquid/gas mass-transfer rates and coefficients in relation to the operational conditions as basis for N_2O harvesting

Initially, conventional gas stripping was intended for N_2O removal, but changed toward the application of a membrane contactor throughout the course of the investigations. Previous studies had successfully demonstrated the application of membrane contactors for CO_2 and CH_4 extraction and provided a good basis for the application of N_2O extraction in the CANDO process. The research was hence based on the following hypothesis:

> “*N_2O extraction for subsequent combustion can be conducted by the use of a membrane contactor device especially regarding the similar gas properties of CO_2 and N_2O.*”

Mass-transfer dynamics and coefficients were successfully determined. The results indicate application potential for the removal of N_2O at expected process conditions in the nitrous denitritation stage of a continuously operated CANDO system. Details on this study are given in chapter 8 (**Paper #3**).

In addition to the peer-reviewed publications presented in this thesis, the information and results were also shared at various national and international conferences as documented earlier.

5. A proposed nomenclature for biological processes that remove nitrogen

The development of more efficient processes for nitrogen removal resulted in inconsistent terminology. The inconsistency became evident throughout a preliminary literature research to this work concerning potential N_2O production pathways and trigger mechanisms to be applied for optimal processing conditions (Weißbach et al., 2015).

During the study, it was evident that biochemically or stoichiometrically distinct processes are termed the same way and vice versa. Hence, a novel and consistent terminology was successfully developed to clearly differentiate processes applied in nitrogen removal technology from each other. The terminology is based on the oxidation reduction regime and predominant reaction pathways and hypothesis 1:

> "*A consistent terminology can be comprised based on the oxidation reduction regime and the determination of reaction regime i.e., oxidation or reduction of nitrogen compounds.*"

was, hence, confirmed.

The results provided the basis for **Paper #1** and was applied in the subsequent publications (**Papers #2-4**).

A proposed nomenclature for biological processes that remove nitrogen

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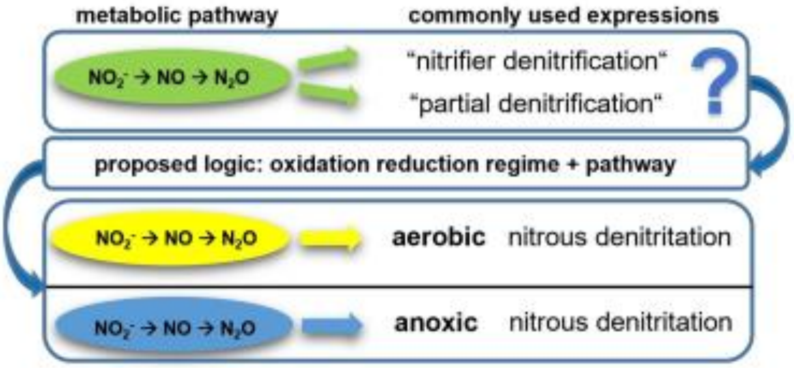


Abstract

For development and optimization of novel biological nutrient removal processes, factors affecting energy and greenhouse gas emissions (e.g. nitrous oxide) are increasingly important. Energy efficient pathways are exploited, such as short-cut processes for treatment of concentrated side streams. These pathways are usually well described, but a consistent nomenclature is still lacking: some functionally equivalent processes are referred to using distinct names, and some functionally distinct processes are referred to with the same name. To address this issue, we propose a standardized terminology, in which a root term designates the end product for oxidation pathways or the reactant for reduction pathways; a qualifier is used to designate the predominant oxidation-reduction regime (ORR) and an optional indicator is used to designate partial turnover rates, e.g. partial ammonia oxidation to nitrite.

1 Introduction

Nutrient removal is required to protect water quality and the environment and is thus a primary objective of wastewater treatment. The conventional activated sludge (CAS) process, an approach invented in 1914, has been optimized for removal of carbon (C), nitrogen (N), and phosphorus (P) (Grady Jr et al., 2011; Jenkins and Wanner, 2014). While these processes are effective, their energy requirements are high, a fact that has spurred interest in strategies that save energy (Englehardt et al., 2016; Gao et al., 2014) and enable resource recovery, avoid greenhouse gas emissions, and decrease operational costs. Wastewater treatment is at a turning point where nutrient removal will be achieved through more sustainable and integrated systems aiming at resource and energy recovery (Grady Jr et al., 2011).

Removal of organic carbon and production of methane by anaerobic digestion of organic waste streams are well established for energy recovery at municipal wastewater treatment plants. Typically, these waste streams contain high levels of nitrogen, and a consequence is the release of high concentrations of ammonia in the digester's effluents and in the reject water from sludge dewatering, respectively. Returning these concentrated streams to the headworks imposes an additional nitrogen load on the mainstream and can potentially drive the CAS to its limits, in which case there is insufficient reducing power for denitrification. To overcome these issues, novel biological nitrogen removal (BNR) processes have been developed in the last decade. These include processes like *Stable High Ammonia Removal Over Nitrite* (SHARON®) (Galí et al., 2006), *Complete Autotrophic Nitrogen removal Over Nitrite* (CANON) (Third et al., 2001) and *ANaerobic AMMonium OXidation* (ANAMMOX®) (2014). All of these technologies are characterized by the use of a short-cut via nitrite (NO_2^-) pathway of microbial metabolism. Such a pathway can reduce the oxygen and carbon demands for reducing power and increasing potential for energy recovery compared to CAS. Although these technical applications have primarily been applied to side stream BNR, ongoing research is presently directed to investigating mainstream implementation as well (Laureni et al., 2015; Tan and Shuai, 2015). Despite their energy benefits, these processes could potentially release significant GHG emissions such as nitrous oxide (N_2O), when compared to conventional BNR processes (Aboobakar et al., 2013; Daelman et al., 2013a; Domingo-Félez et al., 2014; Pijuan et al., 2014) (N_2O has a global warming potential 310 times higher than that of CO_2)(Ravishankara et al., 2009). Research is on-going to prevent greenhouse gas emissions through improved process stability and control (Bartrolí et al., 2010).

Novel alternative strategies are also being developed and came to the fore recently. One approach consists of bioelectrochemical hybrid-systems to substitute the necessary amount of carbon with an electrical current to gain sufficient reducing power (Rodríguez Arredondo et al., 2015; Tejedor-Sanz et al., 2016). Another strategy is nitrogen removal coupled with energy recovery. In this respect, the *Coupled Aerobic-Anoxic Nitrous Decomposition Operation* (CANDO) (Scherson et al.,

·Scherson et al.2013) has been proposed for intentional N_2O production and its subsequent use as an energy-yielding co-oxidant for biogas combustion. The CANDO process might also facilitate P removal and biopolymer production (Scherson et al., 2014a).

The opportunities provided by these state-of-the-art treatment technologies are presently of high scientific relevance underscored by a growing number of peer-reviewed journal articles in this domain. As an example, publications focusing on nitritation (oxidation of ammonia (NH_4^+) to nitrite, $NH_4^+ \rightarrow NO_2^-$) and denitritation (reduction of nitrite to nitrogen gas (N_2), $NO_2^- \rightarrow N_2$) peaked in 2015 with a share of ~25 % within the total publication number from 2006-2015. The results were obtained from an online research in the Web of Science™ Core Collection (Figure A.1). These studies revealed several novel reaction mechanisms and pathways. Some of them were defined coincidentally by different authors resulting in partial ambiguity in the chosen terminology. Two expressions related to N_2O emissions serve as an example of ambiguous terminology. The first is "nitrifier denitrification", a phrase originally coined to describe unintended N_2O generation when NO_2^- is reduced via nitric oxide (NO) (Wrage et al., 2001a). Problems become clear when this phrase is parsed into "nitrifier" and "denitrification". The term "nitrifier" is problematic, because it implies active nitrite-oxidizing bacteria (NOB) as a potential key-player in the production of N_2O. So far, the presence of both NH_4^+ and NO_2^- oxidation related genes in a single organism has been reported for only two *Nitrospira* species by (van Kessel et al., 2015a), indicating that there are organisms engaging in both pathways. However, production of N_2O during the oxidation of NO_2^- to NO_3^-, accounting for most NOB, is not known. The use of the term "denitrification" is also problematic because it implies complete denitrification to N_2. The second example is "partial denitrification" (Scherson et al., 2014a). This phrase refers to production of N_2O as the end product of denitrification or to conversion of NO_3^- to NO_2^- and possibly also a partial conversion of NO_2^- or NO_3^- to N_2. Phrases such as "nitrifier denitrification" and "partial denitrification" are thus imprecise and do not adequately differentiate pathways and products. Both expressions demonstrate that denotation has become imprecise and underscore the need for a consistent terminology that differentiates pathways to avoid misunderstanding in the scientific community.

We propose here, a conservative consolidation of existing terminology by including new definitions based upon a comprehensive literature review and transparent logic. The goal is to provide a combination of consistent determinants properly representing distinct reaction conditions and underlying processes. We do not claim that the approach in its present form is perfect, but we hope that our contribution could engage a discussion in the scientific community towards a standardization of the terminology facilitating communication in the future.

2 Scientific Approach

We begin by compiling and comparing current terminology for all known BNR metabolic pathways in terms of singularity and distinctiveness. We then propose terminology that establishes a uniform nomenclature for biotechnology and process engineering. Our aim is to integrate novel elements into historically accepted terminology (such as “nitrification” and “denitrification”) so as to establish an unambiguous nomenclature. Names for oxidation sequences are formulated by identifying a root term based upon the reaction product. Oxidation of NH_4^+ to NO_2^- is therefore designated “NITRITation”; oxidation of NO_2^- to NO_3^- is designated “NITRATation”. Analogous historical logic is applied for denitrification, with preservation of the prefix “de” for reduction sequences. In this case, however, the focus is on the initial reactant. Reduction of NO_3^- to NO_2^- is designated “DENITRATation”; reduction of NO_2^- to N_2 is designated “DENITRITation”. With respect to NO_2^-, the metabolic intermediates, i.e. nitric oxide and nitrous oxide, require an additional indicator - the final reaction product - in order to differentiate them from nitrogen removal via N_2.

For determination of reaction pathways, mechanisms and dominant organisms, the oxidation-reduction regime (ORR) is used as a qualifier. We initially considered ranges of dissolved oxygen (DO) concentration for each ORR, but, to the best of our knowledge, specific threshold values have yet to be defined, except for the fully anoxic case where DO is zero. Further research is needed to elucidate the DO ranges for oxidoreductase activities and DO profiles within biofilms so as to differentiate such processes from the mean DO values commonly applied to the bulk mixed liquor. For now, we define just two ranges within the DO constrained ORRs: (1) low (sub-oxic) and (2) high DO (aerobic). Finally, “partial” is applied as an optional second determinant to designate incomplete conversion of reactants. An example would be “partial nitritation” for the case where <100% of the initial NH_4^+ load is converted to NO_2^-. Based on these results, the logical combination of our terminology consists of two highly recommended and one optional quantitative indicator:

Oxidation: *quantitative indicator** + *ORR* + *Reaction pathway (product specific)*

Reduction: *quantitative indicator** + *ORR* + *Reaction pathway (educt specific)*

*only if necessary

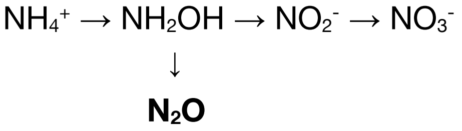
This approach represents a flexible nomenclature for complete integration of the presently relevant processes and to potentially derive the terms for novel treatment steps in the future.

3 Proposed terminology

3.1 Pathways of conventional BNR processes

Mainstream nitrification/denitrification (N/DN) processes are still the most widely applied techniques for nitrogen removal in conventional biological wastewater treatment. The underlying pathways are complete oxidation of NH_4^+ during nitrification and reduction of NO_3^- to N_2 via denitrification (Figure 1) resulting in NO_3^- and N_2 as desired products, respectively. The underlying pathways are well understood and have been the basis for design and operation of conventional BNR processes for decades (Metcalf, 2003):

Nitrification (aerobic oxidation):



$$\begin{tikzcd}[column sep=small] NH_4^+ \arrow[r] & NH_2OH \arrow[r] \arrow[d] & NO_2^- \arrow[r] & NO_3^- \\ & \mathbf{N_2O} & & \end{tikzcd} \qquad (1)$$

Denitrification (anoxic reduction): $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow \mathbf{N_2O} \rightarrow N_2$ (2)

Within N/DN, some metabolites are shared intermediates for oxidation and reduction pathways. The major intermediates are nitrite (NO_2^-), nitrate (NO_3^-) and nitrous oxide (N_2O) (Eqs. 1, 2). During nitrification, N_2O is both a stable by-product of chemical oxidation via hydroxylamine (NH_2OH) (Wrage et al., 2001a) and a product of microbial denitrification pathways. Figure 2 provides an overview of the discussed microbial pathways together with the proposed nomenclature. Nitric oxide (NO) can also be generated chemically by disproportionation of nitrous acid (HNO_2) in wastewater treatment systems, however, appreciable quantities are only released under acidic conditions (Schreiber et al., 2012b) and such conditions are uncommon in BNR systems.

3.2 Role of the ORR

The key process parameter commonly manipulated to control reaction pathways in engineered BNR processes is the DO, a parameter that sets the predominant ORR (Holman and Wareham, 2003; Lackner et al., 2012a; Lackner and Horn, 2012). The ORR in BNR processes can typically be characterized as aerobic (oxidation pathways), anoxic (reduction pathways), and sub-oxic (reduction and oxidation pathways occur simultaneously (Holman and Wareham, 2003)). These categories capture the predominant biochemical dynamics within a particular process. The ORR also constrains and defines the structure and function of the microbial community and dominance of key organisms. Ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria are predominant under aerobic conditions; denitrifying bacteria (DB) are predominant under anoxic conditions. Under sub-oxic conditions, a process phase quite typical for contemporary treatment technologies, such as the SHARON or CANON processes, AOB are the dominant organisms (Wunderlin et al., 2013; Zeng et al., 2010) and can catalyze N reduction reactions (Wrage et al., 2001a; Wunderlin et al., 2012).

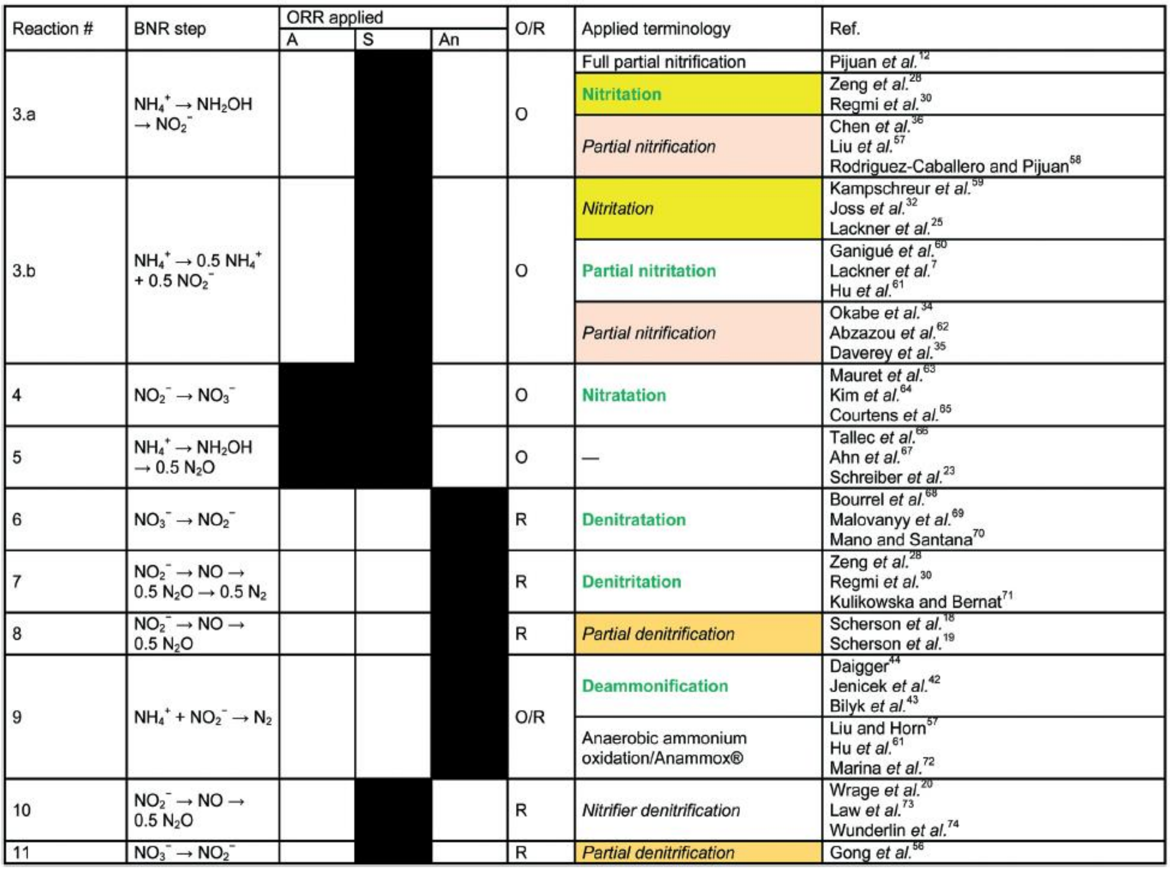
3.3 Pathways of novel BNR processes

In contrast to the terminology used for conventional nitrification/denitrification, the terminology that is used to describe metabolic pathways in contemporary BNR processes is diverse and inconsistent. The typical first step is the oxidation of NH_4^+ to NO_2^-. Nitrite is the precursor for subsequent reduction in several well-established processes (e.g., denitritation, SHARON®, CANON, DEMON® and ANITAMOX®). Nitrogen elimination as N_2 is carried out by anaerobic ammonia oxidizing (so-called Anammox) bacteria(Laureni et al., 2015) (*planctomycetes*) or by DB (Regmi et al., 2015). Frequently, these treatments are carried out simultaneously in single-stage systems (Joss et al., 2011; Joss et al., 2009b). From a biochemical point-of-view, however, the seemingly “simultaneous” reactions are a chain of oxidation and reduction reactions at different micro-scale ORRs.

3.4 Aerobic ORR

In contemporary practice of aerobic and sub-oxic systems, the adjective “full” refers to processes that are designed to achieve essentially 100% conversion of reactants (e.g. NH_4^+) to products (e.g. NO_2^-, NO_3^-). In contrast, the adjective “partial” (Daverey et al., 2015; Okabe et al., 2011a, 2011b) is used to refer to processes designed to achieve less than 100% conversion (Chen et al., 2013; Regmi et al., 2015; Regmi et al., 2014) e.g. “partial nitritation” or is used to differentiate short-cut technologies from conventional treatment e.g. “partial nitrification” ($NH_4^+ \rightarrow NO_2^-$ instead of $NH_4^+ \rightarrow NO_3^-$) (Table 1, Eqs. 3.a, 3.b). Frequently, the intended use is not specified and has to be assessed by the context of the particular publication. In order to enable a distinctive definition, we propose use of the word “partial” to designate all processes that bring about partial oxidation or reduction of educts, respectively.

Table 1. Literature compilation of BNR steps, according to ORR (A: aerobic, S: sub-oxic, An: anoxic), change in the oxidation state of nitrogen (O: oxidation, R: reduction) and currently used terminology with qualitative indicators. The same background color represents ambiguity; italicized letters represent imprecise terms; and adopted terms are listed in bold green font).



Reaction #	BNR step	ORR applied			O/R	Applied terminology	Ref.
		A	S	An			
3.a	$NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^-$		■		O	Full partial nitrification	Pijuan *et al.*[12]
						Nitritation	Zeng *et al.*[28] Regmi *et al.*[30]
						Partial nitrification	Chen *et al.*[36] Liu *et al.*[57] Rodriguez-Caballero and Pijuan[58]
3.b	$NH_4^+ \rightarrow 0.5\ NH_4^+ + 0.5\ NO_2^-$		■		O	*Nitritation*	Kampschreur *et al.*[59] Joss *et al.*[32] Lackner *et al.*[25]
						Partial nitritation	Ganigué *et al.*[60] Lackner *et al.*[7] Hu *et al.*[61]
						Partial nitrification	Okabe *et al.*[34] Abzazou *et al.*[62] Daverey *et al.*[35]
4	$NO_2^- \rightarrow NO_3^-$	■	■		O	**Nitratation**	Mauret *et al.*[63] Kim *et al.*[64] Courtens *et al.*[65]
5	$NH_4^+ \rightarrow NH_2OH \rightarrow 0.5\ N_2O$	■	■		O	—	Tallec *et al.*[66] Ahn *et al.*[67] Schreiber *et al.*[23]
6	$NO_3^- \rightarrow NO_2^-$			■	R	**Denitratation**	Bourrel *et al.*[68] Malovanyy *et al.*[69] Mano and Santana[70]
7	$NO_2^- \rightarrow NO \rightarrow 0.5\ N_2O \rightarrow 0.5\ N_2$			■	R	**Denitritation**	Zeng *et al.*[28] Regmi *et al.*[30] Kulikowska and Bernat[71]
8	$NO_2^- \rightarrow NO \rightarrow 0.5\ N_2O$			■	R	*Partial denitrification*	Scherson *et al.*[18] Scherson *et al.*[19]
9	$NH_4^+ + NO_2^- \rightarrow N_2$			■	O/R	**Deammonification**	Daigger[44] Jenicek *et al.*[42] Bilyk *et al.*[43]
						Anaerobic ammonium oxidation/Anammox®	Liu and Horn[57] Hu *et al.*[61] Marina *et al.*[72]
10	$NO_2^- \rightarrow NO \rightarrow 0.5\ N_2O$		■		R	*Nitrifier denitrification*	Wrage *et al.*[20] Law *et al.*[73] Wunderlin *et al.*[74]
11	$NO_3^- \rightarrow NO_2^-$		■		R	*Partial denitrification*	Gong *et al.*[56]

Oxidation of nitrite to nitrate (Table 1, Eq. 4), a typical side reaction in nitritation processes (Egli et al., 2003; Jianlong and Ning, 2004), is carried out by nitrite oxidizing bacteria (NOB) via “nitratation” (Bagchi et al., 2010). This terminology is in accordance with the proposed scheme and is adopted as is. In contrast, generation of N_2O via NH_2OH under aerobic and sub-oxic conditions, an important reaction for greenhouse gas emissions at wastewater treatment plants (Daelman et al., 2013a; Itokawa et al., 2001b; Ravishankara et al., 2009) and energy recovery options (Scherson et al., 2014a; Scherson et al., 2013b), does not follow an agreed-upon terminology (Table 1, Eq. 5). Processes that generate N_2O clearly need better definition. Previous investigations (Daelman et al., 2013a) revealed, that the share of N_2O within the total carbon footprint can be as high as 78 %. However, N_2O is also a potential energy recovery option as co-oxidant in biogas combustion processes (Scherson et al., 2013b). To systematize the nomenclature, we propose the term “nitrousation” for its aerobic microbial generation of N_2O (Figure 2).

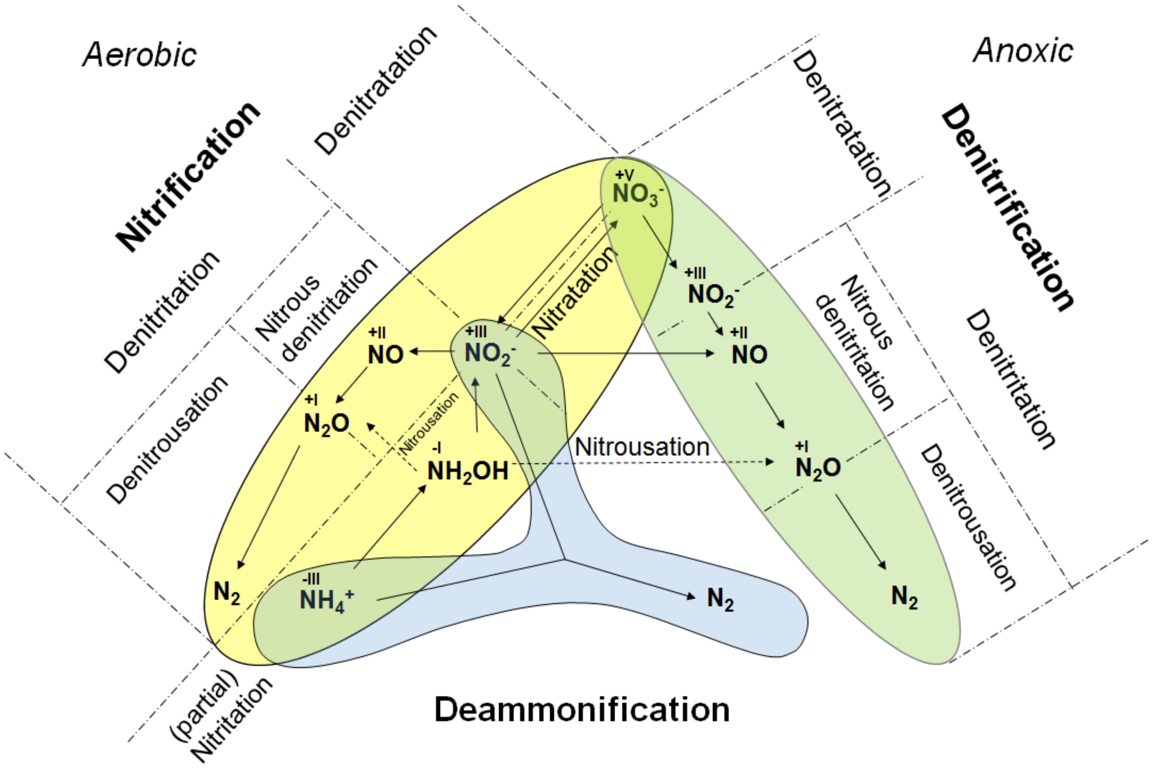


Figure 2. Schematic representation of the proposed terminology. The continuously lined arrows indicate microbially catalyzed reaction pathways,the dashed lined arrows chemical reactions, reaction pathways are indicted by dash-dotted lines. In yellow: The aerobic and sub-oxic reaction pathways summarized in the “aerobic” regime. In green: Reductive pathways including the intermediate steps in nitrite reduction to N_2O. Denitrousation is included for both aerobic and anoxic regimes with respect to the mitigation of N_2O emissions. In blue: The deammonification pathway.

3.5 Anoxic ORR

For the reductive pathways, historically the prefix “de-” has been commonly used (e.g., “DEnitrification”). To be conservative, we propose to generally maintain the logic, i.e. initial nitrogen compound plus prefix “de-“. This will result in two terms, “denitratation” (Table 1, Eq. 6) and “denitritation” (Table 1, Eq. 7), which have already been applied in this context and are adopted for the novel terminology framework.

In studies by Scherson et al. (Scherson et al., 2014a; Scherson et al., 2013b), an anoxic carbon-limited biochemical reaction pathway for N_2O production (Table 1, Eq. 8) has been referred to as “partial denitrification”. Considering the historical interpretation of “denitrification”, the expression is misleading as it relates to the production of N_2. However, applying the scheme for reductive pathways to the CANDO process causes the problem that “denitritation” already refers to nitrogen removal via N_2. As a qualitative indicator for a clear product specification, we propose “nitrous” in addition - resulting in the expression “nitrous denitritation” (Figure 2).

Independent from N/DN, the oxidation reduction reaction carried out under anoxic conditions by *planctomycetes* is known as “deammonification” (Bilyk et al., 2012; Daigger, 2014; Jenicek et al., 2007) as well as “anaerobic ammonium oxidation”

(Domingo-Félez et al., 2014; Jensen et al., 2007; Okabe et al., 2011a; Rosenwinkel and Cornelius, 2005; Tao et al., 2013; Wett, 2007) or "ANAMMOX®" (Table 1, Eq. 9). The process is based on an alternative pathway (Strous et al., 1999) in the biological nitrogen metabolism and decoupled from the reaction chains in N/DN. In contrast to the potential bi-directional reaction pathways in N/DN, deammonification is uni-directional (Figure 1). In contrast to nitritation or denitritation, deammonification is an intracellular reaction pathway without any transition links within the metabolic web. Due to its isolation, the one-directional redox-reaction and lack of available intermediates, the process is integrated independently (Figure 2). However, since ANAMMOX® is a licensed process by Paques (EL Balk, NL), we continue using the term "deammonification" in order to distinguish between the technical process as a registered trademark and the underlying metabolic pathway.

3.6 Sub-oxic ORR

During sub-oxic conditions, AOB catalyze reduction and oxidation reactions "simultaneously" (Leix et al., 2016a). Depending upon reactor conditions, different products can be produced under reductive conditions (i.e., NO, N_2O, N_2). The production of N_2O in this respect represents the same reaction pathway as in the second stage of the CANDO process (Table 1, Eq. 10), but the mechanism is related to the sensitivity of nitrous oxide reductase to oxygen (Berks et al., 1993b; Bertrand, 2015; Lu and Chandran, 2010) instead of carbon limitation. A commonly used phrase in this context is "nitrifier denitrification" (Kool et al., 2010; Wrage et al., 2001a; Wunderlin et al., 2012; Zhu et al., 2013), but with respect to the microbial community as well the terminal reduction step, this expression is misleading. The term "nitrifier" implies the participation of nitrite oxidizing microorganisms, and denitrification historically implies N_2 as the final product (Metcalf, 2003). In order to specify this particular reaction pathway, the term "nitrous denitritation" is proposed. To distinguish between the CANDO process and this reaction, a proper definition of the ORR is necessary. According to the previous explanations, the determinant "sub-oxic" is adequate resulting in "sub-oxic nitrous denitritation".

A comparison of Gong et al. (2013) and Scherson et al.(Scherson et al., 2014a; Scherson et al., 2013b) elucidates another ambiguous expression. Both authors use the phrase "partial denitrification", but very different processes are described: Scherson et al.(Scherson et al., 2014a; Scherson et al., 2013b) refer to N_2O production, while Gong et al. (Gong et al., 2013) refer to sub-oxic reduction of NO_3^- to NO_2^- (Table 1, Eq. 11). According to our proposed scheme, the pathway of Gong et al. can be distinctly described as "sub-oxic denitratation". Moreover, because the dominant organisms (i.e. AOB and NOB) under sub-oxic conditions are the same as under "aerobic" conditions, it is clear that the reduction reaction mechanisms are related to "aerobic" organisms and the "sub-oxic" determinant can thus be omitted from the terminology.

4 Conclusion

This work has shown that the structure of different combinations of metabolic pathways applied in BNR processes has become more complex and inconsistent, leading to ambiguity and confusion within the research community and industry. For future communication and development of BNR processes, we therefore propose to apply a consolidated terminology that enables a more exact definition of the metabolic pathways.

The presented approach enables a distinct identification of every possible step that is important for BNR technologies (Figure 2). Because of its flexibility it can also be adapted for novel derivatives of state-of-the-art BNR technologies. For a process removing nitrogen as nitric oxide, for example, application of the proposed nomenclature would give the phrase “nitric denitritation”. For the reduction of NO_3^- to N_2O “nitrous denitrification” can be derived. While such processes have not been technically applied yet, it illustrates the flexibility of the terminology. Additionally, presently emerging bioelectrochemical technologies also fit into the scheme, since their catalyzed oxidation reduction reactions are the same as in biological systems. Hence, with the adoption of our presented approach a more exact communication about all known biological and bioelectrochemical technologies can be facilitated and will potentially avoid further misunderstanding. However, we do not claim that the presented approach is perfect, but it hopefully triggers further discussion within the community to agree on a commonly accepted terminology.

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Supplementary information

Supplementary data for this work is given in *Appendix A*.

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6. Nitrogen removal and intentional nitrous oxide production from reject water in a coupled nitritation/nitrous denitritation system under real feed-stream conditions

To mediate knowledge transfer and process development, essential questions are usually related to the technical applicability and feasibility of processes and technology. Concerning the CANDO process, fundamental research was been conducted earlier (Gao et al., 2017; Scherson et al., 2014; Scherson et al., 2013) and elucidated the general potential of microbial N_2O production under controlled conditions. These previous results were considerably biased by the supply of readily degradable carbon sources (i.e. acetate) and excess supply of stripping gas to generate high N_2O yields. Hence, regarding potential scale-up and application, real feed-stream conditions need to be investigated to judge about the technical potential of the CANDO in addition to very controlled lab-scale operations. The first objective of the following research was therefore dedicated to investigating the feasibility of real feed-stream conditions, operational stability and to derive future design parameters for viable sludge-specific COD and nitrogen loads according to hypotheses three and four:

> "*Primary effluent can be used as a cost-efficient and locally available alternative carbon source to acetate.*"
>
> "*The correlation of maximum biomass specific PHB storage and carbonaceous substrate added can be expressed in a specific coefficient which will improve operational control. This coefficient might consist of a resulting COD/N/MLVSS ratio that could be applied and substitute a given COD/N ratio for continuous operation in order to assure a full conversion of external carbon into intracellular PHB at the beginning of the anoxic period while also preventing N_2O degradation.*"

Both hypotheses could be positively tested in a continuous long-term operation of the CANDO process applying PE. Additionally, a COD/N/MLVSS ratio could be successfully determined facilitating stable N_2O production.

Further investigations concerned N_2O production and harvesting strategies. As N_2O removal requires additional effort, the assessment of removal strategies and their effects on N_2O yields are essential for the future process development, economic viability and integration into present treatment configurations. To derive suitable integration concepts, different N_2O harvesting strategies and their impact on the process performance were, hence, compared as a second research objective.

Nitrogen removal and intentional nitrous oxide production from reject water in a coupled nitritation/nitrous denitritation system under real feed-stream conditions

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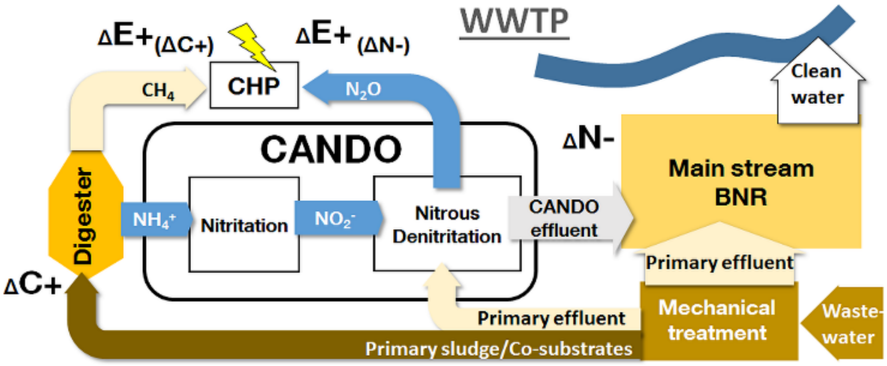


Abstract

A Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO) was performed over five months to investigate the performance and dynamics of nitrogen elimination and nitrous oxide production from digester reject water under real feed-stream conditions. A 93% conversion of ammonium to nitrite could be maintained for adapted seed sludge in the first stage (nitritation). The second stage (nitrous denitritation), inoculated with conventional activated sludge, achieved a conversion of 70% of nitrite to nitrous oxide after only 12 cycles of operation. The development of an alternative feeding strategy and the addition of a coagulant ($FeCl_3$) facilitated stable operation and process intensification. Under steady-state conditions, nitrite was reliably eliminated and different nitrous oxide harvesting strategies were assessed. Applying continuous removal increased N_2O yields by 16% compared to the application of a dedicated stripping phase. These results demonstrate the feasible application of the CANDO process for nitrogen removal and energy recovery from ammonia rich wastewater.

1 Introduction

A solution for the integration of energy recovery from nitrogen into wastewater treatment schemes is potentially a crucial step toward the development of more sustainable and self-sustaining wastewater treatment processes. Considering organic compounds in untreated wastewater, the chemical energy of organic matter is estimated to account for up to 1.93 kWh/m³ (McCarty et al., 2011). In addition, the chemical energy of nitrogen compounds can be estimated to approximately 0.3 kWh/m³ (Horstmeyer et al., 2017). However, while energetic valorization by anaerobic digestion is well established and may be a key technology towards sustainable base-load electricity generation, conventional nitrogen removal is always energy consuming. Additionally, operational challenges for sufficient nitrogen removal potentially arise during intensive use of anaerobic digestion for the treatment of organic loads and co-substrates (Koch et al., 2016). In this respect, it is worth noting, that conventional denitrification requires organic reducing equivalents. Hence, the potential for biogas production from organic matter by anaerobic digestion is always limited by the amount of nitrogen which needs to be treated. The effluent from digesters imposes additional nitrogen loads to the main stream by ammonification of fixed nitrogen loads, which are contained in organic substrates.

In this respect, autotrophic nitrogen removal via deammonification is a promising side-stream treatment technology to decouple nitrogen removal from organic carbon demand. However, considerable N_2O emissions, have been observed, which are released during aerobic and suboxic conditions during nitrogen removal (Jia et al., 2013; Rathnayake et al., 2015). Because N_2O has a CO_2-equivalent of approximately 300 (Ravishankara et al., 2009) and because of its potential to catalyze stratospheric ozone depletion, N_2O emissions should be prevented as much as possible (Domingo-Felez et al., 2014; Kampschreur et al., 2009). Accordingly, operational strategies to mitigate N_2O emissions from deammonification systems are part of ongoing research activities (Leix et al., 2016c).

In addition to N_2O formation under aerobic conditions, N_2O is also a biological metabolite under anoxic conditions. The N_2O yields can be significantly higher than during aerobic processes (Liu et al., 2015b). In this respect, the activity of endogenous substrate (e.g. poly-3-hydroxybutyrate) respiration has been correlated with considerable N_2O yields in anoxic famine phases, when extracellular electron donors are depleted (Ribera-Guardia et al., 2016; Tallec et al., 2008; Zeng et al., 2003b) – an effect, which is suspected to significantly contribute to N_2O emissions from conventional nitrification/denitrification systems (Liu et al., 2015b; Liu et al., 2015a; Read-Daily et al., 2016).

However, next to the global warming potential, N_2O is a well-known oxidant in fuel-combustion. At a stoichiometric ratio of four (N_2O/CH_4) for instance, approximately 37% (Scherson et al., 2013a) more energy can be generated in methane combustion with N_2O compared to oxygen. Additionally, the climate impact is reduced by the formation of climate-neutral N_2 during combustion.

For intentional biological production and energetic valorization of N_2O from ammonium loads, the so-called Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO) has been proposed (Scherson et al., 2013a). It is a biological treatment train comprising two stages: (1) a nitritation stage (N) to oxidize NH_4^+ to NO_2^- and (2) an anoxic nitrous denitritation stage (ND) (Weißbach et al., 2017b) for N_2O production from NO_2^-. In a third stage, energy recovery is intended by combustion with biogas.

So far, only synthetic organic substrates (e.g., acetate) were applied for investigation of the CANDO in bench-scale studies (Gao et al., 2017c; Scherson et al., 2013a). Under these conditions, approximately 74% of NH_4-N was converted to N_2O-N. While these considerable turnover rates are promising, several concerns remain about the technological and economic feasibility. As, for instance, acetic acid is costly, the application of locally and readily available substrates for the heterotrophic N_2O formation would considerably reduce operational costs. In this respect, primary effluent (PE) is potentially a viable option as it is readily available and typically easily degradable. Also, considerable N_2O emissions were observed during simultaneous denitrification and phosphorous removal from domestic wastewater under similar alternating anaerobic/anoxic conditions (Wang et al., 2015). However, the bio-degradable share in real-wastewater is estimated to account for only 65% and concentrations are typically low (500 mg_{COD}/L) compared to synthetic substrates (McCarty et al., 2011). Hence, higher organic loads and exchange volumes are likely necessary under real feed-stream conditions, potentially resulting in shorter hydraulic retention times and washout of active biomass. Additionally, the effect of the wastewater matrix in terms of organic-matter make-up i.e., bio-availability of particulate and dissolved fractions to produce microbial carbon storage compounds should be assessed in addition to viable COD/N ratios applying real feed-streams.

Also, sludge characteristics (e.g., growth rates, flocculation potential, sludge volume index) as well as the necessary sludge-specific and volumetric loads of continuous operation applying PE should be investigated as a basis for subsequent design and scale-up of reactors. In this respect, it is noteworthy, that. a mixed-liquor volatile suspended solids (MLVSS) concentration of 0.2 g_{MLVSS}/L, representing active biomass, was reported in the continuous operation of the CANDO process (Scherson et al., 2014a); this figure is comparatively low considering biological-phosphorous-removal systems operating at similar conditions (i.e., alternating feast/famine conditions) and mixed-liquor concentrations of 2-3 g_{MLVSS}/L (Chen et al., 2004; Lesjean et al., 2002; Pijuan et al., 2008). An intensified operation at higher concentrations could severely reduce the reported reaction time of the CANDO (48-h cycle) (Scherson et al., 2014a) and possibly yield process intensification. Therefore, operational strategies should be investigated to increase the MLVSS concentration to assess the process performance in comparison to conventional systems.

For future process design, a certain COD/N/MLVSS ratio is desirable beyond a COD/N ration to determine the respective loads of COD and nitrogen in relation the biomass for stable operation. In this respect, the COD/MLVSS ratio could determine the sludge-specific uptake capacity during anaerobic phases. The N/MLVSS ratio however, determines the nitrogen removal capacity. Hence, combining organic and nitrogen loads in relation to the biomass concentrations facilitates the determination of the sludge-specific loads of nitrogen and COD for operation and control as well as for the design and scale-up of a nitrous denitritation stage.

Besides the need to utilize real-feed streams in the CANDO, an efficient N_2O production and extraction strategy will be key for the technical viability of the process and the proposed subsequent combustion (Scherson et al., 2014a). In the approach by Scherson et al. (Scherson et al., 2014a), constant removal of N_2O was accomplished via excess helium stripping. Recently, a technically more viable approach for continuous removal of N_2O from aqueous phases was demonstrated applying a membrane contactor device (Weißbach et al., 2018). However, a potentially more feasible approach also achieving higher concentrations in the gas phase is the continuous accumulation of N_2O within the system and an additional dedicated stripping phase at the end of each ND batch. This approach would mitigate the operational costs by reducing the amount of dedicated sweeping gas and stripping.

To address the illustrated challenges of the CANDO process and to develop more feasible operational strategies compared to the application of synthetic substrates, a long-term investigation was conducted to investigate operational stability and N_2O yields, as well as process rates and dynamics, and necessary process stoichiometries for the application of real wastewater feed-streams.

2 Materials and Methods

2.1 Reactor design

A SCADA-controlled bioreactor system has been designed and constructed for the operation of N and subsequent ND stage (Figure 1). The two similarly designed reactors constructed out of PVC had a maximum reaction volume of 12 L. Both configurations comprised a stirrer (IKA-Werke GmbH, Staufen, Germany), peristaltic feed and dosing pump (Watson-Marlow, Falmouth, Great Britain), and heat exchanger (Huber Kältemaschinenbau AG, Germany). Three additional peristaltic pumps (Heidolph Instruments, Schwabach, Germany) were operated in the second stage for the addition of PE and coagulant (5% iron-III-chloride), as well as effluent withdrawal.

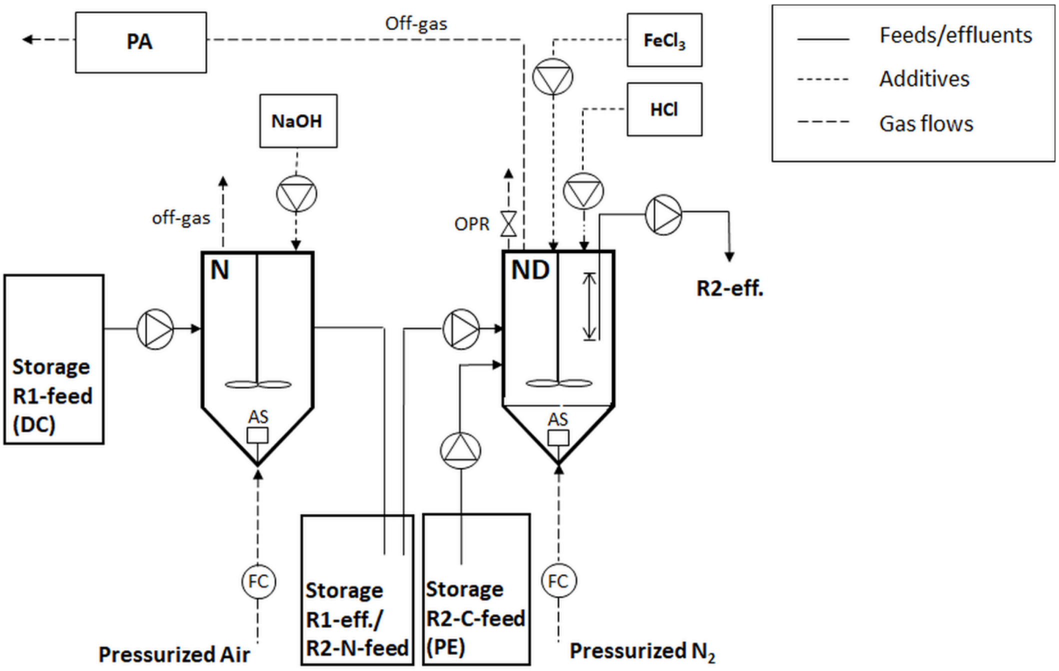


Figure 1. Schematic of the N and ND laboratory-scale reactors and peripherals (Note: The feed and substrate or additive tanks are indicated as follows: digester centrate (DC) as the feed for the nitritation stage, primary effluent (PE) as the carbon feed, and the nitrite feed for the nitrous-denitritation stage. Fine bubble-aeration stones (AS) were installed for aeration during N and N_2O stripping in the ND stage. A strip gas line was connected to the ND stage, guiding N_2O-enriched off-gas through a photoacoustic cell (PA) that was employed for gas-phase N_2O-concentration measurements.).

2.2 Substrates and inocula

Carbon and nitrogen feeds were collected from the municipal wastewater treatment plant in Garching, Germany, designed for a population-equivalent of 31,000 (Figure B.1, Supplemental Information). The PE samples of this study were collected once to twice a week from a 4-mm drum screen for mechanical pretreatment and provided average COD and NH_4-N concentrations of 376±141 mg_{COD}/L and 52±7 mg_N/L (n = 32), respectively (Table 1).

Table 1. Characterization of the primary effluent applied as feed for the second CANDO stage regarding COD fractions and BOD_5 as well as ammonia nitrogen.

	tCOD [mg/L]	sCOD [mg/L]	pCOD [mg/L]	sCOD/ pCOD [-]	BOD_5/ tCOD^a [-]	NH_4-N [mg/L]	NO_3-N [mg/L]	NO_2-N [mg/L]
Mean	376 ± 159	144 ± 41.7	225 ± 151	0.60 ± 0.18	0.58 ± 0.03	52.2 ± 7.32	<0.23	<0.6
min	150	86.7	40.0	0.32	0.55	43.2	<0.23	<0.6
max	785	311	593	0.98	0.64	66.6	1.6	<0.6
n	32	32	32	32	25	32	13	13

[a]data provided by the wastewater treatment plant Garching

A BOD_5/COD ratio of 0.58±0.03 (n = 25) indicated well degradability throughout the investigations. The particular feed samples were stored at 4 °C and used to refill the PE-feed tank every two to three days. Residual feed volumes in the tank were discarded.

The nitritation feed was collected once a week from the digester centrate storage tanks at WWTP Garching. A centrifuge (Hiller, Vilsbiburg, Germany) was used for on-site dewatering of the digester sludge. Throughout the operational periods of both stages, the digester centrate contained ammonia concentration of 1,098±169 $mg_{NH_4\text{-}N}$/L and 364±43 mg_{COD}/L (n = 19) resulting in an average COD/N ratio of 0.33.

The inoculum of the N stage originated from the first stage of a partial nitritation/deammonification process that was piloted at WWTP Kempten, Germany (Leix et al., 2016c). It was inoculated more than three months prior to the ND stage to provide the required feed quality for the second stage. For inoculation of the ND stage, seed sludge was collected from the denitrification zone of the activated sludge system at WWTP Garching.

2.3 Operation

Nitritation stage

The N stage was continuously operated as sequencing batch reactor. Low dissolved oxygen concentrations (0.2 mg/L to 0.5 mg/L) in intermittent aeration were applied to suppress nitratation (Bernet et al., 2001). At steady-state conditions, the exchange volume of 1.5 L resulted in an average HRT of 8 d. No sludge was actively removed resulting in a theoretically infinite SRT. Reactor temperature and pH were controlled at 31±1 °C and 7.5±0.1, respectively. The system was initially pulse-fed with digester centrate. A residual ammonia concentration of 20 $mg_{NH_4\text{-}N}$/L was chosen to terminate the ammonia oxidation phase, which was followed by a 30-min settling and draw phase.

Nitrous denitritation stage

The investigations regarding the ND stage comprised 108 SBR cycles, which can be described as follows: the start-up phase covering cycles 1–23 (referred to as “phase A”), an optimization phase covering cycles 24–63 (referred to as “phase B”), and a third operational phase (referred to as “phase C”) at steady-state conditions for another 45 cycles (cycles 64–108).

In phase A, the operation of the ND stage (Figure B.2, Supplemental Information) started with a 24-h cycle with one carbon pulse (0 h) and one nitrogen pulse (11.6 h) at the beginning and half of the SBR cycle, respectively. In phase B, the feeding strategy was changed throughout the course of the experiment, adding a second carbon-feed pulse at 5.8 h of the anaerobic-reaction time to enable higher loading rates at the applied COD/N ratios. Eventually, the anoxic reaction period was prolonged to 24 h, resulting in a total 36-h cycle time in phase C. Additionally, a 45-min settling, and a 10-min draw phase were included. No sludge was actively removed (infinite SRT). Automatic pH control was conducted only during anoxic reaction periods by the addition of hydrochloric acid (1 mol/L) at pH = 6.9±0.05. For N_2O stripping, technical-grade nitrogen gas (Linde, Germany) was chosen at a flow rate of 30 L/h and 15 L/h for removal during the dedicated and continuous-stripping phases, respectively.

2.4 Analytical Methods

Single measurements of feed, effluent, and mixed-liquor concentrations of COD, NH_4-N, NO_2-N, and NO_3-N were conducted using cuvette tests (Hach-Lange, Berlin, Germany) according to the manufacturer's instructions. Samples for nitrogen species and dissolved COD (dCOD) were filtrated (0.45 µm) right after sampling and directly measured or stored for no longer than 12 h at 4 °C before measurement. Total COD (tCOD) was determined by homogenizing samples before measurement. The particulate COD (pCOD) was calculated by subtracting the dissolved fraction from the total COD.

The measurement data were averaged for each phase including all data points and the standard deviation was calculated. Similarly, the number of cycles was considered the number of replicates within each experimental phase under the same conditions for the calculation of removal and production rates, respectively.

Biomass-specific measurements comprised sludge volume (SV), measurement of MLVSS, and effluent volatile suspended solids (VSS). The samples were taken toward the end of the anoxic reaction periods at a reactor fill level of 12 L, and the measurements were conducted in accordance with German standard methods (DEV, 2016).

Intracellular PHB was extracted according to Riis and Mai (1988) by subjecting dried cells to propanolysis. For sample preparation harvested cell-mass was freeze-dried overnight. PHB quantification was carried out with a GC-FID device (7890A, Agilent, Santa Clara, CA, USA) equipped with a DB-5 column according to Wang et al. (Wang et al., 2013).

For control and online-data acquisition, the SCADA software (Citect SCADA, Schneider Electric, Rueil-Malmaison, France) was coupled to a PLC (WAGO, Minden, Germany) collecting the following parameter data with measurement equipment by Endress+Hauser (Reinach, Switzerland): pH, temperature (T), dissolved oxygen (DO), oxidation–reduction potential (ORP), conductivity, ammonia concentration (NH_4-N), and water-level indicators. Additionally, a micro-electrode (N_2O-R sensor, Unisense, Arhus, Denmark) with a measurement range of 25-2,500 μmol_{N2O}/L has been employed to measure liquid-dissolved N_2O concentrations in the second stage. For gas-phase N_2O measurements, a photoacoustic gas analyzer was used (Thaler et al., 2017a).

2.5 Mass balance

A semi-quantitative nitrogen mass-balance over both stages was conducted. For the first stage, average feed and effluent concentrations (n = 21) at steady-state (fixed exchange volume) were taken into account to quantify the mass flow for each dissolved nitrogen compound. For the second stage, the loads were calculated as product of concentrations and respective exchange volumes of carbon and nitrogen feed loads under stable operating conditions (phase C, n = 30). The mass-balance was closed by taking gaseous by-products as residual term into account (i.e., ammonia, nitric oxide and nitrogen gas). The results were normalized to the ammonia feed load (100 %).

3 Results and Discussion

3.1 Nitritation stage

After three months, the N stage was considered adapted. During stable operation (Figure B.3, Supplemental Information), an average conversion efficiency of NH_4-N to NO_2-N of 93% was achieved in the N stage. Additionally, 2% of NO_3-N were produced and 3% of NH_4-N remained in the effluent. Accordingly, about 2% of nitrogen were removed via the gas phase (Figure 5). During the parallel operation of both reactor systems, the nitrogen-feed concentrations had a suitable quality exhibiting 970±69 $mg_{NO_2\text{-}N}/L$.

3.2 Nitrous denitritation stage

Performance during phase A (cycles 0–23)

During phase A, the system was operated with a single PE-pulse at the beginning of each cycle and one NO_2^--pulse after 12 h resulting in a total 24-h reaction time (12 h anaerobic, 12 h anoxic). Operating cycles 1–5 were characterized by complete denitrification. Neither effluent nitrite nor considerable N_2O concentrations (maximum of 6.9% N_2O-N/NO_2-N cycle two) were observed. Starting with cycle six, N_2O production gradually increased. In cycle 12, 70% of NO_2-N was converted to N_2O-N within the anoxic reaction period (Figure 2a) in addition to complete NO_2^- removal. The observed sludge specific N_2O formation rates (rN_2O_{MLVSS}) throughout this period were 1.30±0.20 $mg_{N_2O\text{-}N}/(g_{MLVSS}\cdot h)$ (n = 6) on average and already comparable to the rates observed in the subsequent phases B and C (Table 2).

During cycles 13–16, N_2O production started to decrease. Additionally, up to 7% of the NO_2-N feed loads were released in reactor effluents (Figure 3a). Simultaneously, a considerable sludge loss was observed. However, no settleable matter was detected in the effluent and VSS concentrations in the effluent were negligible (< 0.01 g_{VSS}/L). Hence, the MLVSS decline was suspected to be rooted in sludge adaption processes.

Throughout the subsequent course of the investigations, effluent NO_2^- loads further increased up to 41% of the feed loads and residual NO_2^- concentrations were measured also at the end of anaerobic reaction periods. Simultaneously, the MLVSS concentration had further decreased from initially 2.15 g_{MLVSS}/L to 1.3 g_{MLVSS}/L. To assess nutrient limitation as a reason for the considerable sludge loss, the food to biomass ratio (F/M) as the ratio of COD to MLVSS was investigated. Previous operations of nitritation/denitritation reactors demonstrated viable F/M ratios of 0.05-0.32 also applying a COD/N ratio of three (Frison et al., 2016; Katsou et al., 2015). The operating value at cycle 21 of 0.07 was at the lower end and was potentially limiting as a MLVSS concentration of 1.29 gMLVSS/L would be expected considering the applied COD load of 90 mgCOD/L. As NO_2^- accumulation increased and MLVSS continued to drop, the organic and nitrogen loading rates were increased in operational phase B.

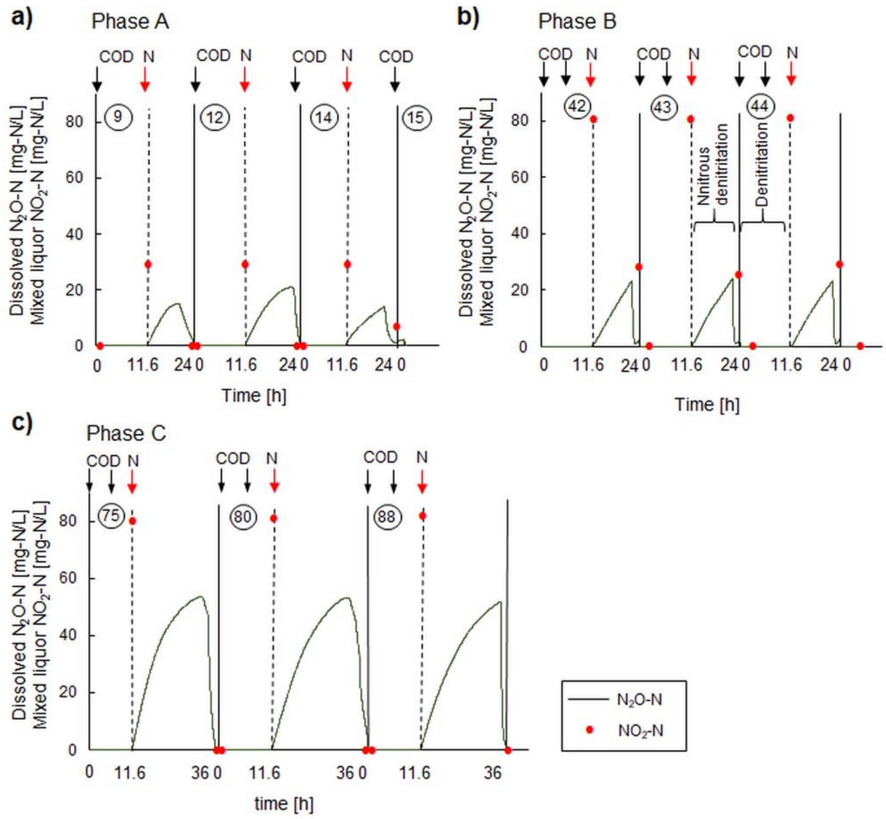


Figure 2. Characteristic deviations of N_2O production and NO_2^- concentrations throughout SBR cycles in phases A-C. a): Transition from increasing N_2O production to decreasing N_2O production and limited NO_2^- removal performance (cycles 9, 12, 14,15); b): stable operation at limited NO_2^- removal performance in phase B (complete nitrite elimination in subsequent cycles) (cycles 42, 43, 44); c): stable operation and reliable NO_2^- removal performance in phase C (cycles 75, 80, 88).

Additionally, to mitigate the increasing NO_2^- concentrations, excess COD was provided by the addition of 10 mL of acetic acid (0.5 mol/L) at the beginning of cycle 21 in addition to the PE pulse resulting in a total concentration of 140 mg_{COD}/L.

Despite the poor performance of cycles 13–23, the N_2O production rates throughout the first cycles were remarkable. A comparably short response time has not been previously described in the operation of a CANDO process and demonstrates the feasibility of more complex substrates e.g., PE, for N_2O production. Especially, reflecting the enormous effort for seeding sludge acquisition for full-scale deammonification plants, the observed start-up behavior is noticeable and should be considered in future decisions for a side-stream nitrogen removal technology.

Performance during phase B (cycles 24–63)

During phase B, the daily loads were initially increased by cycle 24 from 360 $mg_{NO_2\text{-}N}$/d to 1,020 $mg_{NO_2\text{-}N}$/d and from 1,180 mg_{COD}/d to 3,060 mg_{COD}/d targeting more efficient nitrogen removal and sludge growth at an F/M ratio of 0.27. To achieve this operation, the feeding strategy was altered. Because the increase of organic loading would have resulted in exchange volumes of approximately 70%, the PE-feed pulse was split 1:1 into two pulses at reaction times of 0 h and 5.8 h, resulting in an intermediate settling, draw and fill sequence within the anaerobic period (after a 5-h reaction time) (Figure 2b). Applying this strategy mitigated the average exchange volumes to approximately 40% throughout the subsequent investigations.

Despite the increase of organic loads, however, the MLVSS concentration further decreased. Considering that nutrient limitation could be excluded considering the applied F/M ratio, ultimately floc formation limitation was assessed as potential reason for the observed MLVSS decrease. Suspended cells however, have not been accounted for by applying 0.45 µm filters for the VSS measurements according to German standard methods (DEV, 2016). Further measurements for the quantification of suspended cells applying smaller pore sizes and optical density methods (Contois, 1959) did not yield plausible results due to the impurities of the PE matrix. The SVI however, remained at approximately 100 mL/g_{MLVSS} (Table 2), indicating a constant quality of the flocculated biomass. A suspected loss of suspended cells was hence, counter-acted in cycles 34 to 36. $FeCl_3$ was manually added as a coagulation and precipitation agent. The result was an increase of 0.29 g_{MLVSS}/L from cycles 34 to 39 (Figure 3b). By automated addition of $FeCl_3$ to a concentration of 40 mg/L (5%, 10 mL per cycle), the MLVSS loss was resolved and concentrations considerably increased to 1.7 g_{MLVSS}/L until cycle 42, followed by a gradually increasing trend up to cycle 63. Simultaneously, the N_2O production continuously increased over cycles 33 to 42 in correlation with the MLVSS concentration, from 90 $mg_{N_2O\text{-}N}$ to 280 $mg_{N_2O\text{-}N}$. Accordingly, the effluent NO_2^- concentrations decreased and eventually proved the suspected necessity for flocculation support under the applied conditions (Figure 3b). However, full nitrite removal could not be accomplished under the applied conditions.

Throughout cycles 43 and 56, an average of 56.1±15.7% of the initial NO_2^- concentration was removed during anoxic reaction periods. But in contrast to phase A, nitrite accumulation was not observed, and residual nitrite concentrations were eliminated during subsequent anaerobic periods (Figure 3b).

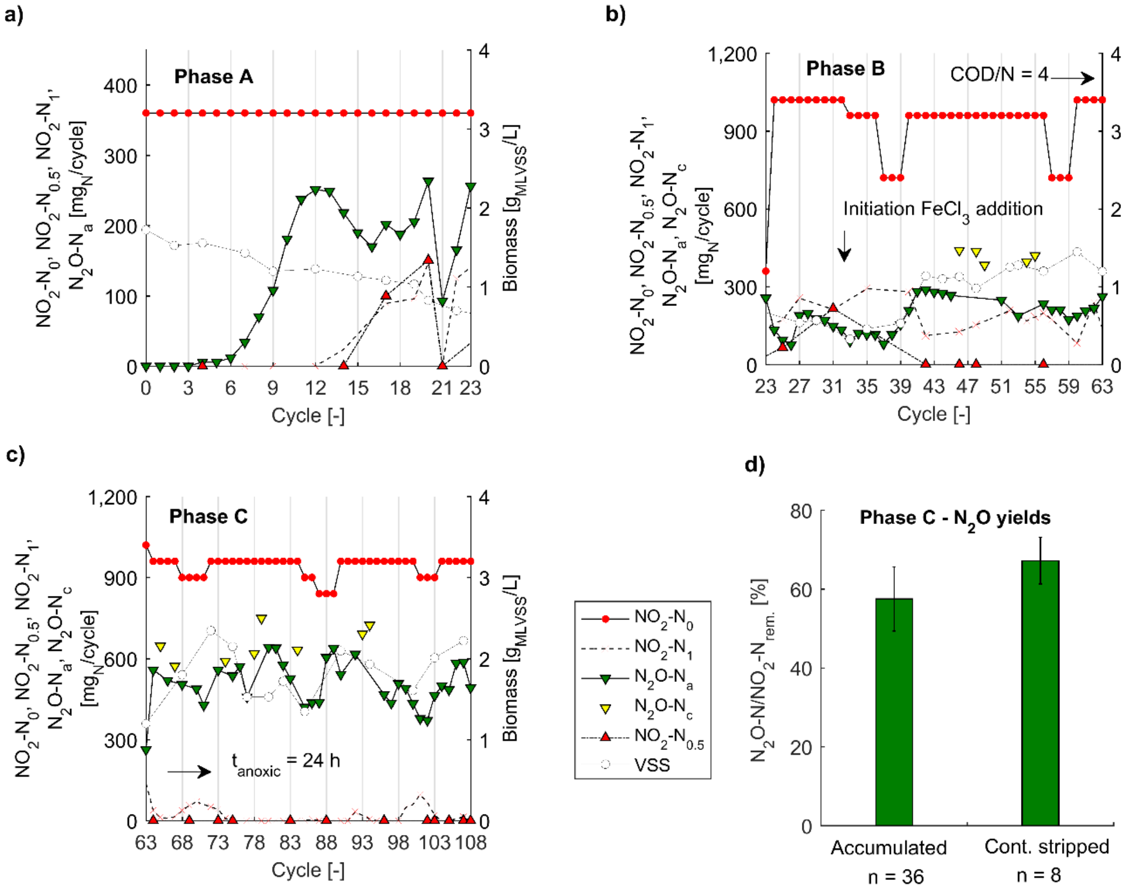


Figure 3. Development of nitrogen loads over the operational phases A-C in the ND stage. Displayed in a)-c) are: cyclic NO_2-N loads (NO_2-N_0), accumulated NO_2-N (NO_2-$N_{0.5}$) at the end of the anaerobic phases in phase A (a)) or anaerobic effluent withdrawal (b), c)), respectively, effluent loads (NO_2-N_1), N_2O-N production by accumulation (N_2O-N_a), N_2O-N production by continuous removal (N_2O-N_c) as well as MLVSS concentration (Note the different scales of left y-axes between a) and, b) and c)). N_2O yields at stable operating conditions applying a dedicated stripping phase and continuous stripping are displayed in d).

Regarding N_2O formation, it was evident that by the addition of an extracellular COD, as observed during anaerobic periods with residual nitrite concentrations, the nitrous denitritation switched to complete denitritation without considerable N_2O formation (Figure 2b). Additionally, considerably higher sludge specific NO_2^- removal rates (rNO_2-N_{MLVSS}) of 6.83±1.79 $mg_{NO_2\text{-}N}/(g_{MLVSS}\cdot h)$ were observed under these conditions compared to the anoxic reaction periods, where rNO_2-N_{MLVSS} were 3.36±1.03 $mg_{NO_2\text{-}N}/(g_{MLVSS}\cdot h)$ (Table 2). The different removal rates especially highlight the necessity of sufficient supply of biologically degradable reducing equivalents in the operation of conventional denitrification systems for both, N_2O emission mitigation and process intensity, which is possibly rooted in the limitation of nitrogen removal via endogenous compared to exogenous respiration (Liu et al., 2015a).

Because N_2O production and efficient NO_2^--removal performance decreased again until cycle 59, the COD/N ratio was increased to 4 by decreasing the load from 1,020 $mg_{NO_2\text{-}N}$/d to 720 $mg_{NO_2\text{-}N}$/d in cycle 60 to increase the NO_2- reducing equivalents. As a result, N_2O production increased in correlation with decreasing NO_2^- -effluent loads until the end of phase A. Complete nitrogen removal, however, was not achieved.

Operational phase B has highlighted the operational challenge of typically relatively low COD concentrations in the applied municipal wastewater and limited flocculation potential of the mixed liquor despite viable F/M ratios. In this respect, viable countermeasures have been applied including the addition of a coagulant, i. e. $FeCl_3$, and temporal separation of feed pulses.

Performance during phase C (cycles 64–108)

Considerably higher conversion efficiency of NO_2^- to N_2O and efficient NO_2^--elimination performance in anoxic phases was achieved by extending anoxic reaction periods (cycles 64-108, Figure 2c). An average volumetric elimination rate of 3.36±1.03 $mg_{NO_2\text{-}N}$/(L·h) in anoxic periods of operational phase B (Table 2) indicated a reaction time of approximately 24 h was sufficient for complete NO_2^- removal. The resulting SBR cycle applied in operational phase C comprised 12 h of anaerobic and 24 h of anoxic reaction (Figure 2c). Under these conditions, nitrite was reliably eliminated (95.3±7.9%) at a nitrogen sludge load of 48±8 $mg_{NO_2\text{-}N}$/(g_{MLVSS}·d). Elevated effluent NO_2^- concentrations were observed only during cycles 68–71 and 99–103 (Figure 3c) resulting in an average effluent concentration of 2.67±5.96 $mg_{NO_2\text{-}N}$/L. Reducing the NO_2^- load to 900 $mg_{NO_2\text{-}N}$/d at observed effluent NO_2^- concentrations re-established complete NO_2^- elimination at the applied COD/N ratio of 4 and the operation was continued at 960 $mg_{NO_2\text{-}N}$/d.

Additionally, a stable MLVSS concentration (1.80±0.33 g_{MLVSS}/L) compared to previous phases could be maintained at hydraulic retention times of 0.97±0.22 d. This resulted in sludge specific COD and NO_2-N loads summarized in a COD/N/MLVSS ratio of 170 mg_{COD}:40 $mg_{NO_2\text{-}N}$:1 g_{MLVSS} for stable operating conditions. Considering sludge properties, similar to the previous phases, the sludge was well settleable throughout this period and exhibited SV and SVI values of 350±70 mL/L and 102±12.9 mL/g_{MLVSS}, respectively.

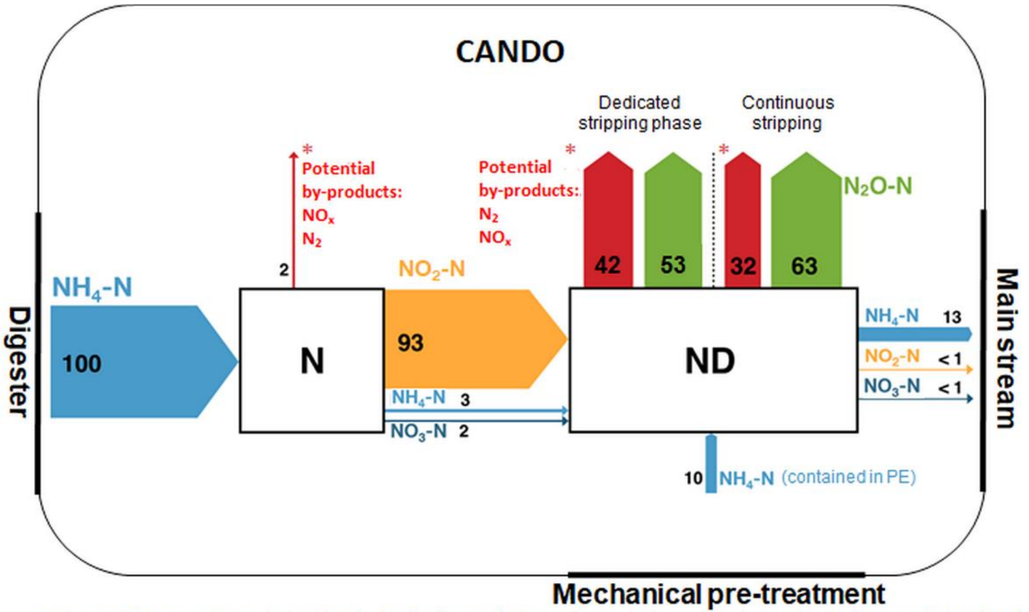
Table 2. Overview of operational settings and executed loads during continuous SBR operation in the investigated phases as well as the observed sludge characteristics, effluent concentrations and average rates of NO_2-N removal and N_2O production throughout the cycles.

Operational condition		Phase A^a	n	Phase B^a	n	Phase C^b	n
SBR cycles	-	23	-	40	-	45	-
N-load	[mg$_{NO2\text{-}N}$/(L·cycle)]	30.0 ± 0.00	23	79.3 ± 7.63	40	78.2 ± 3.04	45
COD-load	[mg$_{COD}$/(L·cycle)]	90.0 ± 0.00		255 ± 40.7		312 ± 13.3	
COD/N	[-]	3		3		4	
F/M	[-]	0.08 ± 0.02	9	0.27 ± 0.09	15	0.18 ± 0.03	25
COD:N:MLVSS	[mg$_{COD}$/mg$_{NO_2\text{-}N}$/g$_{MLVSS}$]	75:25:1		252:84:1		172:43:1	
t_{anoxic}	[h]	12	23	12	40	12	45
$t_{anaerobic}$	[h]	12		12		24	
Sludge characteristics							
MLTSS	[g$_{MLTSS}$/L]	1.57 ± 0.39	9	1.45 ± 0.63	15	3.34 ± 0.50	25
MLVSS	[g$_{MLVSS}$/L]	1.22 ± 0.30		0.95 ± 0.36		1.80 ± 0.33	
SV	[mL/L]	170 ± 50		130 ± 80	9	350 ± 70	12
ISV	[mL/g]	108 ± 5.48		98.3 ± 16.5		102 ± 12.9	
Effluent concentrations							
NO_2-N_1	[mg$_{NO2\text{-}N}$/L]	8.7 ± 12.0	6	34.8 ± 12.1	18	2.67 ± 5.96	43
NO_3-N_1	[mg$_{NO3\text{-}N}$/L]	<0.23		0.60 ± 0.50	6	0.31 ± 0.57	12
NH_4-N_1	[mg$_{NH4\text{-}N}$/L]	57.6 ± 12.8		49.4 ± 4.49		46.4 ± 3.84	6
$tCOD_1$	[mg/L]	170 ± 31.2		239 ± 53.9		236 ± 56.6	
Average process rates applying N_2O accumulation							
rNO_2-N_{MLVSS}	[mg$_{NO2\text{-}N}$/(g$_{MLVSS}$·h)]	2.66 ± 1.03	6	3.35 ± 0.75	13	1.78 ± 0.35	18
rNO_2-N_V	[mg$_{NO2\text{-}N}$/(L·h)]	3.32 ± 1.30	9	3.36 ± 1.03		3.08 ± 0.33	34
rN_2O-N_{MLVSS}	[mg$_{NO2\text{-}N}$/(g$_{MLVSS}$·h)]	1.30 ± 0.20	6	1.74 ± 0.87		1.05 ± 0.20	17
rN_2O-N_V	[mg$_{NO2\text{-}N}$/(L·h)]	1.76 ± 0.32	9	1.41 ± 0.84	36	1.81 ± 0.30	34
Average process rates applying continuous N_2O stripping							
rNO_2-N_{MLVSS}	[mg$_{NO2\text{-}N}$/(g$_{MLVSS}$·h)]	-		3.73 ± 0.61	4	2.03 ± 0.32	7
rNO_2-N_V	[mg$_{NO2\text{-}N}$/(L·h)]	-		4.29 ± 0.28		3.27 ± 0.07	
rN_2O-N_{MLVSS}	[mg$_{NO2\text{-}N}$/(g$_{MLVSS}$·h)]	-		2.52 ± 0.43		1.34 ± 0.30	
rN_2O-N_V	[mg$_{NO2\text{-}N}$/(L·h)]	-		2.94 ± 0.14		2.16 ± 0.22	

^{a}considerable variations of MLVSS concentrations, ^{b}stable operating conditions

The sludge specific NO_2^- elimination rates of 1.8±0.4 mg$_{NO_2\,N}$/(g$_{MLVSS}$·h) were in the lower range, but compare well with reaction rates in conventional heterotrophic denitritation/denitrification processes of 1.25 mg$_N$/(g$_{MLVSS}$·h) to 4.2 mg$_N$/(g$_{MLVSS}$·h) (Chu et al., 2009; Wang et al., 2014). The average sludge specific N_2O production rate during accumulating cycles was 1.05±0.20 mg$_{N_2O\text{-}N}$/(g$_{MLVSS}$·h). In cycles of continuous N_2O removal, higher rates of 1.34±0.30 mg$_{N_2O\text{-}N}$/(g$_{MLVSS}$·h) were observed (Table 2). Accordingly, higher N_2O yields of 68.0±6.14% could be generated applying continuous stripping compared to dedicated stripping phases, which generated 57.5±8.12% (Figure 3d).

The total process performance in terms of N_2O production, which was obtained from a mass balance over both stages, reveals 63% or 53% conversion of NH_4-N to N_2O-N at stable operating conditions applying continuous N_2O removal or dedicated removal phases (Figure 4). Additional shares of 37% and 47%, respectively, are also removed in the gas phase, which most likely mainly consist of nitrogen gas. Regarding the pH of 6.9 during striping periods, the share of possibly stripped ammonia is likely negligible in the mass-balance and residual ammonium loads will have to be treated when the effluent is provided back to the main-stream (Figure 4). The stability of nitric oxide in anoxic famine conditions (Liu et al., 2015a) also indicates a minor share among possible by-products.



- Respective masses were derived by closing the N mass balance. By-products are listed according to the expected order of likelihood

Figure 4. Process dynamics in cycle 108; a): dissolved concentrations of nitrite (NO_2-N), nitrous oxide (N_2O-N), ortho-phosphate (PO_4-P) and polyhydroxybutyrate (PHB); b): gas-phase N_2O concentration ($c(N_2O)_g$) and cumulative mass of N_2O-N removed ($m(N_2O\text{-}N)$); c) deviations of dissolved COD (dCOD) and particulate COD (pCOD) over the SBR cycle and d): online measurement of the oxidation reduction potential (ORP) and pH.

Based on these results, it is evident that aiming for high yields, a continuous removal strategy appears to be more suitable. Possible reasons for the observed difference are the rate constants for production and consumption of N_2O under carbon limited conditions. Recently, this bottleneck has been reported and applied to model PHB-related N_2O emissions at limitation of extracellular carbon (Liu et al., 2015a). In return, it indicates that continuous removal is possibly an effective measure to increase N_2O yields as observed in this study. In contrast to the CANDO process with an intended N_2O production, minimizing stripping effects and addition of excess carbon are potent precautionary measures for emission control in conventional denitrification. However, considering economic feasibility of the CANDO process, the trade-off between the costs of removal aggregates (e.g., stripping towers) and higher yields must be considered in continuous operation.

Regarding the applicability of PE, the generated yields with acetate-fed systems exhibited similar conversion ranges (78±5%) as reported previously (Scherson et al., 2014a). A comparison of the results by statistical means (unpaired t-test: $\alpha = 0.05$) indicated that carbon supply by PE had no significant effect upon the N_2O yield compared to the application of acetate. Considering COD utilization in operational phase C, on average, 196±24.4 mg_{COD}/L and 222±44.3 mg_{COD}/L for the anaerobic withdrawal and final effluent withdrawal indicated a degradation of approximately 60%, which compares well with the BOD measurements. Additional COD, which was introduced by the nitrogen feed, added up to 25.5 mg_{COD}/L and potentially explains the higher concentration in the final effluent. The results underscore that higher COD/N ratios need to be adjusted according to the biodegradable COD in real wastewater applications to achieve stable operating conditions.

Regarding the effluent COD make-up, dissolved COD concentrations were 56±8.9 mg_{dCOD}/L (first withdrawal) and 84±29 mg_{dCOD}/L (second withdrawal). Effluent particulate COD concentrations contained 140±23.7 mg_{pCOD}/L (first withdrawal) and 137±50.2 mg_{pCOD}/L.

Next to the long-term assessment, detailed investigation of process dynamics in a representative cycle (Figure 5) were conducted. The observed deviations are very similar to previously reported observations applying synthetic feeds in the CANDO process (Gao et al., 2017c; Scherson et al., 2014a). The production of PHB was observed simultaneously to the expense of intracellular polyphosphate, which was released as ortho-phosphate in the anaerobic reaction period (Figure 5a). The concentrations of dCOD, however, remained on a constant level (Figure 5c) after two hours of reaction, which indicates complete consumption of readily degradable organic matter (e.g. VFA) contained in the PE. In return, the organic carbon required for PHB production must have been originated from the more complex particulate fraction, which has previously been described for the operation of granular sludge systems under feast/famine conditions (Kreuk et al., 2010; Morgenroth et al., 2002). This assumption is supported by the observed pH decline during the anaerobic phase (Figure 5d), which is an indicator of hydrolysis or acidogenesis of the more complex particulate fraction during anaerobic reaction periods (Wellinger et al., 2013). Most likely, particulate organic matter is hydrolyzed and simultaneously taken up by the mixed liquor biomass, which could explain, why the dissolved fraction remains on a constant level. The exact mechanisms of the decomposition and uptake however, need further investigations for better understanding.

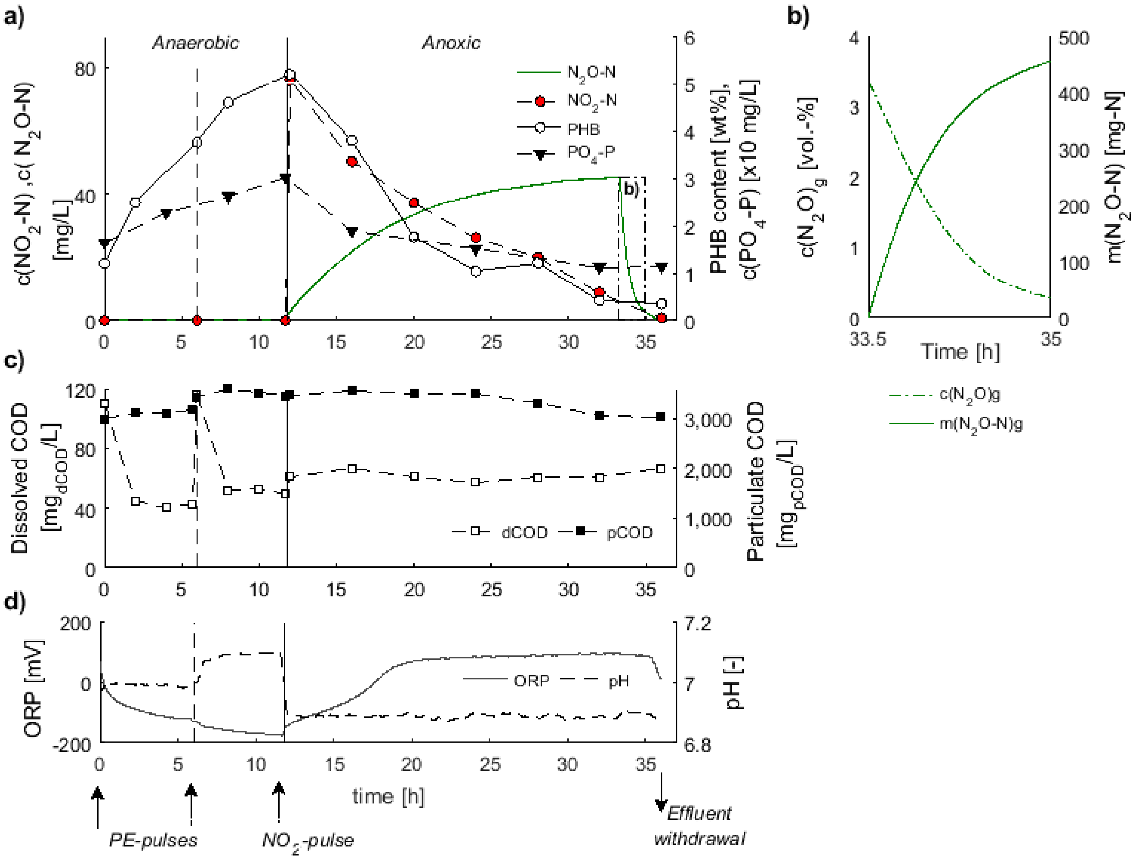


Figure 5. Process dynamics in cycle 108; a): dissolved concentrations of nitrite (NO_2-N), nitrous oxide (N_2O-N), ortho-phosphate (PO_4-P) and polyhydroxybutyrate (PHB); b): gas-phase N_2O concentration ($c(N_2O)_g$) and cumulative mass of N_2O-N removed ($m(N_2O\text{-}N)$); c) deviations of dissolved COD (dCOD) and particulate COD (pCOD) over the SBR cycle and d): online measurement of the oxidation reduction potential (ORP) and pH.

In anoxic reaction period, PHB was consumed as reducing equivalent for NO_2^- reduction and resulted in N_2O accumulation. Additionally, phosphate re-uptake was observed during anoxic conditions, which indicates the P-removal potential of the CANDO process as recently described by Gao et al. (2017).

Next to process dynamics of matter, a characteristic pattern of the oxidation reduction potential (ORP) was observed and appeared useful for judging about the current system state (Figure 5d). It continuously increased in the presence of NO_2^- and N_2O and decreased as soon as both were depleted. These findings indicate the potential of the ORP as a surrogate parameter for nitrogen abundance in the mixed liquor, similar to the "swinging ORP" approach by (Lackner et al., 2012b) proposed for the deammonification process.

N_2O removal in the subsequent stripping phase yielded a maximum off-gas concentration of 3.5 % v/v N_2O at the beginning and continuously decreased over the stripping time (Figure 5b). Previously, a 6.5% higher energy gain was reported applying N_2O/CH_4 mixtures (11% v/v of N_2O) operating a combined heat and power unit (Scherson et al., 2014a) by which the technical potential of dedicated stripping phases is indicated. In contrast, a maximum off-gas concentration of only 0.34% v/v was observed for continuous removal (cycle 93).

4 Conclusions

These results demonstrate the feasible operation of the CANDO process under real feed-stream conditions. This will considerably mitigate operational costs compared to previously applied synthetic feeds. In continuous operation, the CANDO process does not rely on readily degradable organic matter such as VFAs or carbonic acids, which is crucial considering the typical composition and high fractions of more complex and particulate organic matter in domestic wastewater. Additionally, stable N_2O production requires clear separation of anaerobic and anoxic phases. Higher N_2O yields are generated applying continuous removal of N_2O from the mixed liquor during anoxic periods compared to dedicated removal phases.

5 Acknowledgement

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Supplementary information

Supplementary data for this work is given in *Appendix B*.

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7. Application of the oxidation reduction potential for process control and monitoring nitrite in a Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO)

For efficient process operation, reliable control parameters are crucial. Eventually, the application of direct measurement signals and surrogate parameters facilitates process automation. Especially regarding technical implementation and process control of SBRs, robust control variables are essential to serve operational and environmental safety as well as feasibility.

Based on the results of the previous chapter, the following section illustrates the potential of the oxidation reduction regime (ORP) for the operation control of the second CANDO stage motivated by previous findings about the application of the ORP for the control a single-stage deammonification system (Lackner et al., 2012a).

In this respect, the following hypothesis was successfully tested:

> *"The ORP can be used as a surrogate parameter during the anaerobic CANDO II phase controlling the presence of nitrite based on a threshold value which has to be determined. The second stage can be monitored by conducting online headspace and mixed liquor online N_2O-measurements."*

Absolute values, however, were not suitable for process automation, but the ORP gradient in anaerobic and anoxic phases served for automation of the second CANDO stage. Additionally, online N_2O-measurements were confirmed as a means for process control, but considering robustness, investment and maintenance costs, the ORP appears to be the more viable alternative in full-scale applications.

In addition to the process automation strategy of the first stage presented in **Paper #2**, the results provide a complete automation solution over both stages under real feed-stream conditions.

Application of the oxidation reduction potential for process control and monitoring nitrite in a Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO)

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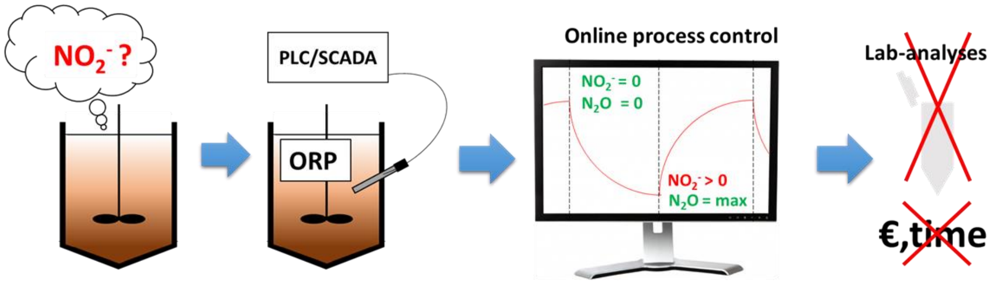

Abstract

The investigations were conducted for the second stage of a continuously operated Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO) over 84 cycles. The oxidation reduction potential (ORP) was investigated in relation to effluent nitrite concentrations, N_2O yields and processing conditions. Characteristic deviations for stable operation conditions, underload and overload could be derived based on representative cycles. Additionally, the ORP gradient was investigated in respect to its potential for process control. The results revealed that an increasing ORP is correlates to the abundance of NO_2^- in anoxic reaction periods and that depletion of NO_2^- correlates with switching from a positive to negative gradient. The results could be underlined be intensive investigation of process dynamics of a single cycle. Ultimately, the reactor was successfully operated at different COD/N ratios and automatically controlled by applying an ORP gradient of -1 mV/min over 20 minutes as termination criterion. The operation resulted in reliable NO_2^- elimination. Additionally, previous observations concerning the necessity of a COD/N ratio of 4 could be confirmed concerning optimal N_2O yields. A COD/N ratio of 5 compromised the N_2O yield to a higher nitrogen removal rate.

1 Introduction

In conventional wastewater treatment, heterotrophic denitrification is widely applied as part the nitrification/denitrification process for nitrogen removal. Throughout this operation, a considerable share of organic loads is utilized as reducing equivalents for nitrate reduction (7.6 kg_{COD}/kg_N) (Sobieszuk and Szewczyk, 2006a). As a result, in conventional treatment configurations the energy recovery potential contained in organic wastewater compounds (on average 1.93 kWh/m^3) (McCarty et al., 2011) is limited to the required denitrification capacity. To decouple denitrification from COD demand and hence, to increase energy recovery potentials, alternative microbial pathways or short-cuts in the microbial metabolism are promising (Weißbach et al., 2017a). Based on these pathways, alternative processes for instance, partial nitritation/deammonification (PN/D) (Joss et al., 2009c), nitritation/denitritation (N/D) (Wang et al., 2016) or the Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO) (Scherson et al., 2013a) have been developed. Next to a lower organic carbon demand, the oxygen demand can be reduced by up to 60% compared to conventional nitrification (Horstmeyer et al., 2018) by constraining the aerobic processes to the oxidation of ammonia to nitrite (NO_2^-) instead of nitrate (NO_3^-). However, tight processing conditions are required to trigger these alternative metabolic pathways. For this purpose, robust measurement signals of control variables and surrogate parameters are essential to maintain stable operation. Full-scale operation of PN/D, for instance, is controlled by online measurements of ammonia, nitrate and oxygen (Joss et al., 2009c). An additional example are full scale granular reactors (Abma et al., 2010) for PN/D operated with continuous aeration and monitoring of effluent ammonia and nitrite concentrations. Considering novel treatment technologies, the ORP has been investigated for the operation of a single-stage PN/D system published as 'Swinging ORP' (Lackner et al., 2012a).

Like in most of the alternative nitrogen removal technologies, the first stage of the CANDO process is a nitritation ($NH_4^+ \rightarrow NO_2^-$) stage. In the second stage, nitrite is biologically reduced to nitrous oxide ($NO_2^- \rightarrow N_2O$) (Scherson et al., 2014b; Scherson et al., 2013a) for subsequent harvesting and energetic utilization. Considering the carbon savings, 4.6 kg_{COD}/kg_N can be saved for anaerobic treatment compared to conventional denitrification in the second stage (Weißbach et al., submitted). Additionally, energetic utilization of nitrous oxide by e.g., co-combustion possibly with biogas, yields additional energy. Considering the chemical reaction enthalpy of the N_2O decay, theoretically 37% more energy can be generated by substituting O_2 with N_2O during combustion (Scherson et al., 2013a).

In a previous study about the CANDO process (Weißbach et al., submitted), dissolved oxygen and effluent ammonia concentrations were successfully applied for automated operation control of the nitritation stage. For the nitrous denitritation stage however, only time control has been used so far (Gao et al., 2017a; Scherson et al., 2014b; Weißbach et al., submitted). Because the SBR cycle is characterized by alternating anaerobic/anoxic conditions, application of the oxidation reduction potential holds promise for operational control. Earlier findings about the 'Swinging ORP' already correlated low nitrite and nitrate concentrations with low ORP values in anoxic or anaerobic reaction periods (Lackner et al., 2012a). Considering the CANDO process, a complete elimination of nitrite in anoxic phases of the nitrous denitritation process is essential for maintaining high N_2O yields and effluent quality (Weißbach et al., submitted). In this respect, it is also worth mentioning that residual NO_2^- concentrations are reduced to nitrogen gas in subsequent cycles by denitritation which increases the demand of organic reducing equivalents in the nitrous denitritation stage (Weißbach et al., 2017a). Hence, efficient monitoring of NO_2^- would facilitate efficient energy recovery, effluent quality control and maintaining stable operation under balanced COD/N ratios. Commercially available NO_2^- electrodes in the typical concentration range of 0 to 300 mg NO_2-N/L (Scherson et al., 2014b) are not available. However, in contrast to industrial electrodes optimized for lower concentrations, the ORP has different advantages. Next to an easy maintenance, ORP probes are cheaper than e.g., ion selective systems, and do not rely on compensating probes for e.g., chloride, potassium or NO_3^-. Additionally, they are robust and have proven suitability for operational control since the 1980s (Fuerhacker et al., 2000; Ra et al., 2000; Ra et al., 1998; Tanwar et al., 2008) and provide the possibility to judge about the anaerobic reaction periods as well. However, absolute operational values are not readily transferable to other systems, because they are very much depending on the feed matrix (Lackner et al., 2012a). Accordingly, characteristic deviations of the ORP were investigated in relation to NO_2^- and N_2O under continuous and real-feed stream conditions in an operating nitrous denitritation stage of a CANDO system in this study. Additionally, deviations for stable operation, overload and underload were derived in time controlled operation. Ultimately, the application of the ORP as control variable was successfully tested. Additional assessments concerning the performance and a nitrogen mass-balances were part of a previously published long-term investigation about the operation of the CANDO process under real feed stream conditions (Weißbach et al., submitted).

2 Materials and Methods

2.1 Reactor setup

An automated bioreactor system has been designed and operated for nitritation and subsequent nitrous denitritation. The reactor bodies were equally designed with a reaction volume of 12 L. The configuration for both systems comprised stirrer (IKA-Werke GmbH, Staufen, Germany) and peristaltic feed and dosing pumps (Watson-Marlow, Falmouth, Great Britain). Additionally, a heat exchanger (Huber Kältemaschinenbau AG, Germany) was installed for the nitritation stage. The second stage comprised three additional peristaltic pumps (Heidolph Instruments, Schwabach, Germany) to add carbon feed and coagulant (5% iron-III-chloride) and to withdraw effluent volumes. For N_2O removal, fine bubble aeration stones were installed at the reactor bottom for gas stripping with technical grade nitrogen gas (purity 99.5%, Linde, Munich, Germany).

2.2 Substrates and inocula

Feed samples were collected at the municipal wastewater treatment plant Garching (Population equivalent: 31,000), Germany. The wastewater is screened behind the inlet of the plant by a 4-mm drum screen. Primary effluent samples were collected weekly behind the screen and exhibited COD and NH_4-N concentrations of 468 ± 55 mg_{COD}/L and 54.7 ± 5.3 mg_N/L, respectively (Table 1). Samples were stored at 4 °C. Digester reject water was collected from storage tanks at the WWTP Garching. The centrate contained ammonia concentration of 1,074 ± 173 $mg_{NH_4\text{-}N}$/L and 359 ± 51.7 mg_{COD}/L (COD/N ratio = 0.33). The nitritation stage was operated in sequencing-batch-operation and served the production of nitrogen feed for the second stage. Detailed information about the operation are given in a previous publication (Weißbach et al., submitted).

Throughout the investigations, the reject water contained 965 ± 65.1 $mg_{NO_2\text{-}N}$/L on average after processing in the nitritation stage (Table 2) and hence, exhibited a suitable quality for the second stage. Both stages were inoculated prior to this study described by (Weißbach et al., submitted).

Table 1. Composition of the primary effluent collected at the wastewater treatment plant Garching as COD feed for the second CANDO stage.

COD feed (primary effluent)		mean	std. dev. (±)
tCOD [mg/L]	(n = 21)	468	155
sCOD [mg/L]	(n = 21)	171	44.3
pCOD [mg/L]	(n = 21)	296	151
sCOD/pCOD [-]	(n = 21)	0.83	0.67
NH_4-N [mg/L]	(n = 21)	54.7	5.30
pH [-]	(n = 21)	7.3	0.10

Table 2. Composition of the nitritation effluent used as nitrogen feed for nitrous oxide production in the second CANDO stage.

Nitrogen feed (nitritation effluent)		mean	std. dev. (±)
NH_4-N [mg_N/L]	(n =18)	30.3	12.5
NO_3-N [mg_N/L]	(n =18)	57.4	33.0
NO_2-N [mg_N/L]	(n =18)	965	65.1
Sum-N [mg_N/L]	(n =18)	1,053	70.0
COD [mg/L]	(n =18)	468	151
pH [-]	(n =18)	6.99	0.25

2.3 Operation

Considering the nitrous denitritation stage, this study refers cycles 39-63 (referred to as ‘phases A’ in this study) and cycles 64-108 (referred to as ‘phase B’) of the previous publication. In both phases, initial nitrogen concentrations of 80 $mg_{NO_2\ N}$/L were applied. The SBR operation in phase A consisted of a 24-h cycle which comprised two pulses of primary effluent (COD) (0 h and 5.8 h) and a NO_2^- pulse of after 11.6 h of reaction at a COD/N ratio of 3 (240 mg_{COD}/L). In phase B, the anoxic reaction period length was extended to 24 h resulting in a total 36-h cycle. The applied COD/N ratio was increased to 4 at initial NO_2-N concentrations of 80 $mg_{NO_2\text{-}N}$/L. Throughout anoxic reaction periods (after the nitrite pulse), control of pH was automatically conducted at pH = 6.9±0.01. In an additional operational phase C (cycles 109–123), the oxidation reduction potential was investigated as operational control variable. For this purpose, the system was operated at varying COD/N ratios (3, 4 and 5) at fixed initial NO_2^- concentrations of 80 $mg_{NO_2\text{-}N}$/L. As a result, of the previous investigations, a continuous ORP gradient of $\Delta ORP \leq -1$ mV/min over 20 minutes was applied as termination criterion of the anoxic reaction period.

For N_2O removal two different strategies were applied and investigated. The first was the integration of a dedicated SBR-phase for gas stripping (flow of 30 l/h) at the anoxic reaction periods. The second strategy consisted of continuous N_2O removal by gas stripping (15 l/h) throughout the anoxic reaction which was applied in operational phase C and 8 cycles in phases A and B. In both operational strategies, the cycles ended with a 45-min settling phase and subsequent effluent withdrawal.

2.4 Analytical methods

The reactors were monitored and controlled by a SCADA system (Citect SCADA, Schneider Electric, Rueil-Malmaison, France) and a corresponding PLC (WAGO, Minden, Germany). Online-data was collected with different probes (Endress+Hauser, Reinach, Switzerland) for: pH, temperature (T), dissolved oxygen (DO), oxidation–reduction potential (ORP), conductivity (Con), and mixed-liquor volume (V). For the measurement of dissolved nitrous oxide, a micro-electrode (N_2O-R sensor, Unisense, Arhus, Denmark) with a measurement range of 25-2,500 μmol_{N2O}/L was employed in the nitrous denitritation stage. Strip gas N_2O concentrations were quantified with a photoacoustic gas analyzer (Thaler et al., 2017a).

For measurements of effluent and feed as well as grab sample concentrations of COD, NH_4-N, NO_2-N, and NO_3-N, photometrical cuvette tests (Hach-Lange, Berlin, Germany) were used according to the manufacturer’s instructions. Nitrogen and dissolved COD (dCOD) samples were filtrated (0.45 µm) and stored for no longer than 12 h at 4 °C before measurement.

PHB sample preparation and extraction was conducted according to Riis and Mai (Riis and Mai, 1988). After freeze-drying over-night, the samples were subjected to propanolysis. PHB concentrations were measured with a GC-FID device (7890A, Agilent, Santa Clara, CA, USA) equipped with a DB-5 column in reference to Wang et al. (Wang et al., 2013).

3 Results and Discussion

3.1 ORP characteristics in continuous operation

The nitrous denitritation reactor was operated in the operational phase A at a COD/N ratio of 3. At the applied conditions (i.e., COD/N ration = 3, 12-h anaerobic and 12-h anoxic reaction time) 45.6±12.7% of the initial nitrite concentration was eliminated throughout the anoxic reaction periods resulting in continuous abundance of residual NO_2^- concentrations at the beginning of the subsequent reaction periods (Figure 1a). Residual effluent concentrations were on average 33.0±9.7 mg NO_2-N/L. In relation to the initial NO_2-N concentration, on average 29.7±11.4% were converted to N_2O-N and accumulated in the mixed liquor before removal. This corresponds to a share of N_2O in the denitritation products of 65.2% applying continuous accumulation. The conductivity throughout the cycle had no distinct correlation to NO_2^- or N_2O (Figure 1b). In contrast, in continuous operation with residual NO_2^- concentrations, the development of the ORP throughout operational phase A was characterized by initially increasing values in intentionally anaerobic reaction phases. Generally, the ORP deviation resembled an amplitude that was dominated by the presence of nitrite i.e., increasing when present, decreasing, when absent. Typically, nitrite was eliminated before the second primary effluent feed pulse. Accordingly, the pH slightly increased by denitritation activity (Figure 1b). Additionally, it was evident that after complete denitritation the ORP values started to decrease continuously with a considerable slope of approximately -2 mV/min (Figure 1a, cycle 42) until -320 mV and then continued with a lower gradient until the end of the anaerobic reaction period. The minimum ORP value at the end of an anaerobic reaction phase was -391 mV observed in cycle 35.

On average, the ORP value was -290 mV before the nitrite pulse. In the anoxic reaction phases, the ORP increased immediately by the addition of hydrochloric acid for pH control. Afterwards the development continued until the stripping phase was initiated for N_2O removal. However, the observed ORP drop throughout the stripping phases is suspected to be caused by CO_2 removal and a subsequent pH increase (Figure 1b). Accordingly, after the stripping period, the ORP values started to increase again in the presence of NO_2^-. Additionally, a considerable drop was always related to the first primary effluent pulse with an average pH value of 7.3±0.1 which lifted the mixed liquor pH at the pulse and eventually resulted in a mitigation of the ORP (Figure 1b).

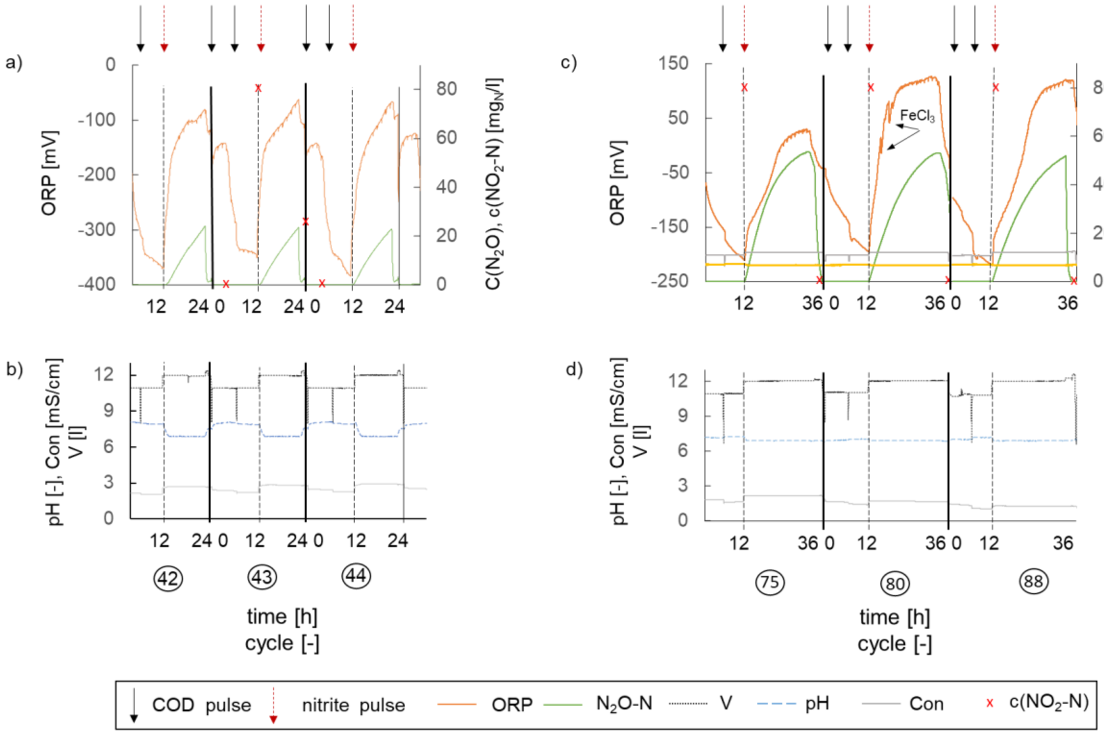

Figure 1. Cycle structure and charcteristic deviations of online-data and NO_2^- concentrations under NO_2^- overload (a-b) and stable operating conditions (c-d) in time-controlled operation.

By increasing the COD/N ratio from 3 to 4 and extending anoxic reaction periods to 24 h, nitrite was reliably eliminated until the end of the cycle in operational phase B (Figure 1c). Additionally, the N_2O yields were higher, because nitrous denitritation was conducted until NO_2^- was completely depleted throughout the anoxic reaction periods. As described in (Weißbach et al., submitted)), the average conversion of NO_2-N to N_2O-N was 58.1±8% and 68.8±8% in a dedicated stripping phase or continuous stripping in anoxic phases, respectively. The stripping strategy however, had no effect upon the ORP deviations.

As observed in operational phase A, the ORP continuously decreased at the beginning of the anaerobic periods (-2 mV/min) until approximately -120 mV, when nitrite was eliminated in a previous cycle. Subsequently, the development continued gradually until the end of the anaerobic reaction phase. The lower boundary of the amplitudes was generally higher than observed in operational phase A. The average minimum value throughout the operational phase was -171 mV (minimum value: -229 mV). The values rapidly increased after the nitrite pulse and the beginning of N_2O production (Figure 1b). As in phase A, the pH control was responsible for the first increase. In all cycles exhibiting complete nitrite elimination, the ORP started to decrease toward the end of the cycle and continued during N_2O removal, settling and withdrawal phases. In both operational phases, small dips in regular intervals were observed caused by $FeCl_3$ addition (Figure 1c).

Main difference between operational phase A and operational phase B in terms of the microbial pathways can be derived by N_2O concentration in the mixed liquor. While in operational phase B complete denitritation occurred in the presence of extracellular carbon provided by the primary effluent, nitrous denitritation took place in anoxic reaction periods of operational phase B, when extracellular or easily degradable carbon is depleted (Weißbach et al., submitted). As in operational phase A, the dominant control variable for the behavior was NO_2^- abundance. Therefore, the ORP can potentially serve as semi-quantitative surrogate parameter for operational control of NO_2^- effluent concentration in a CANDO system. However, absolute values as threshold values appear to be not suitable for operation considering the deviation of maxima and minima values (Figure 2b).

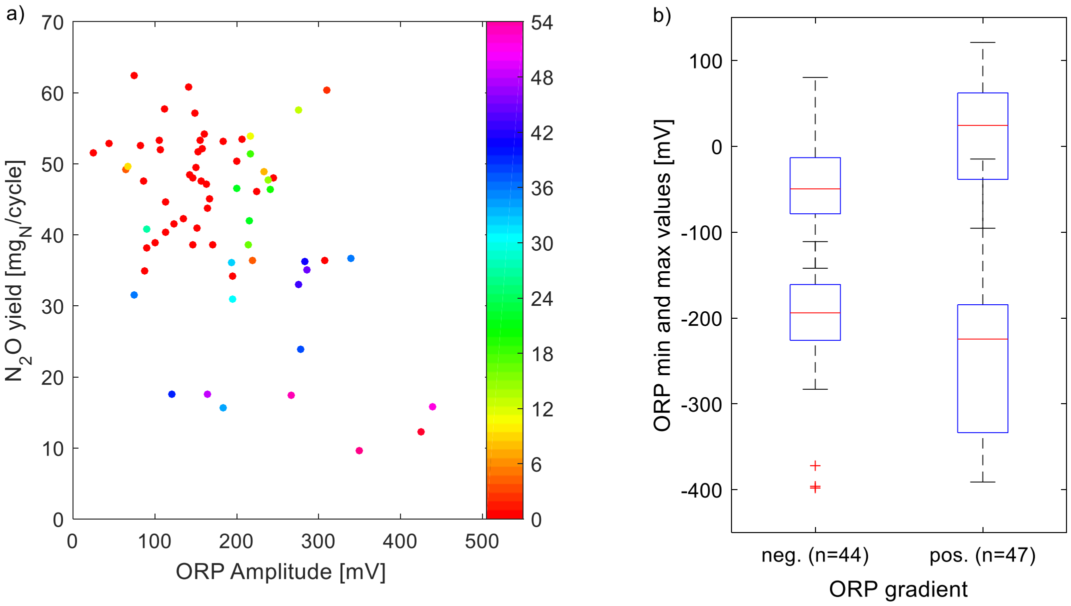

Figure 2. a) Illustration of effluent NO_2-N concentrations displayed in respective colors in relation to the ORP amptlitude and N_2O yields. b) Absolut maxima and minima values of the ORP in relation to negative and positive ORP gradients at the end of anoxic reaction periods.

For better characterization of the ORP development and process performance, the ORP deviations were compared. It was evident that an amplitude in the range 50 mV to 200 mV correlated with reliable nitrite elimination and high N_2O yields (Figure 2a). Taking the ORP gradient over the operational periods into account underlines the observation that negative gradients and therefore, complete NO_2^- elimination correlates with smaller amplitudes (Figure 2b). Despite the differences regarding the absolute ORP values between the cycles in continuous operation, the investigations revealed a distinct correlation between the ORP gradient and the abundance of nitrite within the anaerobic or anoxic reaction periods.

Considering the desired operational conditions of the CANDO i.e., aiming for high N_2O yields and low effluent concentrations, an amplitude of approximately 150 mV in the range of -200 mV and -50 mV enables high N_2O production and NO_2^- elimination performance under the investigated conditions. Additionally, a negative ORP gradient is a suitable surrogate parameter for effluent quality control in terms of residual NO_2^- concentrations. In this respect, increasing ORP values toward the end of an SBR cycle are an indicator for an unbalanced COD/N ratio eventually resulting in a system overload or underload regarding NO_2^- or COD, respectively.

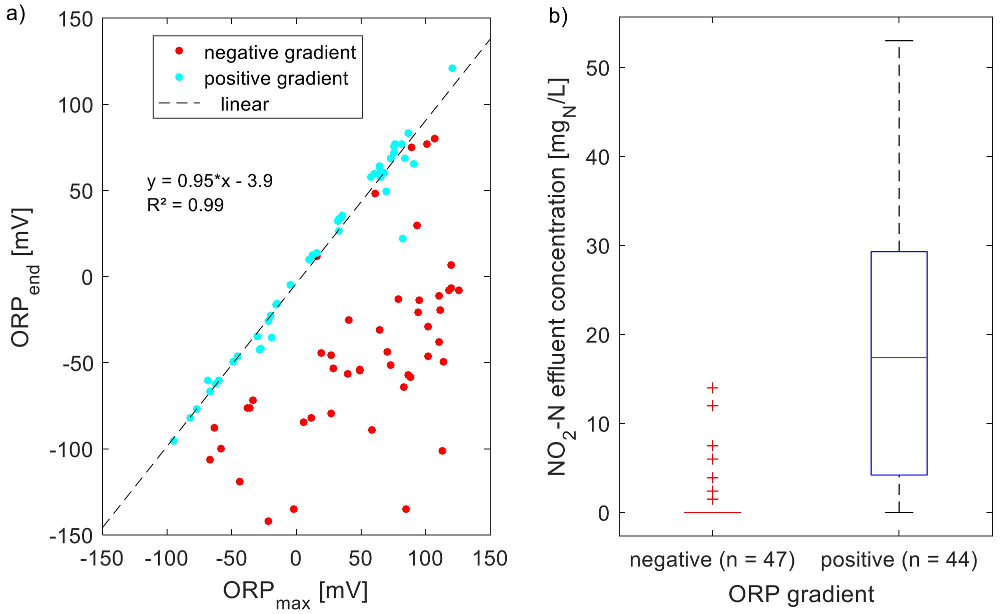

Figure 3. Illustration of the correlation of final ORP values and maximum ORP values in relation the the final ORP gradient displayed in a); illustration of effluent NO_2-N concentrations and final ORP gradient displayed in b).

3.2 Relation of effluent quality, N_2O yield and ORP

To investigate the robustness of the ORP gradient for operation control, a correlation of the maximum ORP value and the final ORP value was conducted. The results yield a linear correlation with a slope of 0.95 for maximum and final ORP values, indicating nearly agreement of both, when the gradient was positive. Lower maximum values than the final ORP values were observed for negative gradients (Figure 4) without distinct correlation. These observations underscore that continuously increasing deviations are a strong indicator for the presence of, NO_2^- in the system. Additionally, out of 47 observations with negative gradient, 7 effluent concentrations were positively tested regarding NO_2^-. However, these observations relate to an observed ORP slope that was considerably lower than observed for deviations, when NO_2^- was depleted.

To obtain a better resolution of the process dynamics in relation to the ORP, a single cycle in the operational phase B was intensively studied. The reaction dynamics were similar to previous findings (Gao et al., 2017a; Scherson et al., 2014b; Weißbach et al., submitted).

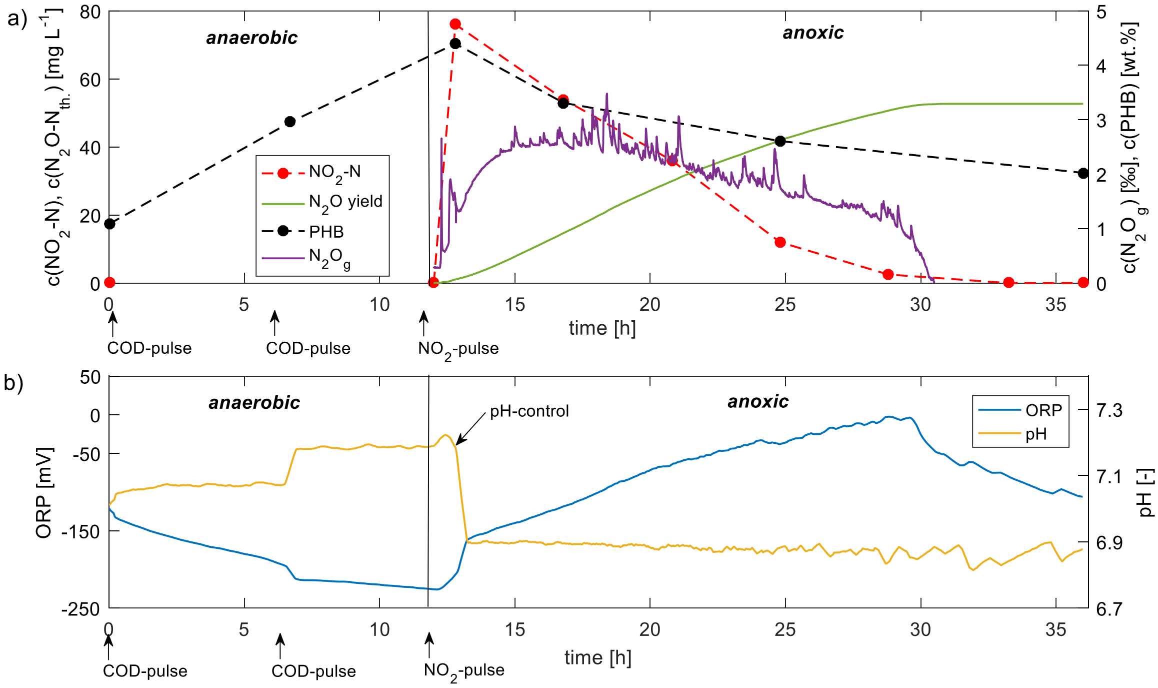

Figure 4. Process dynamics over an SBR cycle: a) development of nitrite, intracellular storage compounds (PHB), N_2O off-gas concentration (N_2O_g) production. In b), development of pH and oxidation reduction potential (ORP) are displayed. (Note: The N_2O yield is displayed as theoretical mixed-liquor concentration to illustrate the relation of the initial NO_2^- concentration, although it was continuously stripped out of the mixed-liquor.)

The PHB concentration increased from initially 1.1 wt.% to 4.4 wt.% in the anaerobic period indicating continuous accumulation of intracellular storage compounds for subsequent endogenous respiration. Simultaneously, the oxidation reduction potential continuously decreased. Together with the PE pulses (pH of the PE 7.6), the ORP and the pH decreased and increased respectively. At the end of the anaerobic reaction period, the pH value was 7.19 and the ORP -224 mV. Initiation of the pH control to 6.9 resulted in an ORP increase at the beginning of the anoxic reaction period. At controlled pH, the ORP further increased along with the degradation of NO_2^- and N_2O production, respectively. Continuous N_2O removal resulted in a maximum off-gas concentration of 2.7‰ after 16 h of reaction. Simultaneously, the intracellular PHB concentration decreased indicating the activation of microbiological endogenous substrate respiration. After approximately 30 h of reaction, NO_2^- was depleted. Simultaneously, the ORP gradient switched distinctly from positive to negative and the N_2O production faded to zero. The results support the previously described observations described in 3.1 and underline the potential of the ORP for the application in CANDO process as a surrogate parameter for NO_2^-. Furthermore, it was evident that the investigated cycle exhibited excess reaction time regarding the NO_2^- depletion after 30 h. Previously, similar deviations were reported by Gao et al. (2017a) applying continuous accumulation of N_2O in the mixed liquor and a dedicated stripping phase. The nitrous oxide production in the second CANDO stage also ceased before the end of representative cycles and subsequently decreased in the mixed liquor toward the end of the anoxic reaction period indicating system underload or excess reaction time. In this respect, it is worth noting that dissolved N_2O is suspected to be prone to microbial respiration, when accumulated in the mixed liquor (Weißbach et al., submitted). Hence, applying the ORP as termination criterion would mitigate microbial N_2O consumption after complete reaction in favor of higher N_2O yields and possibly higher turnover rates by applying continuous accumulation and subsequent stripping as removal strategy.

Based on the results, characteristic deviations for the respective operational conditions for operational phase C were derived by fitting the data of representative cycles for stable operation, overload and underload in relation to the ORP (Figure 5).

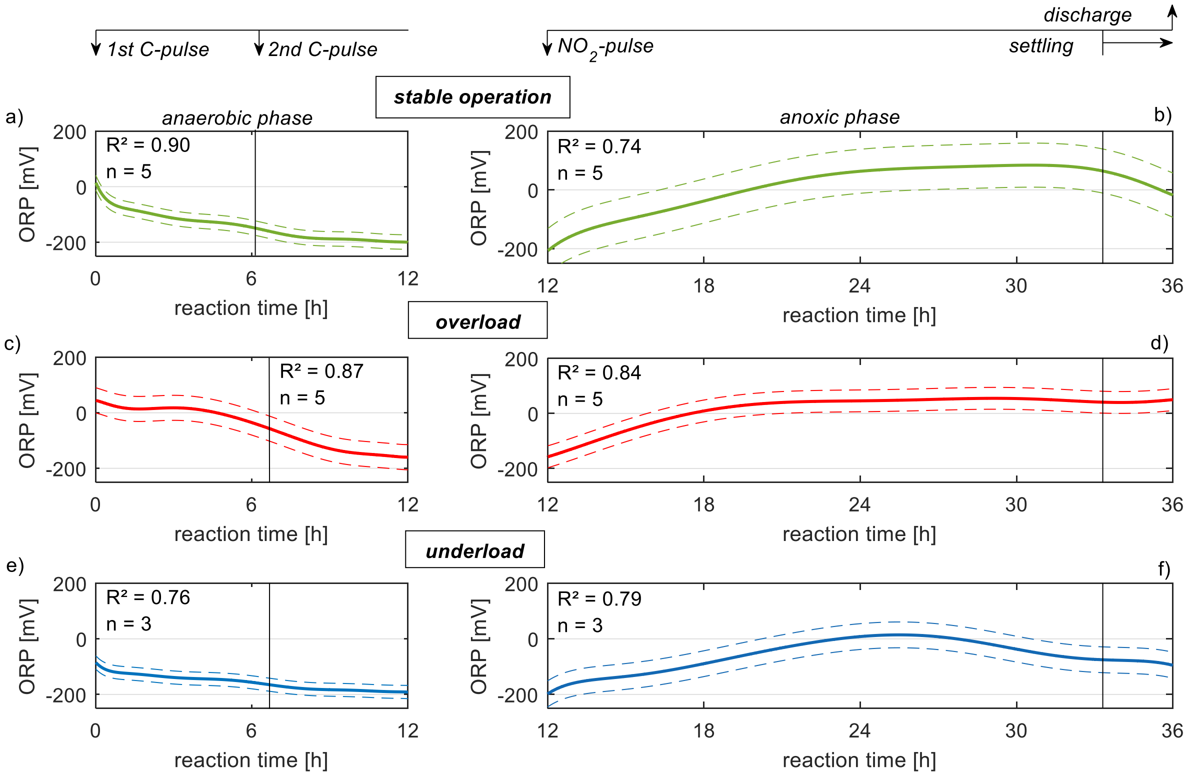

Figure 5. Characteristic mean ORP deviations in 95% confidence bounds (dashed lines) throughout SBR cycles under stable operating conditions (a-b), NO_2^- overload (c-d) and NO_2^- underload (e-f).

Considering stable operation, the deviation of the ORP is characterized by steep decline at the beginning of the anaerobic period followed by a continuous decline until approximately -200 mV (Figure 5a). Subsequently, the ORP rises after the addition of the NO_2^- pulse. The cycle should be terminated at an observed continuous ORP decline for more than 30 minutes (Figure 5b). An overload is evident, when the ORP increases after the addition of the first PE pulse indicating residual NO_2^- concentrations (Figure 5c). Additionally, the ORP continuously increases until end of the anoxic reaction periods (Figure 5d). An underload is characterized by an initial comparably low ORP value (Figure 5e) and an ORP decline which begins more than approximately 30 minutes before settling time (Figure 5f). However, the ORP is depending on the operational conditions as well as substrate composition e.g., salts, pH. Hence, the absolute values are not readily transferable to other systems. The deviation however, is expected to be similar.

3.3 Validation

To validate the automation potential of the ORP, the nitrous denitritation reactor was operated in nitrogen overload at a COD/N ratio of 3 for one cycle (Figure 6a, cycle 109). The settling phase initiated after the ORP value had decreased with a slope of ≤ 1mV/min for 20 minutes continuously (Figure 6b-c). The procedure was repeated for another two cycles (Figure a, cycles 113 and 114). It was evident that the reaction time extended considerably to an average of 38.2 h of anoxic reaction, but successful elimination of NO_2^- was maintained (Table 3).

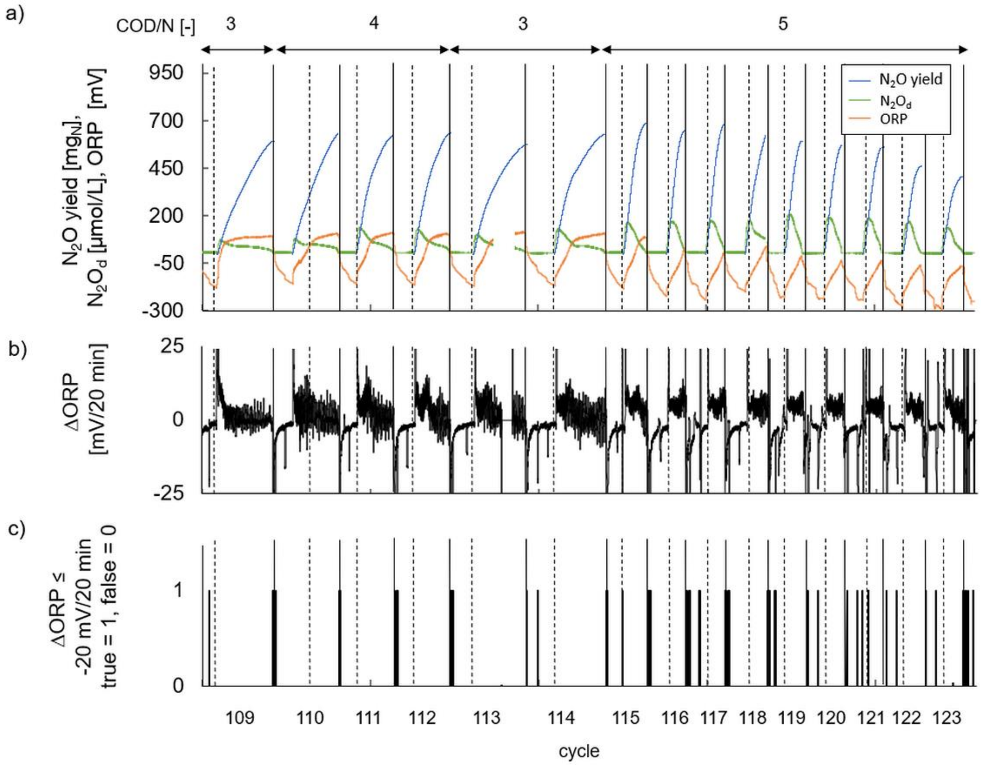

Figure 6. Validation of the ORP as control variable in continuous operation at different COD/N ratios. Displayed are online-data of mixed liquor N_2O concentration (N_2O_d), cummulative off-gas N_2O yield and ORP as well as NO_2-N concentration at the beginning of anoxic reaction periods and in the effluent in a). The ORP gradient over 20-min intervalls is depicted in b) and times, when the ORP gradient was equal or lower than the termination criterion of -1mV/min over 20 minutes is indicated by values of '1' in c).

Table 3. Anoxic reaction times, effluent NO_2^- concentrations and N_2O yields at different COD/N ratios during automated operation of the second CANDO stage depending on the ORP gradient.

COD/N		time	std (±)	NO_2-N	N_2O yield	std (±)	N_2O-N/NO_2-
3	(n = 3)	38.2	0.83	<0.015	600	28.0	62.5
4	(n = 3)	28.6	4.69	<0.015	540	153	56.2
5	(n = 9)	15.2	2.03	<0.015	584	95.7	60.8

Throughout the following cycles which were operated at COD/N = 4, the operation resumed to approximately 24 hours of anoxic reaction and average N_2O-N/NO_2-N conversion of 62.5%. Subsequently, a COD/N ratio of 5 was applied for another 9 cycles. As a result, the N_2O yield was initially higher, but consecutively decreased continuously to a conversion of 42.7%. However, no residual NO_2^- was detected in the effluent. The results suggest that a COD/N ratio greater 4 comprise the nitrous denitritation to denitritation and should be avoided aiming for high N_2O yields.

3.4 Technical implementation potential

The results have demonstrated the implementation potential of the ORP for the control of a nitrous denitritation stage. The ORP is known for its robustness [32] and the presented application does not require compensation for e.g., NO3-, pH or chloride. In contrast to ion-selective measurement technology for nitrite, the ORP is a well-established and flexible technology in wastewater treatment [32] and integration into technical process environments during scale-up of a CANDO system can be easily conducted.

For dissolved N2O measurements, amperometric probes are typically sensitive to temperature and require intensive maintenance and control [33]. Additionally, instruments as applied in this study are relatively expensive and have a shorter life-time compared to ORP probes. Moreover, the measurement of N2O concentrations during production by accumulating N2O in the mixed liquor cannot be conducted by standard measurement equipment, because the observed ranges often vary extremely. Presently, the ORP appears to be an economically and technically viable surrogate parameter to optimize N2O yields and NO2- elimination in a CANDO system. Further preliminary results also indicated its application potential in denitritation systems for effluent and process control (Figure B.2).

4 Conclusion

The ORP is an applicable surrogate parameter for the automation of the second stage of the CANDO process i.e., the nitrous denitritation stage. Its deviations exhibit characteristics that can be utilized for judging about the system state, when operated time controlled. However, application of absolute minima and maxima values cannot be applied due to the considerable variations. Instead, under the investigated operational conditions, an ORP amplitude of approximately 150 mV correlated with high N_2O yields and complete NO_2^- elimination. Additionally, the presence of nitrite can be assessed be the ORP gradient. Applying the gradient for process automation is a feasible means for optimizing the reaction under variable processing conditions e.g., variable COD concentrations applying real wastewater feed streams. Its application facilitates quality control concerning effluent NO_2^- concentrations and can be applied as termination criterion in sequencing-batch-operation. Further research should be conducted to assess correlations in anaerobic reaction periods. A more detailed understanding of the ORP and anaerobic processes of e.g., hydrolysis and acidogenesis, are key for PHB production which is suspected to be key for efficient heterotrophic N_2O production.

5 Acknowledgements

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Supplementary information

Supplementary data for this work is given in *Appendix C*.

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8. Separation of nitrous oxide from aqueous solutions applying a micro porous hollow-fiber membrane contactor for energy recovery

Membrane technology is already widely applied in the wastewater treatment sector for liquid-liquid processes e.g., membrane bioreactors or water purification. For gas separation processes, or liquid-gas applications, the removal of volatile organic compounds has also been demonstrated. In addition, presently emerging research concerns the development of biogas purification processes and the removal of residual methane from digester effluents to potentially increase the energy recovery potential of anaerobic digestion processes on WWTPs.

The application conditions are similar to the ones expected for the application in a running CANDO system. For instance, real digester reject water has been previously treated as demonstrated by Cookney et al. (2013). Typically, porous hollow-fiber membrane contactors are applied for these gas-liquid separation processes because of their higher permeability compared to dense membranes.

Considering the successful application for methane removal under comparable conditions, the application of hollow-fiber modules holds promise for harvesting N_2O from a CANDO system. In addition to environmental conditions, however, also the gas properties play a considerable role as a naturally limiting factor for diffusive mass transport. In this respect, especially the solubility of N_2O, expressed by the Henry constant, is considerably lower compared to e.g., methane (Sander, 1999).

However, to assess the potential and derive operational strategies for the removal of N_2O under conditions representative of a running CANDO system, a hollow-fiber membrane contactor has been studied. Mass transfer analyses and the study of dimensionless numbers to describe the mass transport behavior under varying processing conditions have been conducted and successfully validated the corresponding hypothesis:

> *“N_2O extraction for subsequent combustion can be conducted by the use of a membrane contactor device especially regarding the similar gas properties of CO_2 and N_2O.”*

Extraction efficiencies above 90% in relation to initial nitrogen loads have indicated the potential for N_2O removal for technical implementation. Additionally, the stripping gas demand could be reduced compared to conventional gas stripping (**Paper #2**) by 70% underlining the implementation potential also in relation to operational costs.

Separation of nitrous oxide from aqueous solutions applying a micro porous hollow fiber membrane contactor for energy recovery

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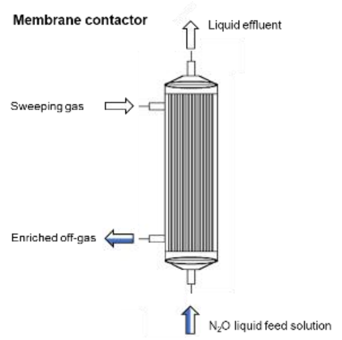

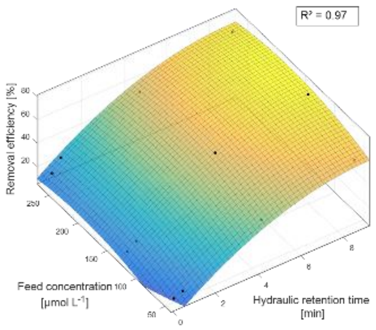

Abstract

Nitrous oxide (N_2O) is a potent green-house gas, but has also recently been acknowledged as sustainable energy source that can intentionally be produced by bioreactor systems such as the **C**oupled **A**erobic-anoxic **N**itrous **D**ecomposition **O**peration (CANDO). In this study, the application potential of a porous hollow fiber membrane contactor has been assessed at expected conditions of an operating CANDO system in shell-side liquid feed operation for N_2O removal. At varying feed concentrations, liquid and gas flow rates, off-gas concentrations, removal efficiencies, trans-membrane fluxes and mass-flows have been assessed and compared to previous studies on CO_2. Under the applied operational conditions, the gas flow rate had no significant effect on the results. Removal efficiencies up to 77% of the module feed load demonstrated a high application potential for the CANDO process and indicate a promising removal potential greater 90% in reference to typical initial substrate loads in an operating CANDO system. Additionally, considerably higher off-gas concentrations compared to recently published results applying conventional stripping technology of 3,700 ppmv were generated reducing sweeping gas demand by 70%. The mass transfer was dominated by the superficial velocity on the liquid side and its behavior could be well described by Sherwood correlations. However, previously published mass transfer correlations over-estimated the results. Hence, a more appropriate correlation is presented to describe the observations of this study.

1 Introduction

Environmental concerns regarding climate-change and pollution have motivated the development towards climate-friendly and sustainable technologies. In this respect, resource and energy recovery from wastewater and organic waste are potential key technologies in the water-climate-energy nexus (McCarty et al., 2011). Considering energy recovery from organic waste and biomass, anaerobic digestion for biogas production and subsequent combustion in a combined heat and power unit (CHP) is a well-established process for sustainable electricity and heat generation. However, an inherent drawback of anaerobic digestion is the ammonia-rich digestate effluent, which requires further treatment to meet effluent quality restrictions.

State-of-the-art biological post-processing technologies for ammonium removal are partial nitritation/anammox (Joss et al., 2009a) or nitritation/denitritation (Fux et al., 2006) among others. However, these processes have recently come to the fore due to considerable emissions of N_2O (Kampschreur et al., 2009). These emissions are especially critical considering the CO_2-equivalent of N_2O of approximately 300 and hence, its global warming potential (Ravishankara et al., 2009). Additionally, stratospheric N_2O catalyzes the decay of ozone and is suspected to have considerably contributed to the destruction of the stratospheric ozone layer in the last century (Ravishankara et al., 2009). Its emissions should hence, be mitigated as far as possible.

Presently, various mechanisms are being discussed, which are related to N_2O emissions and specific processing conditions in biological nitrogen removal systems. Among others, ammonium and nitrite concentration gradients, the oxidation reduction regime as well as chemical oxidation of biological metabolites are investigated to derive process control strategies to mitigate N_2O emissions from wastewater treatment processes (Kampschreur et al., 2009).

An alternative approach for biological post-treatment combines the removal and energetic valorization of ammonia loads i.e., the so-called Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO) (Scherson et al., 2014a; Scherson et al., 2013a). This process comprises two biological stages facilitating conversion of (1) ammonia (NH_4^+) to nitrite (NO_2^-) (a.k.a., nitritation) and (2) NO_2^- to nitrous oxide (N_2O) (a.k.a., nitrous denitritation (Weißbach et al., 2017c)). Investigations concerning the CANDO process at real processing conditions have demonstrated its technical potential with a conversion efficiency of approximately 65% of NH_4^+ to N_2O (Weißbach et al.). In a third stage, N_2O is combusted in combination with methane in a CHP and thereby reduced to climate-neutral nitrogen gas. Additionally, in contrast to oxygen, N_2O is not only an oxidant for methane combustion, but also decays exothermally releasing additional 82 kJ mol^{-1} N_2O (Scherson et al., 2014a). The energy yield of methane combustion can thereby potentially increase by 37% by solely using N_2O as oxidation agent.

In continuous operation, low N_2O effluent concentrations from a CANDO system are desired for efficient energy recovery and environmental protection. However, so far, the demand for feasible and technically applicable extraction methods for harvesting N_2O from the CANDO mixed liquor has not been addressed. Due to dipole-dipole interactions with water, N_2O is highly soluble and its Henry constant is comparatively high ($H^{cp}_{N_2O} = 2.4 \cdot 10^{-2}$ mol L^{-1} bar^{-1} (Sander, 1999b)) compared, for instance, to methane ($H^{cp}_{CH_4} = 1.4 \cdot 10^{-3}$ mol L^{-1} bar^{-1} (Sander, 1999b)). Hence auto-diffusion, as applied for methane separation in anaerobic digestion, is not viable for a technical application. Potential technologies to enhance the liquid-to-gas transport are e.g., gas-stripping, vacuum degasification or membrane-contacting processes. Previously, off-gas concentrations of 0.09% and 0.35% were produced applying continuous gas and discontinuous gas removal (i.e. in a dedicated phase at the end of each batch) during the CANDO process (Weißbach et al.) by gas stripping. It was observed that total NH_4^+/N_2O conversion increased by 18% applying continuous gas removal making it the preferable removal strategy (Weißbach et al.). Considering feasibility and off-gas quality, gas extraction by membrane contactors appears to be more viable compared to conventional gas stripping. By establishing a non-dispersive boundary layer between the liquid and gas phase, individual control of gas and liquid could potentially reduce sweeping gas demand and simultaneously increase off-gas N_2O concentrations for efficient CH_4 combustion. Additionally, packing densities of hollow fiber membrane contactor modules facilitate higher process intensity (Atchariyawut et al., 2007) and hence, reduction of process scale. Concerning energy recovery applying bio-technology, previous studies already demonstrated the application potential of membrane contactors for e.g., residual methane from digester effluents (Cookney et al., 2016) and biogas upgrading (i.e., removal of biogas contained carbon dioxide, CO_2) (Atchariyawut et al., 2007; Kim et al., 2016; Li and Chen, 2005). Regarding the physical similarities of CO_2 and N_2O e.g., Henry constants ($H^{cp}_{CO_2} = 3.4 \cdot 10^{-2}$ mol L^{-1} bar^{-1}) as well as of the applied feed matrices, the viability of membrane-contactors for the CANDO process holds promise. However, considering mass transfer of N_2O and the application of membrane contactors for N_2O removal, no information is available in the peer-reviewed literature. Considering the membrane fabric, in general, hydrophobic porous membrane contactors exhibit higher mass transfer and removal efficiencies (Atchariyawut et al., 2006) compared to non-porous membranes (Cookney et al., 2016). However, they are more prone to clogging and fouling (Cookney et al., 2016). Considering the quality of the CANDO mixed liquor, potentially containing e.g., biomass flocs, particulate and dissolved organic matter, these issues need to be considered. In this respect, shell-side operation of the liquid phase is favorable to eventually avoid the necessity of a pretreatment. In this regard, Cookney et al. (Cookney et al., 2016) have successfully demonstrated shell-side operation of real digester effluents for methane removal to mitigate fouling and clogging potential within the lumen of hollow fibers.

Hence, in this study, the N_2O removal potential of a hydrophobic porous hollow fiber membrane contactor (HFMC) was investigated in shell-side operation to assess the viability of an implementation into the CANDO process for N_2O extraction. All experiments were conducted under controlled conditions for an unbiased and general assessment of mass transfer dynamics and removal efficiencies as a basis and benchmark for further investigations (e.g., process integration). Dominant control parameters and operational ranges for liquid and gas phase sides at expected feed concentrations were derived by the means of a design of experiment (DoE) and a subsequent analysis of variance (ANOVA). Additionally, detailed analyses were conducted on removal efficiencies at varying operational conditions as well as mass transfer coefficients and resistances with respect to N_2O removal.

2 Experimental methods

2.1 Experimental set-up

For the investigations, a conventional polypropylene/polyurethane (membrane/potting material) hollow fiber porous membrane contactor (HFMC) type Liqui-cell® 1.7x5.5 MiniModule® (Membrana, Charlotte, USA) (Table 1) was employed. The operation was conducted in counter-current flow mode in shell-side liquid feed operation.

Effluent and sweeping gas left the membrane module at ambient pressure conditions. Two pressure indicators were installed, one for the gaseous phase and one for the liquid phase just in front of the inlets to the MC. A maximum liquid feed flow rate (Q_l) of $6.5 \cdot 10^{-6}$ m³ s⁻¹ was determined for an intended maximum pressure drop of 0.05 bar for energy conservation. The pressure drop in the gas phase did not exceed 0.01 bar throughout all conducted experiments. Hence, no pore-wetting was expected at the applied trans-membrane pressures (max. 0.04 bar) according to the manufacturer's technical specifications (Membrana, 2005). Ambient air was used as a potentially technically applicable and locally available sweeping gas at a temperature of 17±1 °C. The air was conducted from outside the laboratory to the setup to prevent potential recycling of yet enriched off-gas.

Feed solutions for the DoE and mass-transfer analyses were prepared in a separate laboratory to prevent N_2O contamination of the experimental environment. For this purpose, a N_2O saturated stock solution was prepared and diluted according to Weiss and Price (Weiss and Price, 1980) in 0.5 l of deionized water. The saturated solution was prepared in a sealed glass bottle comprising inlet and outlet ports as well as a thermometer with an evacuated gas bag attached (Figure D.1, Supplemental Information). For saturation, synthetic 3.5 grade N_2O (purity 99.95%, Linde AG, Munich, Germany) was continuously sparged into the water for 30 minutes. Subsequently, the exit port was closed and the gas bag inflated with N_2O to maintain

N_2O filled headspace for constant saturation at ambient pressure conditions and to compensate for withdrawal volumes.

Feed and calibration solutions were prepared by dilution of the stock solution according to the solution temperature and ambient pressure conditions (Weiss and Price, 1980). The applied amperometric nitrous oxide sensors (type: N_2O-R, Unisense, Aarhus, Denmark) were calibrated by linear regression over four points in the range of 0-500 µmol L^{-1} in a 1 L glass round bottom flask and a magnetic stirrer applied during calibration (Figures D.1-2, Supplemental Information).

Table 1. Geometric and material specifications of the investigated hollow fiber membrane contactor

HFMC specifications		
fiber characteristics		
membrane type		X50 Fiber
membrane material		poly-propylene (PP)
outer/inner diameter	[µm]	300 / 220
contacting length	[cm]	12.3
bubble point	[bar]	16.5
porosity	[%]	40
pore size	[µm]	0.04
tortuosity		2.5
module design		
length	[cm]	18.2
diameter	[cm]	4.3[a]
shellside volume	[mL]	78
lumenside volume	[mL]	53
typical membrane surface	[m^2]	0.72[b]
number of fibers	[-]	6350
packing	[m^{-1}]	3,500
potting material		polyurethane (PU)

[a] outer housing

[b] based on outer diameter

Feed solutions applied in the experiments were prepared by dilution of the stock solution in a 5 L cylinder. During all experiments, the concentrations of the feed solutions were monitored with the N_2O sensor. During the experiments including recycling over the feed tank (Figure 1), the concentrations were continuously monitored and controlled by the N_2O sensor and the SCADA system. A magnetic valve opened as soon as the concentration dropped below the target concentration in order to sparge 3.5 grade N_2O (purity 99.95%, Linde AG, Munich, Germany) through a needle-valve into the feed tank. This way, extracted N_2O was replaced and enabled continuous recycling over the feed tank. During DoE and mass-transfer related experiments, the effluent solutions were not recycled but discarded to mitigate even small deviations in the feed solution observed in the preliminary experiments. Gas-exchange to the environment was minimized by a floater covering the water level.

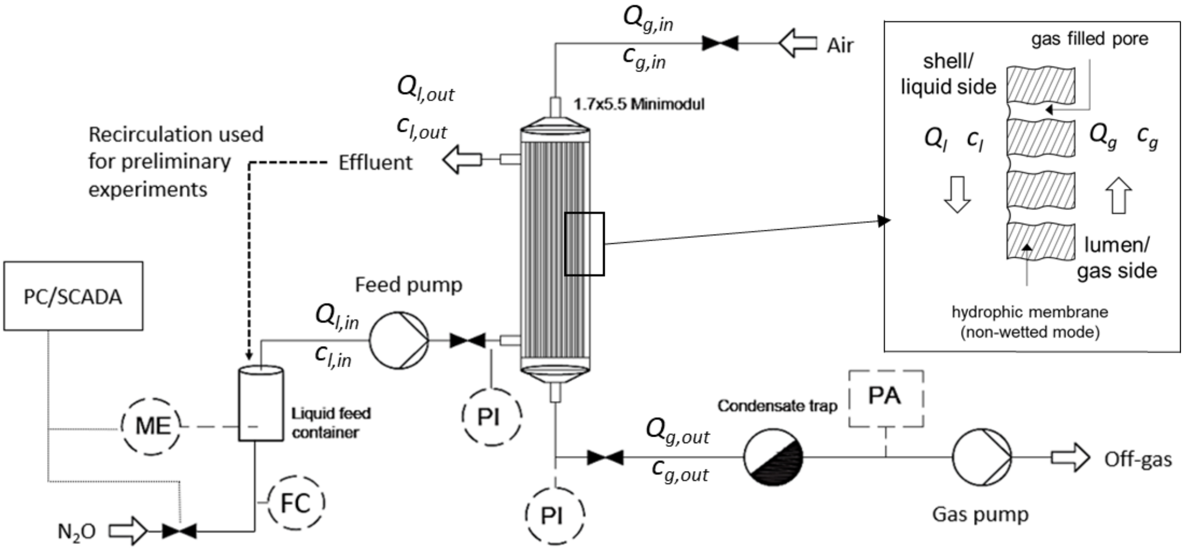

Figure 1. Schematic illustration of the membrane contactor system and peripherals as used for the experiments. ME = micro-electrode for N_2O measurements, FC = rotameter, PA = online photo-acoustic N_2O gas concentration measurement, PI = pressure gauge. Flows (Q) and concentrations (c) at the respective ends of the module are additionally indicated as used for the computation of the mass balance.

Liquid pumps (RGL 85 Heidolph Instruments, Schwabach, Germany) and gas pumps (Ecoline, VC-280, Ismatec, Wertheim, Germany) were calibrated and liquid and gas flow rates were regularly measured during experiments by manually metering volumes over time at the particular outlets of the gas phase and the liquid phase, respectively. Off-gas concentrations ($c_{g,out,ppmv}$) were measured by an opto-thermal photoacoustic measurement device (PA) with a measurement range of 10 ppmv to 10,000 ppmv N_2O (Thaler et al., 2017b) at measurement intervals of 10 s and 5 s during preliminary and detailed mass transfer analyses, respectively.

2.2 Experimental design

The main objective for all experiments was the assessment of N_2O removal during continuous operation of the membrane throughout anoxic reaction periods of a CANDO reactor. By targeting continuous N_2O removal, higher N_2O yields in the gas phase in relation to the initial substrate (i.e., nitrite) are expected (Weißbach et al.). Throughout this operation, dissolved N_2O feed concentrations are typically low (Figure D.3, Supplemental Information). For the investigations, possible feed concentrations in the range of 50 μmol_{N2O} L^{-1} (1.4 $mg_{Nitrogen}$ L^{-1}) to 320 μmol_{N2O} L^{-1} (9 $mg_{Nitrogen}$ L^{-1}) were chosen to test removal efficiencies of 90% to 98.5% in relation to a typical initial nitrite-nitrogen load of 90 $mg_{Nitrogen}$ L^{-1}, respectively.

Two preliminary experiments were conducted to generally assess the suitability of the applied membrane contactor module for N_2O extraction, and derive process optimization strategies for consecutive experiments. For this purpose, the relative saturation (S) (%) in the off-gas phase was determined to assess the separation efficiency in relation to the partition coefficient ($H^{cp}{}_{N2O}$) as:

$$S = \frac{p_{g,N2O},\cdot H_{N2O}^{CP}}{c_{l,in}} \tag{1}$$

where p_{g,N_2O} is the partial pressure (bar) of N_2O in the off-gas, $H^{cp}{}_{N2O}$ the Henry constant of N_2O (24 mol m^{-3} bar^{-1}). The particular N_2O feed concentration is indicated by $c_{l,in}$ (mol_{N_2O} m^{-3}). Additionally, the mass flow rate $m_{g,out}$ (mg s^{-1}) was calculated based on the measured off-gas concentration and the gas flow rate defined as Q_g (m^3 s^{-1}):

$$m_{g,out} = Q_g \cdot \frac{c_{g,out,ppmv}}{1{,}000{,}000\ ppmv} \cdot \frac{P_g}{P_{STP}} \cdot \frac{T_{STP}}{T_g} \cdot \frac{M_{N2O}}{V_{mol,STP}} \tag{2}$$

where P_g (bar) and T_g (K) are ambient pressure and gas temperature at the outlet of the photoacoustic measurement device. P_{STP}, T_{STP} and $V_{mol,STP}$ are the standardized pressure (1 bar) and standardized temperature (273 K) as well as the molar volume at STP-conditions ($2.27 \cdot 10^{-2}$ $m^3 mol^{-1}$). M_{N_2O} represents the molar weight of N_2O ($4.4 \cdot 10^4$ mg mol^{-1}). Finally, the removal efficiency (η (%)) defined as the ratio of $m_{g,out}$ and $m_{l,in}$ (-) was determined as:

$$\eta = \frac{m_{g,out}}{m_{l,in}} \cdot 100 \tag{3}$$

, where $m_{l,in}$ is the mass flow of N_2O in the feed solution to the module (mg s^{-1}) calculated as:

$$m_{l,in} = Q_l \cdot c_{l,in,mol} \cdot M_{N_2O} \tag{4}$$

with Q_l (m^3 s^{-1}) as the feed flow rate.

The effluent mass flow $m_{l,out}$ (mg s^{-1}) from the module on the liquid phase was calculated by closing the mass balance over the module considering the N_2O concentration in ambient air is only 300 ppb (Flückiger et al., 1999) and hence, negligible:

$$m_{l,out} = m_{l,in} - m_{g,out} \tag{5}$$

In both preliminary experiments, the system was operated at steady-state conditions over 2.5 hours. The effluent was recycled to the feed tank and re-enriched automatically to the targeted N_2O concentrations (200 μmol_{N2O} L^{-1} and 320 μmol_{N2O} L^{-1}).

Subsequently, a series of 8 experiments was conducted in a factorial 2^3 design of experiment (DoE) to assess the impact of gas flow rate, liquid flow rate, and feed concentrations in the investigated ranges (Table 2) by an ANOVA in relation to the mass transfer rate and the removal efficiency (significance level: $p = 0.05$). Based on the ANOVA results, further experiments were conducted for a detailed mass transfer analyses.

Table 2. Overview of the applied 23 design of experiment with two factors for each variable i. e. superficial gas velocity (v_g) or gas flow rate (Q_g), superficial liquid velocity (v_s) or liquid flow rate (Q_l) and feed concentration ($c_{l,in,mol}$).

DoE #	gas phase			liquid phase			concentration	target variables
	v_g	or	Q_g	v_s	or	Q_l	$c_{l,in,mol}$	
	[m s^{-1}]		[m^3 s^{-1}]	[m s^{-1}]		[m^3 s^{-1}]	[mol m^{-3}]	
1	$2.4\cdot10^{-3}$		$1.01\cdot10^{-6}$	$1.6\cdot10^{-4}$		$1.00\cdot10^{-7}$	0.05	
2	$5.4\cdot10^{-3}$		$2.33\cdot10^{-6}$	$1.6\cdot10^{-4}$		$1.00\cdot10^{-7}$	0.05	
3	$2.4\cdot10^{-3}$		$1.01\cdot10^{-6}$	$4.0\cdot10^{-3}$		$2.55\cdot10^{-5}$	0.05	a) m_g
4	$5.4\cdot10^{-3}$		$2.33\cdot10^{-6}$	$4.0\cdot10^{-3}$		$2.55\cdot10^{-5}$	0.05	[mg s^{-1}]
5	$2.4\cdot10^{-3}$		$1.01\cdot10^{-6}$	$1.6\cdot10^{-4}$		$1.00\cdot10^{-7}$	0.13	b) η
6	$5.4\cdot10^{-3}$		$2.33\cdot10^{-6}$	$1.6\cdot10^{-4}$		$1.00\cdot10^{-7}$	0.13	[%]
7	$2.4\cdot10^{-3}$		$1.01\cdot10^{-6}$	$4.0\cdot10^{-3}$		$2.55\cdot10^{-5}$	0.13	
8	$5.4\cdot10^{-3}$		$2.33\cdot10^{-6}$	$4.0\cdot10^{-3}$		$2.55\cdot10^{-5}$	0.13	

2.3 Mass transfer analyses

The overall (log mean) mass transfer coefficient (K_o (m s^{-1})) has been calculated from the experimental results as follows (Ismail and Matsuura, 2016):

$$K_o = \frac{Q_l(c_{l,out} - c_{l,in})}{A\Delta c_{l,average}} \tag{6}$$

where Q_l is the liquid flow rate (m^3 s^{-1}), A is the contacting area (m^2), and $c_{l,in}$ and $c_{l,out}$ are the feed and effluent solute gas mole-fractions (mol mol^{-1}), respectively. The logarithmic mean of the solute trans-membrane concentration is determined by (Ismail and Matsuura, 2016):

$$\Delta c_{l,average} = \frac{(H^{cc}c_{g,in} - c_{l,out}) - (H^{cc}c_{g,out} - c_{l,in})}{\ln\left(\frac{H^{cc}c_{g,in} - c_{l,in}}{H^{cc}c_{g,out} - c_{l,out}}\right)} \tag{7}$$

The dimensionless Henry constant of N_2O ($H^{cc} = c_l/c_g = 1.75$, at 17°C (Sander, 2015)) serves as partition coefficient. Feed gas and off-gas concentrations in mole fractions are indicated by $c_{g,in}$ and $c_{g,out}$, respectively.

Individual mass transfer resistances of hollow fiber membrane contactors are determined based on the common resistance-in-series-model (Wu and Chen V., 2000):

$$\frac{1}{K_o} = \frac{1}{k_l} + \frac{H^{cc} \cdot d_o}{k_m \cdot d_{ln}} + \frac{H^{cc} \cdot d_o}{k_g \cdot d_i} \tag{8}$$

where the total membrane resistance can be expressed as $1/K_o$ (s m^{-1}) with d_o (m), d_i (m) and d_{ln} (m) as outer, inner and logarithmic mean fiber diameter. Typically, the gas phase resistance (k_g) becomes negligible in a gas-liquid membrane contacting process, because gas phase diffusion coefficients are considerably higher (Gabelman and Hwang, 1999). As a result, the process can be characterized by the sum of membrane (k_m) and liquid-side resistances (k_l). To quantify the membrane-resistance, the Wilson-plot method can be applied (Wilson, 1915). The liquid-side mass transfer resistance can then be computed accordingly:

$$\frac{1}{k_l} = \frac{1}{K_o} - \frac{H^{cc} \cdot d_o}{k_m \cdot d_{ln}} \tag{9}$$

Additionally, the membrane resistance can be computed by theoretical gas kinetics based on the flow regime. Depending on the ratio of the nominal pore size d_n (m) and the mean path length l (m) during molecular diffusion, the flow regime can be determined by the Knudsen number Kn (-) (Drioli et al., 2011):

$$Kn = \frac{l}{d_n} \tag{10}$$

Unrestricted gas flow can be assumed, if Kn is << 1 and Knudsen diffusion can be neglected under these conditions. The membrane resistance of a gas-filled pore is determined by the effective diffusion D_{eff} ($m^2\ s^{-1}$) in the membrane pore (Mavroudi et al., 2006):

$$D_{eff} = \frac{1}{\left(\frac{1}{D_m} + \frac{1}{D_{Kn}}\right)} \tag{11}$$

, where D_m ($m^2\ s^{-1}$) is the molecular diffusion and D_{Kn} the Knudsen diffusion ($m^2\ s^{-1}$) (Drioli et al., 2011). Based on the effective diffusion, porosity ε (%) and tortuosity τ (-), the membrane mass transfer coefficient is calculated accordingly (Prasad and Sirkar, 1988):

$$k_m = \frac{D_{eff} \cdot \varepsilon}{\tau \cdot d_n} \tag{12}$$

Additionally, Sherwood (*Sh*) correlations are used to describe the mass transfer. The *Sh* correlation is a function of the Reynold (*Re*) and Schmidt (*Sc*) numbers (Gabelman and Hwang, 1999). Specific correlations can be obtained by fitting experimental results (Sh_{ex}) to theoretical Sherwood numbers (Sh_{th}) by a factor *a* as well as respective exponents α and β:

$$Sh_{th} = a \cdot Re^{\alpha} \cdot Sc^{\beta}, \quad Sh_{ex} = \frac{k_l \cdot d_h}{D} \tag{13, 14}$$

Where,

$$Re = v_l \cdot \frac{d_h}{\nu}, \ Sc = \frac{\nu}{D} \tag{15, 16}$$

with d_h (m) as the hydraulic diameter, ν ($1.08 \cdot 10^{-6}\ m^2\ s^{-1}$, at 17°C) as the kinematic viscosity and *D* as the diffusion coefficient of N_2O in water ($1.92 \cdot 10^{-2}\ m^2\ s^{-1}$) (Thomas and Adams, 1965). For gas-liquid processes, a common β-value was chosen ($\beta = 0.33$) (Costello et al., 1993; Prasad and Sirkar, 1988; Wickramasinghe et al., 1993) and the data were fitted to *a* and α, respectively.

The hydraulic diameter d_h (-) was calculated as the difference of the squared inner shell diameter d_S (m) and the outer fiber diameter divided by the product of number of fibers *N* (-) and the outer fiber diameter (Zheng et al., 2005):

$$d_h = \frac{d_S^2 - d_o^2}{N \cdot d_o} \tag{17}$$

Graetz numbers (*Gz*) are also dimensionless numbers to describe the effect of convective and diffusive mass transfer as a function of *Re* and *Sc* (Drioli et al., 2011):

$$Gz = Re \cdot Sc \left(\frac{d_h}{L}\right) \tag{18}$$

, where *L* is the length of the contact area in flow direction.

3 Results and Discussion

To evaluate the general applicability of the chosen membrane contactor, preliminary experiments were conducted. Because the results demonstrated potential viability for N_2O removal, the effect of each control variable was tested to derive more specific operational conditions for efficient N_2O removal. For this purpose, dynamics of off-gas concentrations, transmembrane flux and removal efficiency in potential technical operational ranges were investigated. Based on these results, further analyses were conducted concerning the mass transfer coefficients and resistances regarding the applied module.

3.1 Preliminary assessment

During the preliminary experiments, the membrane contactor was succesfully operated for more than 2.5 h in contiuous operation applying a superficial liquid velocitiy of $1.0 \cdot 10^{-2}$ m s^{-1} and a superficial gas velocity of $4.3 \cdot 10^{-4}$ m s^{-1}. The applied volumetric gas-to-liquid flow rate ratio was 0.03. At the applied feed concentrations of 200 μmol_{N_2O} L^{-1} and 320 μmol_{N_2O} L^{-1}, constant off-gas concentrations (coefficient of variation ≤ 0.1) of 2,400 ppmv and 3,700 ppmv on average (Figure 2) could be observed. These concentrations were considerably higher than previously reported concentrations of 900 ppmv by applying conventional gas stripping in an operating CANDO system (Weißbach et al.). Accordingly, relatively high saturations of N_2O of 29.0% and 27.8%, respectively, were determined in the off-gas.

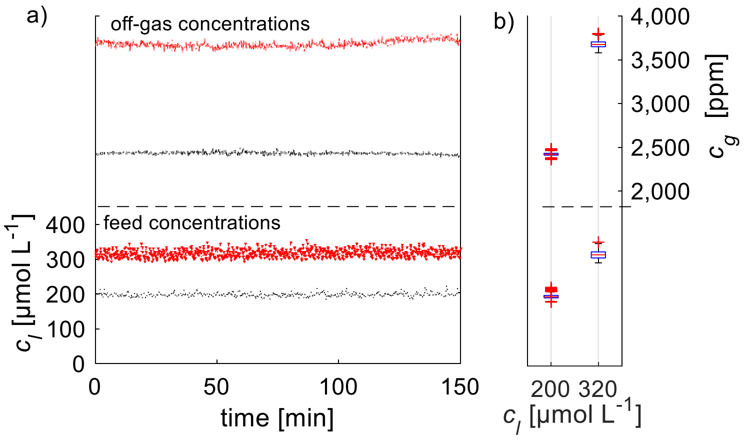

Figure 2. Measurements of a) off-gas (c_g) and and feed phase (c_l) concentrations (200 μmol/L (black dots) and 320 μmol/L (red dots)) and b) average concentration and standard deviations from the preliminary assessment of the applicability of the chosen MC for N_2O removal. The experiments were conducted over 150 minutes at steady-state conditions.

The observed off-gas concentrations are relatively low and potentially enable the off-gas utilization in the present design of CHPs without retrofitting to higher energy gas engines. However, further investigations are necessary for a better determination of necessary gas flows at real operating conditions and integration of the CANDO process, a digester, and a CHP unit.

In contrast to the relatively high, low removal efficiencies of 2.25% and 2.13% were observed resulting in considerable N_2O effluent concentrations (approximately 9 $mg_{N2O\text{-}N}$ L^{-1}). Given the CO_2-equivalent of N_2O of approximately 300 (Ravishankara et al., 2009), these concentrations need to be considered. To reduce effluent concentrations, recirculation of the reactor mixed liquor for removal in the membrane contactor could be an option. Furthermore, the addition of an external carbon source toward the end of the process is a potential alternative. In this respect, it was demonstrated that the addition of extracellular carbon triggers microbial elmination of residual N_2O to nitrogen gas and would hence, also reduce the greenhous gas potential of the effluent (Weißbach et al.).

However, in order to investigate operational means for the reduction of effluent N_2O concentrations, the operational variables were altered. The superficial velocity on the feed side was decreased in subsequent experiments based on previous findings (Cookney et al., 2016; Mansourizadeh and Ismail, 2011; Prasad and Sirkar, 1988), to increase removal efficiencies. Additionally, the gas flow rate was increased to 140 mL min^{-1} (superficial gas flow velocity of $5.4 \cdot 10^{-3}$ m s^{-1}) to mitigate a potential mass transfer resistance in the gas phase possibly triggered by the observed relative saturations (Drioli et al., 2011).

3.2 Effect of operational variables on N_2O removal performance

To determine process conditions, which would enable higher removal efficiencies throughout the operation, the particular effect of each operational variable was investigated based on a series of experiments according to a 2^3 factorial design. Within an experimental series at equal concentrations, similar values of off-gas N_2O mass flows, transmembrane fluxes and removal efficiencies were observed. Considerable differences were only evident for off-gas concentrations under varying gas flow rates at equal superficial velocites. The concentrations were approximately twice as high at a gas flow rate of $2.4 \cdot 10^{-3}$ m s^{-1} compared to the results at $5.4 \cdot 10^{-3}$ m s^{-1}.

The results obtained from a conducted ANOVA underlined that feed concentrations and gas flow rates have no significant effect on the removal efficiency in the investigated ranges (*p*-value > 0.05, Table 3). Hence, mass transfer resistances in the gas phase are insignificant under these conditions.. In contrast, a significant correlation ($p = 0.001$) between liquid flow rate and removal efficiency was evident by the statistical results. In this respect, a maximum of 77% (DoE 2, $c_l = 130$ μmol_{N_2O} L^{-1}, $v_s = 1.6 \cdot 10^{-5}$ m s^{-1}) of N_2O could be removed from the N_2O mass flow to the module applying the lowest superficial velocity. Increasing the superficial liquid velocity to $4 \cdot 10^{-3}$ m s^{-1} (DoE 3 and DoE 4) reduced removal efficiency in relation to the N_2O mass flow in the feed to approximately 10%. These results compare well with previous findings of shell-side operation of microporous hollow fiber membranes. For instance, Park et al. (Park et al., 2009) reported removal efficiencies between 15% and 45% investigating the removal of nitrogen dioxide from aqueous phases. Cookney et al. (Cookney et al., 2016) achieved removal efficiencies for methane in the range of 10% to 90% at similar variations of the feed flow rate.

Table 3. ANOVA results for a) the mass flow in the gas phase and b) the removal efficiency showing non-significant influence of either operational variable on the mass transfer, but significant influence of the liquid flow rate in the tested operational ranges.

Factor	F-value	Prob > F (p)*
a) target variable: mass-flow (*m)*		
Q_l	11.8	0.03
Q_g	0.16	0.71
c_f	7.86	0.049
b) target variable: removal efficiency (η)		
Q_l	85.4	0.001
Q_g	2.37	0.199
c_f	1.04	0.365

**significance level $p < 0.05$*

In addition to the significant effect of the superficial liquid velocity on the removal efficiency, significantly higher N_2O mass flows and fluxes indicated by *p*-values of 0.026 and 0.049, were determined. In contrast, no significant correlation could be determined in relation to the flow rate of the sweep gas ($p = 0.71$).

The observed results illustrate that a reduction of sweeping gas flows produces higher off-gas N_2O concentrations, but does not considerably compromise removal efficiencies at the investigated operational conditions. However, aiming for high removal efficiencies and high off-gas concentrations is challenging, because higher superficial velocities significantly increase the trans-membrane flux, but result in low removal efficiencies. From a design perspective, in-series operation of modules would compensate for the natural mass transfer limitation i.e., the Henry constant, and increase N_2O removal efficiencies and mass flows (Gabelman and Hwang, 1999). Additionally, mass-tansfer could be enhanced by e.g., salt addition or heating. In this respect, Kosse et al. (Kosse et al., 2017) have recently demonstrated efficient salt-induced N_2O stripping.

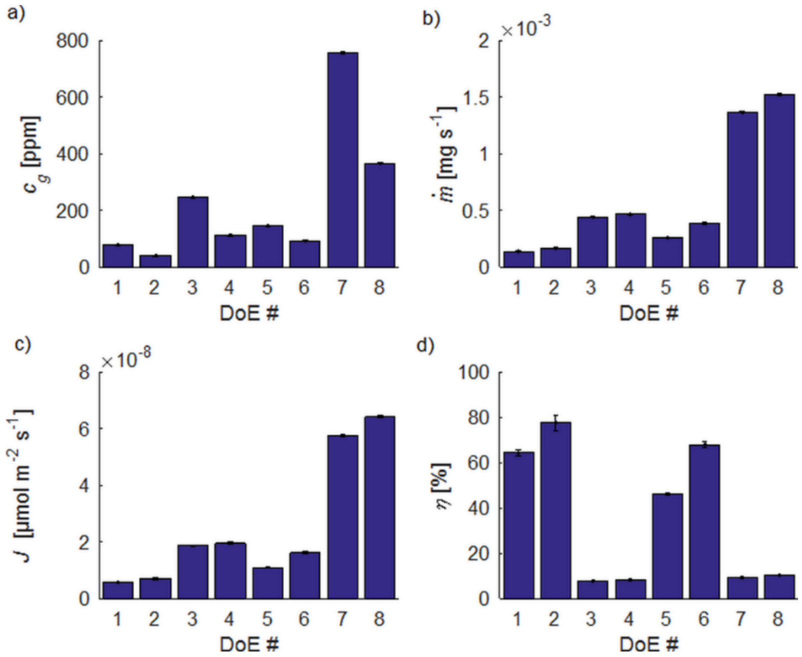

Figure 3. Comparison of DoE results illustrating a) N_2O off-gas concentration (c_g), b) mass flow (m), c) trans-membrane flux (J) and d) removal efficiency (E) as well as standard deviations (n = 60 for each experiment).

However, the addition of for instance sodium bromide or magnesium sulfate would result in increasing operational costs and could also potentially effect the microbial community of the CANDO process as well as the effluent quality. Increasing the feed temperature for mitigation of the Henry constant could also be considered, but this will likely compromise the energy-recovery potential of the CANDO process. Alternatively, fixed surfacting membrane coatings could be a potential solution as recently applied for enhanced carbon dioxide separation (Shen et al., 2016).

However, at the investigated conditions, a N_2O removal efficiency greater than 90% regarding typical batch nitrogen loads of 90 mg_N L^{-1} (equivalent to 3.2 mmol N_2O) can be achieved (Weißbach et al.). Additionally, the sweeping gas (140 mL min^{-1}) demand compared to recently proposed gas stripping (500 mL min^{-1} (Weißbach et al.)) can be reduced by 70%, indicating a considerably higher feasibility applying membrane contactor technology compared to gas stripping.

3.3 Mass transfer dynamics

For a better understanding of the mass transfer behavior to changes of the significantly relevant parameters determined in the ANOVA, additional experiments were carried out. To obtain a higher resolution of the expected operational conditions, additional measurements were conducted. Feed concentration of 260 μmol_{N_2O} L^{-1} at superficial liquid velocities of $1.5 \cdot 10^{-4}$ m s^{-1} to $4 \cdot 10^{-3}$ m s^{-1} as well as concentrations of 130 μmol_{N_2O} L^{-1} and 50 μmol_{N_2O} L^{-1} at superficial velocities of $3.2 \cdot 10^{-4}$ m s^{-1} and $1.8 \cdot 10^{-3}$ m s^{-1} were investigated. To investigate the mass transfer, additional experiments were conducted at the superficial liquid velocity of $1.02 \cdot 10^{-2}$ m s^{-1}.

No distinct correlation was observed between removal efficiency and feed concentration. The observed efficiencies were in a similar range at fixed flow rates, but exhibited varying feed concentrations. In accordance to the DoE results, low superficial liquid velocities correlated to low N_2O off-gas concentrations and accordingly low transmembrane fluxes were generated under the investigated conditions. By increasing the superficial liquid velocity or the feed concentrations, off-gas concentration, mass flow and membrane fluxes are elevated in a non-linear correlation (Figure 4a-c). These findings are in agreement with previous studies for different gases, e.g. CO_2 (Zhang et al., 2006), NO_2 (Park et al., 2009), and CH_4 (Cookney et al., 2016)..

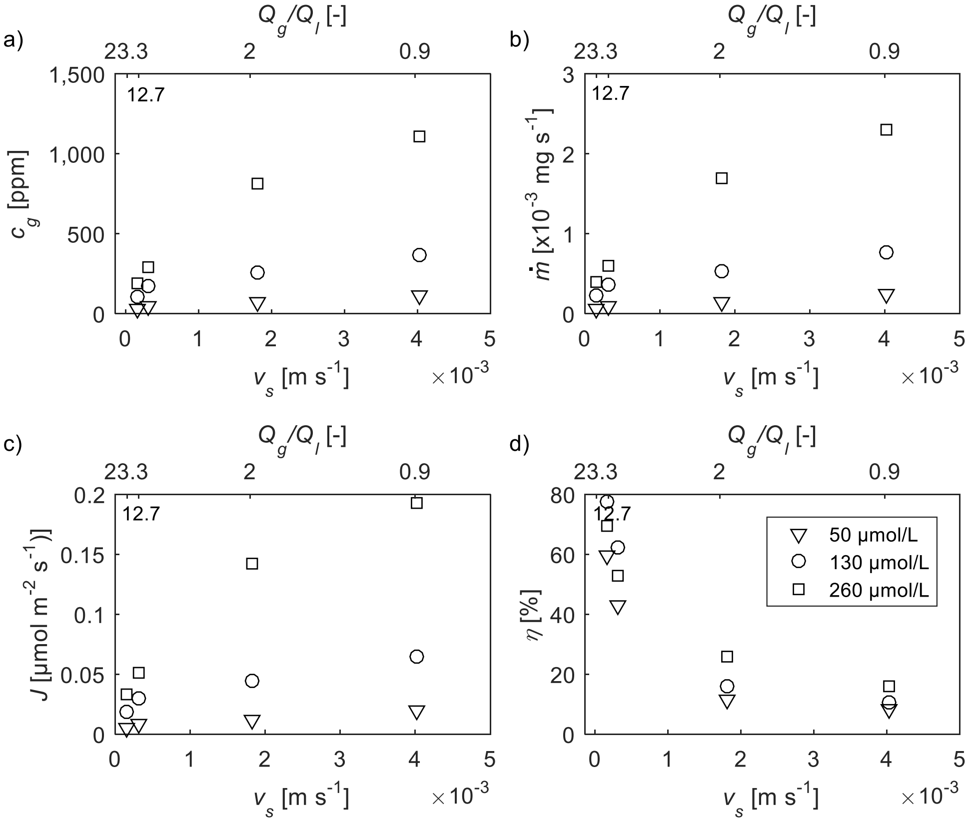

Figure 4. Comparison of the measurements regarding a) N_2O off-gas concentration ($c_{g,off,ppm}$), b) N_2O mass flow (m) of N_2O, c) trans-membrane flux (J) and d) removal efficiency (η) in relation to the liquid flow rate at feed concentrations of 50, 130 and 260 µmol L-1 and the gas-to-liquid flow ratio (Q_g/Q_l).

At a particular superficial liquid velocity, off-gas concentrations, mass flow and transmembrane fluxes increased in non-linear correlation with higher feed concentration. Hence, aiming for high N_2O gas concentrations, high feed concentrations and superficial velocities need to be applied, which is also supported by the investigation of the gas-to-liquid flow ratio (Figure 4d). However, in contrast to the observed correlations concerning the mass transfer, the removal efficiency decreased with increasing superficial liquid velocities. The development is characterized by a continuous exponential concentration drop over the applied range

The results illustrate that lower removal efficiencies correlate with higher respective N_2O mass flows in the off-gas. According to the results, removal efficiencies over 50% can be expected at superficial liquid velocities above $3.2\cdot10^{-4}$ m s^{-1} for concentrations between 50 µmol L^{-1} and 260 µmol L^{-1}. The highest off-gas concentration of 1,200 ppmv and respective mass flow of $4.6\cdot10^{-3}$ $mg_{N_2O\text{-}N}$ s^{-1} however, was obtained at a concentration of 260 µmol L^{-1} and a feed flow rate of 153 mL min^{-1}. With a removal efficiency of 16%, approximately 218 µmol L^{-1} would remain in the membrane contactor effluent. Focusing on small scale application of a membrane contacting process in the CANDO for N_2O extraction, a high superficial liquid velocity will reduce the necessary membrane surface at a given treatment objective i.e., meeting the N_2O production rates of the biological process. For instance, considering recently published production rates of approximately 2.35 $mg_{N2O\text{-}N}$ L^{-1} h^{-1} in the CANDO reactor (Weißbach et al.) at real processing conditions, theoretically 0.10 m^2 of membrane surface per liter reactor volume are necessary to meet the required removal performance. Aiming for low effluent concentrations, considerably larger membrane surfaces at the same operational conditions of the biological process are required. Regarding operational feasibility, lower superficial velocities will potentially reduce operational costs of peripherals e.g., pumping units, but in contrast, the membrane-lifetime will likely be increased by fouling mitigation that can be expected from higher shear stresses at higher superficial velocities (Choi et al., 2005).

In addition to the investigations of mass flows and efficiency at specific operating conditions, the overall mass transfer coefficient was investigated for a general description of N_2O mass transfer with respect to the applied module. The measurement data exhibited a similar deviation of K_o for each concentration series at respective superficial velocites. The best fit to the measurement data was obtained from the following exponential function:

$$K_o = 3.75\cdot10^{-5}\cdot v_s^{0.91} + 1.17\cdot10^{-8} \quad , R^2 = 0.99 \qquad (19)$$

Similar to Costello et al. (Costello et al., 1993), applying a log-log plot (Figure 5) yielded a linear deviation over the investigated range. The average values were in the range of $1.4\cdot10^{-7}$ m s^{-1} to $5.8\cdot10^{-6}$ m s^{-1} at superficial velocities of $1.5\cdot10^{-4}$ m s^{-1} to $1.2\cdot10^{-2}$ m s^{-1}. Considering previous studies investigating mass transfer for pure water and pure CO_2 systems, Wu and Chen (Wu and Chen V., 2000) as well as Wang et al. (Wang et al., 2005) reported values for higher liquid flow rates (0.05 m s^{-1} to 0.5 cm s^{-1}). The respective mass transfer coefficients ($2.1\cdot10^{-5}$ cm s^{-1} to $5.2\cdot10^{-5}$ cm s^{-1}) were also higher by approximately an order of magnitude, which complies with the linear correlation of K_o and liquid flow rates (eqn. (5)). Especially considering that Wang et al. (Wang et al., 2005) applied the same module supports this observation. Heile et al. (Heile et al., 2014) presented mass transfer coefficients ($5\cdot10^{-6}$ m s^{-1} to $1.1\cdot10^{-5}$ m s^{-1}) in a more similar range for methane. The values for K_o are a bit higher, which is most likely based on the lumen-side liquid operation in their study. However, regarding these studies on CO_2 and CH_4, the mass transfer of N_2O using the applied membrane can be considered similar.

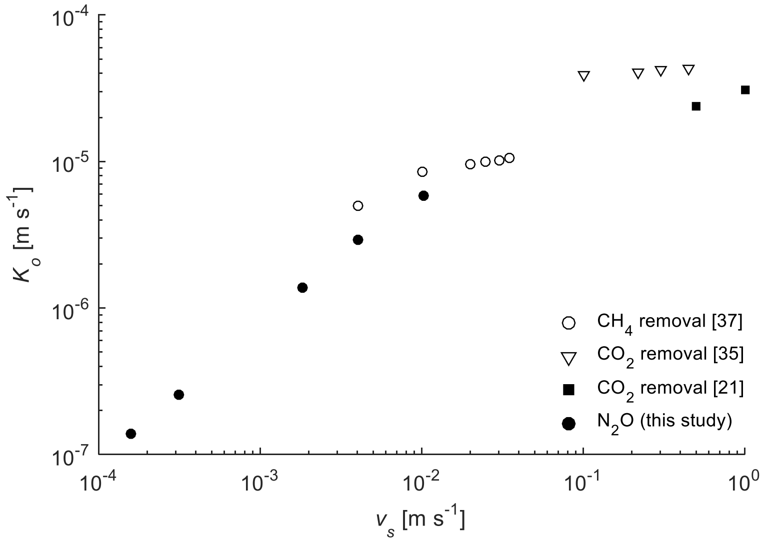

Figure 5. Comparison of different studies on overall mass transfer (K_o) coefficients applying micro-porous hollow fibers membrane contactors for the removal of CO_2 and CH_4 under similar operating conditions in comparison to the results of this study.

Further investigation to characterize the mass transfer were conducted. Membrane and liquid side resistances were determined by applying the Wilson-plot method. The best fit for the linearization of the overall mass transfer resistance yielded an α_W-value of 0.94:

$$\frac{1}{Ko} = 18240 \cdot v_s^{-0.94} + 9475, \qquad R^2 = 0.99 \tag{20}$$

The results plotted in Figure 6 illustrate that the membrane resistance is negligible under the applied conditions, especially the comparatively low superficial velocites, which is in agreement with previous findings. The absolute resistance accounted for 9,475 s m^{-1} and hence accounts for a relative share of 0.7% at the highest applied superficial velocity. Previously reported membrane resistances for CO_2 removal under similar conditions, however, were lower. Wang et al. (Wang et al., 2005) and Mavroudi et al. (Mavroudi et al., 2006) applied the same membrane type and reportet 4,700 s m^{-1} and 5,500 s m^{-1}, respectively.

To further investigate the membrane resistance, theoretical values of the membrane resistance for N_2O were determined considering non-wetted operation. For this purpose, Knudsen diffusion had to be taken into account (Kn = 0.67). The results yielded a resistance of 134 s m^{-1}, which is comparable to the value given by Cookney et al. (Cookney et al., 2016), who reported 124 s m^{-1} for CH_4.

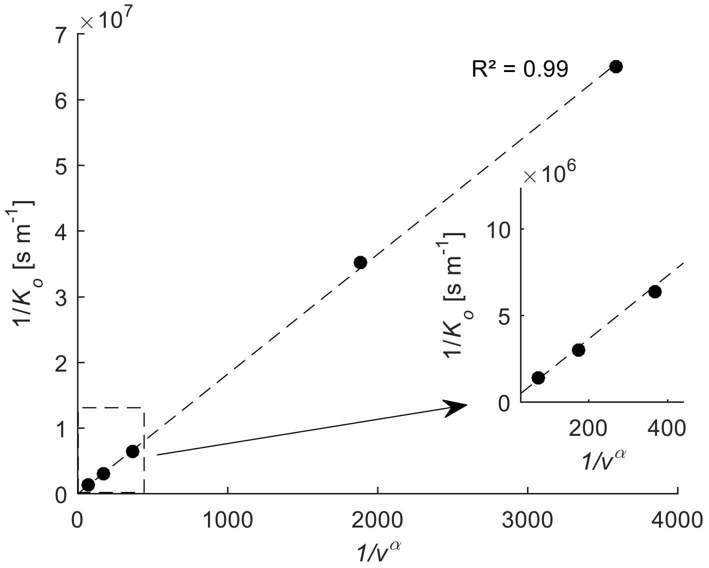

Figure 6. Wilson plots based on the overall mass transfer coefficient and an alpha-value: $\alpha_w = 0.94$.

Considering the differences between the theoretical and the empirical membrane resistances, however, it is likely that pore wetting influenced the membrane mass transfer and caused a considerably higher empirical value. This could be possibly due to capillary condensation (Fisher et al., 1981). It was reported that capillary condensation potentially occurs from the liquid side during the initial time of operation of a membrane and it cannot be ruled out that this has played a role. Atchariyawut et al. (Atchariyawut et al., 2006) have reported a similar effect in the operation of polyvinylidene fluoride membranes for CO_2 removal. In their study, the membrane resistance also increased from <0.5% to up to 40% of $1/K_o$, but at a much higher superficial velocity of 0.25 m s^{-1}. Additionally, the application of ambient air used in this study instead of vapor-free synthetic gases might have contributed to this effect from the lumen-side.

In this respect, it should be noted that pore wetting is also likely to take place under real processing conditions by organic compounds measured as COD in digester and CANDO effluents. Organic surfacting chemicals can potentially trigger pore wetting already at trace concentrations (Gabelman and Hwang, 1999). The particulate and soluble fractions of COD (pCOD and dCOD) in the CANDO effluent have been reported to be 137 mg_{pCOD} L^{-1} and 84 mg_{dCOD} L^{-1} from a CANDO system operated with digester reject water and primary effluent (Weißbach et al.). CH_4 removal from an anerobic bioreactor applying the same fiber type was succesfully conducted at similar COD concentrations of 124 mg_{pCOD} L^{-1} and 45 mg_{pCOD} L^{-1} (Cookney et al., 2016). Additional pretreatment as presented by Henares et al. (Henares et al., 2016) by sieving or advanced membrane technology could also be an option to mitigate this effect.

Table 4. Different Sherwood correlations given in the literature in comparison to the results of this study.

Author	Equation	Operation	*Re* [-]			*Sh* [-]		
Costello et al. (1993)[b]	$Sh = (0.53-0.58\ \varphi)Re^{0.9}\ Sc^{0.33}$	shell-side	21	-	234	1.03	-	9.01
Lévêque (1928)[a]	$Sh = 1.62d_h^2\ v_s/L\ D)^{1/3}$	lumen-side		-		1.50	-	6.01
Prasad & Sirkar (1988)[b]	$Sh = \beta\ [d_h(1-\varphi)/L]\ Re^{0.6}Sc^{0.33}$	shell-side	0	-	500	0.08	-	0.92
This study	$Sh = 0.002\ Re^{0.66}Sc^{0.33}$	shell-side	0.14	-	9.0	0.01	-	0.37
Zheng et al. (2005)	$Sh = 2.15\ Re^{0.42}\ Sc^{0.33}$	shell-side	0.8	-	20	8.29	-	47.8

[a] *generally predicts lumen-side flow*

[b] *equations based on packing density (φ)*

For a general characterization of the mass transport under the investigated conditions, different previously published Sherwood correlations were examined (Table 4). Along with the general Lévêque equation(Leveque and Ann, 1928), different shell-side correlations were taken into account. The Lévêque equation as well as correlations proposed by Zheng et al. (Zheng et al., 2005) as well as Costello et al. (Costello et al., 1993) exhibited similar deviations, but considerably over-estimated the mass transfer (Table 4). The best fit ($R^2 = 0.99$, Figure 7) was obtained with $\alpha = 0.007$ and $\beta = 0.8$:

$$Sh = 0.007 \cdot Re^{0.8} \cdot Sc^{0.33},\quad R^2 = 0.99 \tag{21}$$

The equation by Prasad and Sirkar (Prasad and Sirkar, 1988) covered a similar range of *Sh*, but the values are approximately an order of magnitude higher (Figure 7). Previously, over-estimation of shell-side results was supposed to be caused by polydisperse flow-channels, especially at low superficial velocities which possibly explains the observations in this study. Additionally, flow channeling within the shell-side of the applied module in the outer regions (close to the housing) of the fiber package might have played a role, too. The correlation proposed by Prasad and Sirkar (Prasad and Sirkar, 1988) accounts for this effect by taking the packing density into account and hence, compares better to the presented results. Additionally, the impact of an operation at low *Gz* numbers on Sherwood should be noted. Wang et al. (Wang et al., 2017) recently reported a considerable over estimation of *Sh* at *Gz* values below 20 for the lumen-side operation of hollow-fiber membranes. Other studies have also reported an over-estimation of K_o in comparison to local mass transfer coefficients at low Graetz numbers [44-45]. The authors have described this behavior for low Graetz ranges and postulated also poly-dispersity and non-uniform flow as possible reasons [44-45]. Regarding the applied *Gz* range in this study (0.8 ≤ *Gz* ≤ 50), this effect is likely to be accounted for the observed mass transfer limitation compared to the published *Sh* correlations (Figure 7).

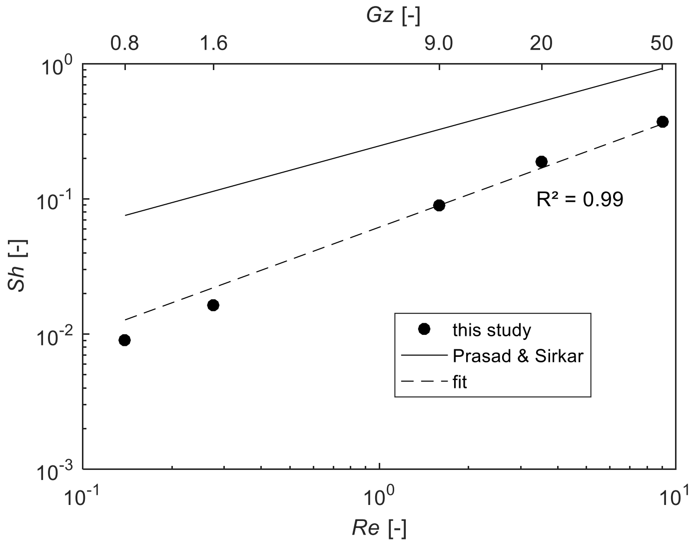

Figure 7. Illustration of theoretical Sherwood values of this study and fitted correlation as well as Sherwood correlation according to Prasad and Sirkar [27].

4. Conclusion

The results demonstrate the potential of a poly-propylene micro porous hollow fiber membrane contactor to enhance liquid-to-gas mass transfer of N_2O in shell-side operation for N_2O recovery. The applied module and poly-propylene fabric appear to be suitable for N_2O removal at the expected operational conditions of an operating CANDO system and also holds promise to mitigate N_2O emissions from other biological nitrogen removal systems.

Relative saturations of N_2O in the gas phase up to 30% regarding the Henry constant can be achieved at the applied conditions. However, removal efficiencies are compromised by targeting high off-gas concentrations and saturations. As a result, potentially harmful N_2O emissions from the reactor effluents are potentially generated. For emission mitigation, the operation of the membrane contactor at lower superficial liquid velocities as well as operational strategies concerning the bio process are mitigation measures. Considering the mass transfer behaviour under the investigated operational conditions, the mass transfer is dominated by the superficial velocity of the liquid phase. Membrane and gas phase resistances are negligible. However, pore-wetting will potentially increase the relative share of the membrane resistance in the overall resistance at higher superficial velocites. Additionally, a higher potential of pore wetting due to wastewater and anaerobic digester effluent constitutuents can be expected under real processing conditions. In this respect, the investigation of thin surfacting coatings should be investigated during long-term operation in a biological process environment to derive mass transfer behaviour, membrane lifetime and subsequently operational feasibility. Also the fiber spacing or pre-treatment measures should be adressed since clogging is also likely to occur in shell-side operation in densly packed modules similar to the investigated one.

In addition, alternative materials or selective surfacting coatings should be investigated to potentially compensate for the relatively high Henry constant of N_2O, as well. This would improve mass transfer coefficients and removal efficiencies at low superficial velocities and facilitate both, high removal efficiencies and off-gas concentrations.

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Supplementary information

Supplementary data for this work is given in *Appendix D*.

Nomenclature

Computation variables

α	fitting factor of *Re* in Sherwood correlation [-]
α_W	fitting factor for Wilson-plot [-]
β	fitting exponent of *Re* in Sherwood correlations
$\Delta C_{l,average}$	mean logarithmic concentration gradient [-]
$c_{g,in}$	feed gas concentration [mol%]
$c_{g,out}$	off-gas concentration [mol%]
$c_{g,out,ppmv}$	off-gas concentration [ppmv]
$c_{l,in}$	feed concentration [mol%]
$c_{l,in,mol}$	feed concentration [mol m^{-3}]
$c_{l,out}$	effluent concentration [mol%]
$c_{l,out}$	effluent concentration [mol m^{-3}]
η	removal efficiency [%]
Gz	Graetz number [-]
J	molar flux [μmol m^2 s^{-1}]
K_o	overall mass transfer coefficient [m s^{-1}]
k_l	liquid-side mass transfer coefficient [m s^{-1}]
k_m	membrane mass transfer coefficient [m s^{-1}]
$m_{g,out}$	off-gas N_2O mass flow [mg s^{-1}]
$m_{l,in}$	dissolved mass flow into the module [mg s^{-1}]
$m_{l,out}$	dissolved effluent mass flow [mg s^{-1}]
p_g	partial pressure [bar]
P_g	ambient pressure [bar]
Q_g	gas flow [m^3 s^{-1}]
Q_l	feed flow [m^3 s^{-1}]
Re	Reynold number [-]
S	relative gas saturation [%]
Sc	Schmidt-number [-]
Sh	Sherwood-number [-]
T_g	gas temperature [K]

v_s	superficial velocity [m s^{-1}]

Computation constants

A	membrane surface (0.72 m^2)
d_h	shell-side hydraulic diameter ($9.5 \cdot 10^{-4}$ m)
D	diffusion coefficient of N_2O in water ($1.92 \cdot 10^{5}$ m^2 s^{-1}) (Thomas and Adams, 1965)
d_i	inner fiber diameter ($2.2 \cdot 10^{-4}$ m)·
d_{ln}	logarithmic mean fiber diameter ($2.6 \cdot 10^{-4}$ m)
d_o	outer fiber diameter (3 10^{-4} m)
d_s	inner shell diameter (3.8 10^{-2} m)
ε	porosity [%]
H^{cp}	Henry constant of N_2O (24 mol m^{-3} bar^{-1})(Sander, 1999b)
H^{cc}	Dimensionless Henry constant (1.75)
l	mean path length of N_2O at 290.15 K and 1 bar ($5.99 \cdot 10^{-8}$ m)
M_{N_2O}	molar weight of N_2O (44,000 mg mol^{-1})
N	number of module fibers (6,350)
P_{STP}	pressure at STP conditions (1 bar)
T_{STP}	temperature at STP conditions (273 K)
τ	tortuosity (2.5)
$V_{mol,STP}$	molar volume at STP conditions ($22.7 \cdot 10^{-3}$ m^3 mol^{-1})
ν	kinematic viscosity of water ($1.08 \cdot 10^{-6}$ m^2 s^{-1}, at 290 K)

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9. Discussion, conclusion and perspective

Indicated by the recent developments and supported also by this study, the conventional approach for nitrogen removal i.e., nitrification/denitrification, will be possibly slowly phased out. Especially regarding the higher oxygen demand in nitrification and the carbon demand of denitrification, emerging technologies e.g., the CANDO process, deammonification or nitritation/denitritation, have considerable advantages. In relation to decision-making on subsequent research and application of the CANDO process, the document-in-hand allows for a first preliminary assessment regarding the technical and economic viability of the CANDO process in comparison to the other state-of-the-art treatment technologies.

The conducted research was directed toward the extension of previous fundamental research with the intention of subsequent knowledge transfer and a potential scale-up. Throughout the course of the investigations, the viability under real feed-stream conditions could be demonstrated for the first time and key parameters and processing conditions were derived to enable future site-specific process design (**Paper #2**). Beyond this, an operational strategy was developed enabling the process automation of both stages in continuous operation, which could be employed in future experimental and technical applications (**Paper #4**).

Further investigations addressed different N_2O production and removal strategies and technologies (**Papers #2 and #3**) as well as their impact on process performance. The results provide the first basis for the integration of potentially technically viable solutions for N_2O production and harvesting at wastewater treatment facilities. In combination with the development of the biological stages, the results provide a new perspective considering the technical application potential of the CANDO process, possibly in the biogas sector in general.

An additional scientific merit at the theoretical level is the proposed novel terminology, which provides a consistent nomenclature for the presently known processes occurring in nitrogen removal technology, also beyond yet investigated processes (**Paper #1**).

Based on these results, a differential preliminary assessment on the competitiveness of the CANDO process compared to other state-of-the-art technologies is given in the following sections. In this respect, the feasibility in relation to carbon and oxygen demand, but also regarding start-up behavior and operational stability is discussed. Additionally, the various knowledge gaps on the mechanistic level and regarding the operation and integration of the CANDO process are highlighted to provide a future research and application perspective.

9.1 Scientific merit of the proposed terminology

The necessity of the presented terminology remains evident to enable a clear mechanistic comparison of the biological processes applied in nitrogen removal technology. Especially with respect to the increasing number of processes in different configurations and applications, the proposed terminology (**Paper #1**) provides a basis for a consistent scientific course of discussion now and in the future. Especially regarding on-going investigations (Ma et al., 2017; Sun et al., 2017) concerning N_2O or potentially NO related pathways and emissions, the novel expressions “nitrous nitritation”, “nitrous denitritation” or “nitric denitritation” now precisely define respective pathways involved.

So far, the terminology still lacks acceptance. Throughout the literature research during the composition of this document, the term “nitrifier denitrification” is still commonly used (Jensen et al., 2017; Su et al., 2017) to describe N_2O emissions under sub-oxic conditions despite the distinct mechanisms which are involved (cf. Chapter 3.1). Further previous misleading terminology could also be identified. As for instance, “denitratation” (Johansson et al., 1998) is used in the meaning of “denitrification”.

Considering **Paper #1** has only recently been published, it is possibly too early to judge about its acceptance. However, scientific interest in the terminology is remarkable as it has been among the top 10 of requested publications in *Environmental Science: Water, Research & Technology* in 2017.

9.2 Technical application potential of the CANDO process

Both stages of the CANDO were successfully operated, and eventually automated treating digester reject water by applying for the first time a real carbon source i.e., primary effluent. The applicability of primary effluent is hence, a promising option for the integration of the CANDO process on wastewater treatment plants and will potentially considerably reduce the operational costs in comparison to the application of acetate (Scherson et al., 2014) or propionate (Gao et al., 2017).

The results published in **Papers #2-4** serve as a basis for reactor design and operation of a subsequent scale-up. In this respect, especially the determined sludge-specific reaction rates as well as the ratio of COD/N/MLVSS enable preliminary assessments for site-specific design of reactors and peripherals. Furthermore, comprehensive data about the start-up behavior, system performance e.g., N_2O yields and NO_2^- elimination rates in addition to the elucidation of process dynamics have underlined the application potential as side-stream treatment technology for digester reject water.

9.2.1 Start-up

An important issue concerning the technical applicability of processes is on-site process implementation. Based on the presented results (**Paper #2**), a start-up of the CANDO process in technical scale appears to be feasible. Considering the first stage, although adapted seed sludge was used for the inoculation in this study, typically, nitritation systems can be well established on-site from conventional activated seed sludge. Various applications of partial nitritation and nitritation systems and respective selection mechanisms were described earlier. Main selectors - starting with an inoculum from a conventional activated sludge process - promoting AOB growth are: mesophilic temperature conditions (Hellinga et al., 1998), low DO concentrations (Hanaki et al., 1990; Laanbroek and Gerards, 1993) and nitrite accumulation (Fux et al., 2003; Lai et al., 2004). In combination with low SRTs (1 to 2 d), the predominance of AOB within a nitritation system can be expected after approximately 20 days of operation (Galí et al., 2007; van Dongen et al., 2001).

The start-up behavior of the nitrous denitritation stage had not yet been described in detail and was unexpectedly fast. Turnover rates of NO_2^- to N_2O of 70% were observed within two weeks after inoculation with conventional activated sludge. Hence, the adaption potential of conventional activated sludge to the applied conditions appears to be very high.

Denitritation systems have been described to exhibit even faster adaption, potentially for a similar reason as observed for the CANDO (Torà et al., 2011). In contrast, anoxic ammonia oxidation, which is necessary for deammonification, the capability for heterotrophic reduction of NO_2^- is typically already inherited within a high share of the denitrifying microbial community of a conventional activated seed sludge. This possibly also enabled the observed complete NO_2^- elimination throughout the start-up period and transition from denitritation to nitrous denitritation. To further elucidate the start-up mechanisms, more detailed studies involving next-generation sequencing tools and metagenomic analyses should be conducted to identify key players and functions as part of the process under real feed-stream conditions. Additionally, the role of phosphate availability regarding the predominance of glycogen or polyhydroxyalcanoates accumulating organisms (Zeng et al., 2003) will possibly enhance the start-up and stability of the process during continuous operation.

In comparison to the deammonification systems re-inoculation can be conducted from a local sample, which is especially beneficial for two reasons. The considerable costs, which are typically related to the acquisition of seeding sludge for deammonification processes can be spared. Additionally, potential fines in the case of an operational failure and, consequently potentially exceeded discharge standards, can be avoided by the inoculation with local seeding sludge (**Paper #2**).

9.2.2 Process stability

As presented in **Paper #2** and **Paper #4**, the previously described process dynamics and mechanisms in the second stage of the CANDO process applying synthetic substrates are reproducible applying real wastewaters e.g., primary effluent, in continuous operation. Accumulation of PHB and utilization as reducing equivalent for NO_2^- could be observed in anaerobic and anoxic periods, respectively. The N_2O yields were not significantly different from the ones achieved with synthetic feeds (Gao et al., 2017; Scherson et al., 2014). Hence, it is evident that the more complex organic substrates contained in municipal wastewater are technically and economically viable substitutes. Additionally, it could be demonstrated, that pre-conditioning measures by e.g., hydrolysis or acidification under anaerobic conditions, as proposed earlier (Scherson et al., 2014) are potentially unnecessary.

The continuous operation under real feed-stream conditions is challenging for various reasons. Aiming for a constantly high biomass concentration, challenges are especially related to comparatively low COD concentrations of untreated municipal wastewater (**Paper #2**). The necessary high organic loading rates for efficient nitrogen elimination result in critical cyclic exchange volumes at competitive daily nitrogen loads (>50%). Although coagulant application is an efficient measure to counter-act biomass washout and hence, retain active biomass in the system, alternative operational strategies or reactor setups might also be feasible means and facilitate operation without the addition of chemicals. A membrane-bioreactor (MBR) setup is a potentially viable alternative. The application of membrane technology enables the retention of flocs or even single cells and hence, complete biomass retention. The retention efficiency in terms of biomass accumulation is controlled by the membrane pore size. Commercially available systems are often operated applying ultrafiltration or microfiltration membranes. While ultrafiltration enables complete biomass and cell retention (0,01 µm < nominal pore size < 0.3 µm), microfiltration (0.3 µm < nominal pore-size > 100 µm) will potentially mostly increase the retention of flocs (> 10 µm) and particulate matter. In this respect, the effect of the membrane type on biomass growth and accumulation i.e., the start-up potential, should be investigated as a preliminary study for the design of a MBR as second CANDO stage.

Regarding ultrafiltration, the retention of more complex organic substrates will potentially support the hydrolysis and further anaerobic substrate degradation processes (Wellinger et al., 2013) supporting the production of precursors for the incorporation of COD and intracellular production of polyhydroxyalcanoates (PHAs). Reflecting the necessity of PHAs for efficient N_2O production, increasing the substrate retention time by the application of membrane technology potentially enhances the accumulation of intracellular carbon. Ultimately, higher N_2O production rates will theoretically be triggered. Regarding the deviations of N_2O production presented in **Paper #2** and **Paper #3** assuming first-order reaction kinetics appears reasonable. Hence, it is likely that membrane operation accelerates the N_2O production rates exponentially, especially during the initial anoxic phase under higher PHB concentrations. In turn, process intensification is a likely result and would contribute to decreasing process scales.

During implementation and continuous operation of an MBR, the repetitive fill-and-draw strategy, as described in **Paper #2**, should be altered to continuous feeding and withdrawal via the membrane during anaerobic phases, which would simultaneously provide the option to increase the organic loading rate and MLVSS concentrations

(Wei et al., 2014). With conventional membranes in full-scale treatment of municipal wastewater mixed-liquor biomass concentrations up to 15.0 g_{MLVSS}/l are reported applying ultrafiltration membranes (Sabia et al., 2013). In addition to the smaller process scale, an increase and further degradation of organic substrates will potentially result in higher effluent quality regarding COD loads, too. Especially regarding decentralized treatment and direct discharge of e.g., industrial wastewaters, this effect would be beneficial and should be addressed in future research. Furthermore, as the PHB accumulating organisms exhibit a high potential for the degradation of trace organic chemicals (TOrCs) e.g., *comamonas testeronii* (Scherson et al., 2014), the biomass accumulation coupled to higher substrate retention times will possibly yield high degradation rates of TOrCs as well.

During experimental investigations, tight monitoring of process dynamics and effluent concentrations of nitrogen compounds and COD should be conducted. In addition to PHB measurements, the abundance of alternative intracellular polymeric substances e.g., poly-hydroxyvalerates and glycogen, but also polyphosphate as conducted by Gao et al. (2017) should also be considered for better process understanding regarding the effect of the feed quality. Mixed liquor measurements of volatile fatty acid concentrations and carbonic acids will potentially support the understanding of substrate degradation and the mechanistic understanding of necessary microbiological steps and bottlenecks in the PHB production pathway under real feed-stream conditions. Ultimately, monitoring of TOrCs will elucidate the given hypothesis concerning a high degradation potential.

Similar mechanisms as described for ultrafiltration membranes can be expected by the application of a microfiltration step. However, the substrate retention time of dissolved non-particulate matter (< 0.3 μm) cannot be expected to increase to the same extend due to the larger pore size. However, efficient retention of flocculated biomass can be expected enabling higher sludge concentrations as well. For the operation of anaerobic MBRs applying microfiltration, biomass concentrations up to 10 g_{MLVSS}/L are technically viable (Zheng et al., 2018; Gurung et al., 2016). Referring the COD:N:MLVSS concentration, elaborated in **Paper #2**, higher organic and nitrogen loading rates are likely to be applicable for increasing process efficiency. The specific loads, however, should be determined as they also potentially depend on the process scale, substrate composition, and other factors such as the feeding regime.

From an economic perspective, the operational and investment costs of a membrane unit will also need to be assessed and should be compared to the presented approach. Based on a previous life-cycle assessment, the minimum operational costs of an anaerobic membrane reactor treating moderate and high-loaded municipal wastewater have been assessed to 0.14 €/m³ (Pretel et al., 2016). However, these costs strongly depend on the membrane applied. Applying the microfiltration membranes, lower operational costs are expected due to lower driving pressures employing smaller pore sizes. The operational costs of an ultrafiltration approach might hence, compensate for the process efficiency. In both cases, membrane fouling needs to be investigated to assess the particular membrane lifetime.

Independent of future investigations, it is likely that microfiltration membranes are related to higher economic feasibility, but are possibly limited regarding effluent quality. Hence, for the proposed concept in **Paper #2**, the microfiltration technology appears to be more suitable. However, considering applications, which require higher effluent quality as in decentralized treatment processes (without an option of main stream post-treatment) or water reuse applications, ultrafiltration should be considered, especially aiming for advanced treatment objectives.

With respect to process integration (i.e., coupling N_2O production, removal and combustion), the MBR setup will likely increase the performance and lifetime of the proposed membrane contactor for gas removal (**Paper #3)**. By retaining biomass from the HFMC, membrane fouling will most likely be mitigated. In this respect, the ultrafiltration membrane, which is hypothesized to achieve higher substrate degradation and biomass retention, is possibly superior to the microfiltration. In both cases, a continuous feeding throughout the anoxic reaction phase is proposed to increase the concentration gradient of product and educt (NO_2^- and N_2O) and hence, increase the reaction rate according to the common mass-action law. A recirculation over the reactor should be targeted to minimize NO_2^- effluent concentrations.

Further attention should be given to the design parameters of future plants, too. Considering effluent concentrations, in comparison to synthetic feeds, the degradability of the COD is lower and the refractory part of the COD will remain in the effluent. In addition to the low COD concentrations of the primary effluent, its lower degradability results in higher COD/N ratio under real feed-stream conditions compared to synthetic feeds. For design of technical applications, it is likely that the specific COD/N ratio can be generally assessed by the BOD_5/COD ratio for the design of a CANDO system (**Paper #2**). In this respect, it is likely that the COD/N ratio of approximately 3, as given previously (Scherson et al., 2014), can be used as a reference for the necessary BOD_5/N ratio for real feed-stream applications. Ultimately, this will possibly decrease the energy recover potential from organic matter in comparison to readily degradable substrates.

The previously published results for readily degradable substrates were in the range of 2.8 for propionate (Gao et al., 2017) to 3.1 for acetate (Gao et al., 2017). As proposed in **Paper #2** and confirmed in **Paper #4**, a COD/N ratio of 4 is required at the applied operational conditions. The energy yield from organic substrates thereby theoretically decreases by approximately 2.25 kWh/kg_N removed (Horstmeyer et al., 2018). In this respect, it remains unclear, whether the non-bioavailable share of the COD, which increases the COD/N ration under real feed-stream conditions is readily degradable for biomethane production. This issue needs further investigation for a more exact assessment of the energy balance.

It should be evaluated, whether the energy yield could possibly compensate for the costs of synthetic feeds in future investigations. In this respect, different carbon sources should be also considered. Especially regarding the viability of treating industrial wastewater or digestates from agricultural biogas or composting plants, methanol and ethanol, as the most applied synthetic carbon sources, represent cheaper solutions (Torà et al., 2011) and viable options for future investigations and decentralized applications.

In contrast to the obvious impact of degradability, under steady-state operation the effect of the high variance of particulate and soluble fractions in the available COD while using municipal wastewater appears to be negligible. Soluble as well particulate matter are utilized for PHB formation, although it is evident that soluble COD is the preferable source (**Paper #2**). In relation to the particulate COD, it remained unclear throughout the investigations which parts of the particulate matter is consumed and how it is utilized. Hence, additional research should potentially address the relevance of anaerobic processes i.e., hydrolysis and acidogenesis, involved in the degradation of complex matter and the respective formation of intracellular storage compounds during anaerobic reaction phases. Previous investigations toward this direction have been conducted by investigating the production of volatile fatty acids in correlation to the oxidation reduction potential. The findings demonstrated that VFA production was optimal at ORP values lower than -100 mV (Ding et al., 2016). These results hold also promise for further development of the operational strategy presented in **Paper #4** by the integration of anaerobic process control.

The observed dynamics of intracellular PHB formation and subsequent N_2O production are in agreement with previously published results (Liu et al., 2015; Zeng et al., 2003). They prove that process dynamics under controlled conditions are reproducible under real feed-stream conditions. Additionally, these findings underline the correlation of endogenous substrate respiration and considerable N_2O yields under anoxic conditions. Especially in comparison with the low N_2O emissions from denitritation processes operated at higher COD/N ratios (Liu et al., 2015), it is likely that the accumulation of N_2O is rooted in the degradation rate of storage compounds rather than metabolic rate constants of NosZ or qNOR and cNOR. To increase the mechanistic understanding on N_2O accumulation, further studies on endogenous substrate respiration in relation to the respiratory mechanisms of the nitrogen metabolism should be conducted. Possible assessments to address this question about the rate limiting step could involve biomolecular techniques. In this respect, the investigation of gene expression by mRNA isolation and qPCR evaluation throughout the different process steps could elucidate the regulatory mechanisms involved in N_2O accumulation. Formulating and investigating a negative hypothesis e.g., no correlation assumed between nitrogen metabolism related gene expression and N_2O accumulation, would be one option. In combination with genetically modified organisms e.g., by gene knockouts of NosZ, would enable the determination of nitric oxide reduction rates under CANDO specific alternating conditions. In addition, supply of N_2O as substrate would facilitate the determination of N_2O reduction rates in the non-genetically-modified organisms. Finally, the addition of different substrates as conducted by Gao et al. (2017) in parallel experiments will elucidate, whether the degradation of the intracellular storage compound (e.g., PHB, PHV or glycogen), will result in different process rates. From the present point of view, these rates should vary based on the hypothesis that N_2O is only depending on the degradation of these intracellular compounds.

The experimental procedure need to be executed at laboratory scale under very controlled conditions and should involve the isolation and cultivation of representative organism, e.g., *comamonadaceae*, from an operating CANDO system. The DNA sequences of qNorB, cNorB and NosZ have earlier been identified and are available in common databases such as NCBI BLAST®. They should serve for the design of primers. After confirming the suitability of primers and the abundance of the respective gene within the model organism, gene knockout applying site specific nucleases by e.g., clustered regularly interspaced short palindromic repeats (CRISPR) (Cong et al., 2013), transcription activator-like effector nucleases (TALENs) (Joung et al., 2012) or zinc-fingers (Klug et al., 2010), can be performed.

The subsequent experimental series should be conducted preferably in triplicates. The proposed design procedure includes the following combinations of factors resulting in 36 runs:

- two cell cultures (with and without gene knockout)
- three organic substrates targeting different storage compounds:
  - acetate → PHB (Gao et al., 2017)
  - propanol → PHV (Gao et al., 2017)
  - glucose → glycogen (Jeon and Park, 1999)
- nitrogen sources (N_2O, NO_2^-)

Tight monitoring of process dynamics should be conducted involving the measurement of nitrogen compounds, especially online N_2O measurement and grab-sampling for the analyses of storage compounds, gene expression measurements, dissolved substrates and biomass concentration/cell density to derive reaction kinetics throughout representative cycles.

9.2.3 N_2O removal and utilization

Considering the targeted combustion of N_2O, either enrichment of ambient air or biogas would enable process integration possibly without further gas-treatment. Reflecting on the results presented in **Paper #2** and necessary process conditions, elucidates the related challenges, especially aiming for high N_2O yields. As it was evident that continuous removal yields higher N_2O yields, a dispersive boundary layer as for instance, during gas stripping, will need to be continuously established to enable N_2O mass-transfer. This will result in the removal of N_2O on the one hand, but on the other hand, strip gas constituents will diffuse into the mixed-liquor. In the case of ambient air, oxygen diffusion will likely lead to process failure. Considering the resulting aerobic conditions, the respiratory systems will potentially switch from reduction to oxidation (cf. Chapter 5). In contrast to the utilization of ambient air, biogas stripping would conserve anoxic processing conditions. However, methane diffusion into the mixed liquor, will possibly be utilized as extracellular reducing equivalent resulting in denitrousation as observed in the case of extracellular carbon availability (**Paper #2**).

Considering gas removal during a dedicated stripping phase, previous findings (Gao et al., 2017) have demonstrated the viability of air stripping. The expected N_2O yields are potentially lower by 10%, but depending on the energy demand of stripping towers, this will possibly be compensated. Biogas stripping could be an option, but – similar to continuous biogas stripping – result in the availability of extracellular carbon with could potentially mitigate N_2O yields by denitrousation in the mixed-liquor during stripping phases. Therefore, from the present state-of-knowledge, N_2O removal with ambient air in a dedicated stripping phase appears to be most feasible.

A general disadvantage that needs to be considered in terms of in-situ stripping is water vapor contained in the off-gas after passing the bioreactor. Water vapor is of general concern in the operation of CHP units, but can be typically easily removed from feed-gas. A potential alternative technology to reduce water vapor and potentially operational expenses as well as simultaneously increases off-gas concentrations is a membrane-contactor (**Paper #3**). The hydrophobic nature effectively retains water and water vapor from entering the sweeping-gas stream. Additionally, the operational costs of a membrane-contactor are considerably lower than for conventional gas stripping towers (Cookney et al., 2016).

The results of **Paper #3** illustrate the potential for N_2O removal in gas membrane contactors. The determined mass-transfer coefficients provide a basis for a scale-up and potential process integration. However, different questions concerning the application of the membrane contactor should be assessed in advance. Application in the mixed-liquor will possibly lead to considerable membrane fouling or clogging. Hence, the vulnerability to fouling and clogging should be investigated along with potential mitigation measures.

An alternative approach could possibly be the pre-treatment of the mixed-liquor by e.g., membrane-technology. However, as feasibility plays an important role in wastewater treatment, the different approaches should be compared to each other. Especially, the pre-treatment could considerably increase operational costs. However, as mentioned earlier depending on process configurations and desired effluent quality, additional costs can possibly be accepted. Therefore, the investigation of the different process combinations should be taken into consideration during subsequent research.

9.2.4 Nitrogen elimination, N_2O production and emission mitigation performance

During continuous operation, N_2O yields above 50% are presently realistic considering the operation of both stages, nitritation and nitrous denitritation. In continuous operation, the N_2O yields were 63% and 53% applying different N_2O extraction strategies, i.e., continuous removal and removal in a dedicated stripping phase, respectively (**Paper #2**). Hence, the operational strategy has a direct impact on the respective yield. In this respect, further investigations should be conducted at the mechanistic level to possibly increase the yield applying a dedicated removal phase. If it is possible to increase the yield to a range comparable to continuous removal, the operational efforts of running peripheral units (cf 7.2.3) could also be reduced.

The off-gas N_2O concentrations were in the range of 0.3% to 3.5% for continuous stripping and dedicated removal phase. Considering both, the energetic benefit in the operation of a CHP needs further investigation. However, regarding that the given values are comparably low, significant impact on the energy yield is potentially unlikely. In general, the energy recovery potential is mostly driven by the savings of reducing equivalents. Based on the typical concentrations of nitrogen and COD in municipal wastewater, the energy potential can be increased by approximately 7.73 kWh/kg_N removed compared to conventional treatment with nitrification/denitrification (Horstmeyer et al., 2018). The energy recovery of nitrogen via N_2O in this respect has a comparably low share of only 0.53 kWh/kg_N i.e., 6.7%. However, this value strongly depends on the composition of the feed matrix. Accordingly, higher values can be expected for the treatment of wastewater with lower COD/N ratios.

Regarding N_2O emission mitigation strategies, the CANDO process is still the most promising technology in theory (Horstmeyer et al., 2018). However, further research is required to quantify the exact mitigation potential in technical applications. For this purpose, an assessment should be conducted based on the integration of bioreactors, N_2O removal and combustion unit. Related investigations should comprise measurements of the CHP off-gas concentrations of nitrogen oxides and potential diffusive losses of N_2O throughout the entire process chain. Drawing an analogy between the emission potentials of N_2O from the CANDO process and methane emissions from anaerobic digestion, diffusive N_2O losses are probably negligible, if N_2O can be efficiently removed from the mixed liquor. Methane losses are especially related to methane saturated or even over-saturated digester effluents (Wellinger et al., 2013). Diffusive emissions from gas-tight or concrete reactors are negligible (Wellinger et al., 2013). Small amounts are emitted by gas slipping in CHP units (0.44-2.43%). Considering the GHG potential of N_2O these data indicate that exhaust gas of CHP units are a potentially critical step in the CANDO concept. The effective N_2O slip should therefore be assessed in future studies.

A crucial step considering N_2O emission mitigation will be the integration and hence, elimination of N_2O emissions from the nitritation stage as well. Presently, efficient N_2O generation in aerobic processing conditions are not feasible (cf. section 3.2). Hence, further research should be conducted on stable aerobic N_2O generation in the nitritation stage as well as harvesting strategies to potentially increase the overall N_2O yield and mitigate emissions.

9.2.5 Comparison to other state-of-the-art technologies

Based on the conducted research, the CANDO process is an another potentially technically and economically feasible option for the treatment of ammonia rich feed streams like digester reject water. The investigated operation, which has been independent from costly synthetic substrates, has demonstrated a potential on-site application, similar to deammonification or nitritation/denitritation systems considering substrate related operational expenses.

Considering previous (Fux et al., 2003; Galí et al., 2007) and observed findings regarding nitritation and nitrous denitritation systems under real feed-stream conditions, especially the startup of the CANDO process appears advantageous, especially in comparison to the deammonification process. The fast startup potential is beneficial in terms of operational safety as it provides an easy restart option from a locally available inoculum and substrate. In comparison to the denitritation, it appears to be comparable.

However, in contrast to the deammonification process, its operation is depending on organic carbon supply. In this respect, despite the comparably low COD/N ratio of 4 under real processing conditions, the deammonification process will be more feasible regarding its entirely autotrophic nature. Considering the typical composition of municipal wastewater, the additional recoverable energy potential from organic substrates is 10.9 kWh/kg_N removed, when nitrogen elimination is conducted by the deammonification process (Horstmeyer et al., 2018). Compared to nitrification/denitrification, however, the COD/N ratio is considerably lower. The amount of readily degradable substrates required for reliable denitrification is 7.6 kg_{COD}/kg_N removed and hence, more than twice as much as for nitrous denitritation (COD/N = 3). The COD/N ratio considering readily degradable substrates required for denitritation was also 3 (Torà et al., 2011). However, the stoichiometries of the particular reduction processes indicate a higher efficiency of nitrous denitritation:

Denitrification: $NO_3^- + 5\ H^+ + 5\ e^- \rightarrow 0.5\ N_2 + 2\ H_2O + OH^-$ (9)

Denitritation: $NO_2^- + 3\ H^+ + 3\ e^- \rightarrow 0.5\ N_2 + 1\ H_2O + OH^-$ (20)

Nitrous denitritation: $NO_2^- + 2\ H^+ + 2\ e^- \rightarrow 0.5\ N_2O + 0.5\ H_2O + OH^-$ (21)

According to equations Eq. (20) and Eq. (21), the necessary reducing equivalents, expressed in electrons, of the denitritation process are theoretically 33% higher. In this respect, further investigations are necessary to quantify the extent to which the endogenous substrate respiration as part of the nitrous denitritation possibly has an effect on the consumption of reducing equivalents.

Considering that both processes, denitritation and nitrous denitritation, require a COD/N ratio of 3, the energy recovery from carbon savings are equal. Additional 7.2 kWh/kg_N removed can potentially be generated compared to denitrification. A preliminary case study on the wastewater treatment plant Garching has shown that this could result in approximately 11.6% more energy compared to the presently applied main-stream treatment (Schweizer, 2015).

In addition to the carbon savings, the CANDO process offers the energetic valorization of N_2O. Based on the typical composition of municipal wastewater, approximately 0.52 kWh/m^3 of wastewater can be recovered via N_2O combustion. In the case study, which considered the treatment of the digester reject water, approximately 0.6% can be generated additionally by N_2O combustion. This figure is relatively low and possibly cannot compensate for the additional units that need to be operated. However, considering main-stream treatment for nitrogen removal will increase the energy recovery potential to 0.52 kWh/kg_N removed and hence, offers a recovery potential of approximately 6.8% based on the assumption that all organic matter is treated anaerobically (Horstmeyer et al., 2018).

Considering emissions, the CANDO process holds promise to mitigate the N_2O related emissions and related concerns in the water-energy-climate nexus (cf. Chapter 2). Its application will likely mitigate N_2O emissions and reliably eliminate nitrogen in combination with the possibility for energy recovery from waste and wastewater. However, further research is necessary in technical applications. For this purpose, a subsequent pilot-scale application will potentially facilitate the quantification of the emission mitigation potential. Beyond this, additional effort has to be spent on treatment or mitigation of emissions from the nitritation stage.

In summary, the CANDO process is an attractive application for nitrogen removal. Presently, the application for side-stream treatment on wastewater treatment plants is most likely the best option for process implementation and further areas of research. Beyond this application, main stream application, possibly in combination with alternative treatment schemes, offers a great potential for additional energy generation and hence, lower operational costs related to electrical power acquisition. Ultimately, an implementation of the CANDO process is a potential step in further leveraging energy recovery potentials and is therefore, another option for the transition of wastewater treatment plants to resource recovery units. In addition, if N_2O emissions by any means (e.g., slipping, effluent, nitritation stage) can be controlled, the CANDO process will be potentially also key for efficient N_2O emissions control and enable more sustainable operation of wastewater treatment plants, especially regarding nitrogen removal.

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Appenidx A – Supplemental information for Chapter 5

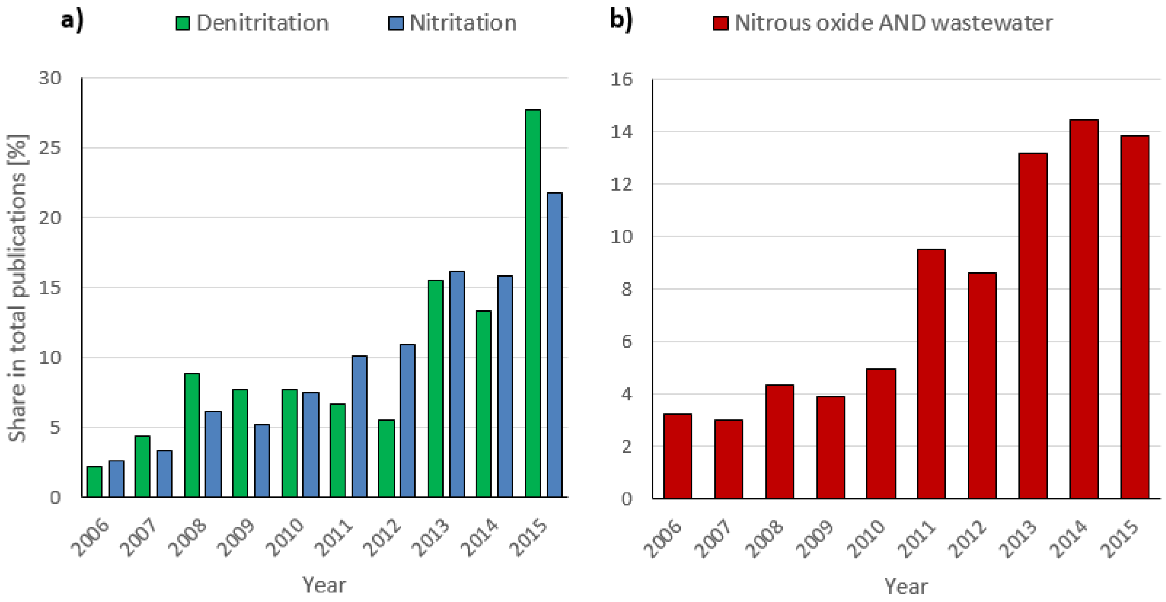

Figure A.1: Illustration of the publication trends in the core collection of Web of Science™ of a) nitritation, denitritation and b) publications including both terms nitrous oxide and wastewater in the topic.

To illustrate the present relevance of short-cut biological nitrogen removal processes the shares of total publications in the core collection of Web of Science™ per year from 2006 to 2015 are presented. As examples for short-cut BNR, the search was executed for the expressions (a) "*nitritation"* and "*denitritation*" and (b) "*nitrous oxide* AND *wastewater*". The increasing trends in a) and b) illustrate the historical development and underline the development of such processes towards central techniques in biological wastewater treatment. However, no consistent nomenclature exists for their description so far.

Appendix B - Supplemental information for Chapter 6

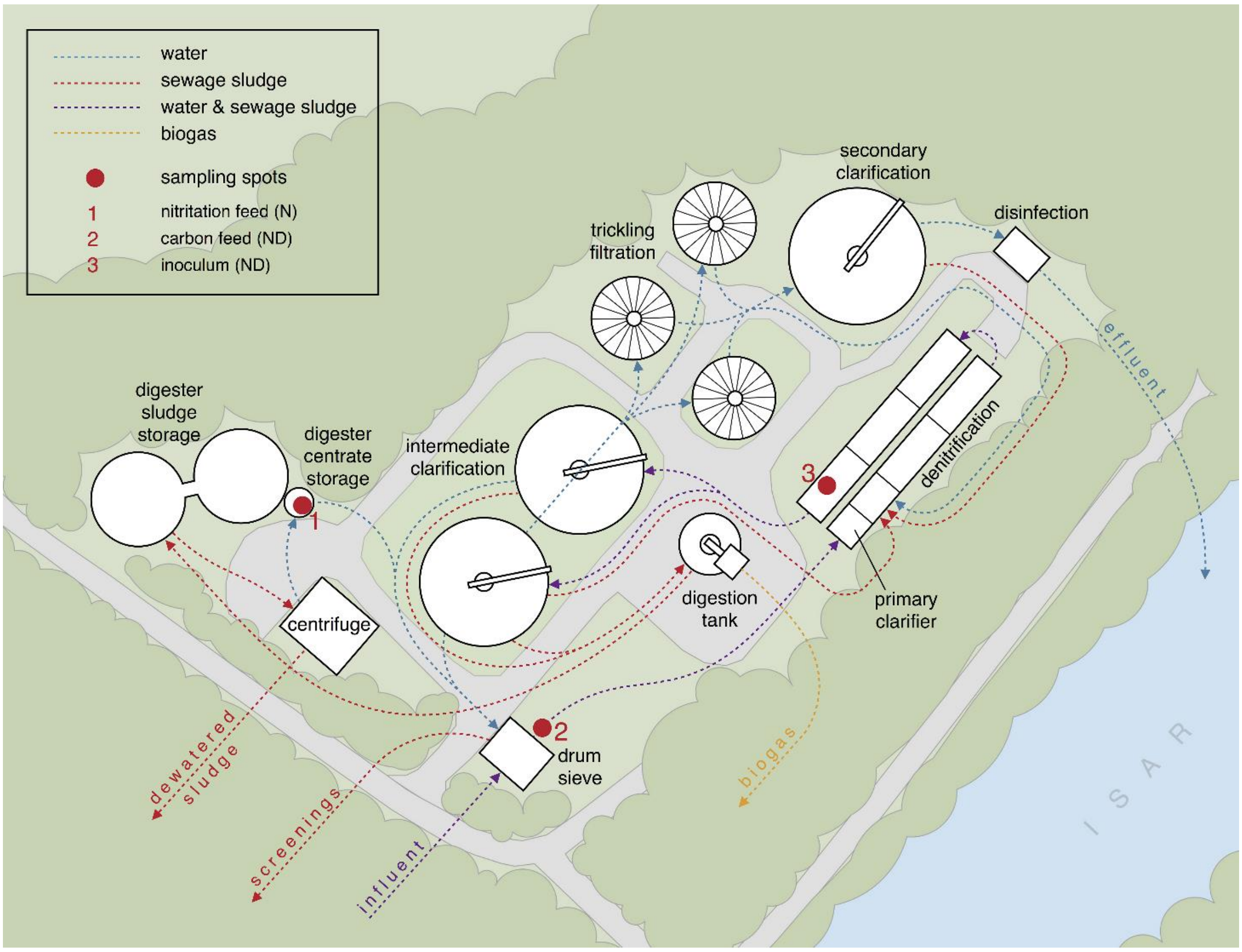

Figure B.1. Overview of WWTP Garching, including treatment units and flow connections. Additionally, the sampling points or the feed streams to the (1) nitritation and (2) nitrous-denitritation stages as well as for (3) the inoculum of the nitrous denitritation are indicated.

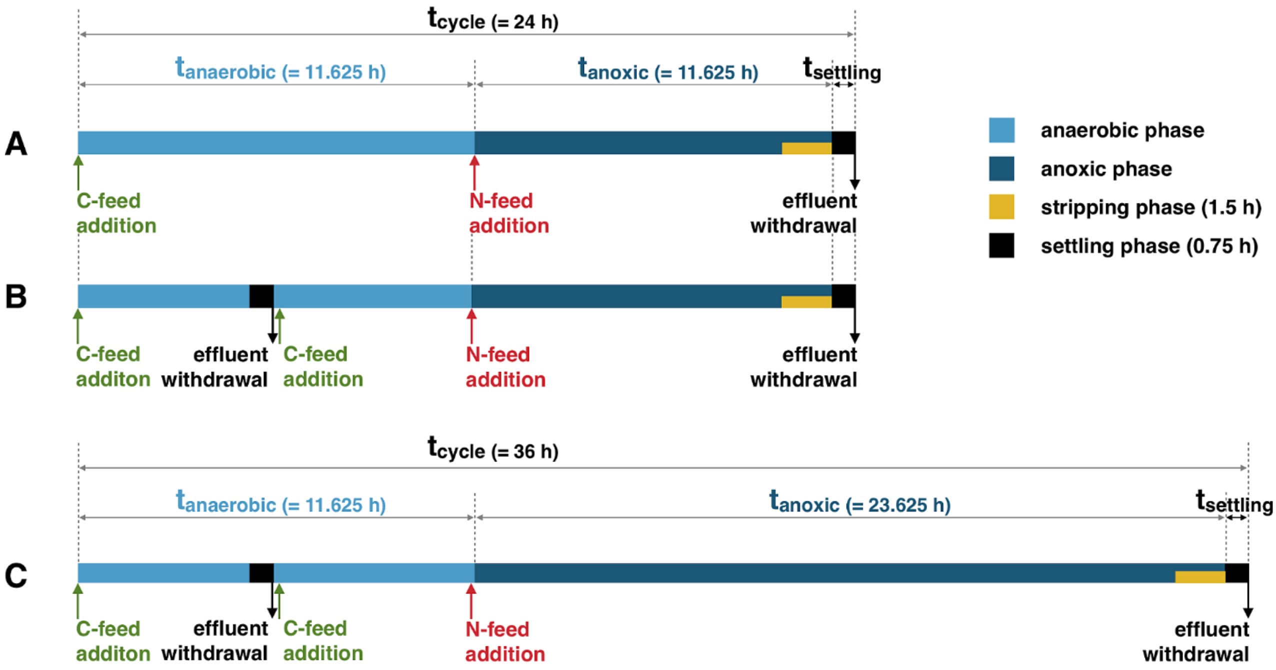

Figure B.2. Development of SBR programs for operation of the ND stage during investigational periods A–C. A (cycles 0–23): Operation with a single carbon feed and a 24-h cycle duration; B (cycles 24–63): integration of a second carbon feed to enable reactor-load increase; C (cycles 64–108): prolongation of the anoxic reaction period for complete nitrite reduction in the anoxic phase to 24 h.

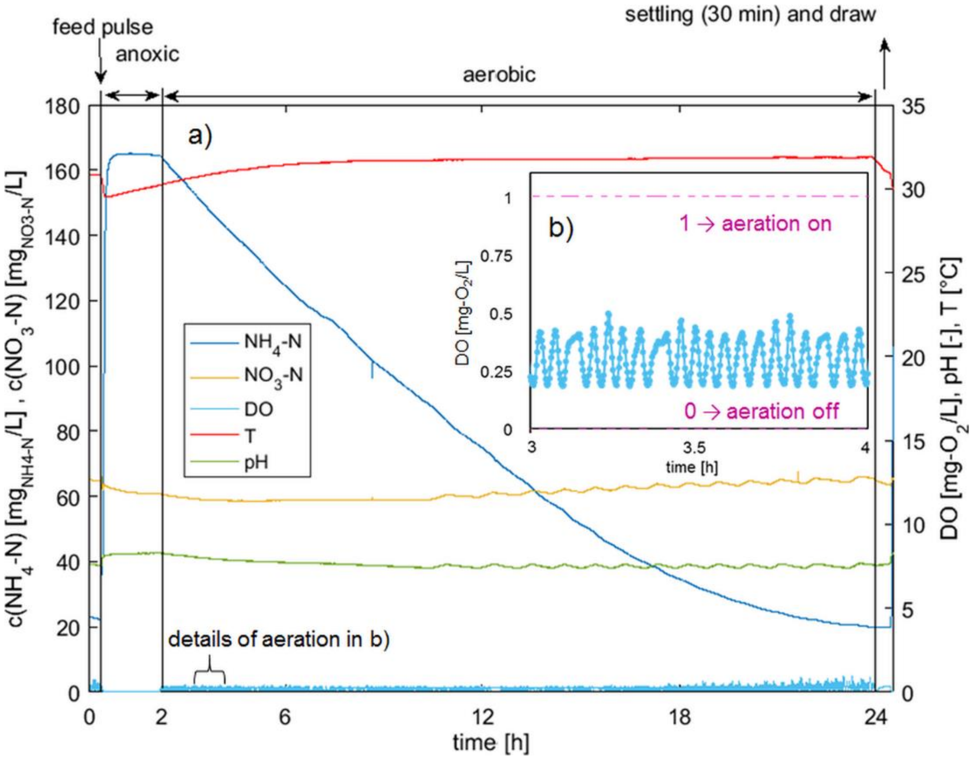

Figure B.3. Online-process diagram of a typical SBR cycle in the N stage, displaying in a) the mixed-liquor deviations of c(NH_4-N), c(NO_3-N), dissolved oxygen (DO), temperature (T) and pH. The first two hours of the cycle are kept anoxic to mitigate unintended nitrate concentrations; the following reaction period is intermittently aerated at 0.2 mg O2/L < DO < 0.5 mg O2/L. The batch is automatically stopped at a residual concentration of 20 mg NH_4-N/L, followed by a 30-min settling time and a subsequent draw phase. In b), the applied aeration strategy and DO concentrations are displayed in detail.

Appendix C – Supplemental information for Chapter 7

1 Quantification of intracellular poly-hydroxy butyrate

For the quantification of intracellular poly-hydroxy butyrate (PHB), methods proposed by Riis and Mai [1] and Wang et al. [2] were applied. For sample preparation, 100 mL of mixed liquor samples were taken per sampling point. The samples were centrifuged at 3,000 rpm (4K15, Sigma, Osterode am Harz, Germany) and the supernatant subsequently discarded. The harvested biomass was stored at -20 °C over night and subsequently freeze-dried (Alpha 1-4, Martin Christ, Osterode am Harz, Germany) for 24 h.

After freeze-drying, samples were grinded, and 80 mg were used for subsequent analyses. The samples were prepared in borosilicate vials (10 mL). For cell lysis and depolymerization of intracellular PHB and subsequent synthesis of 3-hydroxy-propyl ester, 1.5 mL HCl/Propanol (ratio 1/4), 0.5 mL of benzoic acid (BA) as internal standard and 1.5 mL of 1,2-di-chloroethane were added to the sample. Afterwards, the vials were stored for 2 h at 100 °C and shaken every 20 min.

At room temperature, the hydrophilic phase was discarded and 3 mL of deionized water were added to the sample. Subsequently, the samples were treated on a vortex mixer (Vortex Genie, neoLab, Heidelberg, Germany) for 10 min and the hydrophilic phase discarded again. The procedure was repeated twice. Afterwards, sodium sulfate was added. After settling of residual cell mass, the organic phase was transferred into 1.5 mL glass vials and sealed for subsequent gas chromatographic analysis.

The quantification was conducted with a gas chromatography device (7890 GC, Agilent, Santa Clara, California, USA), which was equipped with a DB-5 column (Agilent, Santa Clara, California, USA). The nitrogen flow rate was 1 mL/min and temperature started at 50 °C, was held for 3 min and subsequently, continuously increased at a gradient of 15 °C/min to 325 °C. The final temperature was held for another 15 min. Target compound and internal standard were expected at retention times of 7.69 min and 10.28 min, respectively.

The calibration (Figure C1) was conducted for standards of propyl benzoate (PB) (Table C1) and the synthesized products of 3-hydroxybutyric acid (3HB) (Sigma Aldrich, St. Louis Missouri, USA) with propanol, and benzoic acid (Sigma Aldrich, St. Louis Missouri, USA) and propanol (Table C2), respectively.

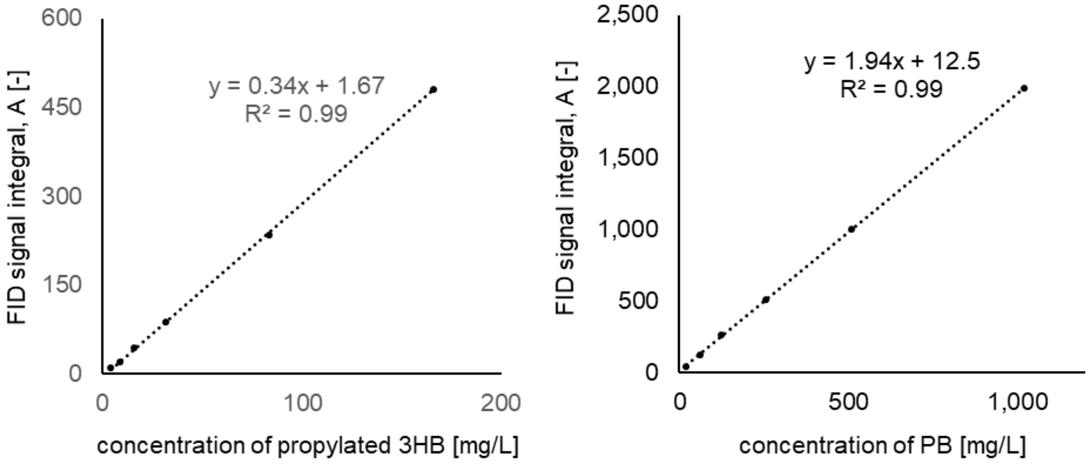

Figure C.1. Calibration results for the quantification of propylated 3-hydroxybutyric acid (3HB) displayed in a) and propyl benzoate (PA) displayed in b) by flame ionisation detection(FID).

Table C.1. Calibration data for the quantification of the propylated benzoic acid (PB) and integral of measurment signal (A) as well as the respective concentration of benzoic acid (BA) as internal standard of the conducted syntheses.

calibration BA	level	c(PB) [mg/L]	A_{PB} [-]	c(BA) [mg/L]
	1	20.4	43.2	15.2
	2	61.3	129	45.6
	3	123	261	91.2
	4	255	508	190
	5	510	1005	380
	6	1,020	1991	759

Table C2. Calibration data for the quantification of the propylated 3-hydroxybutyric acid (3HB). The integrated measurment signal (A_{3HB}) for 3HB as well as the propylated benzoic acid (A_{PB}) as internal standard, the reaction efficiency (η) and the respective concentration (c_{eff}(3HB)) of the propylated 3HB are given.

calibration PHB	level	c(3HB) [mg/L]	A_{3HB} [-]	A_{PB} [-]	efficiency η [%]	c_{eff}(3HB) [mg/L]
	1	12.5	9.2	93.6	34.2	4.3
	2	25	20.0	95	34.8	8.7
	3	50	43.2	87.3	31.9	16.0
	4	100	87.2	87.1	31.9	31.8
	5	250	234	91.3	33.4	83.5
	6	500	1,208	90.7	33.2	165.8

2 Measurement of dissolved compounds

For measurements of effluent and feed as well as grab sample concentrations of COD, NH_4-N, NO_2-N, and NO_3-N, photometrical cuvette tests (Hach-Lange, Berlin, Germany) were used according to the manufacturer's instructions. Nitrogen and dissolved COD (dCOD) samples were filtrated (0.45 µm) and stored for no longer than 12 h at 4 °C prior to analysis.

3 Preliminary results

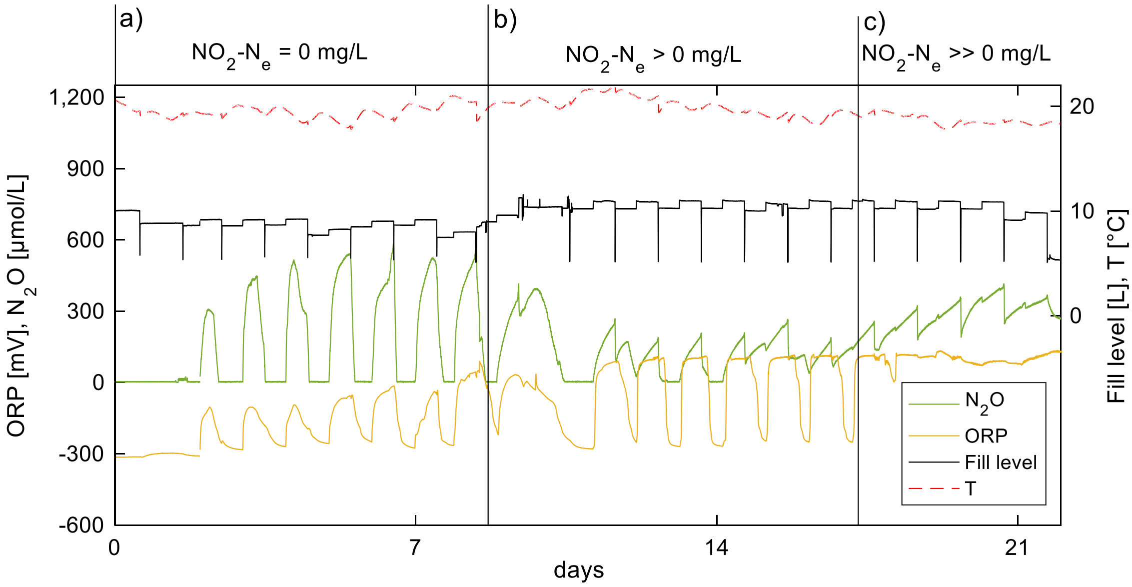

Figure C.2. Online data of dissolved mixed liquor N_2O concentrations (N_2O), the oxidation reduction potential (ORP), reactor fill level (Fill level) and reactor temperature (T) observed during preliminary investigations for the operation of a nitrous denitritation stage under real feed-stream conditions. In phase a), the reactor performance developed over the first eight days indicated by the increasing dissolved N_2O concentrations. Simultaneously, the ORP amplitudes increased with increased N_2O production. In phase b), effluent nitrite concentrations were constantly observed. However, the ORP potential decreased as soon as the mixed liquor nitrite was mostly removed. Simultaneously, the ORP amplitudes remained at values above 280 mV and the N_2O production decreased. In phase c), a system failure occurred, and nitrite constantly accumulated within the system. Accordingly, the ORP remained at the upper level of the amplitudes observed in phase b).

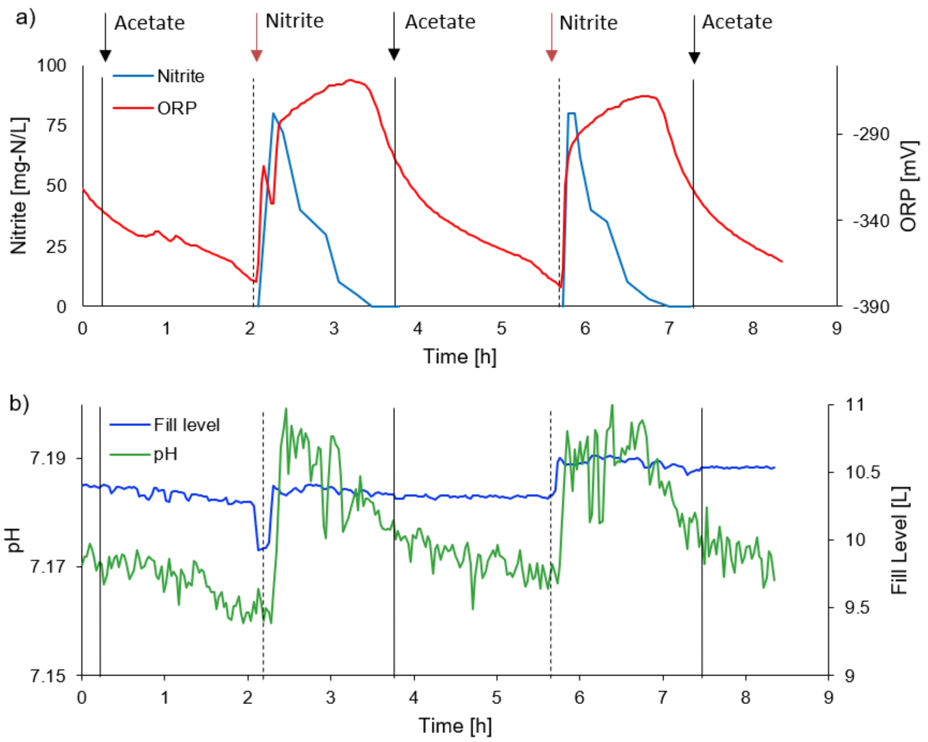

Figure C.3. Preliminary study on the application of the ORP as control variable in a denitritation reactor. Representative cycles of a continuously operated system in sequencing-batch-operation are displayed. Acetate and nitrite-rich effluents from the first stage of the investigated CANDO system were applied as feeds at a COD/N ratio of 6. As observed in the nitrous denitritation reactor, the oxidation reduction potential (ORP) increased after the nitrite pulse (a)) and decreased as soon as nitrite was depleted. The pH (b)) even increased slightly after the nitrite pulse simultaneously to the ORP. This observation indicates that the effect of nitrite addition on the ORP is potentially even more dominant than the change in pH in denitritation systems, as pH and ORP are typically negatively correlated. They support the findings in the nitrous denitritation reactor presented in the manuscript, and additionally indicate the application potential for operation and control of denitritation stages.

References

[1] V. Riis, W. Mai, Gas chromatographic determination of poly-β-hydroxybutyric acid in microbial biomass after hydrochloric acid propanolysis, J. Chromatogr. A 445 (1988) 285–289.

[2] B. Wang, R.R. Sharma-Shivappa, J.W. Olson, S.A. Khan, Production of polyhydroxybutyrate (PHB) by Alcaligenes latus using sugarbeet juice, Ind. Crop. Prod. 43 (2013) 802–811.

Appendix D – Supplemental information for Chapter 8

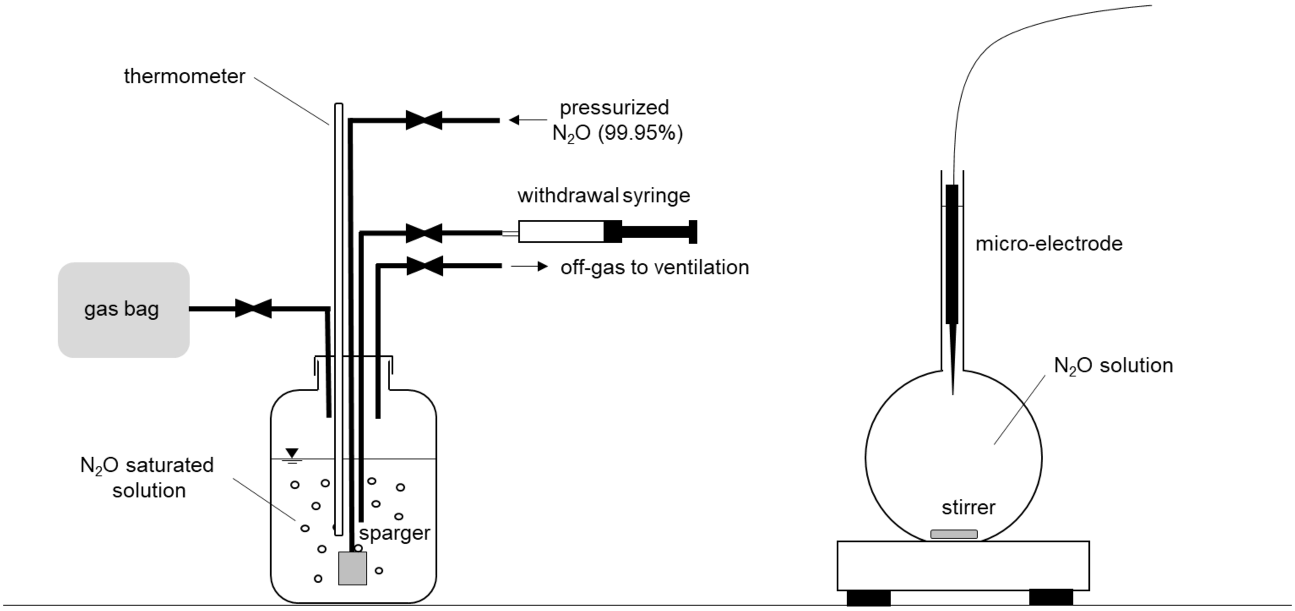

Figure D.1. Setup for the preparation of N_2O saturated stock solution (left) and calibration of the micro-electrode for dissolved N_2O measurements (right).

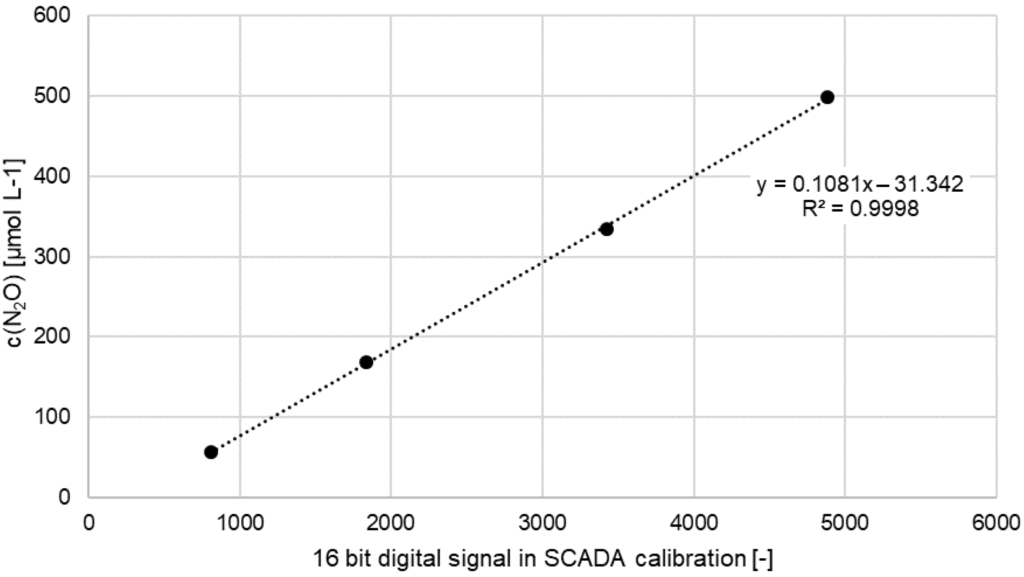

Figure D.2. Example of a four-point calibration and linear regression to the 16-bit measurement signal of the SCADA system for the application of the N_2O-microelectrodes.

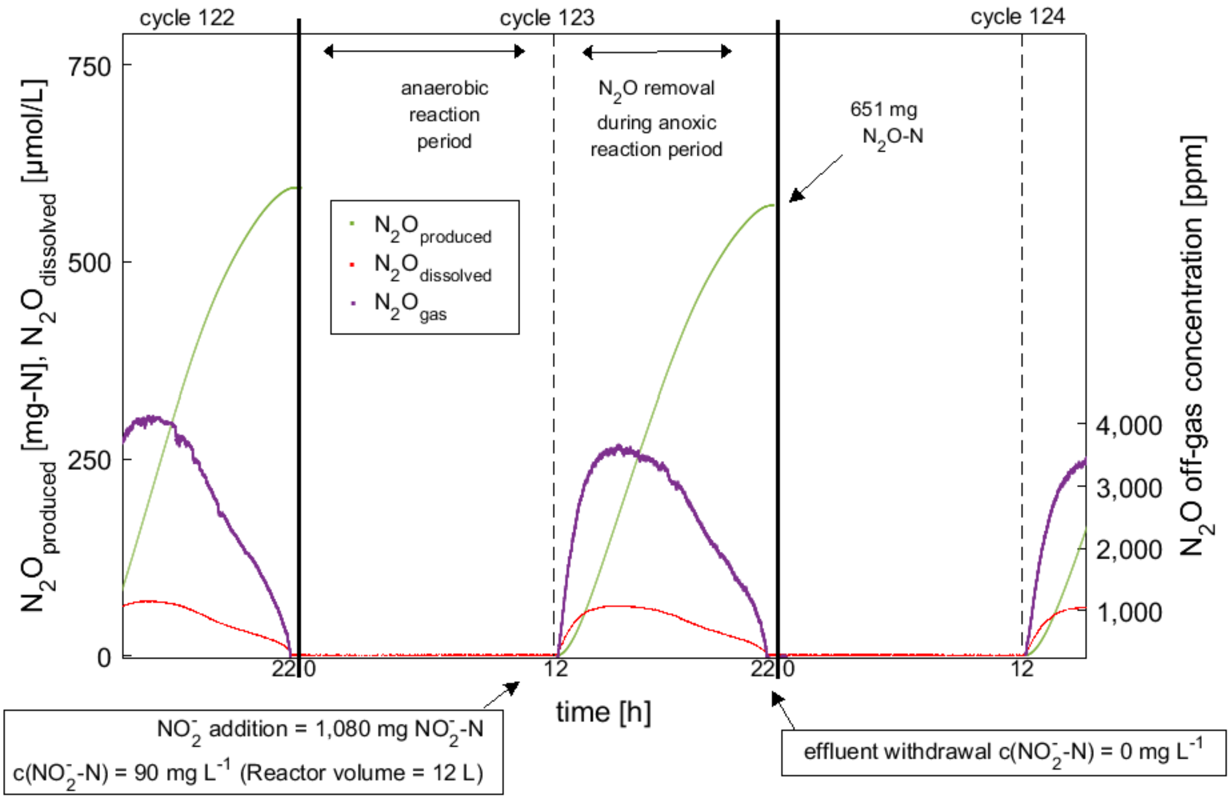

Figure D.3. Deviations of dissolved N_2O ($N_2O_{dissolved}$) concentrations and cummulative N_2O production ($N_2O_{produced}$) in consecutive cycles during SBR operation of a lab-scale coupled aerobic-anoxic nitrous decomposition operation (CANDO) with initial nitrite concentrations of 90 mg NO_2-N L^{-1}. The detailed SBR cycle (cycle 123) consisted of a 12-h anaerobic reaction period and a subsequent 10-h anoxic period in which N_2O is continuously produced and removed by gas stripping. Throughout the presented cycles, N_2O was continuously removed from the mixed liquor by gas-stripping at a gas flow rate of 500 mL min^{-1}.