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Opportunities and challenges of shortcut nitrogen removal in membrane-aerated biofilm reactors (MABRs)

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A B S T R A C T

The membrane-aerated biofilm reactor (MABR) has attracted increasing attention from both academia and water practitioners due to its potential to enhance aeration efficiency and intensify treatment capacity. However, emerging evidence highlights a critical challenge in achieving shortcut nitrogen removal within MABRs because nitrite-oxidizing bacteria (NOB)—key enemies of shortcut nitrogen removal—reside in the innermost biofilm layer and are consequently well-protected. In response, extensive efforts over the past two decades have focused on developing strategies for stable NOB suppression to facilitate shortcut nitrogen removal in MABRs. This work offers a comprehensive review of the advancements made in this field, providing an in-depth analysis of the kinetic mechanisms that underpin the NOB suppression in MABRs. We propose that the current NOB suppression strategies in MABRs could be categorized into three groups according to their different kinetic mechanisms, while most were tested in laboratory with synthetic or high-strength wastewater and under controlled conditions. We present various strategies for the start-up, long-term maintenance and performance recovery after deterioration for shortcut nitrogen removal in MABRs. We also highlight MABRs as a potential platform for enriching and applying new nitrogen-cycling microorganisms. Finally, future research directions are suggested in both fundamental and engineering aspects, including exploring microbial ecology in biofilms and demonstrating NOB suppression strategies in real-world conditions. These efforts could further advance the understanding and application of MABRs in nitrogen removal processes.

1. Introduction

The water industry has set aspirational targets for sustainable wastewater management, requiring the development of intensified, cost-effective, and low-carbon footprint technologies [1]. Membrane-aerated biofilm reactor (MABR) is a novel and promising solution with multiple merits, including intensified capacity for pollutant removal, compact reactor designs, and less fugitive greenhouse gas emissions [2,3]. Moreover, by delivering oxygen through the pure diffusion process, MABRs can theoretically achieve oxygen transfer efficiencies of 100 % [4]. In MABR biofilms, substrates diffuse in an opposite direction (i.e., counter-diffusion biofilms). Specifically, oxygen is supplied from the biofilm substratum while other substrates, such as ammonium and organics, are fed from the bulk liquid. This counter-diffusion mode supports the coexistence and collaboration of diverse microorganisms across different biofilm layers.

The discovery of novel microbial processes has brought increasing attention to shortcut nitrogen removal, which directly converts nitrite to dinitrogen gas, bypassing nitrate [5]; [6–8]. Partial nitritation and anammox (PN/A, or deammonification) and nitrite shunt are two shortcut nitrogen removal processes. Compared to conventional nitrification-denitrification, the shortcut nitrogen removal can significantly reduce aeration, organic carbon consumption, and sludge production [9]; [6], which could be further amplified in combination with MABRs. However, this integration remains challenging and contentious. Specifically, recent studies have highlighted the difficulty of achieving stable shortcut nitrogen removal in MABRs, primarily due to the challenge of suppressing nitrite-oxidizing bacteria (NOB) in this unique

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counter-diffusion biofilm [10]. This challenge highlights the need for a deeper understanding of the kinetics governing microbial interactions within MABR biofilms and the development of effective strategies to control NOB activity.

This review aims to provide a kinetic perspective to explain why achieving shortcut nitrogen removal in MABRs is challenging and summarize the strategies developed so far to overcome this obstacle. More importantly, future research directions to address this challenge and to advance the field are proposed and discussed.

2. NOB suppression is more challenging in MABRs than in other systems

The key challenge of achieving a stable shortcut nitrogen removal is the proliferation of NOB. Studies have indicated that suppressing NOB in MABRs poses greater challenges compared to conventional co-diffusion biofilm systems and flocculent biomass [11]; [10,12]. From the perspective of kinetic principles, the suppression of NOB requires ensuring that the term $\frac{dX_{NOB}}{dt}$ is zero or negative (Equation (1)). Specifically, $\frac{dX_{NOB}}{dt}$ represents the variations of NOB concentration over time, which is equal to the net growth rate of NOB (i.e., growth rate minus decay rate) minus the NOB loss via biofilm detachment. Thus, $\frac{dX_{NOB}}{dt}$ is expressed as follows:

$$\frac{dX_{NOB}}{dt} = (\mu_{NOB} - b_{NOB} - k_d) \times X_{NOB} \quad (1)$$

where X_{NOB} is the area-specific concentration of NOB (g/m^2), t is time (d), μ_{NOB} is the NOB growth rate (1/d), b_{NOB} is the NOB decay rate (1/d), and k_d is the detachment coefficient (1/d).

In co-diffusion biofilms (Fig. 1a), the dissolved oxygen (DO) level inside biofilms is typically lower than the DO in the bulk liquid. The middle layer benefits from sufficient oxygen availability and reduced shear force, resulting in a net growth rate of NOB exceeding the NOB detachment ($\mu_{NOB} - b_{NOB} > k_d$), serving as a favourable niche for NOB development within co-diffusion biofilms. In contrast, the favourable conditions for the NOB development in MABRs are considerably different (Fig. 1b). In MABRs, oxygen diffuses from the biofilm base toward the surface, resulting in a substantially higher DO concentration in the inner biofilm layers compared to the bulk liquid. This means that if the bulk liquid DO is controlled at the same level as that of co-diffusion biofilms, the inner biofilm in MABRs would have a much higher DO profile compared with co-diffusion biofilms, making the NOB suppression in MABRs more challenging and complicated. Even when the bulk liquid DO concentration is maintained at zero in MABRs, the majority of the biofilm remains oxic, leaving only a small fraction of the outer biofilm in an anoxic state:

- **Layer I (Outer Layer):** The outermost layer of MABR biofilms is also subjected to strong shear forces, resulting in a high NOB detachment rate ($\mu_{NOB} - b_{NOB} < k_d$), thus suppressing the development of NOB.
- **Layer II (Middle Layer):** The DO and nitrite are restricted in the middle layer, suppressing the net growth of NOB ($\mu_{NOB} - b_{NOB} < k_d$), thereby hindering their development within this region.
- **Layer III (Inner Layer):** The innermost layer, in proximity to the oxygen source, experiences high oxygen and nitrite availability and an extremely low NOB detachment. These conditions create an optimal niche for the NOB growth ($\mu_{NOB} - b_{NOB} - k_d > 0$). Once NOB establish in this layer, their suppression and washout become exceedingly difficult due to the protective environment provided by the biofilm matrix.

Comparing the two different biofilms highlights that suppressing NOB in MABR biofilms is considerably more challenging, because in the innermost layer 1) μ_{NOB} is high as this region has the highest concentrations of oxygen and nitrite; 2) b_{NOB} is relatively low because NOB are protected from unfavourable external conditions; and 3) k_d is extremely low due to minimal biofilm detachment. These conditions collectively

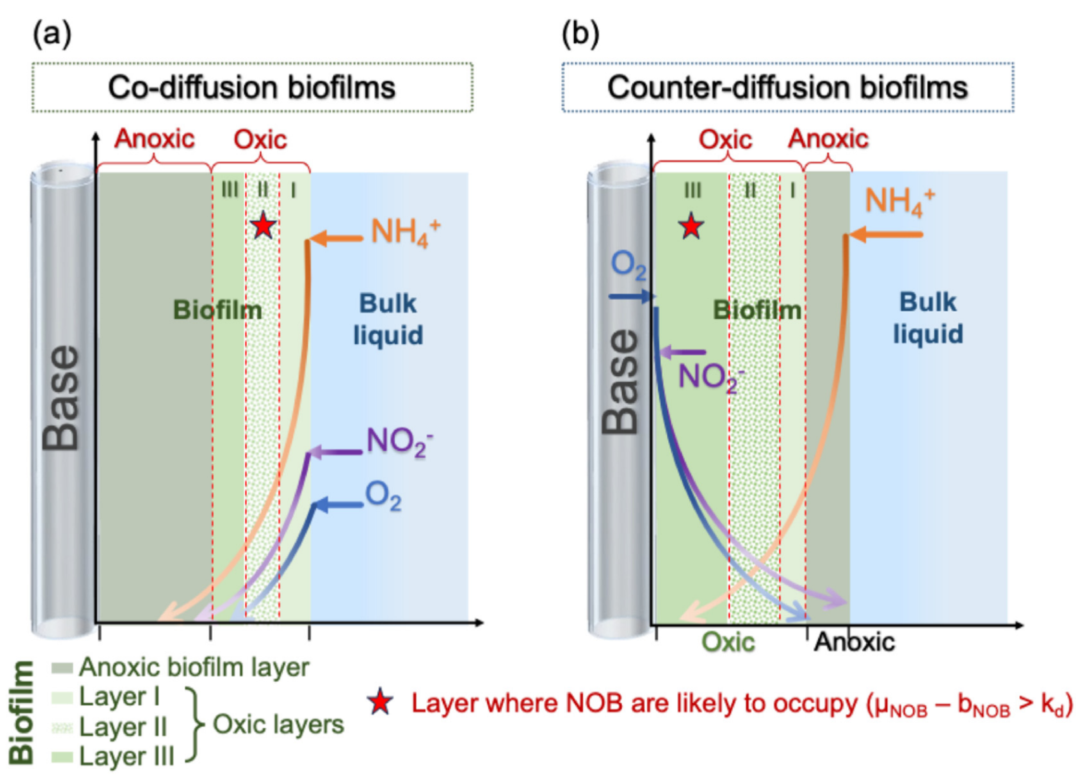

Fig. 1. Comparison between (a) co-diffusion and (b) counter-diffusion biofilms. The oxic zone of both biofilms can be stratified into three sub-layers. Layers likely inhabited by NOB are marked with stars.

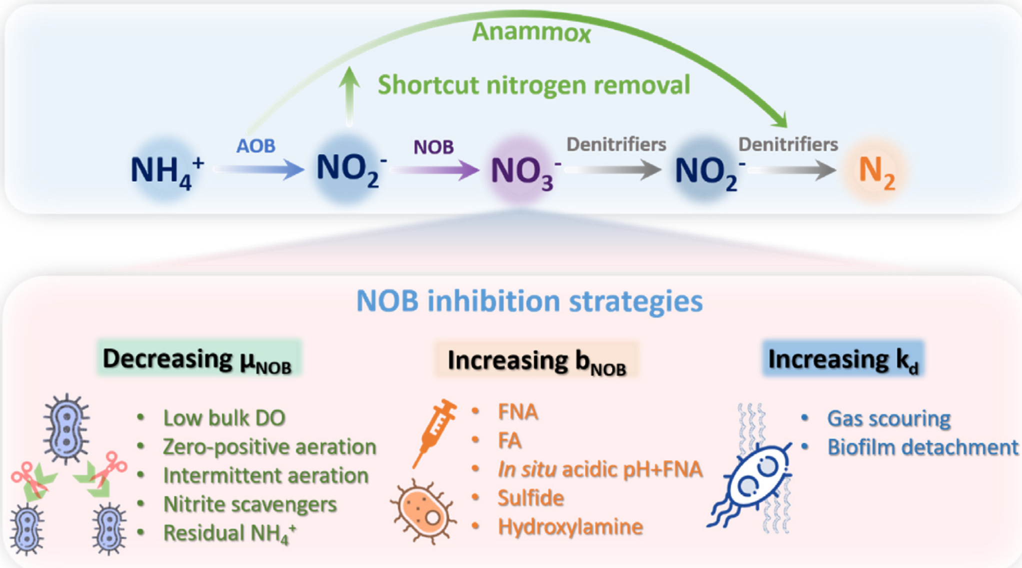

Fig. 2. Schematic summary for NOB suppression strategies in MABR. The strategies are classified into three groups: decreasing μ_{NOB}, increasing b_{NOB}, and increasing k_d.

establish the innermost layer of counter-diffusion biofilms as a particularly favourable niche for NOB proliferation. Therefore, the implementation of precise and sustained strategies is essential to inhibit NOB development in this critical region.

3. Strategies to suppress NOB in MABR

From a kinetic perspective, the suppression of NOB can be only achieved if $\frac{dX_{NOB}}{dt}$ is zero or negative, as described by Equation (1). This principle thus classifies various strategies developed to suppress NOB in MABRs into three categories: reducing μ_{NOB}, increasing b_{NOB}, and extending k_d. In this context, we systematically categorized the numerous strategies that have been developed thus far into three groups, providing an overview of recent advancements and highlighting the challenges associated with each approach (Fig. 2). Unlike traditional biofilms and flocs, which mainly focus on the NOB suppression in low-strength wastewater, many strategies developed for MABRs have been based on medium-strength and high-strength wastewater. This indicates that NOB suppression in MABRs is already more challenging even in medium- and high-strength wastewater, and suggests that the difficulty of applying these strategies to low-strength sewage will be even more pronounced.

3.1. *Strategy 1: Decreasing NOB growth* (μ_{NOB})

The growth rate of NOB (μ_{NOB}) is determined by the maximum growth rate, substrate concentrations, and other environmental factors (e.g., temperature). While the maximum growth rate and environmental factors could be considered constant and uncontrollable, to decrease the growth rate of NOB, limiting substrate concentrations is important. Therefore, strategies have been developed to limit oxygen, nitrite, or both to decrease the growth rate of NOB.

Low DO control is undoubtedly one of the most widely used strategies in practice, due to the ease of regulating DO levels during the real-time operation of treatment processes. In fact, for most of other techniques mentioned above and later, low DO control is often applied in combination to achieve stable NOB suppression, emphasizing the significance of low DO in shortcut nitrogen removal. Specifically, limiting oxygen to suppress NOB in MABRs can be achieved in various approaches, such as lowering bulk DO level (usually <0.5 mg/L) [10,13], intra-membrane pressure control [14], zero-positive aeration [15], and intermittent aeration [16–18].

Limiting the availability of nitrite is another approach to controlling the growth rate of NOB in MABRs. This can be achieved by introducing nitrite-scavenging microorganisms, such as heterotrophic denitrifiers and anammox bacteria, into the biofilm. As a result, the implementation of shortcut nitrogen removal in a one-stage configuration—where both aerobic and anaerobic processes occur within the same biofilm—has been considered more favourable in terms of NOB suppression. Recent studies have corroborated this hypothesis, demonstrating that without the presence of anammox, the NOB suppression was not maintained at DO of 0.3–0.6 mg/L, but with anammox, the NOB suppression could be well-maintained at DO of up to 0.8 mg/L [10]. This is because anammox bacteria can reduce the nitrite availability for NOB, thereby relaxing the requirement of strict oxygen control. To promote the competition between nitrite-scavenging microorganisms and NOB for nitrite, it is essential to ensure that organic and ammonium concentrations are sufficient to support heterotrophic denitrifiers and anammox bacteria, respectively. Particularly, controlling the residual ammonium levels is a critical strategy in MABRs, as it not only helps limit oxygen availability (by promoting AOB), but also restricts nitrite availability (by promoting anammox bacteria) [19]. This dual role of residual ammonium in shortcut nitrogen removal has been demonstrated in a pilot-scale flocculent system [20]. For mainstream sewage with total nitrogen of ~50 mg N/L, controlling residual ammonium level of 10 mg N/L was critical to the shortcut nitrogen removal performance.

Reducing the growth rate of NOB, while essential, is often insufficient to achieve stable and long-term shortcut nitrogen removal. First, limiting DO levels (usually <0.5 mg/L) also negatively affects the activity of AOB. This unintended consequence conflicts with the primary objective of using MABRs for process intensification. Second, NOB are phylogenetically and functionally diverse, with recent studies uncovering novel NOB species that exhibit differentiated physiological traits and remarkable metabolic versatility [21]. These characteristics contribute to the adaptability of NOB to a wide range of environmental conditions. For instance, members of the genus *Nitrospira* have demonstrated the ability to persist under both low-DO and low-nitrite conditions, underscoring their resilience to such control strategies. Third, while the general principle of lowering substrate availability to suppress NOB is straightforward, its practical application is system-specific. Factors such as biofilm thickness and wastewater strength significantly influence the effectiveness of these strategies, meaning that the DO threshold required to suppress NOB may vary considerably across different systems. For instance, a mathematical model revealed that when the biofilm thickness increased from 100 to 1000 μm, the DO threshold to suppress NOB increased from 0.01 to 0.1 mg/L [10]. This variability necessitates individualized determinations for each operational setup, which may explain the contradictory results regarding the NOB suppression reported under seemingly similar DO conditions in literature. Finally, and most critically, the MABR configuration presents a unique challenge. Since oxygen is supplied directly from the membrane, aerobic ammonia oxidation primarily occurs in the innermost biofilm layer, where nitrite is also produced. Consequently, both DO and nitrite are concurrently available in this layer. As such, achieving complete NOB suppression solely by limiting DO and nitrite concentrations in bulk liquid is theoretically unlikely. This underscores the need for a multifaceted and carefully integrated approach to NOB management in MABRs. It is important to note that most of the previous studies referenced in this section were conducted using medium-strength and high-strength wastewater. Applying this approach to low-strength wastewater would, in theory, require even more stringent operational controls. Additionally, we observed that all the studies cited here used synthetic wastewater under well-controlled laboratory conditions. However, it should be emphasized that demonstrating the shortcut nitrogen removal process in real wastewater is crucial, as it is typically more challenging to maintain performance under dynamic feeding conditions.

3.2. Strategy 2: Increasing NOB decay (b_{NOB})

The decay of NOB (b_{NOB}) can be expedited by exposing biofilms to harsh treatments. While both *ex situ* and *in situ* treatments are applicable for flocculent biomass, MABRs are limited to *in situ* treatment only. This is associated with the fact that it is challenging to separate portions of biofilms of MABRs for external treatment. Consequently, various strategies have been developed to enhance the NOB decay in MABRs while preserving the AOB activity, primarily by exploiting the different resilience of AOB and NOB to adverse conditions.

One widely studied approach is the use of free ammonia (FA) and free nitrous acid (FNA), which have been effectively applied to NOB suppression in flocculent biomass and adapted for use in MABRs [22]. For instance, Wang et al. [23] utilized FA (0.4–2.2 mg N/L) during the startup of partial nitritation in MABRs and subsequently applied FNA (>1 mg N/L) to stabilize the NOB suppression. In their study, a lab-scale MABR was operated in sequential batch mode, achieving a pH below 5 to obtain sufficient FNA in wastewater containing 100 mg TN/L. Similarly, Pourbavarsad et al. [24] demonstrated the application of this strategy in high-strength wastewater with nitrogen concentrations of up to 4900 mg N/L. Following temporary FA/FNA treatments, Chen et al. [15] reported the successful transformation of fully nitrifying MABR biofilms into stable partial nitritation biofilms. Similarly, transient pH increases during intermittent aeration have been shown to generate FA that contributes to the NOB suppression [16]. However, the limited duration and intensity of such pH shifts caused by intermittent aeration reduce their overall effectiveness compared to more deliberate interventions. In a recent study, sustained NOB suppression and a nearly 100 % nitrite accumulation ratio were achieved in an MABR operated at an acidic pH of 5 and FNA of 1–2 mg N/L [25]. Such low pH condition was formed due to insufficient alkalinity in chemically enhanced primary treatment (CEPT) effluent, rather than the addition of external acids. More importantly, the study reported the highest mainstream partial nitritation rate to date (2.3 kg N/m^3/d) because NOB were suppressed by FNA, while low oxygen control was no longer needed.

Han et al. [26] further enhanced the NOB suppression by combining low oxygen supply, pH control, and sulfide addition. Their results highlighted the critical role of intermittent sulfide dosing at a high pH (8.0–8.5), which created transient shocks of high sulfide concentrations to facilitate the decay of NOB. At lower pH values (<7.0), sulfide primarily existed as H_2S, which volatilized from the liquid phase, leading to insufficient sulfide concentrations. Continuous low-concentration sulfide dosing was also found ineffective due to the rapid sulfide oxidation by oxygen or sulfide-oxidizing bacteria (SOB). The addition of sulfide not only inhibited NOB but also promoted the activity of denitrifying SOB, which acted as nitrite scavengers, further limiting nitrite availability for NOB. Similar observations were reported previously [27,28], while they also noted the occurrence of dissimilatory nitrite reduction to ammonium (DNRA) and increased greenhouse gas emissions in sulfide-supplemented MABRs.

Despite these advancements, challenges persist in implementing harsh treatment strategies to facilitate the NOB decay. The use of external chemical reagents, such as acids or alkaline agents, raises cost concerns and requires careful evaluation of dosing rates and frequencies to ensure cost-effectiveness and overall performance. Additionally, most of inhibitors introduced from the bulk liquid must penetrate the biofilm to reach NOB, particularly in the innermost biofilm layers, necessitating higher dosages compared to treatments for flocculent biomass or conventional biofilms. Further, some NOB strains exhibit remarkable tolerance to harsh treatments. For instance, *Nitrobacter* and '*Candidatus* Nitrotoga' have shown high tolerance to FNA and FA. In a previous study, FNA concentrations up to 1.37 mg HNO_2–N/L failed to inhibit NOB, with the relative abundance of '*Ca.* Nitrotoga' increasing significantly [29]. These findings underscore the limitations of relying on a single strategy for the NOB suppression, highlighting the need for combined approaches. For example, alternating FNA and FA treatments on a monthly basis has been shown to outperform individual applications in maintaining the NOB suppression [30].

Future research is expected to adapt more strategies developed for flocculent biomass to MABRs. Starvation, where the biofilm is deprived of substrates, could promote the NOB decay, as AOB typically recover more rapidly than NOB after such treatment [31]. Physical processes like ultrasound application may also facilitate bacterial decay [32]. Unlike most inhibitors that suppress both AOB, NOB, and other bacteria, hydroxylamine has been shown to selectively inhibit NOB while enhancing the activity of AOB and anammox bacteria [33]. Moreover, similar to Strategy 1, most of the previous studies using Strategy 2 were also conducted using synthetic wastewater under well-controlled laboratory conditions. Larger-scale demonstration under real-world conditions is highly expected.

3.3. Strategy 3: Increasing NOB detachment (k_d)

Several studies have explored strategies to enhance NOB detachment (k_d) in MABR biofilms as a method to remove the established NOB. One of the widely adopted biofilm control approaches in MABRs is gas scouring, which introduces coarse gas bubbles from the bottom of the reactor to control biofilm growth and detachment. Bunse et al. [34] demonstrated that high-frequency gas scouring could limit NOB and promote AOB abundance in MABR biofilms by increasing biomass detachment, even at the membrane surface. Similarly, Zhang et al. [35] proposed a strategy involving biofilm detachment and reattachment to suppress established NOB in MABRs. In this study, NOB released from the detached biofilm were exposed to higher concentrations of FA in the bulk liquid. Lacking the protection of the biofilm matrix, NOB were inhibited and subsequently washed out from the reactor, while AOB recovered more rapidly and re-colonized the membrane surface.

Despite these findings, implementing biofilm detachment strategies in MABRs presents notable challenges, which is indeed inapplicable in many scenarios. Unlike flocculent systems, where sludge removal can be precisely controlled, the heterogeneous nature of biofilms makes it difficult to achieve a precise biofilm detachment. Second, as NOB reside predominantly in the innermost biofilm layers, substantial disruption of the biofilm is required to effectively remove them. Such disruption risks damage to other essential functional bacteria, including AOB and

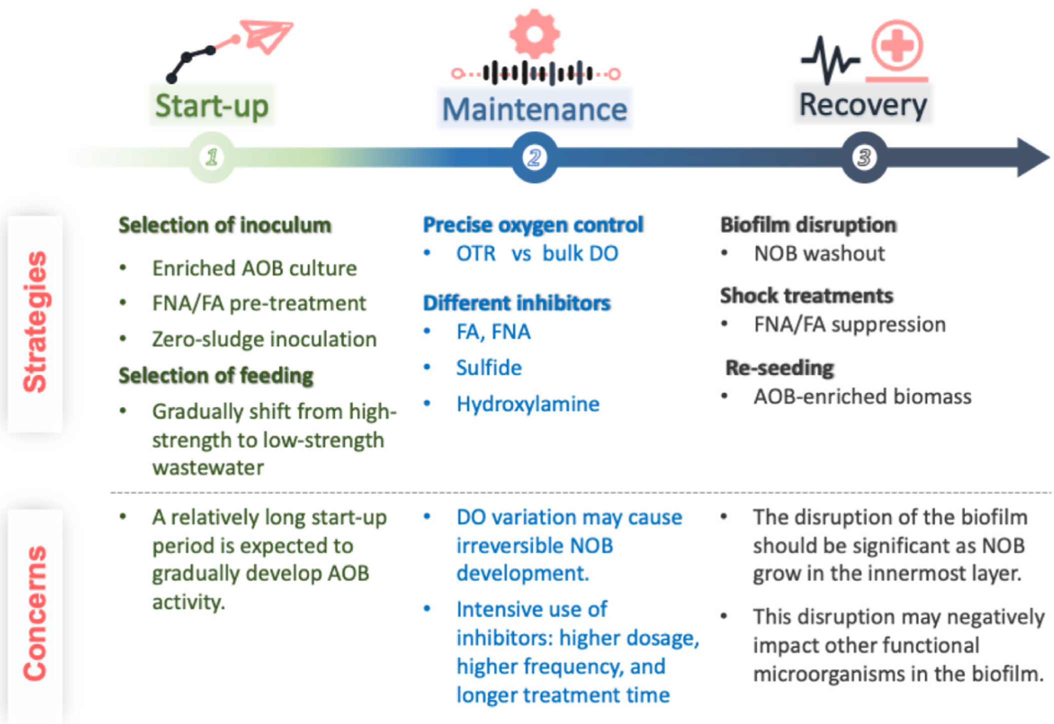

Fig. 3. Startup, maintenance, and recovery of shortcut nitrogen removal in MABRs. OTR denotes oxygen transfer rate.

anammox bacteria, which are crucial for shortcut nitrogen removal processes. For example, Bunse et al. [34] reported that the total nitrogen removal efficiency was reduced by approximate 50 % during high-frequency scouring operations compared to lower-frequency scouring operations, likely due to excessive biofilm removal. Particularly in low-strength wastewater systems with low surface loads, overly aggressive scouring or high biofilm detachment rates may destabilize the system and compromise overall performance. Third, a prolonged exposure to high shear forces can lead to the development of denser, more compact biofilms, which may impede substrate diffusion. Mehrabi et al. [36] highlighted that shear stress strongly influences biofilm structure, thickness, and the associated mass transfer dynamics. With these, the Strategy 3, at the current stage, is not a favoured choice for achieving shortcut nitrogen removal in MABRs.

4. Future perspectives

4.1. It is critical to get it right from the start

Given that NOB are easily protected within biofilms, it is critical to minimize their presence from the outset of sludge inoculation (Fig. 3). Inoculating the biomass with highly enriched AOB could be beneficial and relatively straightforward for lab-scale studies. For the startup of large-scale systems, establishing AOB-enriched plants to produce sufficient biomass would be helpful. This usually requires a stable source of high-ammonium wastewater (e.g., anaerobic digestion liquor) and the development of suitable conditions for AOB cultivation. In scenarios where AOB-enriched biomass is unavailable, the pre-treatment of inocula prior to inoculation to suppress NOB is a viable alternative. For instance, FA and FNA shock treatments have proven effective in selectively inhibiting NOB [30]. Since AOB are more tolerant to these treatments and recover more rapidly, the pre-treated sludge can then serve as an inoculum for MABRs, enabling a fresh start toward the shortcut nitrogen removal. Another potential method to avoid the inoculation of sludge carrying NOB is zero-sludge inoculation [37]. Without any sludge inoculum, this approach requires favourable conditions for the growth of AOB, which are merely introduced from the liquid influent. However, this method may result in a long start-up time and further investigation is needed to confirm its practical applicability. After carefully selecting the seeding sludge, adopting a high ammonium concentration in the influent during the initial phase can enhance AOB activity while suppressing NOB (Wei et al., 2018). For systems targeting mainstream low-strength wastewater, the influent ammonium concentration can be gradually reduced (in combination with other control strategies) once the desired startup performance has been achieved.

4.2. Strategies learnt from conventional biofilms/flocs need to be re-designed to maintain NOB suppression in MABRs

Counter-diffusion biofilms have now been studied for decades, leading to many unique applications that differ from those of co-diffusion biofilms, for example, the application of MABRs to intensify treatment capacity and reduce greenhouse gas emissions. However, in the research field of achieving shortcut nitrogen removal in MABRs, particularly regarding strategies to suppress NOB, we found that the majority developed so far are parallel to those employed in traditional co-diffusion biofilms. Thus, strategies for suppressing NOB in conventional biofilms and flocculent systems provide valuable insights, but their direct application to MABRs often proves inadequate due to the intrinsic differences in biofilm structure, oxygen delivery mechanisms, and operational dynamics. Conventional approaches to maintain and recover NOB suppression, such as low DO control, FA/FNA treatment, and biofilm detachment, must be re-evaluated and tailored to address the unique challenges posed by MABRs. For instance, the counter-diffusion nature of MABR biofilms necessitates more precise oxygen management strategies to prevent the NOB proliferation in the innermost biofilm layers. Unlike bulk DO control employed in conventional systems, MABRs require a focus on controlling the oxygen transfer rate (OTR) (Fig. 3), which can be theoretically regulated through adjustments to air flux and intra-membrane pressure. This is critical because the DO concentration in the innermost layer where NOB reside is typically higher than in the bulk liquid and cannot be easily measured in practice. However, the actual

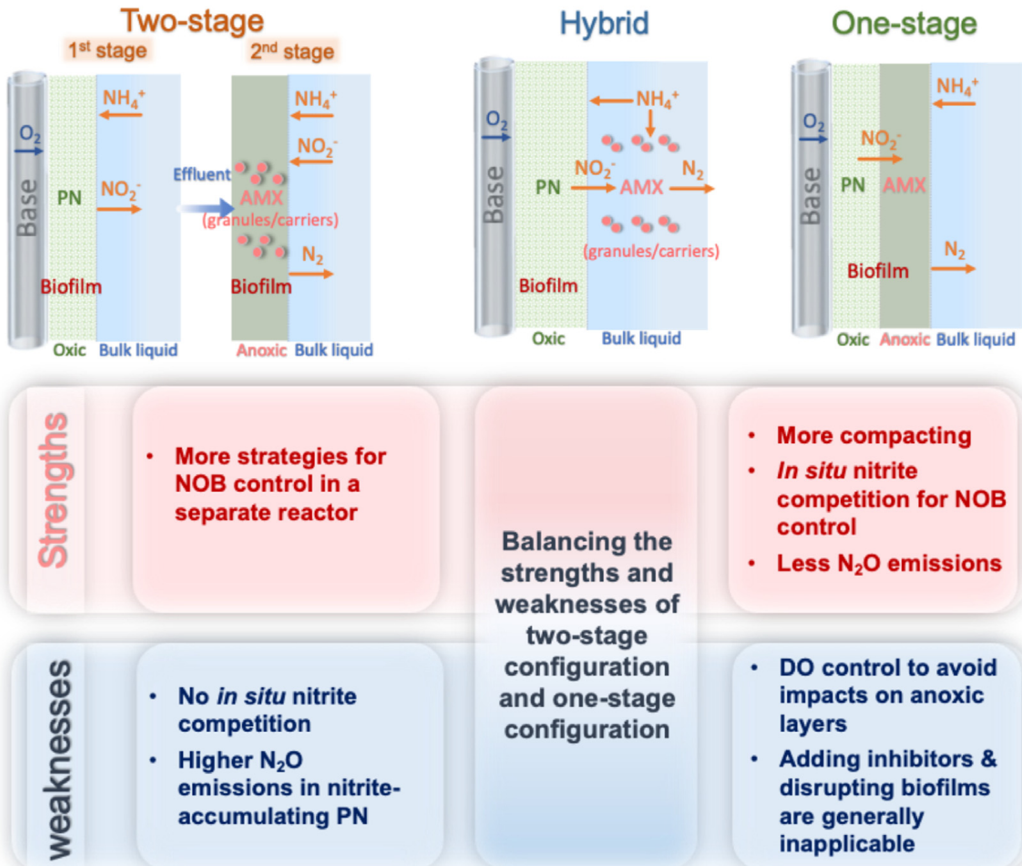

Fig. 4. Comparisons of (a) two-stage, (b) hybrid and (c) one-stage MABR systems. PN denotes the partial nitritation process and AMX denotes the anammox process.

OTR is influenced by additional factors, e.g. membrane material properties and the presence or absence of biofilms. Consequently, the relationship between OTR and controllable parameters like air flux or intra-membrane pressure is often empirical and subject to variation over long-term operation.

Furthermore, while harsh treatments are effective in conventional systems, their application in MABRs must account for the protective biofilm matrix, which shields NOB and reduces the uniformity of chemical exposure. This fact often necessitates higher dosage, higher dosing frequency and longer exposure time of inhibitors (Fig. 3). A notable exception is FNA. The activity of AOB, which converts ammonia into nitrite and releases protons, results in higher nitrite concentrations and lower pH levels within the biofilm compared to the bulk liquid. As a result, the highest FNA concentration occurs in the innermost biofilm layers, meaning that the bulk FNA levels to suppress NOB in MABRs are lower than those reported for other systems. A recent study showed that an FNA of 1–2 mg N/L in bulk liquid, lower than the reported level of 2–5 mg N/L in the flocculent system, was sufficient to maintain NOB suppression in MABRs [25].

Moreover, in response to the potential risk of NOB proliferation during long-term MABR operation [10], it is critical to prepare a prompt and robust control in place. Given the difficulty of re-suppressing active NOB in the innermost layers of MABR biofilms, more intensive approaches, such as system-wide biofilm disruption, sludge wastage, shock treatments, or re-seeding with AOB-enriched biomass, should be employed (Fig. 3). These strategies, applied individually or in combination, can effectively re-suppress active NOB in biofilms. However, other microorganisms, such as AOB and anammox bacteria, may also be affected, necessitating a recovery period for the system.

4.3. Comparisons of one-stage, two-stage and hybrid MABR systems

The one-stage MABR integrates aerobic and anaerobic processes within a single reactor, providing a platform for spatial stratification, with anammox and heterotrophic denitrifiers thriving in the outer layer and AOB inhabiting the inner layers [38]. This system inherently offers a natural competition between anammox bacteria, heterotrophic denitrifiers and NOB for nitrite, facilitating NOB control (Fig. 4). However, in a one-stage configuration, many of the strategies used to facilitate the NOB decay (Section 3.2) are not applicable, as these measures could disrupt other key functional bacteria, such as AOB and anammox bacteria, ultimately diminishing the overall nitrogen removal efficiency. A potential exception is hydroxylamine, which can exclusively suppress NOB while promoting the growth of AOB and anammox bacteria, when it is applied at the appropriate dosage [33].

In contrast, the two-stage configuration allows for a targeted inhibition of NOB without adversely affecting other functional bacteria, particularly anammox bacteria. In a two-stage system, the thinner biofilm facilitates better oxygen and ammonium penetration while enhancing the effectiveness of chemical inhibitors in suppressing NOB (Fig. 4). This two-stage approach has proven successful in achieving effective NOB suppression [39,40]. However, the two-stage system lacks the natural competition between NOB and other nitrite scavengers. Additionally, the emissions of N_2O, a potent greenhouse gas, are often significantly higher in nitrite-accumulating reactors of two-stage configurations [41]; [42–44].

Hybrid systems offer a practical and scalable solution for enhancing the nitrogen removal capacity of existing wastewater treatment facilities, which also support the development of new facilities with improved sustainability compared to traditional methods. Most hybrid processes involve submerging MABR cassettes within unaerated anoxic zones and/or aerated oxic zones containing suspended biomass [45]. While hybrid systems have been proven with multiple benefits, including robust treatment performance, capacity intensification, and energy savings, their application for achieving shortcut nitrogen removal remains less explored. In hybrid configurations designed for shortcut nitrogen removal, a partial nitrifying biofilm is cultivated on membranes for ammonia oxidation, while anammox bacteria (often present as granules or on plastic carriers) and heterotrophic denitrifiers perform nitrogen removal in the bulk liquid (Fig. 4). This setup positions the hybrid system as an intermediary between one-stage and two-stage configurations, balancing the advantages and limitations of both. For example, similar to two-stage systems, the membrane cassettes in hybrid configurations can be removed and subjected to targeted treatments, allowing for effective NOB control without adversely affecting anammox bacteria or heterotrophic denitrifiers in the bulk liquid. Analogous to one-stage systems, competition for nitrite also exists within the hybrid system, and N_2O emissions are expected to be relatively low, as bacteria in the bulk liquid can partially consume and mitigate these emissions before they are released into the atmosphere. Additionally, integrating MABR cassettes into anoxic tanks to form hybrid systems is likely to introduce some degree of oxygen intrusion, potentially disturbing the full denitrification. This, in turn, may facilitate the occurrence of partial denitrification coupled with anammox (PdNA). The partial denitrification process occurs either in suspended biomass or in the outer layers of MABR biofilms. The presence of partial denitrification can convert nitrate produced by NOB and anammox bacteria into nitrite, thereby not only alleviating the requirement of NOB control but also contributing to improved effluent quality by enhancing total nitrogen removal efficiency. Moreover, the presence of denitrifiers also acts as a barrier for N_2O emissions that are produced in the inner layer of biofilms.

4.4. Opportunities to enrich and apply new N-cycling microorganisms in MABRs

The innermost layer of MABR biofilms is uniquely featured by the extremely low ammonium and long SRT. This suggests that MABRs can be fundamentally used as a good platform for the enrichment of new nitrogen-cycling microorganisms, such as comammox *Nitrospira*, ammonia-oxidizing archaea (AOA), and acid-tolerant AOB, that thrive in oligotrophic environments and grow slowly [1]. From the engineering perspective, opportunities also exist to apply these new nitrogen-cycling microorganisms in MABRs for shortcut nitrogen removal. Their unique physiological traits position them as potentially valuable contributors to maintaining the robustness and efficiency of shortcut nitrogen removal. For example, comammox *Nitrospira* and AOA possess superior affinities for ammonium and oxygen compared to canonical AOB, making them advantageous partners to supply nitrite for anammox bacteria under low DO conditions. Recent proof-of-concept studies have demonstrated the feasibility of employing hydrogel-encapsulated AOA and comammox *Nitrospira* in conjunction with anammox bacteria for mainstream PN/A [46,47]. However, their application within MABRs has yet to be explored. Instead, acid-tolerant AOB have been successfully applied in MABRs [25], achieving not only complete NOB suppression under acidic conditions but also an unprecedentedly high ammonia oxidation rate (2.3 kg $N/m^3/d$). While this previous study employed a two-stage configuration to separate the acidic MABR from the neutral-pH anammox reactor, integrating these processes into a one-stage configuration might be also possible. Proton production by AOB within the biofilm naturally creates a pH gradient, ranging from acidic conditions in the inner layers to more neutral conditions in the outer layers. This gradient could establish distinct ecological niches within the same biofilm, allowing acid-tolerant AOB to thrive in the inner acidic layers while supporting the growth and activity of anammox bacteria in the outer neutral-pH layers. Another group of new nitrogen-cycling microorganisms that has been applied in MABR is nitrite/nitrate-dependent anaerobic methane oxidation (n-DAMO) [13,38,42]. Their integration in MABRs performing shortcut nitrogen removal can not only loose the requirement of NOB suppression (because n-DAMO archaea can reduce nitrate back to nitrite), but also contribute to the mitigation of dissolved methane, a hurdle for anaerobic wastewater treatment.

4.5. Densification technology coupling with MABRs

Stable retention of functional microbial communities remains a challenge in mainstream systems. Hydrocyclone-based densification technology offers a potential and promising solution for biomass retention. Densification technology was first proposed for the retention of anammox granules at the Strass WWTP in Austria [48]; [49–51]. In recent years, densification technology has evolved from anammox technologies to conventional biological nitrogen and phosphorus removal systems [52]. These technologies are aim to select densified activated sludge enriched with carbon-storing heterotrophic bacteria, such as polyphosphate-accumulating organisms (PAOs), thereby achieving rapid sludge settling and improved nutrient removal in mainstream systems [52]; [53]. Notably, compared to anammox bacteria and PAOs, AOB and NOB are more prone to detachment from the surface, as they typically grow on the outer layer of the granules [54]; [55,56]. Consequently, while hydrocyclone use centrifugal forces to select functional bacteria, they may cause the detachment and subsequent loss of AOB and NOB due to vortex shear force, though this possibility has not been specifically studied.

Recently, ZeeDENSE® (Veolia) innovatively combined ZeeLung MABR® with inDENSE® process, which could potentially address the issue of retaining AOB and NOB in the membrane-aerated biofilm, regardless of whether they are screened out by the hydrocyclone. Meanwhile, when anammox granules are introduced into the system, although they may be fragmented by vortex shear forces, their preference for nitrite will promote the adhesion of anammox flocs to the membrane-aerated biofilm. This enables rapid competition of anammox bacteria with NOB, thereby effectively suppressing NOB growth [10]. Overall, the coupling of densification technology with MABRs could represent a potential solution for future mainstream systems, enabling simultaneously space-saving and highly effective nitrogen and phosphorus removal.

5. Conclusions and outlooks

Based on this review, it is feasible to achieve shortcut nitrogen removal in MABRs, though it remains more challenging than in traditional biofilms and flocculent biomass. The strategies developed so far are mostly in the early stages, often tested under well-controlled laboratory conditions, while larger-scale demonstrations in real-world settings are urgently needed. We also highlight that compared to flocculent or conventional biofilm systems, our understanding of shortcut nitrogen removal in MABRs remains limited, presenting a wealth of opportunities for future explorations. Fundamentally, intriguing questions persist regarding the counter-diffusion of substrates within biofilm layers, the correlation between substrate gradients and microbial competition and cooperation, detailed pathways for N_2O production and consumption, cross-feeding among functional microorganisms, and the potential enrichment of novel nitrogen-cycling microbes within MABR biofilms. Addressing these fundamental aspects could make significant scientific advancements in the field. From a technical perspective, the pursuit of cost-effective and efficient strategies to achieve shortcut nitrogen removal in MABRs will be continuing. While existing strategies from conventional systems lay a foundation, it is essential to adapt and optimize them to account for the distinct characteristics of MABRs. Furthermore, the unique features of MABR biofilms may lead to innovative solutions. For instance, the bubbleless oxygen transfer mechanism enables precision oxygen supply control by regulating the mass flow in the supply pipeline, with the support of advanced electronic mass flow controllers. By addressing both fundamental questions and engineering challenges, the full potential of MABRs in achieving sustainable and efficient nitrogen removal can be realized.

CRediT authorship contribution statement

Tao Liu: Writing – review & editing, Writing – original draft. **Dongdong Xu:** Writing – review & editing, Writing – original draft. **Yan Lu:** Writing – original draft. **Chenkai Niu:** Visualization. **Daying Chen:** Writing – original draft. **Yanjun Shao:** Writing – original draft. **Yimeng Li:** Writing – original draft. **Yayi Wang:** Writing – review & editing. **Jianhua Guo:** Writing – review & editing.

Declaration of competing interest

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

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