Water Science & Technology

Water Science & Technology Vol 85 No 9, 2539 doi: 10.2166/wst.2022.131

Mainstream short-cut N removal modelling: current status and perspectives

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ABSTRACT

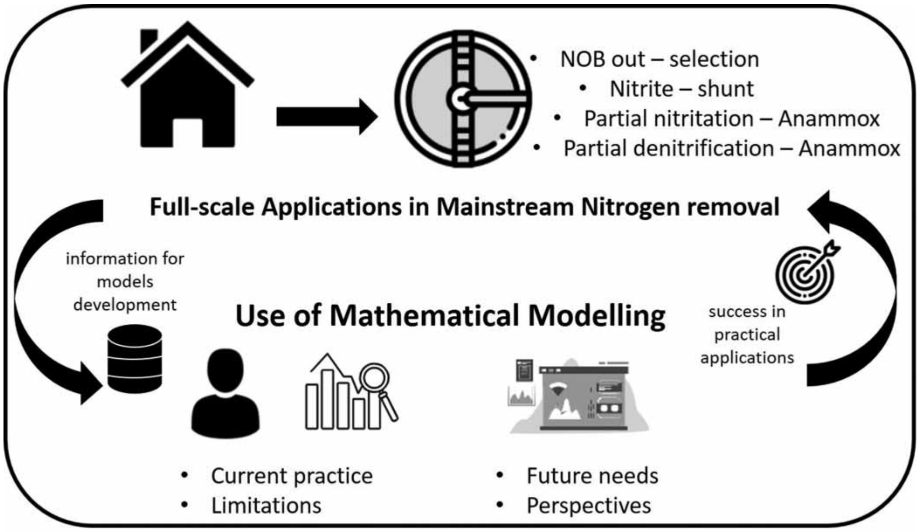
This work gives an overview of the state-of-the-art in modelling of short-cut processes for nitrogen removal in mainstream wastewater treatment and presents future perspectives for directing research efforts in line with the needs of practice. The modelling status for deammonification (i.e., anammox-based) and nitrite-shunt processes is presented with its challenges and limitations. The importance of mathematical models for considering N_2O emissions in the design and operation of short-cut nitrogen removal processes is considered as well. Modelling goals and potential benefits are presented and the needs for new and more advanced approaches are identified. Overall, this contribution presents how existing and future mathematical models can accelerate successful full-scale mainstream short-cut nitrogen removal applications.

Key words: anammox, deammonification, energy optimization, mathematical modelling, partial denitrification, partial nitritation, resource optimization

HIGHLIGHTS

- Models for mainstream short-cut N removal processes are reviewed by considering their current practice and limitations.
- Modelling goals and potential benefits are presented from a modeller perspective to facilitate successful applications.
- More advanced modelling approaches are presented to overcome the addressed challenges and limitations.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Conventional nitrogen (N) removal by nitrification/denitrification is an energy and resource-intensive process: nitrification requires oxygen and alkalinity, and denitrification requires carbon as either influent carbon or supplemental carbon. Compared to nitrification-denitrification, the application of short-cut N removal processes in mainstream wastewater treatment has significant potential to save energy (oxygen demand), resources (carbon demand), and to pursue energy independence for water resource recovery facilities (WRRF). For that reason, short-cut N removal (deammonification and nitrite shunt) has received considerable attention over the last decade from both academia and practice.

Deammonification short-cuts the conventional N removal pathway by directly converting ammonium (NH_4^+-N) to nitrogen gas (N_2) via nitrite (NO_2^--N). The process relies on preventing the oxidation of nitrite to nitrate (NO_3^--N) and making nitrite available for anammox (Zhang *et al.* 2019). The availability of nitrite can be achieved through two pathways: partial nitritation-anammox (PNA) or partial denitrification-anammox (PdNA) (Figure 1). In practice, PNA and PdNA can be combined in full-scale applications for desired N removal performance.

The efficiency of deammonification has been proven for ammonium-rich wastewater such as treatment of side-streams resulting from dewatering of digested sludge, leachate, or industrial wastewaters (van Dongen *et al.* 2001; Wyffels *et al.* 2004; Volcke *et al.* 2005; Wett 2007; Ganigué *et al.* 2009; Lackner *et al.* 2014). Short-cut N removal can also be combined with a pretreatment process for carbon diversion in mainstream applications such as high-rate activated sludge (HRAS), chemically enhanced primary treatment and energy recovery in the side-stream, or even direct anaerobic sewage treatment (Kartal *et al.* 2010; Leal *et al.* 2016). This provides WRRFs with an excellent opportunity to move to energy-neutral or energy-positive operations (Jetten *et al.* 1997; Siegrist *et al.* 2008). However, full-scale applications are currently limited to side-stream treatment and only a few successful mainstream applications are reported so far (O'Shaughnessy 2016; Cao *et al.* 2017; Klaus *et al.* 2020).

PNA is a fully autotrophic process that consists of partial oxidation of NH_4^+-N to NO_2^--N (nitritation) and the anammox process in which NH_4^+-N is oxidized using NO_2^--N as an electron acceptor under anaerobic conditions without the need for carbon (Kartal *et al.* 2010) (Figure 1). Thus, the process requires the cooperation of ammonia-oxidizing bacteria (AOB) and anammox bacteria (AnAOB), and out-selection of nitrite-oxidizing bacteria (NOB). The successful application can reduce the required oxygen input by 60%, eliminate the carbon source demand and reduce the sludge production by 90% in comparison to conventional N-removal (Morales *et al.* 2015; Miao *et al.* 2016).

Partial nitritation-denitritation (nitrite-shunt) relies on partial nitritation of NH_4^+-N into NO_2^--N as the first step, then denitritation of NO_2^--N into N_2 as the second step by heterotrophic bacteria (HB). Thus, it consumes 25% less oxygen than complete nitrification and reduces the organic carbon demand by 40% compared to the full denitrification (Daigger 2014).

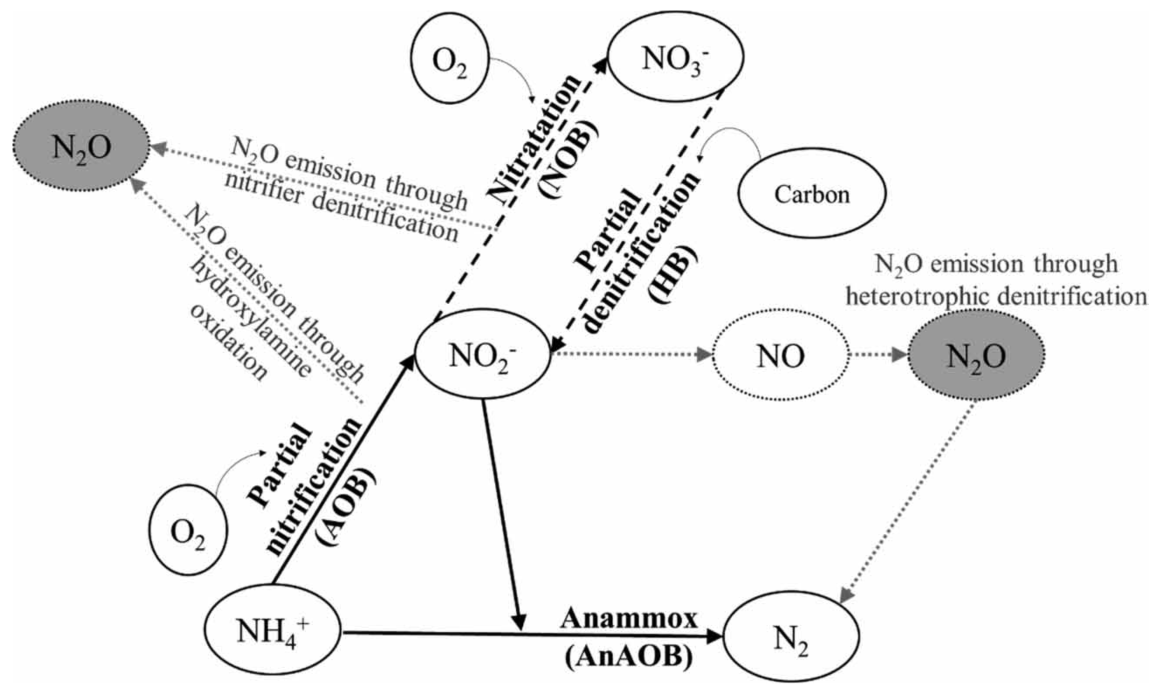


Figure 1 | Deammonification through partial nitrification-anammox and partial denitrification-anammox pathways including potential nitrous oxide emission pathways.

The process requires both out-selection of NOB and also carbon availability for denitritation. The nitrite-shunt process has been successfully implemented in side-stream treatment (e.g. SHARON process (Mulder *et al.* 2001)) and there is an interest to implement it in mainstream treatment in a single reactor (Jimenez *et al.* 2020). However, it is not commonly applied due to the lack of complete understanding of the underlying mechanisms for NOB out-selection and carbon availability for denitritation under these conditions.

During PdNA a portion of the influent NH_4^+-N is aerobically oxidized to NO_3^--N. The NO_3^--N is subsequently reduced to NO_2^--N via heterotrophic denitratation and the resulting mix of residual NH_4^+-N and NO_2^--N serves as substrate for the anammox process (Le *et al.* 2019a; Lu *et al.* 2021b) (Figure 1). This process does not require NOB out-selection and needs a carbon donor to achieve partial denitrification (Zhang *et al.* 2019). The PdNA process consumes slightly more resources (energy for aeration and carbon) than the PNA route; however, the nitrite generating pathway is understood compared to the NOB out-selection pathway (B. Ma *et al.* 2017; Lu *et al.* 2021a). Theoretically, 50% of aeration needs and 80% of carbon demand can be saved and sludge production can be reduced by 60% compared to conventional N removal (Z. Zhang *et al.* 2020). To overcome the external carbon need, recent research efforts aim to take advantage of the slowly biodegradable organics in wastewater or to produce soluble microbial products through fermentation (Ji *et al.* 2020; Liu *et al.* 2022). Also, simultaneous nitrogen and phosphorus removal with lower carbon and oxygen demand can be accomplished by combining endogenous partial denitrification with denitrifying phosphorus removal (X. Wang *et al.* 2019).

Current mainstream deammonification implementations include a variety of processes in laboratory and pilot scales including suspended, attached growth, or hybrid systems in single-stage or 2-stage reactors (e.g. Hoekstra *et al.* 2018; Klaus 2019; Le *et al.* 2019a; Huang *et al.* 2020). Due to the slow growth rate of anammox bacteria (Valverde Pérez *et al.* 2016; Lotti *et al.* 2015), an anammox retention mechanism is required to allow for adequate solids retention time (SRT). In a single-stage process, all biokinetic reactions occur in one basin which decreases both the investment and the operational costs (Pérez *et al.* 2014). Biofilm processes (Lotti *et al.* 2015; Gustavsson *et al.* 2020) or hybrid systems that combine suspended sludge with the biofilm systems such as integrated fixed-film activated sludge system (IFAS) (Cao *et al.* 2017) are used in these single-stage systems (W.-J. Ma *et al.* 2020) to retain AnAOB. In 2-stage systems, partial nitritation or full-nitrification (aerobic environment) and the deammonification processes (anoxic environment) occur in separate basins. Suspended or biofilm processes can be used in the aerated basin and biofilm-based processes may be used in the anammox basin (Regmi *et al.* 2014; Pérez *et al.* 2015).

Despite all the efforts, the mainstream application is still facing challenges due to NOB out-selection, wastewater characteristics, temperature and meeting the strict effluent criteria under dynamic loads (Cao *et al.* 2017). The competition for substrates and growth space between the different functional species is another major application challenge. High influent C/N ratios in the raw wastewater promote the growth of HB in the system, thus hampering deammonification under limiting oxygen concentrations (Gao & Xiang 2021). Also, lower ammonium concentrations and temperature variations make the stable out-selection of NOB very difficult in the PNA systems and lead to competition over oxygen by AOB, NOB and HB (M. Zhang *et al.* 2020).

Strategies are being developed to accelerate successful deammonification process implementation. Microorganisms involved in the short-cut N-removal processes are sensitive to operational and environmental conditions such as pH, dissolved oxygen (DO) level, temperature, SRT and the presence of inhibitors. Several control strategies have been adopted such as low or high DO operation, aerobic SRT, real-time aeration or oxidation-reduction potential control to take advantage of the growth characteristics and the kinetics difference between the microorganisms (Liu *et al.* 2020; Gao & Xiang 2021). However, the shift and adaptation of microbial communities' growth characteristics to mainstream conditions remain a challenge (Agrawal *et al.* 2018; Gao & Xiang 2021). In addition, due to high nitrite accumulation and ammonia conversion rates, the short-cut processes inevitably generate nitrous oxide (N_2O) as a by-product which is one of the most significant greenhouse gases (Castro-Barros *et al.* 2016; Li *et al.* 2020) (Figure 1). Further research is needed under mainstream conditions to achieve long-term process stability (e.g. varying loads or temperatures) and to understand the role of influent characteristics, varying substrates, intrinsic kinetics of the microorganisms involved and competition between them.

Mathematical models and model-based control strategies are under development to overcome implementation challenges and to deal with the complexity of mainstream deammonification (Agrawal *et al.* 2018). Through modelling, it is possible to identify the proper conditions for microbial competition under different operational and environmental conditions and to optimize the processes and implement deammonification successfully (Pérez *et al.* 2014; Liu *et al.* 2017; Shourjeh *et al.* 2021). However, the mechanistic models that are currently being used for the modelling are not sufficiently accurate to model short-cut N removal processes and require specific attention. For example, while the models include the key microbial groups, they do not consider the individual species which are crucial to reflect the competition among them and predict a community shift (e.g. NOB community shift (Liu & Wang 2013)). Also, different process configurations such as biofilm systems require specific sub-models such as the mass transport between the bulk liquid and the microorganisms inside the biofilm (Arnaldos *et al.* 2015; Baeten *et al.* 2019). Thus, the pilot and full-scale applications reported provide invaluable information for models development and to overcome bottlenecks while modelling efforts accelerate the success of practical applications.

The main focus of this paper is pointing out future needs and perspectives to facilitate the technology transfer between the model applications in research studies and accelerate the successful application of mainstream deammonification. The objectives of the presented review article are: (i) to illustrate the current practice in modelling of short-cut N removal processes, (ii) to reveal the challenges in modelling applications with examples, and (iii) to determine the immediate needs and the potential of more advanced modelling approaches and perspectives in the near future.

2. CURRENTLY APPLIED MODELLING APPROACHES AND LIMITATIONS

Modelling plays an essential role in improving process understanding and determining the optimal operating conditions and control strategies for applying short-cut nitrogen removal processes. There has been a concerted effort by academics and practitioners to model mainstream short-cut nitrogen removal recently, but these efforts have not yet resulted in consensus on process models and model parameter values that are transferable to practice. The activated sludge models (ASMs) developed by the IWA task group (Henze *et al.* 2006) are generally being used as default mathematical models for carbon and nutrient removal in wastewater treatment and are available in commercial software packages. For modelling short-cut nitrogen removal processes, ASMs have been used as well with extensions and modifications such as 2-step nitrification, 4-step denitrification, N_2O emission pathways and inhibition mechanisms. Despite its limitations, modelling can serve as a useful tool to improve process knowledge, screen technologies, and develop preliminary designs in a short-cut process. In this section, the required model selection mechanisms and current modelling perspectives for mainstream short-cut N removal processes are given.

2.1. NOB out-selection

Out-selection of NOB is crucial, especially for PNA or nitrite-shunt systems, and is widely recognized as one of the major challenges to mainstream application. The out-selection of NOB has been proven to be quite effective in warm nitrogen-rich wastewater streams (Lackner *et al.* 2014); due to the effect of elevated temperatures on growth rates for AOB and NOB (Hellinga *et al.* 1999), and also the high free ammonia (FA) and free nitrous acid (FNA) concentrations in side-stream liquors which inhibits the growth of NOB (Lackner & Agrawal 2015). However, FA inhibition is not possible in mainstream treatment due to the lower influent ammonium concentration (Cao *et al.* 2017). There are also reports of NOB out-selection achieved through side-stream generated FNA exposure (D. Wang *et al.* 2016) and alternating the sludge treatment strategy between FA and FNA can result in a stable nitrite-shunt with nitrite accumulation above 95% in the mainstream (Duan *et al.* 2019a).

The operating conditions to favour AOB and wash out NOB are thoroughly investigated in literature based on DO, pH, temperature and inhibitors. The intrinsic kinetics of these two groups of microorganisms including maximum growth rate and substrate half-saturations are crucial (Liu *et al.* 2020). DO affects the diversity and kinetics significantly, thus DO control to manipulate the competition for oxygen between AOB and NOB is one of the main strategies in mainstream conditions (Pérez *et al.* 2014; Jimenez *et al.* 2020). The oxygen half-saturation constant for AOB is generally accepted to be lower than for NOB which creates a disadvantage for NOB to compete for oxygen at low concentrations (Sin *et al.* 2008; Cao *et al.* 2017). On the other hand, the predominance of *Nitrobacter* or *Nitrospira*, which are the two main genera of NOB, affect the performance of NOB out-selection through DO control. The systems enriched with *Nitrospira* rather than *Nitrobacter* have a higher oxygen affinity, thus have lower oxygen half-saturation than AOBs and can be well adapted to low DO conditions (Regmi *et al.* 2014; Al-Omari *et al.* 2015). The use of transient anoxia is another approach by providing a lag-time for NOB to transition from anoxic to aerobic condition or nitrite limitation (Zekker *et al.* 2012; Gilbert *et al.* 2014). By consuming nitrite in anoxic conditions, heterotrophs restrict substrate availability for NOB in the aerobic phase (Regmi *et al.* 2014). Moreover, in mainstream treatment under limited DO, the AOB growth rate is higher than the NOB's at high temperatures (above 20°C) (Regmi *et al.* 2014; Yang *et al.* 2016). This allows operating the system at the SRT that is suitable for the growth of AOB and wash out the NOB (Blackburne *et al.* 2008). In addition, there are lab-scale works that support that NOB out-selection can be achieved at lower temperatures depending on the dominant NOB species in the system and the reactor configuration (De Clippeleir *et al.* 2013; Gilbert *et al.* 2015; Cao *et al.* 2017).

For modelling the short-cut processes, nitrite should be considered as an intermediary step in nitrification and denitrification. Modelling the two-step nitrification process is well established where NOB out-selection can be modelled through distinctly defined growth kinetics, substrate affinities, temperature and pH effect on AOB and NOB (Sin *et al.* 2008; Shourjeh *et al.* 2021). However, most simulation studies so far deal with side-stream conditions associated with high-strength nitrogenous wastewater where NOB out-selection can be achieved much more easily with direct pH and temperature effect on the NOB (Volcke *et al.* 2006, 2012; Van Hulle *et al.* 2007; Wett *et al.* 2010; Hubaux *et al.* 2015). For mainstream processes, community shifts and the changes in biokinetics become important which are not implemented in the ASM-based models yet. Favourable conditions to support the existence of AOB and facilitate NOB out-selection through different control systems could be demonstrated in the limited number of existing modelling works for mainstream treatment (Table 1). NOB out-selection is achievable in the models but there are still limitations to these models such as the calibrated half-saturation constants that can be a function of the environmental conditions, process configuration and operating conditions; thus, posing an issue of not being transferable to other systems.

2.2. Partial nitritation – denitritation

Partial nitritation-denitritation is an efficient biological pathway for N removal, but it has been challenged by the aforementioned difficulties (Section 2.1). The process can be applied as the first step of a 2-stage PNA process where nitrite-shunt is facilitated with controlled aerobic SRT. To improve effluent quality, the anammox process can be applied as a polishing step (Regmi *et al.* 2015a, 2015b; W. Zhang *et al.* 2020). Application of nitrite-shunt in mainstream treatment is desired through simultaneous nitrification denitrification because of the opportunity to enhance the utilization of the organic matter in the influent for denitrification. Note that the goal here is to maximize the use of the influent carbon through denitrification and not oxidation; hence, improving N removal while reducing energy consumption. However, it is not easy because the denitritation relies on utilizing the influent COD solely and thus the efficiency of the carbon pretreatment

Table 1 | Examples of modelling works for mainstream short-cut N removal processes

Modelled process	Reactor configuration	Modelling goal	Key findings	Limitations	Reference
Partial nitritation – denitritation	Lab-scale sequencing batch reactor (SBR)	Investigated the effect of aerobic duration on nitritation and NOB out-selection	AOB obtains more growth opportunity than the NOB which can occur only if the AOB reaction rate is higher than the NOB by considering the substrate concentrations	Simplified model excluding COD limitation, ammonification, assimilation of N	Blackburne *et al.* (2008)
	Lab-scale sequencing bench reactor (SBR)	Created an optimization framework and determined the optimal intermittent aeration profile to minimize energy consumption.	Rapid detection of the optimal aeration policies allowing an appropriate and prompt reaction to changes in the operation conditions in SBR processes	Comparing the results against previous publications due to the different conditions of the problem statement in each study	Bournazou *et al.* (2013)
	Pilot-scale activated sludge systems	Evaluated the performance of different process control strategies to achieve nitrite-shunt	The AvN strategy could improve the total nitrogen removal, sustain the NOB out-selection over the ABAC strategy and significant carbon savings could be achieved in comparison to conventional N-removal	Calibrating the model using dynamic input for pilot system due to the lack of a proper AVN controller in the model	Al-Omari *et al.* (2015)
	Biofilm system (pure modelling study)	Determine the influence of biofilm properties (e.g. water-biofilm interface thickness, substrate diffusivities) on NOB suppression	Increased biofilm thickness poses more resistance to diffusive transport of DO, thus limiting the NOB growth	Pure modelling study based on assumed influent characterization	Liu *et al.* (2020)
Partial nitritation – anammox	Granular sludge reactor (pure modelling study)	Investigated microbial community interactions at low temperatures and sensitive parameters leading to NOB repression.	The nitrite half-saturation coefficient of NOB and anammox bacteria proved non-influential on the model output. The maximum specific growth rate of anammox bacteria proved a sensitive process parameter.	Granule size distribution was not considered. Model excluded heterotrophic growth.	Pérez *et al.* (2014)
	Lab-scale SBR	Described the microbial interaction among ammonia-oxidizing archaea (AOA), AOB and anammox bacteria	AOA outcompete AOB under low ammonium concentration and low dissolved oxygen conditions, indicating a better partnership with anammox bacteria.	Oxygen inhibition coefficient for anammox derived from a marine species (1 g DO m^{-3}).	Pan *et al.* (2016)
	Granular sludge reactor (pure modelling study)	Evaluated control strategies to minimize the impact of influent disturbances, using dynamic model simulations.	Fixed or adaptive ammonium set point control strategy with DO limit enabled PNA.	Model assumed a homogeneous granule size.	Wu (2017)
	Lab-scale granular sludge reactor	Investigated the impacts of C/N ratio, DO concentration and granule size distribution on the process performance.	The granule size distribution should be incorporated in the model to accurately describe the granular anammox system.	External mass transfer boundary layer was not taken into account.	Liu *et al.* (2017)
	CSTR (PN)+ granular sludge reactor (anammox) (pure modelling study)	Investigated the effect of operational conditions on final effluent N concentration.	TN discharge standard of 10 gN m^{-3} is only met for temperatures above 25°C.	Model assumed a homogeneous granule size. Pure modelling study based on assumed influent characterization.	Bozileva *et al.* (2017)

(*Continued.*)

Table 1 | Continued

Modelled process	Reactor configuration	Modelling goal	Key findings	Limitations	Reference
Partial nitritation – anammox	MBBR and IFAS (pure modelling study)	Explored operating conditions in IFAS and MBBR systems.	IFAS can achieve higher nitrogen removal at lower airflow rate than MBBR. PN occurs mainly in the biofilm in MBBR and it is restricted to suspended solids in IFAS.	Pure modelling study based on assumed influent characterization. Steady-state simulations.	Tao & Hamouda (2019)
	Lab-scale SBR	Investigated how the composition of the flocs and the NOB concentration respond to changes in DO, fraction of flocs removed per cycle, and maximum volumetric anammox activity	Selective NOB wash out by controlling the DO-setpoint and/or the flocs removal allowed anammox bacteria to act as 'NO2-sink' in the biofilm.	The oxygen inhibition of anammox bacteria was not explicitly modelled. The biofilm was modelled as zero-dimensional, and spatial gradients were neglected. Perfect biomass segregation between flocs and biofilm.	Laureni *et al.* (2019)
	HRAS-PNA (pure modelling study)	Assessed the feasibility and long-term stability of the granular sludge PNA reactor through dynamic modelling and simulation.	Anammox as a dominant process for N removal. The HRAS-PNA system was more sensitive to temperature compared to the conventional activated sludge system.	Model assumed a homogeneous granule size. Pure modelling study based on assumed influent characterization (BSM2).	Jia *et al.* (2020)
	MBBR and IFAS (pure modelling study)	Assessed the role of external boundary layer resistance with respect to bacterial competition and nitrogen removal capacity, focusing on low temperatures (10°C).	The external mass transfer resistance promoted the metabolic coupling between anammox and ammonia oxidizing bacteria. The effectiveness of the nitrite sink depended on the anammox bacteria sensitivity to oxygen.	Steady-state simulations. Pure modelling study based on assumed influent ammonium concentration (without COD).	Pérez *et al.* (2020)
	Lab-scale SBR	Used bifurcation analysis to assess the co-existence of AOB and NOB and the ideal scenario where NOB is completely removed from the reactor.	Good process performance shown even under sub-optimal conditions (i.e., NOB remain in the reactor).	Pure modelling study using a novel mathematical analysis, based on experimental results from Laureni *et al.* (2019).	Wade & Wolkowicz (2021)
	Granular sludge reactor (pure modelling study)	Evaluated the impact of feeding disturbances on the performance of a single-stage PNA granular reactor.	A cascade control strategy based on DO manipulation to derive the ammonium set-point value proved efficient under dynamic influent conditions.	Pure modelling study based on assumed influent characterization (BSM1).	M. Zhang *et al.* (2020)
	UCT-MBR system (pure modelling study)	Comparatively assessed the anammox process and conventional heterotrophic denitrification in an existing UCT-MBR system.	Anammox process weakens the system's resilience to influent fluctuations.	Pure modelling study neglecting diffusion limitations on MBR systems.	Shao *et al.* (2021)
Partial denitrification-anammox	Pilot-scale MBBR, pilot-scale IFAS, & full-scale biological sand filter	Modify SUMO2 (based on ASM) to describe, parameterize, and calibrate partial denitrification for VFA and methanol substrates with and without the presence of AnAOB.	Nitrite preference needed to be removed from denitrification rates. Nitrate residual (via electron flow regulation) needed to be added to model, with additional parameters. Denitrification rate differentials and competition over nitrite with anammox were handled well by model kinetics.	New parameters in the model required batch-test calibration. New rate equations may not fully capture electron competition.	Al-Omari *et al.* (2021)

TN, total nitrogen; MBBR, moving bed biofilm reactor; PN, partial nitritation; UCT-MBR, University of Cape Town membrane bioreactor system; VFA, volatile fatty acids process. In addition, the mechanisms for achieving controllable simultaneous nitrification denitrification are not well understood yet (Klaus 2019; Jimenez *et al.* 2020) and it is out of the scope of this paper.

Operational strategies for stable nitrite-shunt performance have not been demonstrated yet in large-scale systems (Xu *et al.* 2017; H. Wang *et al.* 2019). Modelling of nitrite-shunt requires a holistic approach by simultaneously monitoring the influent dynamics and using the data for controlling the operational conditions and considering their effect on competition for the different substrates. Recent research efforts mostly deal with the application of deammonification in full-scale mainstream as opposed to nitrite-shunt (Table 1).

2.3. Partial nitritation – anammox

For application of PNA processes for mainstream treatment, a further concern is the competition between HB and anammox bacteria for nitrite. Few modelling studies under mainstream conditions have dealt with the interaction between AnAOB, AOB, NOB and ordinary heterotrophs (e.g., Al-Omari *et al.* 2015). As for side-stream applications, simulations showed that the availability of some influent COD can lower the effluent nitrate concentration (produced by anammox) by heterotrophic denitrification and thus increase the total nitrogen removal efficiency of anammox reactors (Hao & van Loosdrecht 2004; Mozumder *et al.* 2014). In many anammox studies, the presence of HB was ignored, and the COD in the reactor was neither measured nor considered in mass balances (Schielke-Jenni *et al.* 2015). Nevertheless, heterotrophic denitrifiers have been widely found in anammox reactors and can account for up to 23% of the biomass in biofilm reactors even without organic matter in the influent because HB could grow both on soluble microbial products and decay released substrate (Ni *et al.* 2012).

When modelling the anammox stoichiometry, one should be careful because the experimentally determined yield for the overall metabolic reaction ($Y^{Met'}_{X/NH_4}$=0.172 g COD/g NH_4^+-N, Strous *et al.* (1998)) mistakenly has been used in many simulation studies (Hao *et al.* 2002; Volcke *et al.* 2010; Ni *et al.* 2012). Ammonium in the anammox process in ASMs is consumed in both catabolic and anabolic reactions, while the yield coefficient only accounts for the ammonium taken up in the catabolic path. Therefore, the experimentally determined yield cannot be directly implemented. To remedy this, Jia *et al.* (2018) proposed an alternative stoichiometric equation based on the biomass yield per amount of ammonium consumed in the overall metabolic reaction or recalculating the widely model yield (Y^{mod}) from the measured $Y^{Met'}_{X/NH_4}$ as follows;

$$Y^{mod} = 1/1/Y^{Met'}_{X/NH_4} - i_{NXB}$$

where i_{NXB} represents the nitrogen content of anammox bacteria (g N g COD^{-1}).

Modelling outputs of the mainstream PNA process also tend to be strongly affected by the oxygen inhibition coefficient of anammox bacteria, as demonstrated by Pérez *et al.* (2020) for MBBR and IFAS systems. The external mass transfer resistance plays a significant role in this case, as the biofilm can be exposed to lower DO concentrations under thicker boundary layers. Although low oxygen levels benefit anammox bacteria, AOB activity could be hampered too. In this case, ammonia-oxidizing archaea (AOA) could be better coupled to anammox bacteria, as the latter can thrive at lower DO levels compared to AOB (You *et al.* 2009). Nevertheless, few models consider the contribution of AOA to nitrogen conversions (Pan *et al.* 2016).

Steady-state simulations have been used to assess PNA systems (Bozileva *et al.* 2017), and only a few studies address dynamic simulations (Table 1). Under varying influent conditions, a higher sensitivity to temperature oscillation was found compared to steady-state simulations (Jia *et al.* 2020). Therefore, dynamic simulations are recommended to assess process feasibility and long-term stability.

2.4. Partial denitrification – anammox

An early indication of PdNA process in the literature was in a 2-stage pilot-scale PNA system (Regmi *et al.* 2015b). Eliminating the challenge of NOB out-selection has led to significant research interest in the PdNA process (Du *et al.* 2017; Cao *et al.* 2019; Du *et al.* 2019; X. Wang *et al.* 2019; B. Ma *et al.* 2020; You *et al.* 2020; Chen *et al.* 2021) and multiple successful pilot-scale and full-scale implementations (McCullough *et al.* 2021). As complete nitrification is already well established in process models, the primary challenge of modelling PdNA is understanding the reduction of nitrate to nitrite under various process configurations, substrate concentrations, redox conditions, and with/without the presence of anammox.

PdNA is typically implemented as a 2-stage system which can be an integrated process (Le *et al.* 2019a; Li *et al.* 2019) or a post-polishing (Campolong *et al.* 2018). Controlling and maximizing denitratation over denitritation is key to PdNA start-up and performance because full denitrification results in a loss of carbon efficiency and no ammonia removal. Multiple carbon sources have been demonstrated to be effective for partial denitrification such as acetate, glycerol, or methanol (Campolong *et al.* 2018; Le *et al.* 2019b; McCullough *et al.* 2021), ethanol (Du *et al.* 2017), fermentation products (Cao *et al.* 2013; Ali *et al.* 2020) and endogenously stored carbon (Ji *et al.* 2017; X. Wang *et al.* 2019). Beyond carbon source selection, numerous factors can affect the partial denitrification efficiency including influent C/N ratio, pH, sludge retention time and microbial population. Recent literature suggests that under mainstream conditions, the dominant factor impacting partial denitrification efficiency is in fact the nitrate concentration in the reactor (nitrate residual) (Le *et al.* 2019a). Nitrate residual has been demonstrated to be an effective method for controlling PdNA and partial denitrification efficiency during start-up (Schoepflin *et al.* Manuscript in preparation), pilot-scale (Le *et al.* 2019a), and full-scale (McCullough *et al.* 2021). Since it can be well correlated with partial denitrification efficiency and was used successfully for process control, the nitrate residual is found to be a key parameter in modelling PdNA as well (Al-Omari *et al.* 2021).

Modelling PdNA requires accurate description of partial denitrification in the model and the ability to address microbial competition over electron donors and acceptors. While denitrification is included in ASM 1, ASM 2-2d and ASM3 models as a single-step (Henze *et al.* 2006), at least two-step denitrification must be modelled for PdNA. Monod functions were used to model two-step denitrification by Hellinga *et al.* (1999), in ASM3 by Ni & Yu (2008) and for granular sludge by De Kreuk *et al.* (2007). Many multi-step denitrification models included terms to inhibit denitratation in favour of denitritation (referred to here as 'nitrite preference') based on experimental observations where nitrite accumulation was not observed or was impeded. Wett & Rauch (2003) proposed a model using the ASM structure in which the rate of denitritation is elevated and the rate of denitratation is hampered by an inhibition factor based on the ratio of nitrate to nitrite. Similar practices appeared to be common practice, as Hiatt & Grady (2008) state that denitrification models from Gujer and colleagues included a nitrite inhibition term for each step of nitrogen reduction, e.g. Wild *et al.* (1995). Their four-step activated sludge model for nitrogen (ASMN) includes a nitric oxide inhibition factor for denitrification as well as separate anoxic reduction factors for each denitrification rate. More advanced three-step or four-step denitrification models introduce additional complexity and parameters, but do not appear to be necessary to successfully model PdNA. These models are discussed in more detail in Section 4.4.

The nitrite preference applied to all electron donor sources used for denitrification hindered the ability to induce partial denitrification in existing models. Al-Omari *et al.* (2021) introduced a partial denitrification switch based on bench-scale and pilot-scale observations where partial denitrification was observed in batch tests and was controlled in continuously fed systems by maintaining nitrate residual. This was observed when using specific carbon sources such as acetate and glycerol but not methanol. The 'nitrite preference' was eliminated in this case for the nitrate reduction reactions using the volatile fatty acids (VFA) substrate state variable.

2.5. N_2O emissions in short-cut N removal processes

N_2O is produced mainly from three microbial pathways: the NH_2OH oxidation and nitrifier denitrification pathways carried out by AOB, and the heterotrophic denitrification pathway carried out by HB (Duan *et al.* 2017) (Figure 1). N_2O is a potent greenhouse gas with a large global warming potential of 265 times that of CO_2, as well as the single most significant ozone-depleting substance (Edenhofer *et al.* 2014). Accurate assessment, modelling, and mitigation of N_2O emissions is critical due to the large contribution of N_2O to the WRRF carbon footprint (Vasilaki *et al.* 2019). Peng *et al.* (2020) analysed the carbon footprint of the mainstream PNA process, including electricity consumption, N_2O emissions, and reduction in carbon emissions through energy recovery. Based on this study, the N_2O emission factor for mainstream PNA should not exceed 0.78% to achieve a carbon-neutral operation. However, the N_2O emissions from the PNA process could be staggeringly high, with up to 10% of the nitrogen removed emitted as N_2O (Domingo-Félez *et al.* 2014; Staunton & Aitken 2015).

The capability to simulate N_2O emissions by mathematical models allows the consideration of N_2O emissions in the design and operation of short-cut nitrogen removal processes. N_2O models have evolved from simple one-pathway models (from AOB or from 3rd or 4th steps of heterotrophic denitrification) (Hiatt & Grady 2008; Mampaey *et al.* 2013; Pan *et al.* 2013) to two-pathway AOB models (Ni *et al.* 2014; Pocquet *et al.* 2016) that involve both N_2O production pathways from AOB. Thereafter, integrated pathway models were developed that consider both AOB and heterotrophic N_2O generations (Guo & Vanrolleghem 2014; Domingo-Félez & Smets 2016; Spérandio *et al.* 2016), and indicating that N_2O can also be further removed but only by HB (Guo & Vanrolleghem 2014). These integrated pathway models have been applied in conventional systems as a powerful tool to evaluate N_2O mitigation strategies (Chen *et al.* 2019; Duan *et al.* 2020, 2021).

With essentially the same principles of N_2O generation, models developed in nitrification and denitrification systems can be directly applied to the short-cut nitrogen removal processes. For example, Wan *et al.* (2019) integrated the two pathway N_2O model by Pocquet *et al.* (2016), with the heterotrophic N_2O production model by Hiatt & Grady (2008) to describe N_2O emissions in a single-stage PNA reactor. The established N_2O model was used to evaluate the effects of operational conditions on N_2O emissions from the PNA system and identified the potential operational conditions to reduce emissions.

Challenges and uncertainties are present in the modelling of N_2O emissions in mainstream short-cut nitrogen removal systems. The largest uncertainty results from the lack of datasets. N_2O emission data from full-scale mainstream short-cut nitrogen removal systems are limited. On the other hand, the data obtained from lab-scale applications show high emission ratios ($5.2 \pm 4.5\%$ of total inorganic N removed) (Roots *et al.* 2020). In addition, many short-cut nitrogen removal evaluations lack N_2O data. Therefore, adequate nitrogen species profiling data becomes crucial to establish nitrogen mass balances. Another challenge is that N_2O process models tend to overparameterize the description of biological pathways and this impacts the N_2O model calibration (Domingo-Félez & Smets 2020).

3. HOW MODELLING CAN ACCELERATE THE FULL-SCALE MAINSTREAM APPLICATIONS

In the mainstream application of short-cut N removal processes, mathematical models are being mostly used to simulate the behaviour of biological processes under different operating scenarios and cost-effectiveness (Shourjeh *et al.* 2021). Although currently experimental work in laboratory and pilot-scale systems prevails over mechanistic model analysis for short-cut processes (M. Zhang *et al.* 2019; Z. Zhang *et al.* 2020), modelling efforts can help to accelerate mainstream full-scale applications. In this section, several modelling goals and potential benefits are presented from a modeller perspective to reveal how modelling could be useful to achieve successful applications (Figure 2).

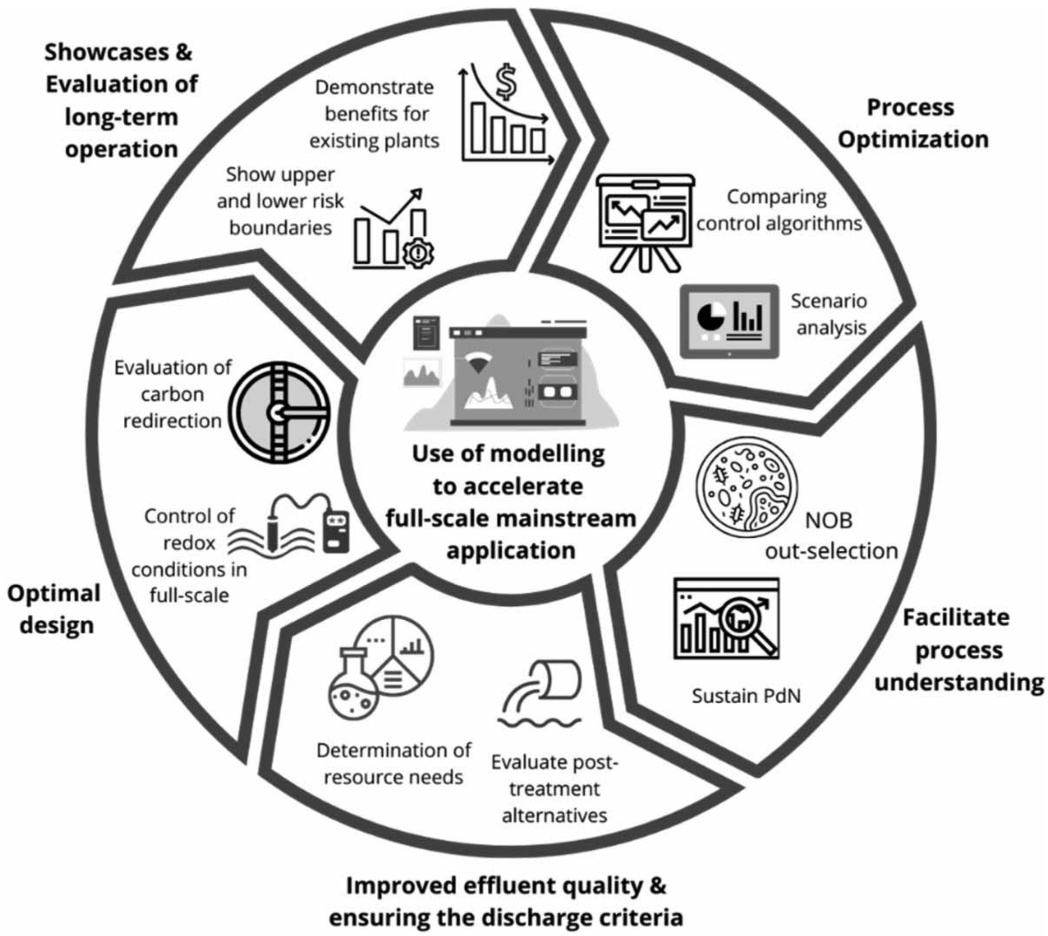


Figure 2 | How to benefit from modelling to accelerate full-scale mainstream application.

3.1. Facilitate process understanding

As presented in Section 2, models are mainly being used to facilitate the process understanding and improve process efficiency through improved operation and process control. Existing models can improve mainstream NOB out-selection applications for PNA processes since it allows assessment of the impacts of stable and dynamic influent conditions, biofilm characteristics and aeration control strategies (Al-Omari *et al.* 2015; Rosenthal *et al.* 2018). With the ability to simulate varying influent conditions such as low or high COD/N ratios, modellers can examine the hydrolysis of biodegradable substrates and impacts on processes (Tao & Hamouda 2019). In addition, models permit the exploration of how different biofilm systems (MBBR, granular sludge, IFAS and filters) would work for mainstream applications (Boltz *et al.* 2011; Rosenthal *et al.* 2018).

Similar to NOB out-selection, existing models can be used to improve the efficiency and nitrogen removal contribution via PdNA pathway by evaluating the impact of feedstock carbon characteristics, biofilm characteristics, process configuration, aeration controls and operational conditions.

3.2. Process optimization and control

Pilot and full-scale applications of different control algorithms are increasing rapidly to provide the operational and environmental conditions to sustain short-cut N removal in mainstream treatment. Modelling can be used to design control strategies to improve the operation of existing plants and serves as a time and cost-saving tool for the evaluation of different control algorithms (Salem *et al.* 2002; Gernaey *et al.* 2004). To fulfil the effluent quality standards, reduce carbon footprint and keep the operational costs at a low level, it is imperative to use control strategies to optimize resource consumption (Ostace *et al.* 2011; Revollar *et al.* 2020).

To achieve mainstream deammonification, several real-time aeration control strategies have been developed and applied such as continuously low DO operation, AvN (NH_4^+-N vs NO_x^--N) with intermittent aeration, ammonia-based aeration control (ABAC) (Regmi *et al.* 2014; Sadowski *et al.* 2015; Poot *et al.* 2016). Together with the real-time aeration control, the application of short aerobic SRT is a robust control strategy that relies on adjusting the wasting rate, aerobic volume and DO setpoints to suppress NOB in PNA systems (Regmi *et al.* 2014; Han *et al.* 2016). Nitrate-based COD dosing control is needed in PdNA systems to ensure nitrite availability for anammox (Le *et al.* 2019a). Finally, yet importantly, dynamic feedforward control of the AvN setpoint to respond to changes in the influent ammonia can be implemented to maximize the PdNA efficiency with savings in carbon dosage (Le *et al.* 2019c). By using the information and experience obtained from lab and pilot-scale studies, modelling can be used to compare features of different process control strategies, provide a proof of concept, and develop the control algorithms (e.g. feedforward control based on the influent dynamics or multi-objective control).

3.3. Evaluation of post-treatment requirement

Depending on the short-cut nitrogen removal process efficiency and the effluent requirements, the post-treatment might be needed for the removal of nitrate, nitrite or residual ammonia. Often in the short-cut N process, nitrate may still be present in the effluent, especially when NOB out-selection efficiency is compromised (Gustavsson *et al.* 2020; Muñoz 2020). This requires post-treatment such as heterotrophic denitrification with external carbon dosage. Effluent ammonia can be an issue at high loading rates and a reaeration zone might be needed. Models can be used to design and test the required post-treatment alternatives coupled with control strategies, determine the reactor volume needed, air consumption or the applicable type of chemical dosage. It can be helpful to ensure the effluent limits and analyse the overall resource consumption in long-term operation.

3.4. Demonstration of the potential benefits of technology add-ons for existing processes under real conditions

Modifying a conventional N removal process with short-cut N removal processes can reduce overall energy and resource consumption of a WRRF. To demonstrate and quantify the gains of such modifications, models can be used for the capacity analysis of a WRRF by identifying the SRT, volume necessity, chemical consumption and stoichiometric needs for the processes (Shao *et al.* 2021). For example, Al-Omari *et al.* (2015) used a calibrated model for a hypothetical scenario and demonstrated the potential of 60% external carbon savings by converting the conventional nitrification denitrification system to mainstream deammonification with the Blue Plains WRRF case study. Moreover, such evaluations provide an assessment of the potential performance to meet the total inorganic nitrogen effluent limits and associated risk in long-term operations. Demonstration of the benefits of technology for existing plants can be achieved when transferable validated process models are available, and the model parameter values are obtained.

Furthermore, from a plant-wide modelling perspective, mainstream short-cut N removal processes are interacting with other biochemical and chemical processes in the WRRF which would be important to capture both the dynamics of the plant and the potential environmental impacts (Arnell *et al.* 2017). For example, through plant-wide modelling, the influence of carbon redirection process on the downstream short-cut N removal process performance and the microbial species could be predicted; including the greenhouse gas emissions, carbon and energy footprint of the WRRF (Mannina *et al.* 2019). Simulating the whole plant would allow determining if the side-stream processes can be used to support the mainstream processes through seeding the bacteria (AOB or Anammox, e.g. Wett *et al.* 2015) or providing FNA or FN exposure (e.g. D. Wang *et al.* 2016; Duan *et al.* 2019a). In addition, combined biological P and short-cut N removal potential can be investigated. Thus, adopting the plant-wide modelling approach can be useful to demonstrate the gains in terms of process performance and stability in full-scale implementations.

4. THE NEED FOR NOVEL MODELLING APPROACHES

Despite its limitations, modelling is proven to be useful to help achieve full-scale mainstream N removal processes (Section 2&3). The applications at different scales provide invaluable information to support and improve the models while the modelling efforts can help to accelerate successful practical application and foresee challenges. Within this section, the needs and future perspectives on model applications are presented. Based on the discussion outputs of the *Workshop* of the *7th IWA/WEF Water Resource Recovery Modelling Seminar Mainstream Short-cut Nitrogen Removal Modelling: From research to full-scale implementation, do we have what we need?*, a feasibility versus importance chart is given in Figure 3 for the future

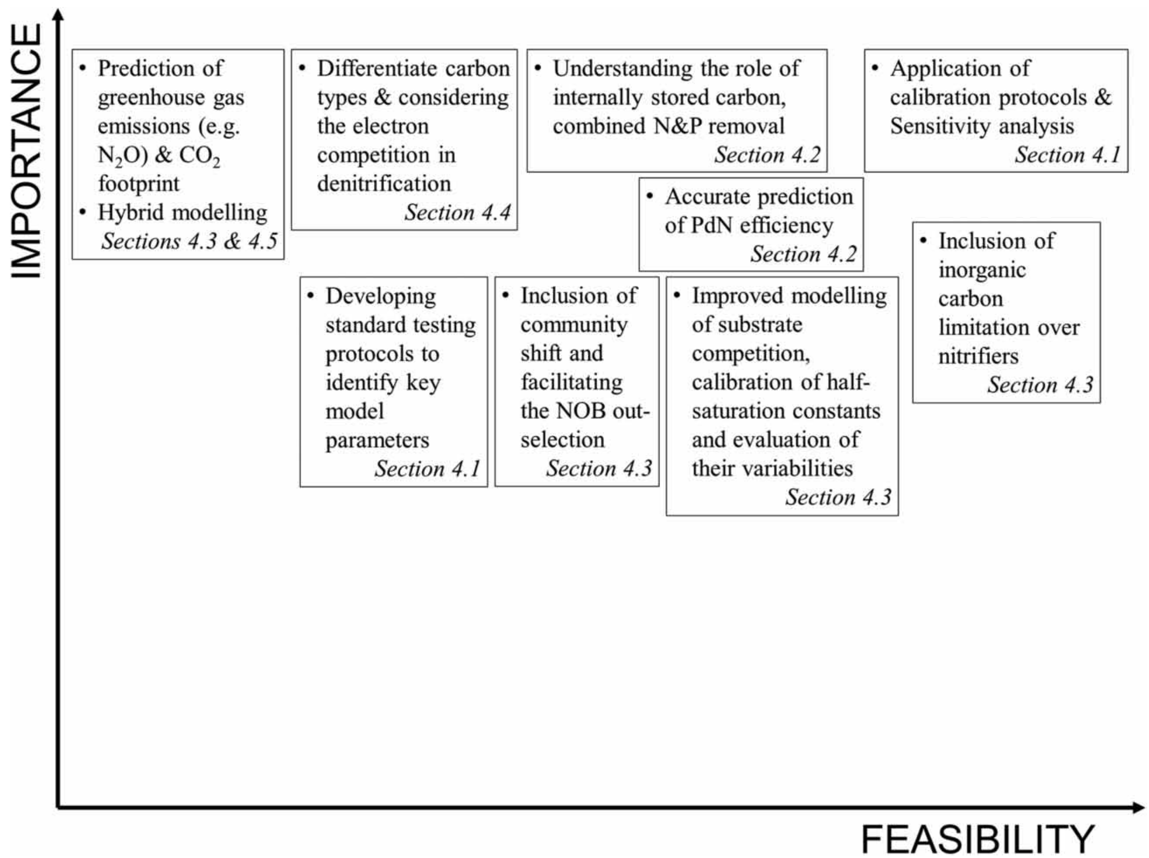


Figure 3 | Importance vs feasibility chart for the future works needed to improve short-cut N removal modelling.

works that are urgently needed (the discussion points with less importance are not included). The main subjects are discussed in detail below.

4.1. Parameter estimation methodology

For the modelling of short-cut processes, robust calibration methodologies and model developments are needed to find the optimal range of influential model parameters and improve process understanding and stability. Existing process models for short-cut nitrogen removal are commonly used for scenario analysis to evaluate process performance under varying operating conditions (e.g. Mozumder *et al.* 2014; Pérez *et al.* 2014). The models are mostly goal-oriented (i.e. applied for a specific purpose and case), but are seldom followed by experimental validation. For example, the degree of stratification of AOB and NOB in nitritating granules (Soler-Jofra *et al.* 2019), or the postulated pH-driven inhibition of NOB in a PNA system (Y. Ma *et al.* 2017) are later confirmed by pH microprofiles which is related to the NOB out-selection in biofilm systems (Ma *et al.* 2021).

Based on the analysis of the reported modelling studies in literature, it appears that the applied calibration methodologies are seldom following good modelling practice which makes the comparison between modelling studies difficult. This is probably due to the complexity and change of the microbial communities studied with lab-scale systems when they are applied to full-scale (Rieger *et al.* 2012). Selection of the parameters is typically based on expert knowledge following the parsimonious principle (i.e. keep the number of parameters to be estimated as small as possible), and the calibration methodology itself mostly is not well described (Al-Omari *et al.* 2015; Trojanowicz *et al.* 2019; Drewnowski *et al.* 2020; Roots *et al.* 2020). One should be aware that this type of model calibration may lead to unrealistic model outputs when the model is run without boundary conditions and is extrapolated for process optimization. Moreover, the model parameter values estimated in this way may not be transferable to other systems. Proper model validation can protect from such unrealistic extrapolation as it would indicate to what extent extrapolation is feasible.

The application of robust sensitivity analysis and the use of more rigorous calibration methodologies would make the comparison between model structures possible and the simulated model outputs would become reproducible and transferable. Importantly, it also enables the modeller to identify the main model factors to pay attention to in terms of model algorithms and the data (Mannina *et al.* 2010; Vangsgaard *et al.* 2013). This identification can reveal which data is more informative for model calibration, thus can guide the experimental efforts (e.g. the higher sensitivity of growth rates regarding performance within the biofilm vs bulk recommends *in situ* microprofiling data is more informative for calibration than the bulk measurements (Y. Ma *et al.* 2017)). This issue may be minimized if long-term consistent data sets can be obtained which is typically the weakest link in modelling of short-cut processes currently.

Sensitivity analysis is the basic method to identify the most appropriate parameter subsets to be calibrated. In short-cut process models, calibration coupled with the use of more advanced approaches has been presented but has remained limited (e.g. normalized sensitivity function (Baek & Kim 2013; Y. Ma *et al.* 2017)). Sensitivity analysis can be done in a simple way through local sensitivity analysis (LSA), for which the number of model simulations that need to be run is low, typically 2^*N+1 runs (N: number of parameters) (De Pauw & Vanrolleghem 2006a). However, the LSA approach does not allow identifying interacting factors, thus may miss some of the influential model parameters and limit the use of the calibrated model. On the other hand, global sensitivity analysis (GSA) provides information on how the model outputs are influenced by the variation on the model inputs and parameters over a wide range of possible parameter values (Cosenza *et al.* 2013). Thus, GSA provides an overall view of the sensitivity and determines the important model parameters much more reliably. In addition, GSA covers interacting parameters much better (Lackner & Smets 2012). Its computational demand is higher than the one of LSA, but it can be optimized by using an appropriate sampling method, e.g. Latin Hypercube Sampling (Vanrolleghem *et al.* 2015).

Sensitivity analysis will further guide the identification of the most influential model inputs and allow for the design of future monitoring campaigns for full-scale applications with the aid of model-based optimal experimental designs (OED) (Ledergerber *et al.* 2019). OED can help design the experimental studies that are highly informative and meaningful for calibration and validation of the models and thus will make models that reliably predict the model outputs (De Pauw & Vanrolleghem 2006b). Finally, standard testing methods/protocols for the measurement of key model parameters would ensure good initial estimates of their values can be assigned since such estimates allow reducing the calibration efforts and making different case studies comparable. Examples of such standard tests are activity tests for nitrification rates and measurements of half-saturation coefficients.

4.2. Role of complex carbon types and their effects on partial denitrification

While the PdNA model from Al-Omari *et al.* (2021) has been calibrated to a range of data from pilot-scale systems and started to be applied to full-scale processes, many questions remain that may require more advanced models (Section 2.4). The developed model can simulate partial denitrification only with methanol and VFA (glycerol or acetate) substrates but have not yet been tested for more complex carbon types. Other electron donors need to be better understood to model PdNA systems that rely on complex influent COD or fermentation products. Fermentation products have been shown to be useful for partial denitrification at pilot-scale (Ji & Chen 2010) and PdNA (Ali *et al.* 2020). Influent COD has also been used to drive partial denitrification, although this may require in-line fermentation (Shi *et al.* 2019; Ji *et al.* 2020; Liu *et al.* 2022) or pH elevation above 9.0 (Qian *et al.* 2019; Shi *et al.* 2019).

Additional work needs to be done to better understand intracellular carbon storage and its impact on PdNA. Simple carbon sources like glycerol and acetate can be stored by heterotrophs (Le *et al.* 2019b), and the resulting denitrification dynamics of storage versus immediate use is not well understood in these systems. Studies have shown that internally stored carbon from influent wastewater is a promising alternative for partial denitrification (Ji *et al.* 2017, 2018). Denitrifying glycogen-accumulating organisms present (dGAO) in enhanced biological phosphorus removal (EBPR) systems may also preferentially perform denitratation (Rubio-Rincón *et al.* 2017; H. Wang *et al.* 2019), leading to a potential synergy between next generation EBPR and PdNA systems.

4.3. Microbial competition modelling

Estimation of half-saturation constants: An efficient deammonification system must ensure both the activity of AOB and AnAOB while the growth of NOB is inhibited (especially in PNA systems). Also, the growth of HB should be controlled since it may shift the process from deammonification to conventional nitrification and denitrification (Cao *et al.* 2019; Gao & Xiang 2021). In low substrate availability conditions such as short-cut N removal processes, microbial competition for substrate becomes important for both application and modelling. The substrate competition might be between AOB and AnAOB for ammonium as electron donor; AOB, NOB and HB competition for oxygen as electron acceptor; and AOB and NOB competition for inorganic carbon (Shourjeh *et al.* 2021). Microorganism growth kinetics and substrate affinities provide a useful tool to understand and model the substrate competition and population shift. Expanding the previous models by accommodating for the competition between main species, we will have to revisit and further study the wide range of kinetic parameters for AOB, NOB, and AnAOB (Cao *et al.* 2017).

Impacts of influent load fluctuation, environmental and operational conditions on model accuracy becomes more important and perhaps more challenging for addressing competition more accurately in the model. On the other hand, strategies and operational conditions are being tested mainly in lab-scale and modelling efforts are often lacking consistent long-term experimental data (Table 1). For example, related to the successful NOB out-selection, the models are mostly applied for the data out of a short-term monitoring period and only reflects the operational conditions that favour the AOB or AnAOB activities while NOB is inhibited (Blackburne *et al.* 2008; Cui *et al.* 2017; Laureni *et al.* 2019; Al-Hazmi *et al.* 2022). Thus, not including the challenging operational conditions that promote NOB growth may lead to an inadequate calibration of NOB activity and their out-selection. Most of the models are calibrated to reflect the periods for stable operation; hence, the half-saturation values would not change drastically except the acclimation and adaptation conditions. Using long-term, where organisms are well adapted to the operational conditions, and consistent performance data for dynamic simulation with robust calibration methodologies could be useful to overcome the calibration challenges related to competing species in the short-cut process (e.g. Vangsgaard *et al.* 2013).

The growth kinetics of microorganisms is substrate-limited based on Monod's formulation used in the ASMs which states a fixed relation between growth rate and bulk substrate concentration (Henze *et al.* 2006). Thus, the substrate half-saturation constant (or affinity constant) is crucial. It represents the substrate concentration corresponding to a half-maximal rate of growth (Riet & Lans 2011). The lack of understanding and proper characterization of half-saturation constant variability between different systems leads to the need for frequent calibration of half-saturation constants. However, calibration may lump the effects of different phenomena in the system (such as mixing, advection and biofilm diffusion limitations) and that may lead to a variation on the apparent values of the half-saturation constants, thus affecting the prediction power of the calibrated model (Arnaldos *et al.* 2015). For example, the current practice for oxygen mass transfer modelling is to apply overly simplified models. These models require multiple assumptions that are not valid for most applications and are highly uncertain (e.g. α-factor). Thus, the calibration of DO half-saturation constants is affected especially in short-cut processes where small deviations in the simulated DO concentration have already a significant impact on the biological conversion processes (Amaral *et al.* 2019). In addition, transport of oxygen within the floc is driven by diffusion where temperature and DO gradient become important factors (Manser *et al.* 2005; Arnaldos *et al.* 2015). A high variation of such factors may require implementing the half-saturation constant as a model variable and calculating its value during the simulation (e.g. K_{NO3} dependence on biomass growth rates, thus the temperature by Shaw *et al.* 2013). Furthermore, the different species of the same genera of microorganisms may have different affinities to the same substrate. For example, *Nitrobacter* or *Nitrospira* are the two main genera of NOB and their predominance in the system is affected by nitrite concentration (Nogueira & Melo 2006). For these reasons, estimation of the half-saturation constants should be handled very carefully by considering the transport phenomena and the effect of biological limitations to model the substrate competition properly. Advanced modelling tools in combination with the biokinetic models such as computational fluid dynamics and population balance models could be useful in full-scale systems (Arnaldos *et al.* 2015). The inclusion of different species in the same genera with their substrate affinities might be considered especially to model NOB suppression (see below: Inclusion of community shift). Also, when the kinetics are under dual limitation, both substrates should be taken into account in the model (Al-Omari *et al.* 2015).

Alternatively, hybrid models are an interesting avenue to pursue in this domain. Hybrid models combine mechanistic models with data-driven techniques and as such leverage both process knowledge and the power of data analytics. As a result, hybrid models can have good extrapolation properties while being less laborious to develop than strictly mechanistic models. Hybrid models can be used in many different configurations and with many different combinations of data-driven tools. However, they have been described to be specifically powerful in situations where the overall process model structure is quite well defined but specific knowledge on variability of subprocesses is missing (von Stosch *et al.* 2014). In these situations, adding a data-driven component to the well-described mechanistic model structure can specifically learn the missing dynamics and compensate for the uncertainties in the mechanistic model which can be much more efficient than going through extensive laboratory experiments to develop in-depth understanding of all the factors contributing to the variability in some subprocesses. The modelling of kinetics in short-cut N removal processes falls exactly within this definition with a lot of process knowledge and corresponding process knowledge available but a lack of mechanistic description of all factors influencing the kinetics (e.g. mixing, biofilm diffusion limitations).

Inclusion of community shift (interspecies competition): It is well-known that at low oxygen concentrations, NOBs are outcompeted because of their relatively low affinity for oxygen. Experimental evidence is available suggesting different reactor operating conditions and control strategies favour the presence of different nitrifying species, which could be different ammonium oxidizers (Bougard *et al.* 2006) and/or different nitrite oxidizers (Dytczak *et al.* 2008). Considering the oxygen concentration as the key variable governing the population shift, Volcke *et al.* (2008) modelled the shift in AOB species in a biofilm reactor. But also NOB could adapt to control strategies by community shifts, resulting in failed NOB control and subsequently disrupting the PNA or nitrite-shunt process. With the low DO (between 0.16 and 0.37 mg O_2/L) NOB control strategy, NOB gradually developed competitive edges over AOB for oxygen uptake by shifting the NOB community to be dominated by *Nitrospira* (Liu & Wang 2013). Similarly, the NOB community could adapt to inactivation from free ammonia (FA) or free nitrous acid (FNA), by shifting to *Nitrobacter*, or *Nitrospira* respectively (Duan *et al.* 2019a, 2019b).

The adaptation of NOB by community shift poses a challenge to the modelling of mainstream PNA or nitrite-shunt processes. The current ASMs with 2-step nitrification could predict the out-selection of NOB under low DO conditions given the commonly higher oxygen affinity constant of NOB than that of AOB. However, as the NOB community adapted to the low DO condition by shifting the dominant NOB genus from *Nitrobacter* to *Nitrospira*, NOB exhibited an increasingly higher affinity to oxygen (lower half-saturation constant). Modelling such NOB activities in the mainstream PNA or nitrite pathway is not feasible with one set of parameters. The inclusion of detailed microbial diversity (interspecies competition) in models for process design and operation may be warranted in cases where it affects the macroscopic reactor performance (e.g. nitrite accumulation). Taking the low DO control scenario as an example, if the model included kinetics parameters/processes for both *Nitrospira* (lower oxygen half-saturation constant) and *Nitrobacter* (higher oxygen half-saturation constant), the model may be able to predict the community shift, and the adaptation of the NOB community to the low DO conditions under long-term operation. The inclusion of the community shift in the model may allow predicting a more reliable NOB control performance, and thus a more stable mainstream PNA or nitrite-shunt process. Note that if the model includes the kinetics parameters/processes for competing interspecies, a complete washout of one the species can be predicted at certain conditions and it would never reappear even if the conditions later shift towards more favourable conditions. However, in reality, this might happen seldom and the two species may always coexist, only switching their predominance depending on the conditions. The reason might be the seeding of NOB from the influent wastewater. It has been reported that different NOB species may be present in raw wastewater reaching the WRRFs (Jauffur *et al.* 2014; Yu *et al.* 2016). This could potentially facilitate the development of the NOB community, which may result in unstable NOB suppression in the mainstream (Duan *et al.* 2019b). To reflect this situation in the model, these interspecies must be included in the input of the model.

Inorganic carbon limitation of AOB: Inorganic carbon can be a crucial factor influencing conversions, as demonstrated by Bressani-Ribeiro *et al.* (2021) for the treatment of anaerobic effluents. Dynamics of AOB have been reported to change significantly under inorganic carbon limitation while the NOB activity remains stable (Guisasola *et al.* 2007; Ma *et al.* 2015; Zhang *et al.* 2016). Biesterfeld *et al.* (2003) showed that nitrification rates are affected by an inorganic carbon shortage (below 45 mg $CaCO_3$ L^{-1}) independently of pH. Instead of inorganic carbon, alkalinity (expressed as bicarbonate) is typically introduced in models to predict possible pH changes and close charge balances (Rieger *et al.* 2012). The ASMs follow that approach, in which alkalinity limitation on (single-step) nitrification is described with Monod kinetics (Henze *et al.* 2006).

The alkalinity limitation considered in these models focuses on unfavourable pH conditions rather than inorganic carbon limitation. Nevertheless, modelling approaches considering inorganic carbon limitation effects on autotrophs have been proposed, described with Monod or sigmoidal kinetics (Wett & Rauch 2003; Guisasola *et al.* 2007; Al-Omari *et al.* 2015; Seuntjens *et al.* 2018). From a fundamental standpoint, NOB is likely less limited by inorganic carbon than AOB, as the latter can up-regulate its anabolism mixotrophically using traces of organic matter (Bock 1976). However, the different behaviour of AOB and NOB under inorganic carbon depleted conditions is typically neglected in models. Moreover, mainstream process models typically assume that influent inorganic carbon content is sufficiently high, meaning hardly any limitation (Sin *et al.* 2008). Bicarbonate as a state variable should be explicitly used to quantify inorganic carbon limitation rather than to simply indicate pH changes. In this case, sigmoidal kinetics are recommended. Furthermore, a higher inorganic carbon limitation for AOB than NOB is essential for modelling nitrogen conversions in trickling filters following direct anaerobic sewage treatment (Bressani-Ribeiro *et al.* 2021).

4.4. Denitrification intermediates (electron acceptors) and electron donors

Denitrification refers to the 4-steps reduction of NO_3^--N to N_2 via NO_2^--N, NO, and N_2O. While formerly considered in denitrification models as a 1-step process, heterotrophic denitrifiers and some phosphate accumulating organisms possess a highly modular microbiome with a diverse distribution of the nar, nir, nor, and nosZ genes (Ekama & Wentzel 1999; Graf *et al.* 2014). External carbon sources such as acetate, ethanol or methanol are being added to enhance denitrification rates (Mokhayeri *et al.* 2009). The metabolic pathways to oxidize each carbon source are different: the tricarboxylic acid cycle for acetate, specific enzymes to convert ethanol to acetate, and two other pathways requiring specific enzymes for methanol degradation (Ribera-Guardia *et al.* 2014). Hence, the chemical composition of the electron donor pool will shape the microbial community yielding significantly different denitrification rates and yields (Hallin *et al.* 2006; Lu *et al.* 2014). In an enriched denitrifying community the individual addition of excess acetate, ethanol, and methanol showed distinctive NO_3^--N reduction rates, and more importantly, different accumulation of the intermediates NO_2^--N and N_2O (Ribera-Guardia *et al.* 2014). Consequently, the oxidation rate of each carbon source can be the limiting step to the overall denitrification rate even at non-limiting organic carbon concentrations (Gao *et al.* 2020).

The distinct reduction and accumulation of denitrification intermediates depending on the carbon source is crucial especially for the PdNA systems since the partial denitrification rate is the key factor for the process performance. Two-step denitrification models fit the purpose of modelling short-cut N removal processes, but the ASMN extended to 4-steps to incorporate NO and N_2O reduction based on Von Schulthess *et al.* (1995). The current structures based on ASMN assume that carbon oxidation supplies all the electrons necessary for the four denitrification steps, leading to include individual maximum denitrification rates, substrate affinity and inhibition constants (e.g. NO, DO). Hence, the complexity of 4-step denitrification models increases significantly while datasets for the intermediates NO and N_2O remain scarce. Thus assumptions need to be made on substrate affinity kinetics for NO and N_2O reduction (Hiatt & Grady 2008). The specific maximum reduction rates for NO_3^--N and NO_2^--N vary substantially depending on the carbon source, and are typically modified during model calibrations to fit denitrification rates (Q. Wang *et al.* 2016; Domingo-Félez & Smets 2020). However, the approach of additive kinetics in ASMN fails to properly describe the electron competition (Pan *et al.* 2015). To overcome this challenge, the ASM-ICE (indirect coupling of electrons) was introduced to explicitly calculate the concentration of internal electron carriers of a methanol-enriched denitrifying community uncoupling the denitrification and carbon oxidation processes over one branch at a cost of higher model complexity (Pan *et al.* 2013). Based on Almeida *et al.* (1997) the ASM−EC (electron competition) calculates denitrification rates and carbon oxidation as an analogy to current intensity flowing through a parallel set of resistors in electric circuits. The ASM−EC was validated with data from batch experiments with four different carbon sources including acetate, ethanol, methanol, and their ternary mixture with fewer parameters than the ASM-ICE (Domingo-Félez & Smets 2020).

4.5. Inclusion of N2O emission

N_2O models have been developed in biological nitrogen removal systems and reached maturity to facilitate process optimization of conventional N removal systems (Section 2.4). However, they have not been widely applied in modelling short-cut processes. The lack of datasets is one of the major challenges to applying N_2O models and particularly the datasets from large-scale demonstrations. For that reason, monitoring the N_2O emissions is essential in pilot and full-scale applications to improve the applicability of N_2O models in mainstream short-cut processes. Hybrid models can also be adopted for short-term laboratory or pilot-scale studies with data scarcity where mechanistic models can be used to simulate the biological process and the data generated can be used in a data-driven model that acts as input to a N_2O prediction algorithm (Mehrani *et al.* 2022).

N_2O modelling in mainstream short-cut N removal systems shares similar challenges as in conventional nitrification and 2-step denitrification systems. For example, since N_2O is an intermediate in the nitrogen transformation pathways, N_2O models require a laborious calibration process under varying operational conditions (Hwangbo *et al.* 2021). Also, microbial communities involved in N_2O production are more complex than conventional systems (e.g. NO is an intermediate of the anammox metabolism and the precursor of N_2O for AOB and heterotrophic denitrifiers). This leads to overparameterized N_2O process models for the description of biological pathways. For example, the aforementioned preferential uptake of carbon sources leads to different patterns of NO_2^- accumulation during denitrification (Ribera-Guardia *et al.* 2014; Zhao *et al.* 2018) and impacts N_2O model calibration (Domingo-Félez & Smets 2020). Thus, while it is important to include the N_2O models for modelling of short-cut processes, model complexity and ease-of-use should be prioritized based on the modelling goals.

5. CONCLUSION AND PERSPECTIVES

A review was presented on models for short-cut N removal processes, in view of mainstream applications, considering the current practice, limitations, future needs and perspectives. Mathematical models are under development to overcome implementation challenges and to deal with the complexity of mainstream deammonification. The pilot and full-scale applications reported provide invaluable information for models development while modelling efforts can accelerate the success of practical applications.

- NOB out-selection is still a major issue for mainstream application. Its dynamics can be captured in individual models but there are still limitations such as the model parameter values that may vary depending on the process configuration and operating conditions. Thus, they may not be transferable to another system.
- Recent modelling efforts mostly deal with the application of mainstream deammonification in full-scale and there appears to be less focus on nitrite-shunt.
- To correctly model the N mass balance and anammox process performance, models should consider incorporation of heterotrophic denitrifiers and anammox bacteria and the yield for the overall anammox metabolic reaction based on ammonium take up.
- While steady-state simulations have been used to model the overall feasibility and performance of short-cut N removal systems, dynamic simulations are especially needed to address issues with capacity evaluations, control strategies for sustained performance, and assessing the risk for meeting effluent limits.
- The application of robust sensitivity analysis is urgently needed and more rigorous calibration methodologies should be adopted to allow the comparison between model structures. The simulated model outputs would become much more reproducible and transferable.
- Modelling PdNA requires accurate modelling of partial denitrification and the ability to address microbial competition among the electron acceptors for various carbon sources. The existing PdNA models were only tested thus far to simulate partial denitrification with simple carbon sources. Other electron donors need to be better characterized and evaluated

using the models to simulate PdNA systems that rely on complex influent COD, fermentation products or internally stored carbon.

- Competition over different substrates and estimation of half-saturation constants should be handled carefully by considering system properties such as mixing, diffusion processes and substrate limitations such as by nitrite and inorganic carbon availability to properly model the substrate competition and out-selection. Hybrid models can be adopted by adding a data-driven component to the well-described mechanistic model structure to model substrate competition properly and compensate the lack of mechanistic description of subprocesses influencing the kinetics of short-cut processes.
- The inclusion of interspecies competition in models by implementing different genera of the same functional group (with different kinetic properties) may be warranted in cases where it affects the macroscopic reactor performance in order to predict a more reliable NOB control performance, and thus find conditions for a more successful process start-up and stable mainstream short-cut process.
- The denitrification intermediate steps are crucial especially for partial denitrification efficiency and N_2O emissions. In model development, 2-step denitrification fits the purpose of modelling short-cut process efficiency while more detailed 4-step models should be adopted if the modelling goal is to predict both process efficiency and N_2O emissions.

ACKNOWLEDGEMENTS

This work is an outcome of discussions at the 'Mainstream Short-cut Nitrogen Removal Modelling: From research to full-scale implementation, do we have what we need?' Workshop of the 7th IWA/WEF Water Resource Recovery Modelling Seminar. It is a collective effort by the practitioners and academics of the wastewater industry who are keen to implement the deammonification process sustainably on mainstream treatment.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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First received 14 January 2022; accepted in revised form 7 April 2022. Available online 20 April 2022