Success of mainstream partial nitritation/anammox demands integration of engineering, microbiome and modeling insights

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Abstract

Twenty years ago, mainstream partial nitritation/anammox (PN/A) was conceptually proposed as pivotal for a more sustainable treatment of municipal wastewater. Its economic potential spurred research, yet practice awaits a comprehensive recipe for microbial resource management. Implementing mainstream PN/A requires transferable and operable ways to steer microbial competition as to meet discharge requirements on a year-round basis at satisfactory conversion rates. In essence, the competition for nitrogen, organic carbon and oxygen is grouped into "ON/OFF" (suppression/promotion) and "IN/OUT" (wash-out/retention & seeding) strategies, selecting for desirable conversions and microbes. Some insights need mechanistic understanding, while empirical observations suffice elsewhere. The provided methodological R&D framework integrates insights in engineering, microbiome and modeling. Such synergism should catalyze the implementation of energy-positive sewage treatment.

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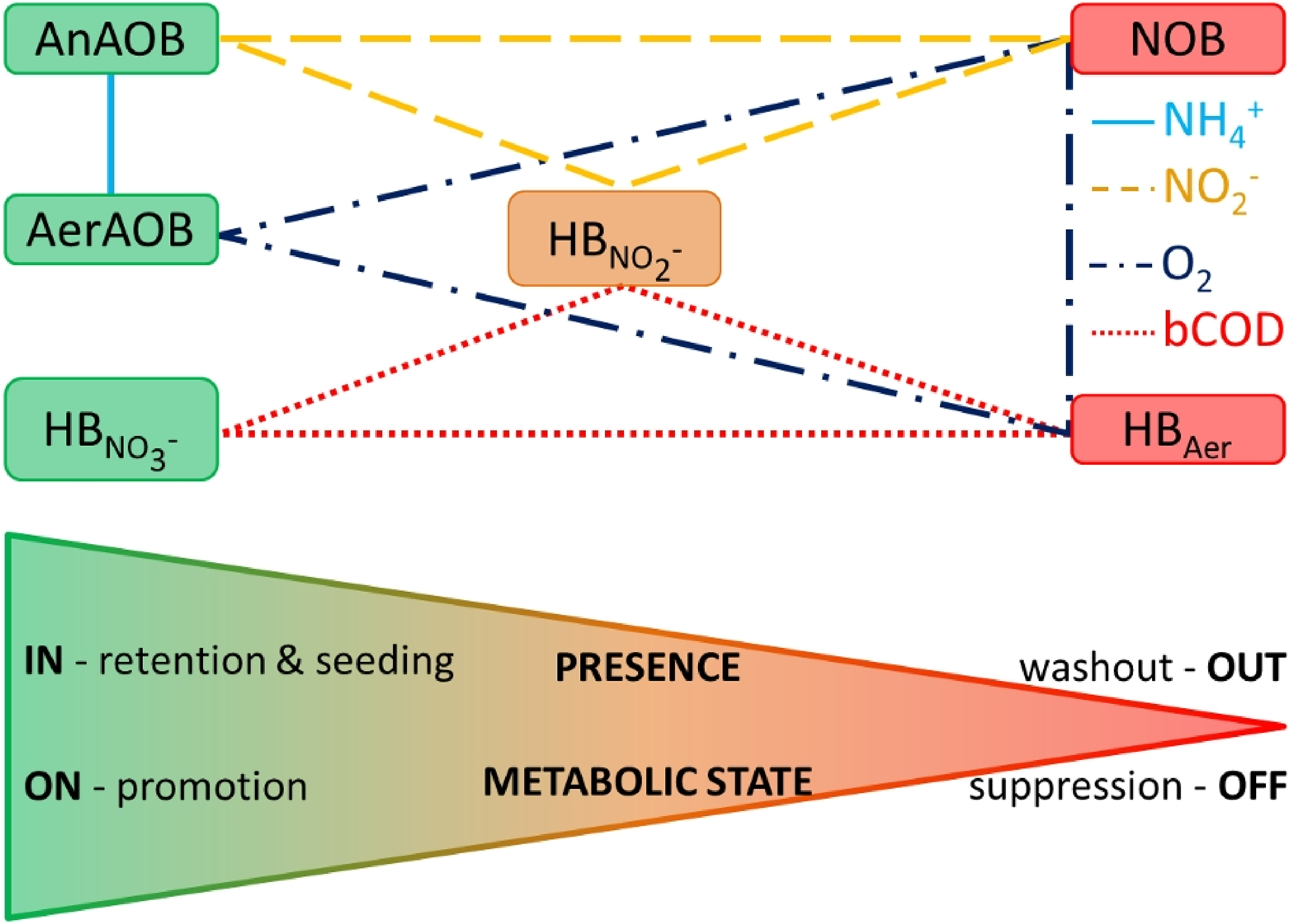
Highlights

- Current design and operational strategies for mainstream PN/A are summarized.
- Combined ON/OFF and IN/OUT strategies are necessary for successful operation.
- A mechanistic framework linking engineering, microbiome, and modeling is proposed.
- Knowledge readiness levels for key process indicators are defined.
- Success relies on integrated research within the framework to boost predictability


AnAOB
NOB
AerAOB
$HB_{NO_2^-}$
$HB_{NO_3^-}$
HB_{Aer}
NH_4^+
NO_2^-
O_2
bCOD
IN - retention & seeding
PRESENCE
washout - OUT
ON - promotion
METABOLIC STATE
suppression - OFF

Full title

Success of mainstream partial nitritation/anammox demands integration of engineering, microbiome and modeling insights

Short title

Strategies for mainstream partial nitritation/anammox

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Abstract

Twenty years ago, mainstream partial nitritation/anammox (PN/A) was conceptually proposed as pivotal for a more sustainable treatment of municipal wastewater. Its economic potential spurred research, yet practice awaits a comprehensive recipe for microbial resource management. Implementing mainstream PN/A requires transferable and operable ways to steer microbial competition as to meet discharge requirements on a year-round basis at satisfactory conversion rates. In essence, the competition for nitrogen, organic carbon and oxygen is grouped into “ON/OFF” (suppression/promotion) and “IN/OUT” (wash-out/retention & seeding) strategies, selecting for desirable conversions and microbes. Some insights need mechanistic understanding, while empirical observations suffice elsewhere. The provided methodological R&D framework integrates insights in engineering, microbiome and modeling. Such synergism should catalyze the implementation of energy-positive sewage treatment.

Introduction to partial nitritation/anammox

It has been 20 years since anaerobic ammonium-oxidizing or anammox bacteria (AnAOB) have been conceptually proposed as game changers for the sustainability of sewage treatment, in so-called mainstream partial nitritation/anammox (PN/A) [1]. PN/A is an autotrophic nitrogen removal process based on two consecutive conversions: ammonium-oxidizing bacteria (AerAOB) oxidize part of the ammonium aerobically to nitrite and AnAOB subsequently oxidize the residual ammonium with the formed nitrite to harmless nitrogen gas. As PN/A does not require organic carbon and lowers aeration (energy) demand, it fits perfectly in a scheme for energy-autarkic treatment of municipal wastewater as secondary (N) stage, enabling a primary (C) stage to maximize carbon capture and redirection for methane production in the sidestream.

Compared to sidestream PN/A, on sludge reject water, it is considerably more complex to achieve sufficiently high nitrogen removal rates and efficiencies for the mainstream process [2]. Particularly winter time in colder climates challenges rates, necessitating a high AnAOB inventory and SRT. Characteristics of the pre-treated sewage impact removal efficiencies, as, besides AerAOB and AnAOB, at least four metabolic types are competing for four substrates, i.e. ammonium, oxygen, nitrite and organic carbon (Graphical abstract), and therefore also for space. Oxygen supports nitrite oxidizing bacteria (NOB) and aerobic heterotrophs (HB_{Aer}) competing with AerAOB; and NOB and anoxic heterotrophs (HB_{NO2^-}) compete for nitrite with AnAOB. In this work, available microbial resource management strategies for mainstream PN/A are compiled, and a comprehensive R&D framework is presented, to catalyze the process' implementation.

Design and operational strategies: the story so far

Until now, several PN/A strategies have been proposed to steer microbial competition, but some are not yet reproduced and lack general consensus. These strategies aimed at (1) promoting growth and activity of AerAOB, AnAOB, and engaging nitrite and nitrate reducing heterotrophs (HB_{NOX^-}) while suppressing NOB, we label this as "ON/OFF" control; and (2) washing-out NOB and heterotrophs from the reactors, while retaining (and seeding) AerAOB and AnAOB, labelled as "IN/OUT" control (Figure 1).

ON/OFF control

Studies based on the ON/OFF control strategy implemented specific oxygen and/or substrate supply patterns. It has been found that sufficient supply of residual ammonium as a baseline needs to be present, i.e. 2-4 mg N L^{-1}, as even sidestream PN/A fails with ammonium limitation [7]. It allows sufficient oxygen limitation in biofilms, and is therefore, the key control parameter to obtain nitritational granular reactors [3,8,9]. This oxygen limitation will also protect AnAOB from oxygen inhibition [2]. In floccular systems, residual ammonium will promote the specific growth rate of AerAOB to ensure that the dissolved oxygen (DO) is the rate limiting parameter during aeration [4,10]. Apart from residual ammonium, aeration is also a key controlled parameter. Continuous low DO-setpoints (< 0.2 mg O_2 L^{-1}) have been reported to minimize AnAOB oxygen inhibition, and increase competition for nitrite in the biofilm to suppress NOB [11,12]. Intermittent aeration on the other hand, balances the periodic supply of oxygen, known as "transient anoxia" by exploiting the nitratational lag (minimum 15-30 min. anoxic) [13,14], complete nitrite consumption in the anoxic phase, and limiting AnAOB inhibition by oxygen (Seuntjens *et al.*, abstract, 5th International Conference on Nitrification and Related Processes (ICoN-5), July 2017). It typically uses higher DO-setpoints (> 1.5 mg O_2 L^{-1}) to maximize activity of AerAOB over NOB [4,10,15,16]. The knowledge gained until now leads to a mixed opinion about which aeration strategy is the best.

Apart from aeration, the introduction of free ammonia (FA) and free nitrous acid (FNA) has been used as an "ON/OFF" approach. As FA and FNA cannot reach inhibitory concentrations in the mainstream, a return-sludge treatment, that exposed thickened flocs from the clarifier, has been proposed. By regularly exposing flocs to inhibitory conditions, it successfully suppressed NOB with 80-90% nitritation in a floccular reactor [5,17].

Municipal wastewater has a high carbon to nitrogen (C/N) ratio, which can promote HB over AnAOB. Therefore, in the past few years, pre-treatment for organic carbon removal (known as "C-Stage") has been proposed to ease the implementation of a mainstream PN/A (known as "N-Stage"). This combination has been adapted to: (1) remove the organic carbon fraction from municipal wastewater, and (2) maximize energy positive wastewater treatment, by methane production. High-rate contact stabilization (HiCS) appears to be the most promising solution because it has low substrate oxidation and efficient removal of organic carbon [18]. Nevertheless, the presence of heterotrophic bacteria (aerobic, HB_{Aer}; HB_{NOX^-}; and other heterotrophs, HB_X) is inevitable in mainstream PN/A processes [19], due to the availability of residual COD in the effluent of the C-stage or soluble metabolic products (SMP) released from AerAOB and AnAOB. However, HB_{NOX} can preferentially participate in: (1) reducing the nitrate concentration in the effluent by maximizing anoxic bCOD utilization [20], (2) and suppressing NOB by reducing nitrite availability along with DO control [4]. Such metabolic potential of a PN/A microbial community has already been observed [21]. However, it requires further activity-based studies.

IN/OUT control

The lower temperatures (10-25°C) under mainstream conditions lower the growth rates and activities of the desired organisms, requiring more selective control of the sludge retention time (SRT) of different sludge fractions. Long biofilm SRT are required to retain AnAOB due to their slow growth rate, especially under low-temperature mainstream conditions (SRT =70d at 15°C, >100d at 10 °C) [11,22]. Therefore, biofilm-based reactors have been used, mainly as granule [12,22], or carrier material [23] configurations, either having high removal rates and lower efficiency or lower rates with higher efficiency. In contrast, a short enough flocculent SRT to selectively washout NOB, yet retain AerAOB ([4,15], Seuntjens *et al.*, unpublished). To bring these conflicting worlds together, one-stage hybrid systems (= granule/biofilm + floc) [11,24] have also been validated to achieve simultaneous, short-floc and long-biofilm SRT, allowing NOB washout from suspension and AnAOB retention in the biofilm. Another strategy might be the separation of nitritation and anammox in a two-stage approach, discussed later.

IN/OUT + ON/OFF control = reactor solution

The possible combination of various strategies belonging to the “ON/OFF” and/or “IN/OUT” approaches have been advocated for NOB out-selection. For instance, in a pilot study [4] based on suspended biomass, operated at 25^{o}C, a combination of short aerobic SRT, intermittent aeration at high DO concentration and residual ammonium was successful for NOB wash-out. In a granular biomass reactor [16] operated at 15°C, shorter SRT of the flocculent fraction with continuous aeration at low DO set-point also demonstrated NOB wash-out. Intermittent aeration at a low DO set-point, and strict SRT to just retain AerAOB and wash-out NOB also worked in a hybrid reactor (suspended and carrier-based biomass) [25]. The information, to date, does present possible design and operation choices. However, criteria for designing such combinations are not clear yet.

Process success: empiricism (empirical observations) where possible, rationalism (mechanistic insights) where needed.

Dynamic characteristic of municipal wastewater (regarding composition, quantity, pH, temperature) make mainstream PN/A processes an ‘open bioprocess’ with high complexity. In the end, a working PN/A process needs to be predictable, i.e. its output needs to be controllable, including this dynamic variation. Despite significant research, focused on (1) reactor engineering; (2) PN/A microbial communities; (3) and modeling to understand the process, more mechanistic insights are needed to unravel the whole complexity of mainstream PN/A. We suggest a mechanistic framework shown in Figure 2 as a tool to summarize knowledge readiness levels of different parameters belonging to various aspects: engineering (operation and design), microbial communities, and process modeling.

Uncontrollable wastewater parameters: process adaptation

The impact of the influent wastewater temperature on the desired microorganisms, i.e. AnAOB and AerAOB, and to some extent on NOB, has been well studied [11,19,22,23]. However, limited information is available regarding the temperature influence on other microbial community members of the PN/A community [19,21,26]. The influence of temperature depends on the morphology of the biomass [27,28] and the microbial community composition (for example, *Nitrobacter* and *Ca. Nitrotoga* are negatively, and *Nitrospira* is positively correlated to temperature) [23,29,30]. This knowledge has however not yet been translated into operational strategies, i.e., automation of adaptive flocculent SRT and DO-setpoints, which are required for stable operation.

Inorganic carbon (IC) concentration and the coupled parameter, i.e., alkalinity also influence the PN/A process. Low IC values significantly limit the activity of AerAOB and AnAOB and contribute to the instability of sidestream PN/A [31,32]. Thus, this needs to be considered for mainstream PN/A as well (Seuntjens *et al.*, abstract, 1st Symposium on Microbiological Methods for Waste and Water Resource Recovery, May 2017). Adaptation of control strategies, and advanced process models [33] that include potential microbial adaptation/selection [34] towards low IC might be solutions for the problem.

Controllable parameters: towards one-stage or two-stage solutions?

Different reactor types (i.e., single or two-stage) have been developed, but to date, neither is ready. Two-stage systems have higher conversion rates [3,22] and high N_2O emission [3,6], whereas, single-stage systems provide extra selection pressure on NOB due to the anoxic removal of nitrite, but the implementation of IN/OUT strategies in single-stage systems is more challenging. This depends on the configuration of the combination; easy separation is possible for a combination of suspended and carrier biomass-based reactors [11,16,24] but complicated in suspended and granular biomass-based combined reactors [15]. For both one- or two-stage solutions, there is lack of definitive information due to the contradicting results, so far. For example which aeration strategy is to employ, continuous or intermittent aeration? The answer to such a question requires knowledge about which microorganisms are present, how they are arranged in the reactor, and how they all do behave at different aeration strategies. We thus require an integrated knowledge of community physiology, morphology, reactor design, with pragmatic mechanistic understanding to model the process and predict optimal reactor performance.

From microbiological understanding towards a working process

AnAOB, AerAOB and NOB, which play the leading role in PN/A systems, have been extensively studied for characterization; abundance/dynamics [19,35]; growth/inhibition [36], as well as spatial organization [28,36,37]. Access to new molecular tools has shed some light on the microbial heterogeneity – the composition (i.e., PN/A biomasses compose a vast diversity of microbial members, many of the dominant members not being AnAOB and AerAOB) [38]. Also, found that complex metabolic synergies exist within PN/A microbial community [26], for example, the nitrate-nitrite loop principle. The current knowledge is not enough, especially for low-temperature mainstream PN/A systems. There is a need for (1) mechanistic understanding of the whole community composition (including HB_{Aer}, HB_{NOX^-} and HB_X, found in PN/A systems); (2) translation of eco-physiological know-how to reactor operation [19].

Due to the high interconnectivity of reactor function and microbiology, a distinct categorization of research objectives is difficult. This complicates the identification of suitable research starting points. As a consequence, both aspects need to go hand in hand. Thus, an ‘information feedback-loop’ in mechanistic understanding is required: (1) incorporating uncontrollable parameters while deciding ON/OFF and IN/OUT strategies; (2) understanding the community and interpreting it’s response to/for reactor function; and (3) performing predictive learning at different levels.

Pragmatic modeling

Process modeling can play an essential role during the transition towards a more mechanistic approach as it allows to couple engineering aspects to microbial ecology and process performance. When fed with quality data on wastewater and sludge characteristics, models can be a powerful tool to map and interpret the multitude of complex physicochemical and biological interactions occurring at different levels of the PN/A process. This knowledge can then be used to asses different design and operational strategies to identify key control parameters (such as morphology [39,40] or DO [41]) and obtain a stable, well-performing process (in terms of both effluent quality [9,32] and emissions [39]). Despite the recent progress in modeling of the PN/A process, more efforts must be made to incorporating dynamic microbial ecology data [26,40–42]. Finally, mechanistic modeling output needs to be coupled back to the engineering approach and microbiology analysis (and *vice versa)* to obtain a predictable and hence transferable process.

Multiscale evaluation of mainstream PN/A sustainability: LCC, LCA, and LCCA

When evaluating the potential of mainstream PN/A as a sustainable alternative to more conventional N-removal processes, one must keep in mind that these processes are to be integrated into the complex, multi-stage structure of a water resources recovery facility (WRRF). Hence, the potential economic and environmental gains must be assessed, not only at the process level but for the entire WRRF as proposed in Figure 3 . It shows how costs (via life cycle costing, LCC) and environmental impact (via life cycle assessment, LCA) could be simultaneously included in process optimization by implementing a proposed superstructure, here called "life cycle and cost analysis" (LCCA). Studies combining dynamic plant-wide modeling with LCA reveal the trade-off between key parameters at a process level (e.g. N_2O emission) and the overall environmental impact when comparing different WRRF scenarios [43,44]. Even though LCA and LCC are broadly adopted methodologies, further but work is still needed to improve the framework, especially when trying to combine both in a superstructure [45,46].

Conclusion

It has become clear that by simply evaluating reactor performances and assessing microbial community composition and its dynamics (mainly focusing on AerAOB, AnAOB, and NOB) our understanding about low-temperature mainstream PN/A will not improve. The knowledge concerning reactor function, microbiology, and mechanistic models is increasing. The methodological framework (Figure 2) suggested here highlights which parameters are less studied right now, and the link between various parameters. The framework also guides a way to connect data between various parameters, to assemble into one useful information. Therefore, the multi-parameter mechanistic approach is advocated. Nevertheless, knowledge gained needs to be transferable to the practical purpose – despite continuous dynamics in municipal wastewater, the PN/A process should, i.e. (1) meet effluent limits, and be (2) easy to manage – operator friendly, (3) overall cost-efficient, (4) and environment-friendly.

Conflict of interest

None.

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The developed DM-LCA platform was able to generate highly realistic information by combining dynamic modelling of wastewater treatment (validated on experimental data) with life cycle analysis. This in turn allowed for improved decision making when comparing the environmental performances of 6 different WRRF scenarios. In addition, the platform also allowed to identify hot spots (e.g. N_2O emissions, infrastructure, heavy metals etc…) which still need optimization to further reduce environmental impact.

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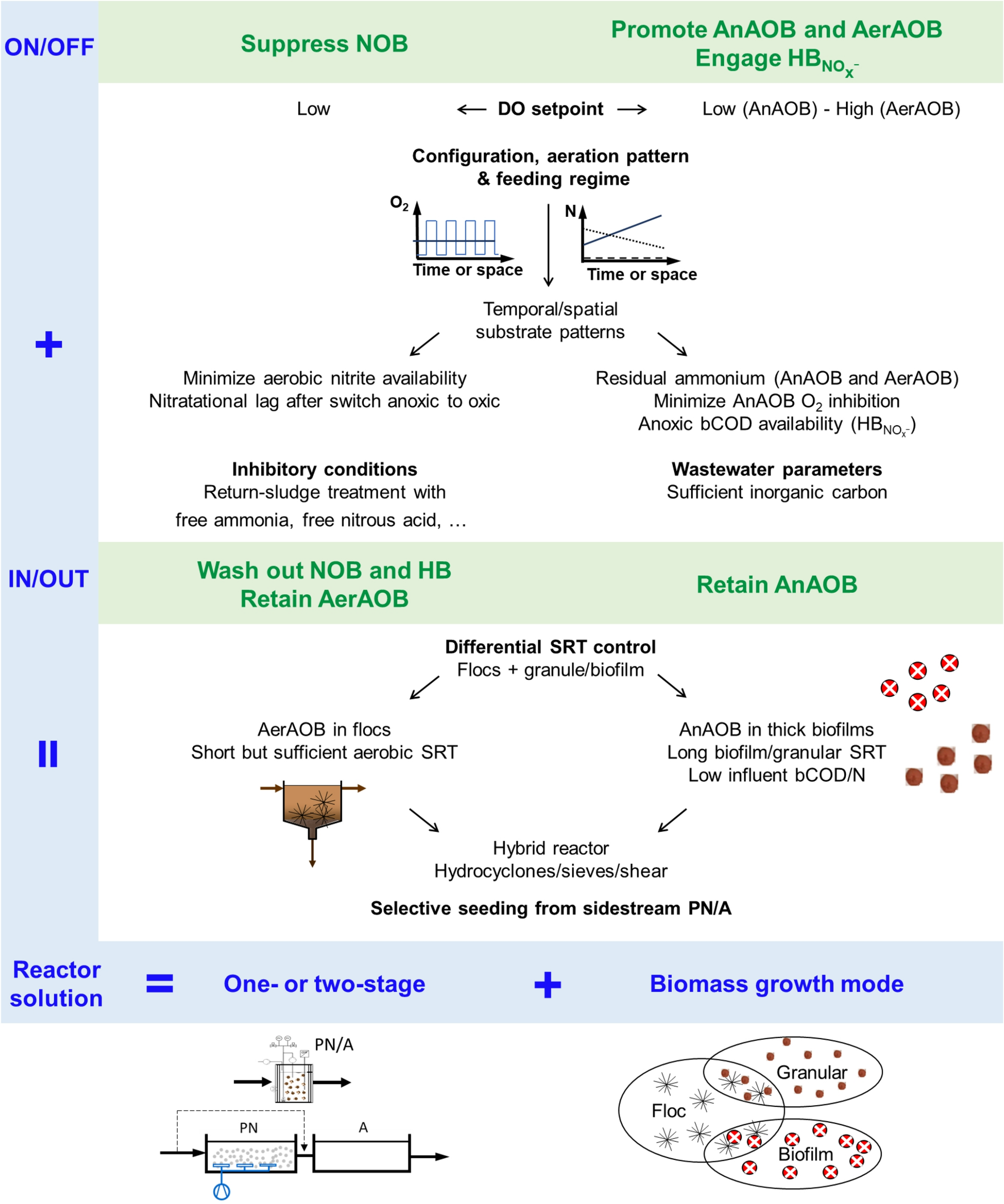
Figure 1: Strategies for design and operation of a one- or two-stage partial nitritation/anammox (PN/A) reactor. NOB: Nitrite oxidizing bacteria. AerAOB: Aerobic ammonium oxidizing bacteria. AnAOB: Anoxic ammonium oxidizing bacteria. HB: Heterotrophic bacteria. HB$_{NOx}^{-}$: Heterotrophic bacteria reducing nitrite or nitrate. SRT: Sludge retention time. bCOD/N: biodegradable chemical oxygen demand over nitrogen.

Figure 2: Methodological framework presenting knowledge readiness level of different parameters related to (1) Process engineering; (2) microbiome; and (3) modelling aspects. The framework links uncontrollable with controllable parameters for reactor design/operation and suggests the integration of physiological data for individual microorganisms into an eco-physiological model covering the whole community. Furthermore, it illustrates how comprehensive modelling at different levels of the process can help consolidate this information in a more mechanistical approach. The arrows indicate the flow of information from one aspect to the other. Colour of the bubbles define the extent of knowledge gained in last 20 years, which can be used for prediction and implementation of low-temperature mainstream PN/A process. The white bubbles signify that there are some unknown parameters, which also require attention in future. The colour of the ring in the microbiome aspect present our current opinion on different microbial groups (i.e. aerobic ammonium oxidizing bacteria, AerAOB; anaerobic ammonium oxidizing bacteria, AnAOB; nitrite oxidizing bacteria, NOB; aerobic heterotrophic bacteria, HB_{Aer}; nitrite and nitrate reducing heterotrophic bacteria, HB_{NOX^-}; other heterotrophic bacteria, HB_X) which are present within the PN/A microbial communities, whether the individual groups contribute to the successful operation of the PN/A process or not.

Figure 3: Role of life cycle assessment (LCA), life cycle costing (LCC) and a proposed superstructure here called "life cycle and cost analysis" (LCCA) in evaluating the sustainability of mainstream PN/A applications. Main end-point criteria are shown for both LCC and LCA. The integration of LCA and LCC in a LCCA superstructure is complicated by their many shared impact points, attention must be paid to avoid double counting. Water resources recovery facility (WRRF); Capital expenditures (CAPEX) and Operating expenses (OPEX).



ON/OFF

Suppress NOB

Promote AnAOB and AerAOB
Engage $HB_{NO_x^-}$

Low ⟵ **DO setpoint** ⟶ Low (AnAOB) - High (AerAOB)

Configuration, aeration pattern
& feeding regime

Temporal/spatial
substrate patterns

Minimize aerobic nitrite availability
Nitratational lag after switch anoxic to oxic

Residual ammonium (AnAOB and AerAOB)
Minimize AnAOB O_2 inhibition
Anoxic bCOD availability ($HB_{NO_x^-}$)

Inhibitory conditions
Return-sludge treatment with
free ammonia, free nitrous acid, …

Wastewater parameters
Sufficient inorganic carbon

+

IN/OUT

Wash out NOB and HB
Retain AerAOB

Retain AnAOB

Differential SRT control
Flocs + granule/biofilm

AerAOB in flocs
Short but sufficient aerobic SRT

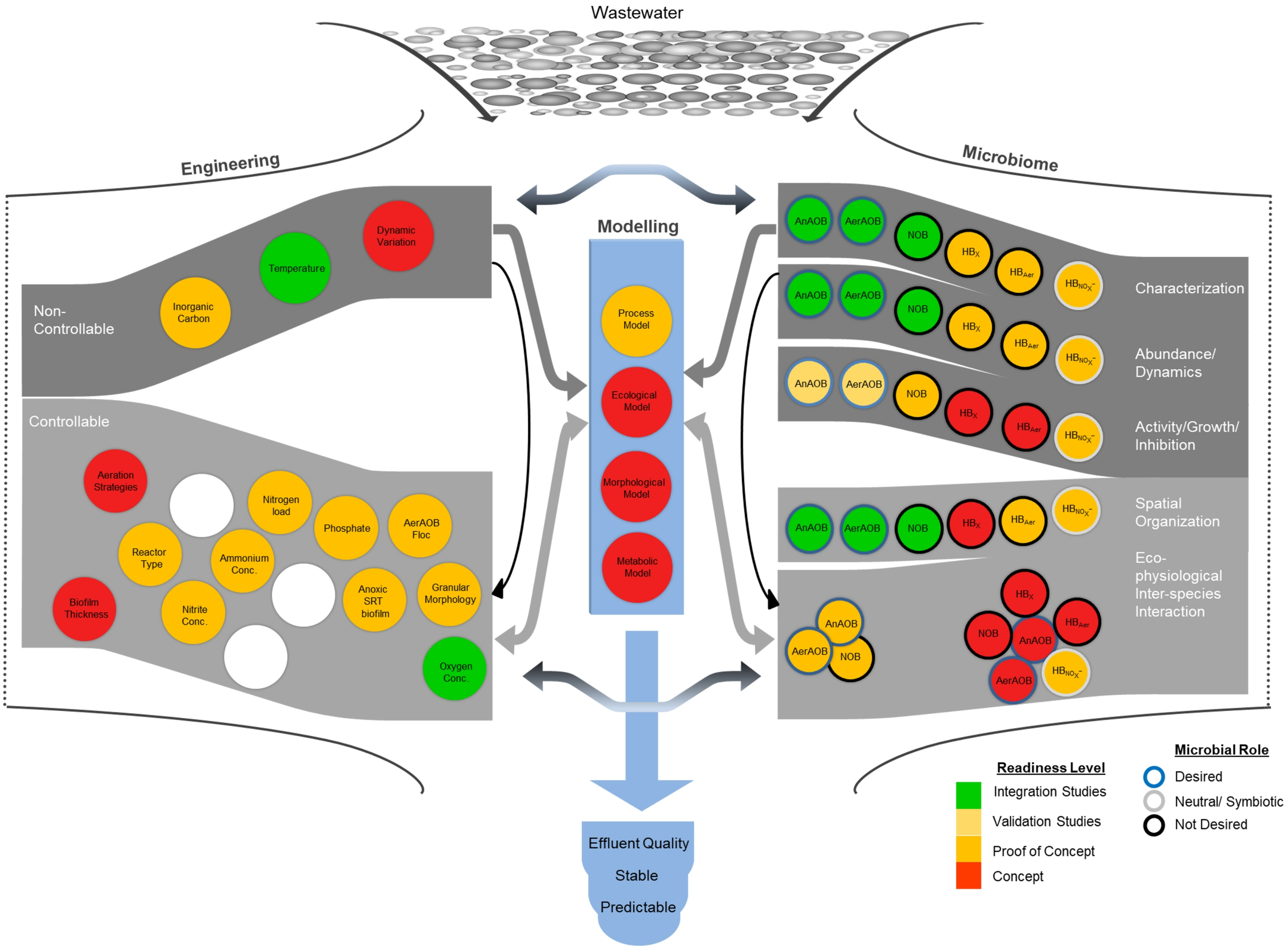
AnAOB in thick biofilms
Long biofilm/granular SRT
Low influent bCOD/N

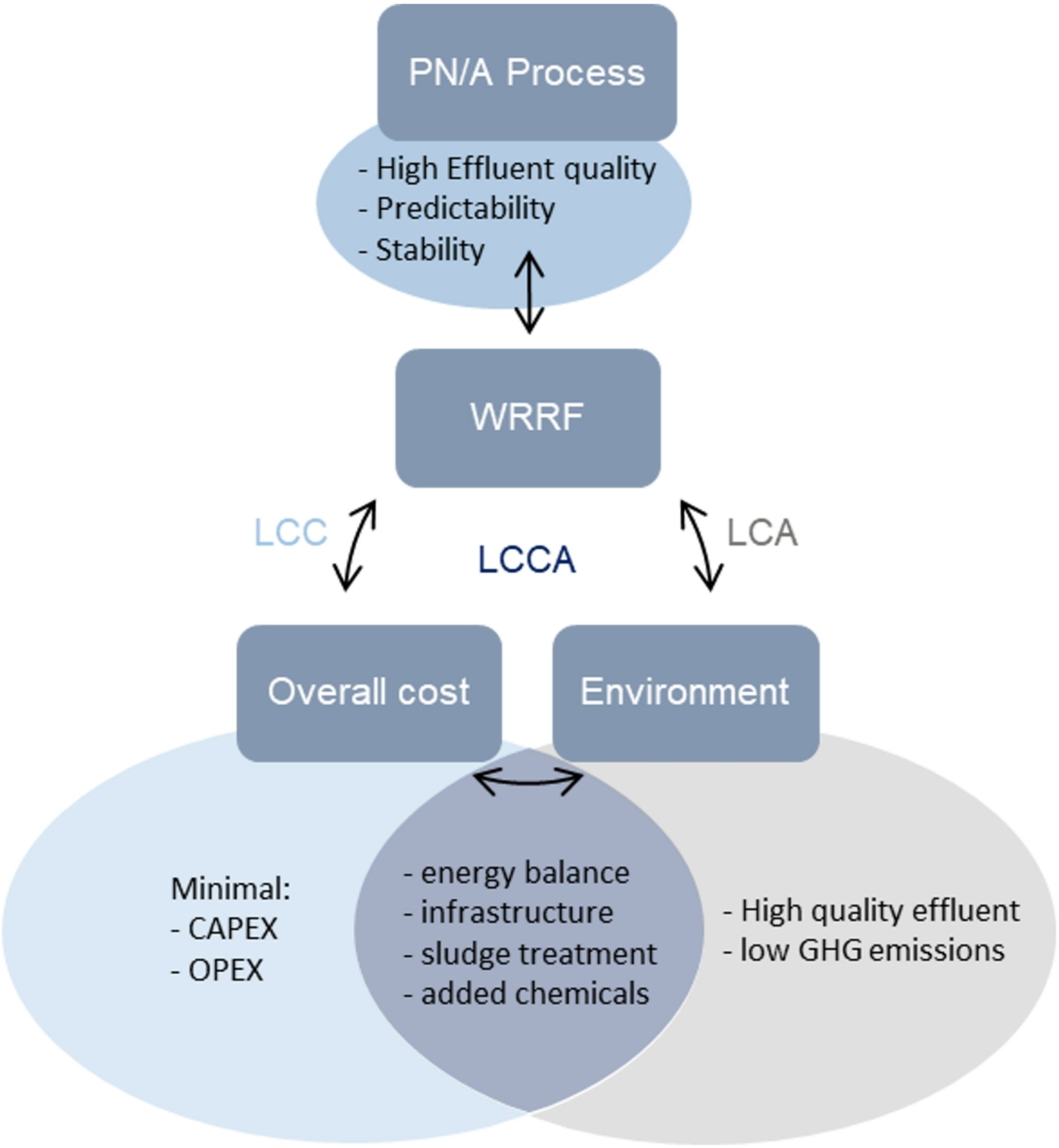
Hybrid reactor
Hydrocyclones/sieves/shear

Selective seeding from sidestream PN/A

=

Reactor solution **=** **One- or two-stage** **+** **Biomass growth mode**


Wastewater
Engineering
Microbiome
Modelling
Non-Controllable
Dynamic Variation
Temperature
Inorganic Carbon
Controllable
Aeration Strategies
Nitrogen load
Phosphate
AerAOB Floc
Reactor Type
Ammonium Conc.
Anoxic SRT biofilm
Granular Morphology
Biofilm Thickness
Nitrite Conc.
Oxygen Conc.
Process Model
Ecological Model
Morphological Model
Metabolic Model
Characterization
Abundance/ Dynamics
Activity/Growth/ Inhibition
Spatial Organization
Eco-physiological Inter-species Interaction
AnAOB
AerAOB
NOB
HB_X
HB_{Aer}
$HB_{NO_X^-}$
Effluent Quality
Stable
Predictable
Readiness Level
Integration Studies
Validation Studies
Proof of Concept
Concept
Microbial Role
Desired
Neutral/ Symbiotic
Not Desired


PN/A Process
- High Effluent quality
- Predictability
- Stability
WRRF
LCC
LCCA
LCA
Overall cost
Environment
Minimal:
- CAPEX
- OPEX
- energy balance
- infrastructure
- sludge treatment
- added chemicals
- High quality effluent
- low GHG emissions