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

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Twenty years ago, mainstream partial nitrification/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 and 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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treatment, in so-called mainstream partial nitrification/anammox (PN/A) [1]. PN/A is an autotrophic nitrogen removal process based on two consecutive conversions: aerobic 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, ammonium, oxygen, nitrite and organic carbon (Graphical abstract), and therefore also for space. Oxygen supports nitrite-oxidizing bacteria (NOB) and aerobic heterotrophs (HB<sub>Aer</sub>) competing with AerAOB; and NOB and anoxic heterotrophs (HB<sub>NO2-</sub>) 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 firstly, promoting growth and activity of AerAOB, AnAOB, and engaging nitrite and nitrate reducing heterotrophs (HB<sub>NOX-</sub>) while suppressing NOB, we label this as ‘ON/OFF’ control; and secondly, washing-out NOB and heterotrophs from the reactors, while retaining (and seeding) AerAOB and AnAOB, labelled as ‘IN/OUT’ control (Figure 1).

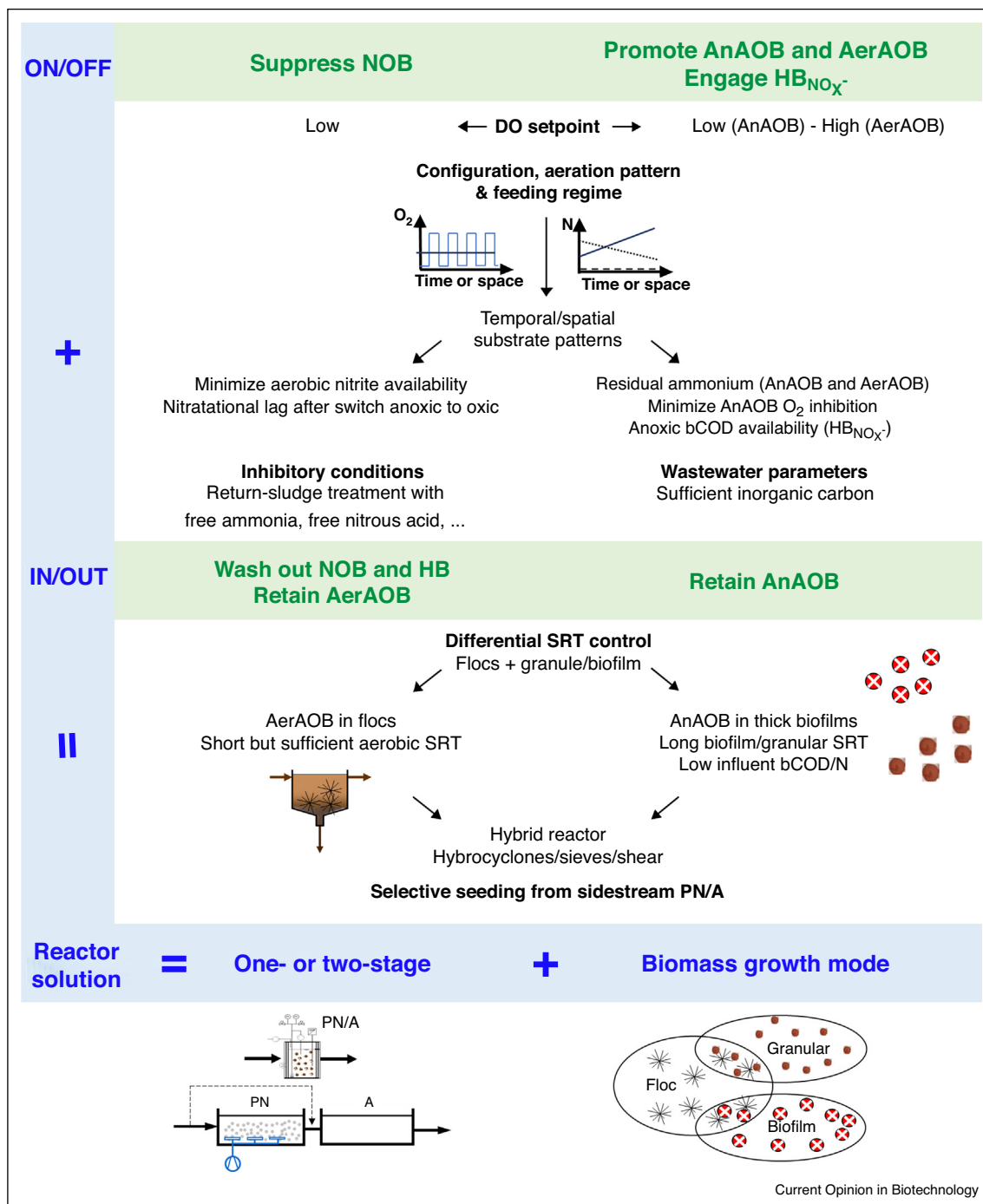
## ON/OFF control

Studies based on the ON/OFF control strategy implemented specific oxygen and/or substrate supply patterns.

## Introduction to partial nitrification/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

Figure 1



Strategies for design and operation of a one-stage or two-stage partial nitritation/anammox (PN/A) reactor. NOB: Nitrite-oxidizing bacteria. AerAOB: Aerobic ammonium-oxidizing bacteria. AnAOB: Anaerobic ammonium-oxidizing bacteria. HB: Heterotrophic bacteria. HB<sub>NOX</sub><sup>-</sup>: Heterotrophic bacteria reducing nitrite or nitrate. SRT: Sludge retention time. bCOD/N: biodegradable chemical oxygen demand over nitrogen.

Maintaining a residual ammonium concentration (i.e. 2–4 mg N L<sup>-1</sup>) is required for efficient NOB suppression in PN/A, even sidestream PN/A fails under ammonium limitation [3]. It allows sufficient oxygen limitation in biofilms, and is therefore, the key control parameter to

obtain nitritational granular reactors [4–6]. 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

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