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Assessing membrane aerated biofilm reactor configurations in mainstream anammox applications

B. M. Wagner, G. T. Daigger* and N. G. Love

Department of Civil and Environmental Engineering, University of Michigan, 1351 Beal Avenue, Ann Arbor, MI 48109, USA
*Corresponding author. E-mail: gdaigger@umich.edu

BMW, 0000-0003-2963-8326; GTD, 0000-0003-3857-8345; NGL, 0000-0002-9184-2451

ABSTRACT

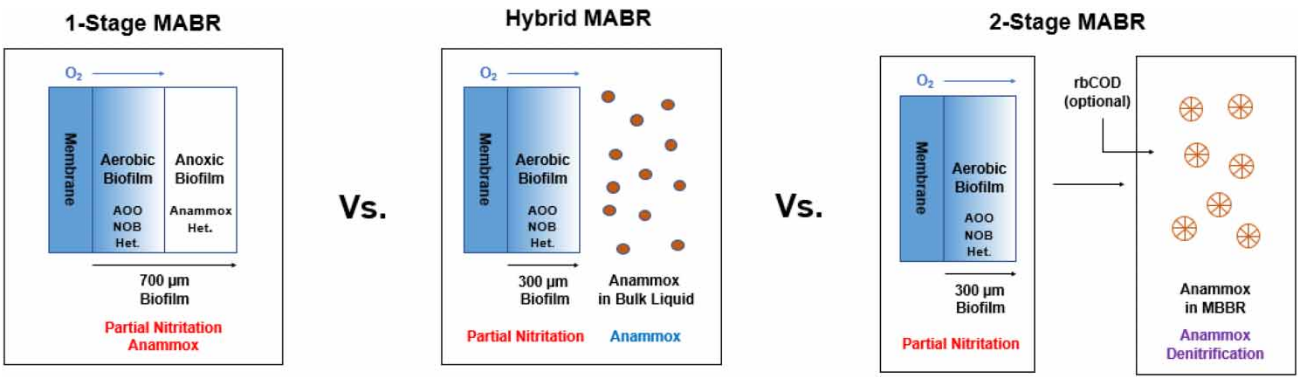
Partial nitritation anammox (PNA) membrane aerated biofilm reactors (MABRs) have the potential to be employed in mainstream wastewater treatment and can drastically decrease the energy and carbon requirements for nitrogen removal. Previous PNA MABR studies have looked at 1-stage systems, but no study has holistically compared the performance of different MABR configurations. In this study, a PNA MABR was mechanistically modelled to determine the impact of the reactor configuration (1-stage, hybrid, or 2-stage system) on the location of the preferred niche for anammox bacteria and the overall nitrogen removal performance. Results from this study show that the 2-stage configuration, which used an MABR with a thin biofilm for nitritation and a moving bed biofilm reactor for anammox, had a 20% larger nitrogen removal rate than the 1-stage or hybrid configurations. This suggests that an MABR should focus on maximizing nitrite production with anammox implemented in a second-stage biofilm reactor to achieve the most cost-effective nitrogen removal. However, the optimal configuration will likely be facility specific, as each facility differs in operating costs, construction costs, footprint, and effluent limits. Additional experimentation is required to confirm these results, but this work narrows the number of viable configurations that need to be tested. The results of this study will inform researchers and engineers how to best implement PNA MABRs in mainstream nitrogen removal at larger scales.

Key words: mainstream nitrogen removal, membrane aerated biofilm reactor, partial nitritation anammox

HIGHLIGHTS

- Membrane aerated biofilm reactors (MABRs) can implement partial nitritation anammox (PNA) in mainstream nitrogen removal.
- 1-stage PNA MABRs are more susceptible to ammonia diffusion limitations and anammox oxygen inhibition.
- A 2-stage PNA MABR had a 20% improvement in its nitrogen removal rate.
- The 2-stage configuration may be best for compliance with strict nitrogen permit limits.

GRAPHICAL ABSTRACT



ACRONYMS

AOO	ammonia oxidizing organisms
AMX	anaerobic ammonia oxidizing organisms
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
MABR	membrane aerated biofilm reactor
MBBR	moving bed biofilm reactor
NOB	nitrite oxidizing organisms
OHO	ordinary heterotrophic organisms
PNA	partial nitritation anammox
rbCOD	readily biodegradable chemical oxygen demand
SRT	solids retention time
TIN	total inorganic nitrogen
WRRF	water resource recovery facility

INTRODUCTION

In order to achieve sustainability goals for water resource recovery facilities (WRRFs), a carbon-efficient biological process such as partial nitritation anammox (PNA) can be combined with energy efficient membrane aerated biofilm reactors (MABRs) to perform energy efficient, carbon-free nitrogen removal in mainstream wastewater treatment. The PNA shortcut nitrogen removal pathway is composed of two major processes: (1) partial nitritation, which uses aerobic ammonia oxidizing organisms (AOO) to convert a portion of the influent ammonia to nitrite and (2) anaerobic ammonia oxidization, anammox, performed by anammox bacteria (AMX), which uses influent ammonia and the nitrite produced from partial nitritation to produce nitrogen gas and 0.11 grams nitrate-nitrogen per gram of nitrogen removed. The combined PNA nitrogen removal pathway reduces the oxygen demand by 60% and the organic carbon demand by 100% when compared to nitrogen removal with traditional nitrification and heterotrophic denitrification. Since oxygen is typically supplied by mechanical equipment, which requires electricity to operate, the reduction in oxygen demand directly correlates to energy savings at a WRRF.

In addition to the energy and carbon benefits achieved via PNA, MABRs can be used to further increase the energy efficiency of nitrogen removal. MABRs use a gas-permeable membrane to diffuse oxygen directly to a biofilm growing on its external surface. This method of aeration is bubble-free, which enables MABRs to transfer oxygen up to four times more efficiently than the oxygen transfer systems generally used in suspended growth biological treatment systems (Ahmed & Semmens 1992; Pankhania *et al.* 1994; Peeters *et al.* 2017). Oxygen in MABRs is supplied by diffusion through the membrane, while ammonia and other substrates diffuse from the bulk liquid, creating a counter-diffusional biofilm. MABRs can also create multiple redox zones within the biofilm, with aerobic AOO, nitrite oxidizing bacteria (NOB), and ordinary heterotrophic organisms (OHO) populating the space closest to the membrane, and anaerobic AMX and OHO populating the space closest to the bulk liquid (Gilmore *et al.* 2013). By combining the oxygen transfer efficiency of MABRs with the low oxygen demand of the PNA nitrogen removal pathway, energy savings up to 76% can be achieved when compared to traditional nitrogen removal through nitrification and heterotrophic denitrification using conventional oxygen transfer systems that transfer oxygen into the process mixed liquor. These carbon and energy advantages provide motivation to implement MABRs incorporating PNA (referred to as PNA MABRs here) for mainstream nitrogen removal.

In this paper, we present a short review of PNA MABR knowledge from published studies and conference proceedings. We then identify a knowledge gap in the comparison of PNA MABR configurations (1-stage, hybrid, and 2-stage) and explore how the performance and the preferred niche (location in the process) for AMX changes with respect to the reactor configuration under mainstream conditions via mechanistic computer modeling. Finally, we propose a set of configurations that should be further explored via experimental validation.

BACKGROUND

PNA MABR research began in the 2000s and greatly increased in the 2010s. The earliest studies utilized both modeling and experimental reactors to first show the feasibility of combining PNA with MABRs. As time progressed and knowledge about MABRs and PNA increased, research shifted to focus on optimizing reactor startup, creating new operational strategies, and transitioning PNA MABRs to larger scales of implementation. To visually show the short history of PNA MABR research, we created Table 1, which lists published scientific papers and other grey literature focused on PNA MABRs of which we are aware. We classified the studies into modeling and experimental research and further divided them based on their influent nitrogen concentrations. In some instances, both modeling and experimentation were used, and some studies tested a range of influent nitrogen concentrations.

Table 1 | Overview of 1-stage PNA MABR studies

	Model	Experimental
High Strength NH_x>100 mg N/L	(Terada *et al.* 2007): A range of 500–750 µm thick biofilms were found to have the best performance. Influent nitrogen = 250 mg N/L. Temperature = 30 °C.	(Gong *et al.* 2007): Biofilm thickness was not reported. Synthetic influent nitrogen = 200 mg N/L. Temperature = 30 °C.
	(Lackner *et al.* 2008): Max biofilm thickness = 1,000 µm. Influent nitrogen = 200 mg N/L. No temperature reported.	(Gong *et al.* 2008): Biofilm thickness was not reported. Synthetic influent nitrogen>200 mg N/L. Temperature = 33–35 °C.
	(Zhao *et al.* 2010): A range of 600–2,500 µm thick biofilms were tested. Influent nitrogen≈200 mg N/L. Temperature = 30 °C.	(Pellicer-Nàcher *et al.* 2010): The biofilm thickness was estimated to be 500 µm. Synthetic influent nitrogen = 530–780 mg N/L. Temperature = 26–32 °C.
	(Ni & Yuan 2013): A 750 µm thick biofilm was used for 4/5 simulations. A range of 250–1,500 µm was used for 1 simulation. Influent nitrogen = 250 mg N/L. No temperature reported.	(Sun *et al.* 2010): Biofilm thickness was not reported. Synthetic influent nitrogen = 540 mg N/L. Temperature = 23–32 °C.
	(Wu & Zhang 2017): A 750 µm thick biofilm was used. Influent nitrogen = 1,000 mg N/L. No temperature reported.	(Terada *et al.* 2010): The biofilm thickness was estimated to be 800–1,000 µm. Synthetic influent nitrogen = 200 mg N/L. Temperature = 33 °C.
	(Ni *et al.* 2013): A range of 750–1,000 µm biofilms were tested. Influent nitrogen = 250 mg N/L. No temperature reported.	(Pellicer-Nàcher & Smets 2012): The biofilm thickness was estimated to be 500 µm thick. Synthetic influent nitrogen concentration not reported, but a high loading of 8 g-N/m^2/d was used. Temperature = 30–33 °C.
	(Peng *et al.* 2015): A 750 µm thick biofilm was used. Influent nitrogen = 500 mg N/L. No temperature reported.	(Gilmore *et al.* 2013): The biofilm thickness was estimated to be 500–600 µm thick. Synthetic influent nitrogen = 659 mg N/L. Temperature = 30 °C.
		(Coutts *et al.* 2020): Biofilm thickness was not reported. Sidestream wastewater with an influent nitrogen concentration = 621 mg N/L was used. No temperature reported.
Low Strength NH_x≤100 mg N/L	(Liu *et al.* 2016): A 200 µm thick biofilm was used for 1 scenario. Influent nitrogen = 30 mg N/L. No temperature reported. A 500 µm thick biofilm was used for a second scenario. Influent nitrogen = 500 mg N/L. No temperature reported.	(Lin *et al.* 2015): Biofilm thickness was not reported. Synthetic influent nitrogen concentration = 30–120 mg N/L. Temperature = 20 °C.
	(Acevedo Alonso & Lackner 2019): Biofilm thicknesses of 200, 500 and 1000 µm were used. Influent nitrogen = 100 mg N/L. Temperatures of 10, 20, and 30 °C were used.	(Ma 2018): Biofilm thickness was not reported. Synthetic influent nitrogen concentration = 75 mg N/L. Temperature = 24–30 °C.
		(Ribeiro Augusto *et al.* 2018): Biofilm thickness not reported. Synthetic influent nitrogen concentration = 50–100 mg N/L. Temperature = 30.6–32.0 °C.
		(Bunse *et al.* 2020): The biofilm thickness was estimated to be 500 µm. Mainstream wastewater with influent nitrogen = 31–120 mg N/L was used. Room temperature was used.

For these studies, we used our best judgement to place the papers into the category that most resembled their research goal. For each study, we listed key factors that are known to impact nitrogen removal performance, which include the biofilm thickness in the MABR, the influent nitrogen concentration, the type of wastewater used, and the wastewater temperature.

Most of the early PNA MABR research tested conditions that are optimal for PNA nitrogen removal performance but may not be fully applicable to the conditions found in mainstream wastewater. 15 of the 21 studies treated high strength wastewater with influent nitrogen concentrations greater than 100 mg N/L. By using concentrated wastewater, the researchers were able to take advantage of the differential impacts of free ammonia and free nitrous acid toxicity on AOOs and NOB, which can inhibit NOB activity by 80–90% while still allowing AOOs to be active (Wang *et al.* 2016a, 2017). NOB inhibition is a key component for optimal PNA nitrogen removal performance, as the nitrate produced by NOB requires organic carbon for its transformation to nitrogen gas via heterotrophic denitrification and decreases the carbon efficiency of the process. Nine of the 12 experimental studies also used elevated temperature ranging from 23 to 33 °C, which has been shown to increase the growth rates of AMX and AOO (Laureni *et al.* 2016). In contrast, mainstream wastewater temperatures are typically lower, ranging from 10 to 25 °C, and have been shown to negatively impact PNA performance (Gilbert *et al.* 2014; Lotti *et al.* 2014; Laureni *et al.* 2016). Six of the 21 studies focused on treating low strength, mainstream wastewaters which commonly experience lower temperatures and do not have ammonia concentrations large enough to experience free ammonia and free nitrous acid toxicity for NOBs. Finally, only one study used real wastewater with ammonia concentrations and temperatures applicable to mainstream wastewater (Bunse *et al.* 2020).

PNA MABR research has thus far primarily focused on 1-stage (MABR only) configurations (Pellicer-Nàcher *et al.* 2010, 2014; Terada *et al.* 2010; Lackner & Smets 2012; Gilmore *et al.* 2013; Ma 2018; Bunse *et al.* 2020), rather than hybrid (MABR + suspended growth) or 2-stage (MABR + traditional biofilm reactor) configurations, shown in Figure 1. Each of the configurations have distinct advantages and disadvantages that will be discussed in the subsequent paragraphs.

The main benefit of 1-stage reactors is that partial nitritation and anammox can be performed within one biofilm. For this to occur, the biofilm must be at least 450 μm thick, and a low oxygen loading must be used to produce the aerobic and anaerobic

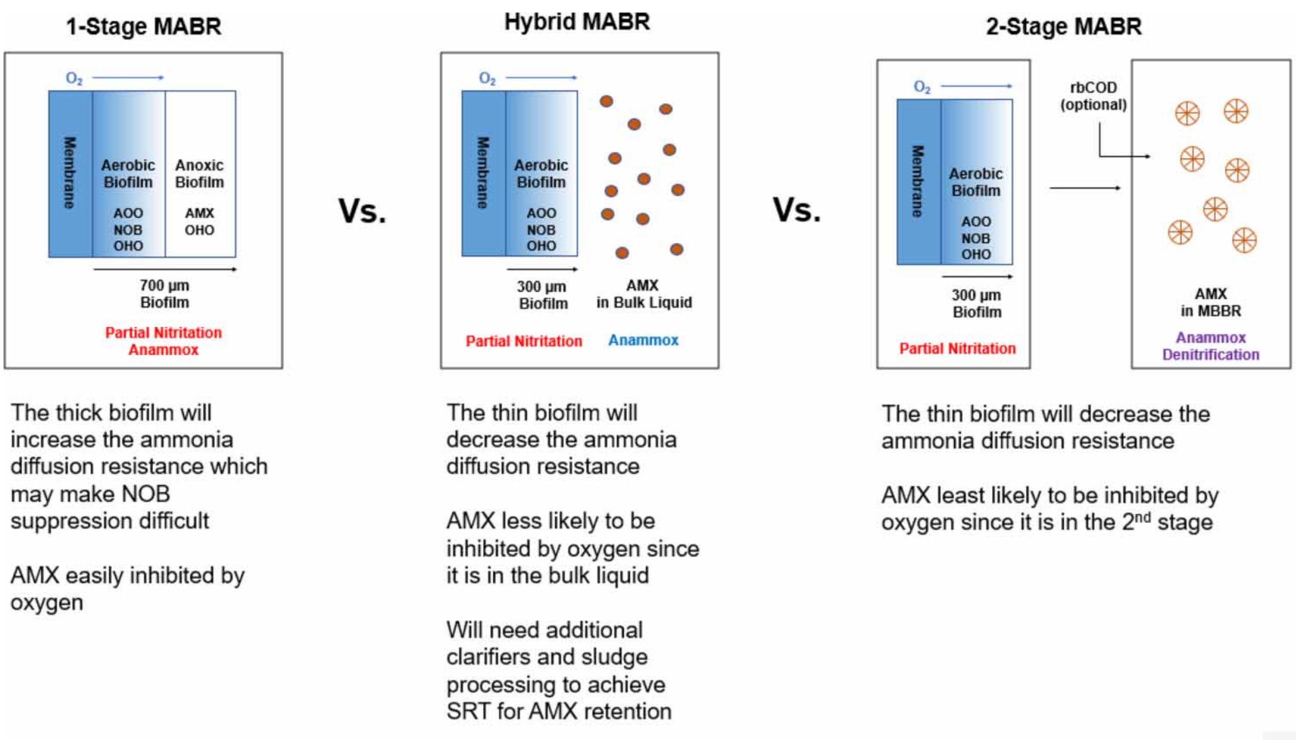


Figure 1 | Overview of PNA MABR configurations that highlight differences in the location of AMX. The color of the metabolisms under each diagram corresponds to the location where each metabolism occurs with red corresponding to the biofilm in the MABR, blue corresponding to the bulk liquid, and purple corresponding to a biofilm in a 2nd stage moving bed biofilm reactor (MBBR). AOO=ammonia oxidizing organisms, NOB=nitrite oxidizing bacteria, OHO=ordinary heterotrophic organisms, AMX=anammox bacteria, and rbCOD=readily biodegradable chemical oxygen demand.

regions within the biofilm required for AOO and AMX (Terada *et al.* 2007). From experimental studies treating both mainstream and high strength synthetic wastewaters, biofilm thicknesses have typically been measured to be between 500 and 1,000 μm in PNA MABRs (Pellicer-Nàcher *et al.* 2010, 2014; Terada *et al.* 2010; Lackner & Smets 2012; Pellicer-Nàcher & Smets 2012; Gilmore *et al.* 2013; Bunse *et al.* 2020). However as the biofilm thickness increases, the diffusion resistance of substrates entering the biofilm from the bulk liquid also increases. This makes it more difficult for ammonia and other substrates from the bulk liquid to diffuse to AOO at the membrane-biofilm interface, resulting in lower ammonia flux. The ammonia concentration at this interface also decreases, which can decrease the AOO specific growth rate. Typical PNA operation relies on AOO to outcompete NOB for oxygen, but if the biofilm thickness is large enough that the AOO specific growth rate is limited by the diffusion of ammonia, NOB may begin to take advantage of the increase in oxygen availability and convert nitrite to nitrate. This would decrease the nitrite availability for AMX and the overall nitrogen removal performance. Therefore in mainstream wastewaters that have low ammonia concentrations, ammonia diffusion resistance resulting from a thick biofilm may limit the performance of 1-stage PNA MABRs. Another drawback of 1-stage PNA MABRs is that AMX can be easily inhibited by oxygen. Since aerobic AOO and anaerobic AMX are located within the same biofilm, it is possible that oxygen transfer can exceed oxygen consumption in the aerobic portion of the biofilm, penetrate the anaerobic portion of the biofilm, and inhibit AMX. Given these drawbacks, it is advantageous to explore other alternative reactor configurations that are less susceptible to diffusion resistance and AMX inhibition.

The hybrid MABR is one possible alternative to 1-stage MABRs. In a hybrid configuration, the MABR is operated to have a thinner, aerobic biofilm to maximize nitrite production with AMX located in the anaerobic bulk liquid. Because this type of operation uses a thin MABR biofilm, AOO will be less likely to experience ammonia diffusion limitations and can potentially suppress NOB more easily than in a thick biofilm. Another benefit of the hybrid configuration is that AMX is less likely to be inhibited by oxygen because it is located further away from the aeration source in the bulk liquid. However, hybrid MABRs require clarifiers and additional sludge processing to maintain a solids retention time (SRT) long enough to grow and retain AMX. Hybrid MABRs have been heavily examined in non-PNA settings, with the MABR biofilm performing nitrification, and the suspended biomass performing heterotrophic denitrification (Downing & Nerenberg 2007, 2008b; Bagchi *et al.* 2018; Pérez-Calleja *et al.* 2020; Carlson *et al.* 2021). However for PNA MABRs, only one study has been reported (Li *et al.* 2016).

Thus far, we are not aware of any studies focusing on 2-stage PNA MABRs. In a 2-stage configuration, an MABR with a thin, aerobic biofilm would be used in the first stage to produce nitrite. In the second stage, a traditional biofilm reactor such as a moving bed biofilm reactor (MBBR) can be used to oxidize influent ammonia and the nitrite produced in the first stage through anammox. Similar to the MABR in the hybrid configuration, the AOO in this MABR are less likely to experience ammonia diffusion limitations which may facilitate NOB suppression, and this configuration is also the least likely to have AMX inhibited by oxygen.

Given the absence of 2-stage PNA MABR research and mainstream PNA MABR research in general, there exists a large knowledge gap that needs to be addressed. When comparing the three PNA MABR configurations, shown in Figure 1, a 1-stage reactor may appear to be the most economical based on its ability to perform PNA in one biofilm, but it requires a low membrane oxygen loading to produce aerobic and anaerobic regions within the biofilm for AOO and AMX. In contrast, because AMX is not located near the aeration source in the hybrid and 2-stage configurations, the membrane oxygen loading in the MABR could be increased without inhibiting anammox. In addition, since the biofilm is less susceptible to ammonia diffusion limitations and the ammonia concentration is larger at the membrane-biofilm interface, AOO may be able to continue suppressing NOB at this increased membrane oxygen loading. MABR units are relatively expensive when compared to other biofilm technologies, so it may be worthwhile to increase the oxygen loading and the nitrite production in a hybrid or 2-stage configuration rather than using a lower oxygen loading that is required to create aerobic and anaerobic regions in the biofilm in a 1-stage configuration. As a result, fewer MABR modules would be needed to produce the same quantity of nitrite. This could create the physical basin space that is required for a second stage reactor, and it may use the same amount of space as a 1-stage reactor. Also, the combined cost for the hybrid or 2-stage configuration could be less expensive than a 1-stage configuration since fewer MABR units would be needed. However, no study has compared 1-stage, hybrid, and 2-stage PNA MABR configurations.

The purpose of this paper is to use mechanistic modeling to identify the preferred niche for AMX and to quantify the performance tradeoffs between 1-stage, hybrid, and 2-stage PNA MABRs under different influent and operating conditions. The impact of increasing the membrane oxygen loading on NOB suppression and AMX inhibition in the hybrid and 2-stage configurations is presented. The ability to reach low effluent total nitrogen concentrations is analyzed. Finally, the most favorable configurations that should be considered for experimental evaluation are identified. This is the first study to compare different configurations of PNA MABRs, and the results of this study will inform researchers and engineers how to best implement PNA MABRs in mainstream nitrogen removal at larger scales.

METHODS

SUMO model description

A mathematical model created in an established wastewater modeling software, SUMO 19.2 (Dynamita 2019), was used to compare the three PNA MABR configurations. The SUMO software combines a 1-dimensional biofilm model, a biokinetic model, and various reactor models to create a complete wastewater modeling package.

The multispecies 1-dimensional biofilm model in SUMO applies user inputs of the biofilm specific mass, density, thickness, and number of layers to set a fixed biofilm thickness that is divided into separate, distinct layers. In the biofilm model, each biofilm layer acts as a completely mixed reactor. Substrates diffuse from the bulk liquid through the user specified hydrodynamic boundary layer into the outermost biofilm layer. The substrates then diffuse into adjacent biofilm layers at half the rate of diffusion that would occur in the bulk liquid. Oxygen substrate from the membrane in the MABR is supplied to the innermost biofilm layer as a fixed loading of oxygen which diffuses outward towards the bulk liquid. Particulate solids within each layer transfer between the biofilm layers at a specified rate. Finally, attachment and detachment occur at fixed, specified rates in the outermost biofilm layer.

The SUMO2 biokinetic model library with 2-step nitrification and denitrification was used in this study, as it was the only library that included AMX. The library accounted for AOO, NOB, OHO, AMX, glycogen accumulating organisms, phosphorus accumulating organisms, anoxic methanol utilizers, acidoclastic methanogens, and hydrogenotrophic methanogens. Given the conditions analyzed in this study, glycogen accumulating organisms, phosphorus accumulating organisms, anoxic methanol utilizers, acidoclastic methanogens, and hydrogenotrophic methanogens were quickly outcompeted and did not significantly affect the results of any configuration or scenario. A temperature of 20 degrees Celsius was used, so temperature corrections were not needed. Half saturation coefficients of organisms within the biofilm were reduced by a factor of 0.1 to account for the fact that diffusion limitations are explicitly incorporated into the biofilm model. The model did not consider the effects of pH, metabolic intermediates for nitrogen removal beyond nitrite, and variations in biochemical kinetic parameters. A table listing all biokinetic and stoichiometric parameters is found in the Supplemental Information.

A SUMO model used by Wagner *et al.* (2021) was expanded to treat mainstream wastewater with an ammonia concentration of 50 mg N/L, a variable COD concentration, and a flow rate of 5 L/d. This correlated to an influent nitrogen loading of 0.25 g N/d and a flux of 4.1 g N/m^2-d when normalized to the membrane surface area in the MABR. The biofilm in the MABR was modeled as five layers with the biofilm thickness and specific mass specified for each configuration. The boundary layer thickness was 100 μm. Although a lab-scale reactor was simulated in this study, important reactor parameters and operating conditions were normalized by the membrane surface area to make the results scale-independent. A clarifier and waste sludge pump were also added to each configuration to control the SRT of the suspended biomass and to allow all configurations to be compared directly. The clarifier effluent solids were set to 0 mg/L, and the suspended growth SRT was modified by altering the sludge wastage rate. However, a suspended growth SRT of 0 days, which represented a condition with no sludge recycled from the clarifier, was found to be optimal for the 1 and 2-stage configurations, which will be described later. Therefore, a suspended SRT of 0 days was used for the 1 and 2-stage configurations in every scenario except when it was explicitly modified in scenario 1. The configurations that were tested are shown in the diagram in Figure 2 and included (1) a 1-stage MABR, (2) a hybrid, and (3) a 2-stage MABR + MBBR.

To create the 1-stage configuration, an MABR unit with a thick 700 μm biofilm was used to form separate aerobic and anoxic zones within the biofilm for AOO and AMX, respectively, in accordance with literature (Pellicer-Nàcher *et al.* 2010, 2014; Terada *et al.* 2010; Lackner & Smets 2012; Pellicer-Nàcher & Smets 2012; Gilmore *et al.* 2013; Bunse *et al.* 2020). The biofilm specific mass was 11.5 g/m^2, and a separate continuous stirred tank reactor (CSTR) was added following the MABR unit. These two reactor units were linked with a recirculation pump that had a flow rate fifty times greater than the influent flow rate to create a pseudo bulk liquid for the MABR, which helped distinguish the impact of suspended biomass on the total inorganic nitrogen (TIN) removal performance when the suspended SRT was modified in scenario 1. For the hybrid configuration, the biofilm thickness in the MABR was decreased to 300 μm in accordance with literature (Downing & Nerenberg 2008a, 2008c; Lackner *et al.* 2010; Wang *et al.* 2016b), the biofilm-specific mass was decreased to 6.78 g/m^2, and all other parameters were unchanged. Finally, to create the 2-stage configuration, an MABR with the same parameters as the hybrid configuration, 300 μm thick biofilm and a 6.78 g/m^2 specific mass was used in the first stage, and the CSTR used in the previous configurations was replaced with a second stage MBBR unit after the clarifier. The combined volume for the

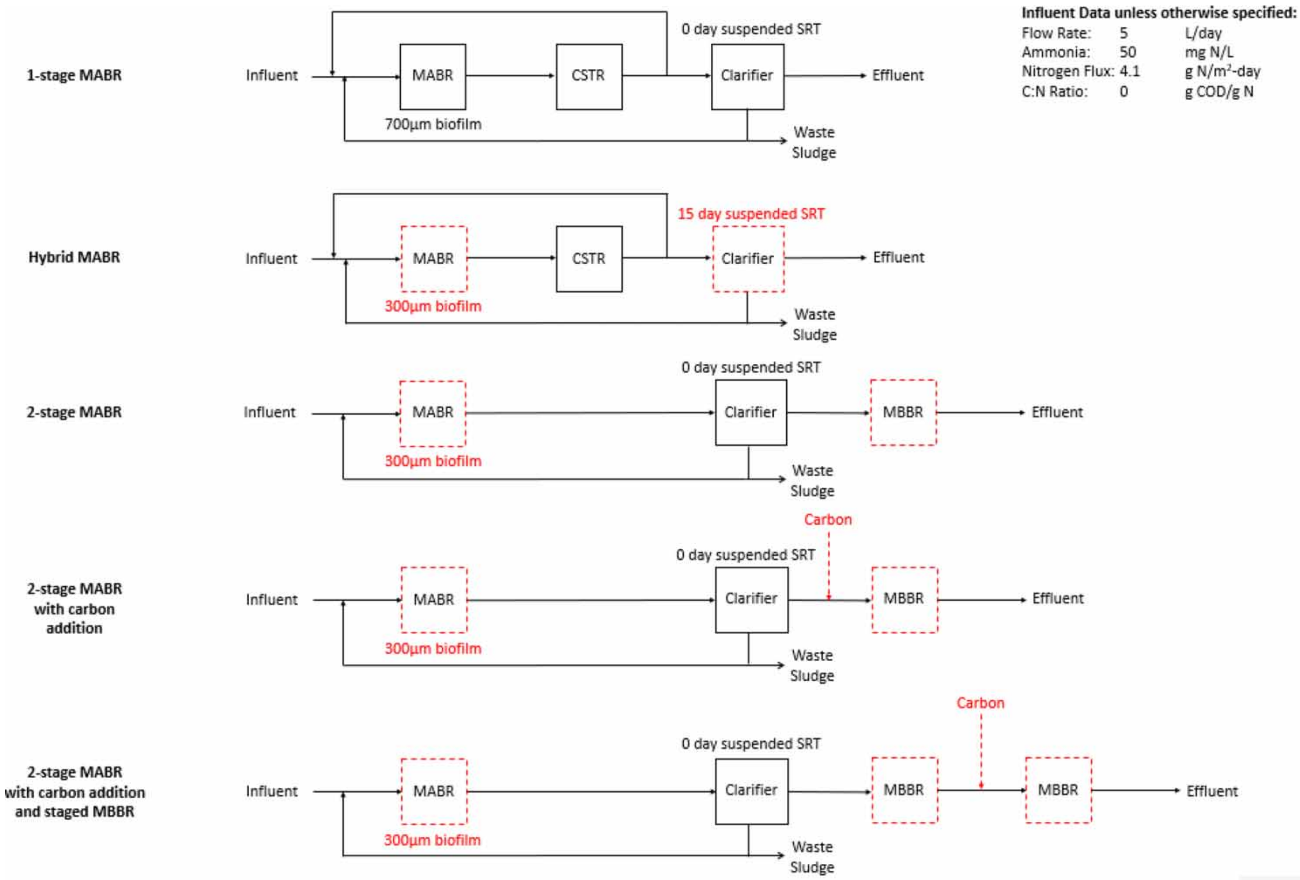


Figure 2 | Diagram of MABR configurations tested. Red, dashed borders highlight significant differences between the configurations.

MABR and MBBR was equivalent to the combined volume of the MABR and CSTR in the 1-stage and hybrid configurations to create equivalent hydraulic residence times. The MBBR biofilm thickness was set to 300 μm, the biofilm specific mass was 6.78 g/m^2, and a boundary layer thickness of 100 μm was used. The MBBR surface area was equivalent to the MABR membrane surface area to allow for easier comparisons of performance.

The modified modeling parameters for each configuration are listed in Table S1, and all other kinetic and state parameters were SUMO default values. Simulations were run for 360 days to achieve steady state effluent concentrations, defined in this study as the moment when the daily difference in the effluent total ammonia/ammonium, nitrite, and nitrate was less than 0.001 mg N/L but, a longer runtime was needed in a few instances to achieve steady state effluent conditions.

Testing scenarios

To determine the performance of the 1-stage, hybrid, and 2-stage configurations under different influent and operational conditions, five modeling scenarios were created which are displayed in a testing matrix in Table 2 and described below. For each scenario, the values of modified parameters are listed in the Table S1. The influent total chemical oxygen demand (COD) and SRT were zero for each simulation, unless otherwise specified, and each reactor's bulk liquid was anaerobic. It is important to note that the performance values for each simulation represent conditions when the effluent nitrogen species reached steady-state but do not represent the performance before steady-state is achieved.

In the baseline scenario zero, the membrane air loading in the MABR that resulted in the best TIN removal for each configuration was determined and then used in each of the subsequent scenarios. In the hybrid and 2-stage configurations, the optimal membrane air loading was found to be larger than the loading used in the 1-stage configuration, which will be discussed later.

The goal of the first scenario was to determine the effect of the suspended growth SRT on the configurations' TIN removal performance and to find the minimum suspended growth SRT for AMX retention in the hybrid configuration. To do this, modeling simulations were run with suspended growth SRT values of 0, 2, 3, 5, 10, 15, and 20 days. A suspended growth

Table 2 | Testing matrix for five modeling scenarios

Scenario	Description	Goal	Manipulated parameters
0 (baseline)	1-stage vs. Hybrid vs. 2-stage	Determine the optimal air loading for the configurations	MABR air loading
1	1-stage vs. Hybrid vs. 2-stage	Determine the effect of SRT on 1 and 2–stage configurations and the minimum SRT for the hybrid configuration	SRT
2	1-stage vs. Hybrid vs. 2-stage	Determine the best configuration across varied C:N ratios	Total COD Loading (C:N ratio)
3	1-stage vs. 2-stage with modified surface areas	Determine if adding more MABR and MBBR surface area can decrease the effluent TIN<10 mg N/L	MABR's membrane surface area, MABR's air loading, and MBBR's biofilm surface area
4	Modified 2-stage MABR with carbon addition to un-staged MBBR	Determine if heterotrophic denitrification and anammox can occur within 1 MBBR unit, decreasing the effluent TIN<5 mg N/L	Carbon loading and MBBR's biofilm surface area
5	Modified 2-stage MABR with carbon addition to staged MBBRs	Determine if heterotrophic denitrification and anammox can occur with MBBR units in series, decreasing the effluent TIN<5 mg N/L	Carbon loading and MBBR's biofilm surface area with the MBBR divided into two reactors in series

SRT of 0 days, which represented a condition with no sludge recycled from the clarifier, was found to be optimal for the 1 and 2-stage configurations and was used in the subsequent scenarios. However, the clarifier was kept for simplicity and was determined to not significantly impact the results in the later scenarios (data not shown). A suspended growth SRT of 15 days in the hybrid configuration was found to retain AMX and was used in the subsequent scenario.

The second scenario's goal was to determine the effect of the influent carbon to nitrogen ratio (C:N) on the configurations' TIN removal performance. Simulations were conducted by modifying the influent total COD concentration inputs of 0, 25, 100, and 250 mg COD/L which correspond to C:N ratios of 0, 0.5, 2, and 5 mg Total COD/mg NH_x-N. A COD fractionation table is shown in Table S2.

After performing scenarios 1 and 2, each configuration was observed to have effluent TIN concentrations greater than 10 mg N/L which would be insufficient for strict effluent nitrogen permits for mainstream nitrogen removal at full-scale. For this reason, scenarios 3–5 tested whether an effluent TIN concentration below 10 mg N/L could be achieved in the 1 and 2-stage configurations. The hybrid configuration was not simulated in scenarios 3–5, as its performance was found to be worse than the performance of the 1 and 2-stage configurations in the previous scenarios.

In scenario 3, the ability to decrease the effluent TIN by increasing the amount of membrane surface area and the membrane air loading in the MABR for the 1 and 2-stage configurations was determined. For simplicity, the membrane surface area and membrane air loading were normalized to their starting values and then increased by increments of 0.05. In the 2-stage configuration, increasing the membrane surface area and air loading was found to be successful at producing additional nitrite, but the 2nd stage MBBR was biomass limited and was not able to remove the extra nitrite. Therefore, simulations were also conducted with increased biofilm surface areas in the MBBR to determine how much additional MBBR media was required to remove the additional nitrite. For simplicity, the MBBR's biofilm surface area was also normalized to its starting value and then increased by increments of 0.25. A normalized membrane surface area and membrane air loading of 1.125 were used in the subsequent scenarios to aid in minimizing the effluent TIN concentration.

The fourth scenario tested whether adding a carbon feed to the MBBR in the 2-stage configuration could lower the effluent TIN below 5 mg N/L to comply with an ultra-low nitrogen permit through complete or partial heterotrophic denitrification and anammox. The non-volatile fatty acid, readily biodegradable carbon feed was added after the clarifier but before the MBBR unit, and the carbon loading was modified by changing the flow rate of a highly concentrated, readily biodegradable non-VFA carbon feed from 1×10^{-12} to 1×10^{-4} m^3/d at order of magnitude increments. Since a normalized membrane surface area and membrane air loading of 1.125 were used here, this scenario also benefitted from increasing the MBBR's biofilm surface area. The MBBR's biofilm surface area was normalized to its starting value and increased by increments of 0.25. To test whether heterotrophic denitrification from the added carbon could allow a designer to decrease the MBBR biofilm surface area, the normalized MBBR biofilm surface area was also decreased by increments of 0.25. However, scenario 4 was not successful; adding carbon to the MBBR let OHO outcompete AMX in the biofilm. This prompted the creation of scenario 5.

Finally, a fifth scenario tested whether staging the MBBR into two MBBRs in series with a carbon feed added to the second MBBR in the 2-stage configuration would prevent OHO outcompeting AMX and could lower the effluent TIN below 5 mg N/L. The carbon feed was modified in the same manner as the feed in the fourth scenario, and the amount of biofilm surface area in both MBBRs in series were normalized to the original, un-staged MBBR's biofilm surface area and modified by increments of 0.25.

RESULTS AND DISCUSSION

The results from the scenarios listed in Table 2 are described below and will be crucial in comparing the PNA MABR configurations. These results will help identify process configurations and general ranges of process loadings that might be most promising to evaluate experimentally, especially for the hybrid and 2-state configurations where little experimental results are currently available. After the results from each scenario are presented, the significance of the biofilm thickness, the membrane air loading in the MABR, and other differences between the three configurations will be discussed.

Air loadings can be increased in the hybrid and 2-stage configurations resulting in increased partial nitritation rates

Results from the baseline scenario, which varied the membrane air loading to maximize the nitrogen removal performance for each configuration, have led to several findings regarding the optimal configuration of PNA MABRs, the ideal niche for AMX, and the impact of the biofilm thickness on NOB suppression. The ideal niche for AMX in each configuration was determined by identifying where AMX was most abundant. For the three configurations, AMX was most abundant in the outer portion of the thick biofilm in the MABR in the 1-stage configuration, in the suspended growth at a suspended SRT of 15 days in the hybrid configuration, and in the biofilm in the MBBR for the 2-stage configuration. Because AMX was located outside the MABR biofilm in the hybrid and 2-stage configurations, the air flux to the membrane of the MABR could be increased from 4.1 to 11.5 g O_2/m^2-d when compared to the 1-stage configuration without inhibiting AMX. Also, since the biofilm was thinner in the hybrid and 2-stage configurations, the ammonia concentration was larger at the membrane-biofilm interface which increased the AOO growth rate and the AOO oxygen demand. This allowed AOO to outcompete NOB for oxygen even though the air loading rate was increased to 11.5 g O_2/m^2-d. The increase in the membrane air loading rate resulted in a 180% increase in the nitrite production rate and an increase in the mass of AOOs by a factor of 2.6 from the 1-stage to the hybrid and 2-stage configurations, shown in Figure 3. The 1-stage configuration did have almost 1.8 times more AMX mass than the hybrid and 2-stage configurations, but it was not able to capitalize on this since it was nitritation (AOO and oxygen) limited. Overall, the ability to increase the membrane air loading rate and nitrite production in the hybrid and 2-stage configurations without impacting NOB suppression is advantageous, and their impact on the TIN removal rate is evaluated in the following scenarios.

Long SRTs are needed for the hybrid configuration

Modeling simulations in scenario 1 analyzed the effect of the suspended biomass's SRT on the reactor performance. This is the only scenario that modified the suspended SRT for the three configurations to determine its effect on the TIN removal rate, shown in Figure 4. The TIN removal rate was used to quantify performance, which represented the combined ammonia, nitrite, and nitrate in the configurations' effluent subtracted from influent nitrogen normalized to the membrane surface area in the MABR. For the 1-stage configuration, the performance was not benefitted by increasing the suspended growth SRT since AOO and AMX resided solely in the biofilm in the MABR. This result conflicts with the results from a study by Bunse *et al.* (2020), which compared a 1-stage PNA MABR that retained detached biomass to a reactor that removed detached biomass. The reactor that retained the attached biomass had a 12% larger total nitrogen removal and a 5% larger ammonium removal. The differences between the study by Bunse *et al.* (2020) and this study could suggest that the model may overestimate the AMX retention in the biofilm in the MABR. For the hybrid configuration, a long SRT of approximately 12 days was required to retain AMX in the suspended biomass in the bulk liquid. At this SRT, the hybrid performance reached a TIN removal rate equivalent to the 1-stage MABR. The 2-stage configuration performed the best of the three, reaching a max TIN removal rate of approximately 3 g N/m^2-d at a suspended SRT of zero days, which represents treatment without sludge recirculation from the clarifier. Similar to the 1-stage configuration, the performance of the 2-stage configuration did not improve as the SRT increased since AOO and AMX were most abundant in the biofilms in the MABR and MBBR, respectively. Because both the 1 and 2-stage configurations had their best performance at a suspended SRT of

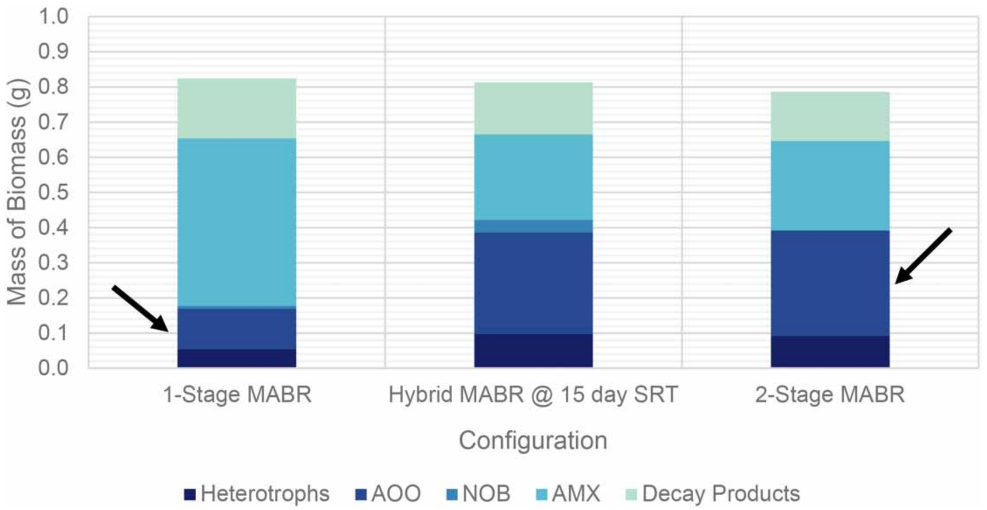


Figure 3 | Biomass comparison for the different MABR configurations. All simulations shown here were run in an anaerobic bulk liquid with no influent COD, and the biomass mass includes all the biomass in the configuration, not just the MABR. Arrows highlight the key differences in the AOO mass between the configurations.

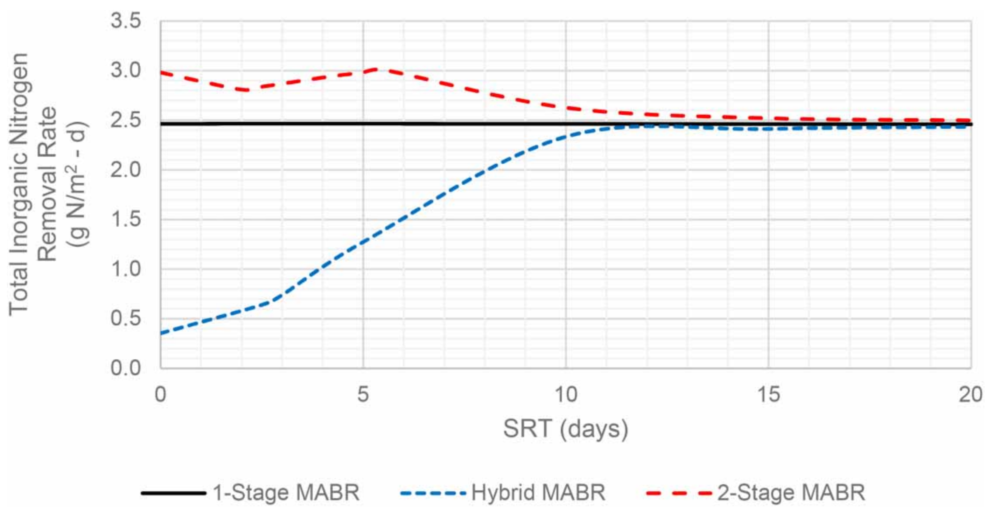


Figure 4 | Comparison of TIN removal rates for the different MABR configurations for variable SRT. All simulations shown here were run in an anaerobic bulk liquid with no influent COD, and the removal rates are for all biomass present in the system, not just the MABR.

zero days, clarifiers and additional sludge processing equipment will not be needed for these configurations in future experimental studies, and a suspended SRT of 0 days will be used in the subsequent scenarios.

NOBs are more easily retained in the hybrid configuration

In scenario 1, the hybrid configuration was observed to have the largest NOB population of the three configurations. This could become problematic in an experimental system where portions of sloughed biofilm inhabited by NOBs are retained in the reactor where they could eventually reattach to the biofilm and decrease the overall nitrogen removal performance. Interestingly, the 2-stage and hybrid configurations had larger increases in their NOB mass within the MABR biofilm and in the suspended biomass at longer SRTs than the 1-stage configuration (Table S3). A possible explanation for this is that the outer portion of the 1-stage MABR biofilm was anaerobic with a low NOB abundance, so the detached portions of the biofilm that were retained in the bulk liquid also had a lower abundance of NOB. The biofilms in the MABRs in the hybrid and 2-stage configurations were thinner and aerobic, however with NOB populations located closer to the outer layer. Thus, when portions of their biofilms detached, the NOB populations suspended in the bulk liquid increased at longer suspended SRTs. The increase in NOB mass and a corresponding increase in nitrate production did not result in significant changes in heterotrophic denitrification rates which could explain why all three configurations approach the same level of performance at longer SRTs in scenario 1 (Table S4). Given the potential for the hybrid configuration to retain NOBs at longer SRTs, it may be less desirable than the 1-stage or 2-stage configurations, which both had their best performances without biomass recycled from the clarifier.

The hybrid configuration's performance is influenced by the basin size more than the other configurations

Since AMX was most abundant in the bulk liquid in the hybrid configuration, the reactor's volume and suspended SRT will control the amount of suspended AMX, unlike the 1 and 2-stage configurations where AMX was most abundant in biofilms. Maintaining a suspended SRT requires a conventional clarifier, so the hybrid configuration may be less desirable than the other configurations if a facility is constrained by its footprint, since the 1 and 2-stage configurations had their optimal performance without biomass recycled from the clarifier. However, suspended AMX could also be retained in the hybrid configuration with granular sludge. This may require additional biomass retention mechanisms such as screens or cyclones to separate granular AMX from the other suspended microorganisms within the reactor, which could decrease the suspended SRT and volume required for AMX retention (Han *et al.* 2016). Additional experimentation should be performed to characterize the performance of a hybrid MABR with granular sludge to confirm this hypothesis.

The 2-stage configuration performed better than the 1-stage and hybrid configurations across varied C:N ratios

The influent carbon to nitrogen ratio has been shown to impact PNA MABRs, so it was important to determine how it impacted the configurations' performance in scenario 2 (Lackner *et al.* 2008). The TIN removal performance for the three configurations is shown for varying influent total carbon to nitrogen (C:N) ratios ranging from 0 to 5 in Figure 5. The 2-stage configuration achieved up to a 20% increase in performance when compared to the 1-stage configuration, with the largest difference in performance occurring at C:N less than 2. At a C:N of approximately 2, the nitrogen removal metabolism in every configuration shifted towards traditional nitrification and heterotrophic denitrification rather than PNA, an observation observed by other studies (Lackner *et al.* 2008; Wagner *et al.* 2021). For C:N less than 2, the increase in performance for the 2-stage configuration was enabled by the increased air loading and partial nitritation rate from the thinner MABR biofilm that was mentioned previously. In summary, nitrogen removal through PNA is maximized when the C:N ratio is below 2. However, mainstream wastewater C:N ratios are typically larger than 2, so an upstream carbon capture reactor will be required for optimal PNA MABR performance.

The 2-stage configuration achieved lower effluent total nitrogen concentrations by adding more membrane surface area and air

The results from scenarios 1 to 2 did not reach effluent TIN concentrations below 10 mg N/L, which could be desirable as WRRF discharge permits further regulate nitrogen. To determine if the 1 and 2-stage configurations could achieve lower TIN concentrations, scenario 3 was performed, which normalized and increased the amount of membrane surface area and the air loading to the MABRs by factors of 1–1.5 as seen in Figure 6. Since this scenario did not modify the influent ammonia mass

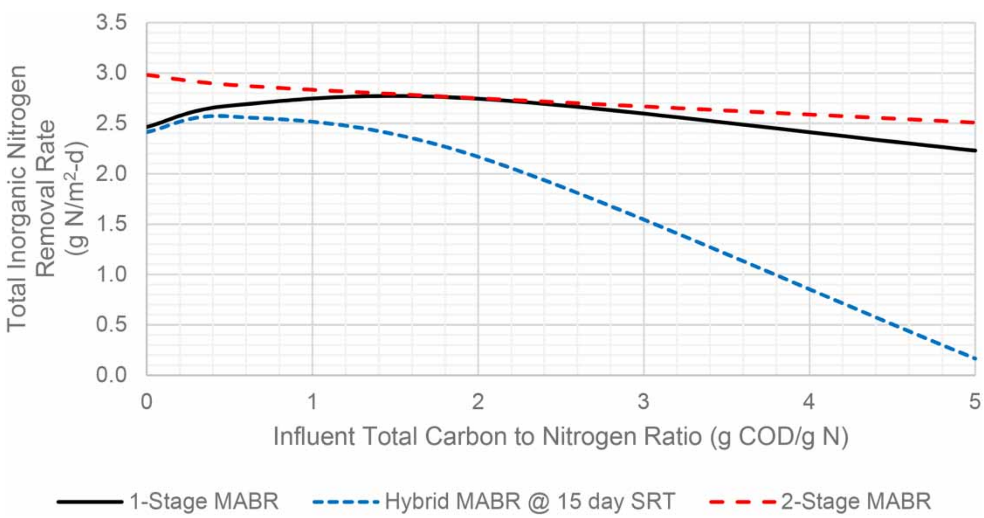


Figure 5 | Comparison of TIN removal rates for the different MABR configurations for variable C:N ratios. All simulations shown here were run in an anaerobic bulk liquid, and the removal rates are for all biomass present in the system, not just the MABR.

loading, the increase in membrane surface area decreased the applied nitrogen flux. The values listed at the intercepts of the membrane surface area and air loading show the TIN concentration in the effluent. The cell color corresponds to this effluent TIN concentration, with darker colors depicting lower concentrations and lighter colors depicting higher concentrations. Additional information describing the effluent ammonia, nitrite, and nitrate concentrations is shown in Figures S1-S3. From these graphs, the lowest TIN concentration achievable for the 1-stage configuration was 15 mg N/L, which was obtained after increasing the membrane surface area by 50%. In contrast, increasing the 2-stage configuration's membrane air loading by 15% produced effluent TIN concentrations below 10 mg N/L, without needing to increase the membrane surface area. Moreover, increasing the membrane surface area led to further decreases in the effluent TIN concentration, making the 2-stage configuration favorable for WRRFs with strict nitrogen permits.

After performing the simulations in scenario 3, the 2-stage configuration was observed to have the ability to produce nitrite beyond what was able to be consumed in the MBBR. Specifically, at a normalized membrane air loading and membrane surface area of 1.125, a nitrite concentration of 2.47 mg N/L and a total ammonia concentration of 2.34 mg N/L were observed in the 2-stage configuration's effluent (Figures S1 and S2). Ideally, the nitrite would be metabolized with total ammonia through anammox in the 2nd stage MBBR to minimize the effluent TIN concentration, but it appeared that the 2nd stage MBBR was biomass limited. Therefore, simulations were also conducted with increased biofilm surface areas in the MBBR at a normalized membrane air loading and membrane surface area of 1.125 in the MABR to determine how much additional MBBR media was required to remove the additional nitrite. The impact of increasing the normalized biofilm surface area in the MBBR is shown in Figure S4. As the MBBR fill ratio increased, the amount of AMX in the reactor also increased, and the effluent total ammonia and nitrite concentrations decreased to approximately 1 mg N/L with effluent TIN concentrations approaching 6.6 mg N/L. Since MBBR media is typically less expensive than MABR units, an experimental system that is testing the performance of a 2-stage configuration should have a biofilm surface area in the MBBR that is larger than the biofilm surface area in the MABR to prevent biomass limitations in the second stage. These findings also emphasize that a 2-stage configuration with increased biofilm surface area in the MBBR can achieve an effluent TIN concentration that is approximately 56% lower than the minimum TIN concentration achieved with the 1-stage configuration, by comparing the results from Figure S4 to a 1-stage configuration with normalized biofilm surface areas and membrane air loadings of 1.5.

MABR Surface Area

MABR Air Loading	1	1.05	1.1	1.15	1.2	1.25	1.3	1.35	1.4	1.45	1.5
1	20.00	19.22	18.49	17.80	17.14	16.54	16.04	15.69	15.44	15.23	15.05
1.05	21.20	20.42	19.69	19.00	18.35	17.73	17.15	16.59	16.07	15.56	15.09
1.1	22.40	21.62	20.89	20.20	19.55	18.94	18.36	17.80	17.28	16.78	16.30
1.15	23.61	22.82	22.09	21.40	20.75	20.14	19.56	19.01	18.49	17.99	17.51
1.2	24.84	24.04	23.29	22.60	21.95	21.34	20.76	20.21	19.69	19.19	18.72
1.25	26.08	25.26	24.51	23.81	23.16	22.54	21.96	21.42	20.90	20.40	19.93
1.3	27.33	26.51	25.74	25.03	24.37	23.75	23.17	22.62	22.10	21.61	21.14
1.35	28.59	27.76	26.98	26.26	25.58	24.96	24.37	23.82	23.30	22.81	22.34
1.4	29.86	29.02	28.23	27.50	26.82	26.18	25.59	25.03	24.51	24.02	23.55
1.45	31.13	30.28	29.49	28.75	28.06	27.41	26.81	26.25	25.72	25.22	24.75
1.5	32.39	31.55	30.75	30.01	29.31	28.66	28.05	27.47	26.94	26.44	25.96

MABR Air Loading	1	1.05	1.1	1.15	1.2	1.25	1.3	1.35	1.4	1.45	1.5
1	13.73	13.59	13.38	13.12	12.85	12.57	12.29	12.00	11.72	11.47	12.20
1.05	11.83	11.75	11.62	11.42	11.16	10.89	10.61	10.33	10.05	9.77	10.46
1.1	10.40	10.36	10.27	10.13	9.92	9.65	9.38	9.10	8.82	8.54	9.10
1.15	8.95	8.91	8.84	8.75	8.60	8.41	8.17	7.91	7.66	7.40	7.31
1.2	10.30	10.22	10.13	10.03	9.92	9.78	9.63	9.45	9.26	9.07	8.67
1.25	13.13	13.05	12.96	12.87	12.78	12.67	12.54	12.39	12.22	12.03	11.47
1.3	15.25	15.18	15.10	15.01	14.92	14.83	14.72	14.59	14.43	14.25	13.49
1.35	15.56	16.43	17.21	17.84	17.78	17.69	17.59	17.48	17.35	17.14	16.15
1.4	15.63	16.49	17.30	18.02	18.64	19.12	19.38	19.38	19.13	18.64	17.97
1.45	19.86	17.36	17.37	18.13	18.81	19.41	19.90	20.24	20.37	20.25	19.90
1.5	24.55	21.69	19.13	18.21	18.88	19.52	20.08	20.55	20.88	21.03	20.95

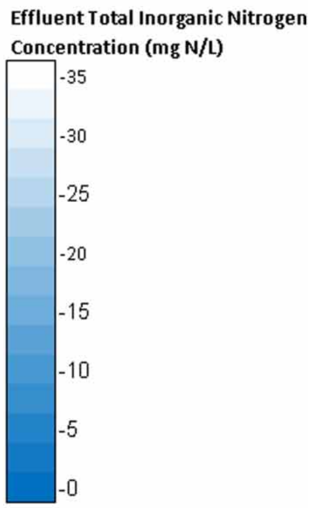


Figure 6 | (A-B): Summary of effluent TIN concentrations for the 1-stage (top, A) and 2-stage (bottom, B) configurations after normalizing and increasing the biofilm surface area and the air loading in the MABR. The color bar is scaled to the minimum and maximum value.

Performance decreases when carbon is added to an un-staged MBBR in the 2-stage configuration

To further evaluate the ability of mainstream PNA MABRs to reach low effluent total nitrogen concentrations (below 5 mg N/L) that could comply with an ultra-low nitrogen permit, readily biodegradable carbon was added to the 2-stage configuration, first to an un-staged MBBR in scenario 4 and later to a staged MBBR in scenario 5. These modifications were chosen to determine if nitrogen could be removed with partial heterotrophic denitrification and anammox, or if traditional heterotrophic denitrification would dominate the nitrogen removal. The effluent TIN concentrations for scenarios 4 and 5 with normalized and modified biofilm surface areas in the MBBR are shown in Figure 7. For both scenarios, a normalized biofilm surface area and membrane air loading of 1.125 in the MABR were used to optimize the nitrogen removal. The mass of carbon added to the MBBR was divided by the mass of nitrogen removed in the MBBR(s) to ascertain its effectiveness in removing nitrogen. For the un-staged MBBR in scenario 4, the addition of carbon did the opposite of its intended goal and hindered the reactor performance for all the biofilm surface areas tested, increasing the total nitrogen concentration from 8 to 14 mg N/L to 20 mg N/L depending on the normalized biofilm surface area in the MBBR. This shift in performance can primarily be attributed to OHO outcompeting AMX for space in the biofilm in the MBBR and a shift from nitrogen removal with anammox to less efficient heterotrophic denitrification (Figure S5). Minimal partial heterotrophic denitrification occurred with anammox, and the effluent total nitrogen concentration also peaked at a value slightly lower than the stoichiometric carbon demand for heterotrophic denitrification, 4.6 g COD/g N, supporting the dominance of heterotrophic denitrification in the MBBR (Figure S6). After this peak, OHOs consumed the added carbon, formed organic nitrogen, and decreased the effluent TIN concentration, seen in the rightmost portion of Figure 7(a). In conclusion, scenario 4 was not successful, and adding carbon to the MBBR let OHO outcompete AMX in the biofilm. This prompted the staging of the MBBRs in scenario 5 to preserve AMX in the first MBBR and to promote heterotrophic denitrification in the second MBBR.

Carbon can be added to a staged MBBR in the 2-stage configuration to promote heterotrophic denitrification to achieve ultra-low effluent TIN concentrations

By staging the MBBR in scenario 5, nitrogen removal by anammox was preserved in the first stage enabling the second stage to be used as a polishing heterotrophic denitrification step. As the carbon loading to the second staged MBBR increased, the effluent TIN concentration correspondingly decreased to concentrations below 2 mg N/L, seen in Figure 7. This improvement in the TIN concentration in the second MBBR was primarily driven by heterotrophic denitrification (Figure S5). The effluent TIN for the staged MBBR configuration peaked at a lower ratio of carbon added to nitrogen removed, closer to 1 g COD/g N, as low nitrate concentrations began limiting the heterotrophic denitrification rates. Using equal amounts of membrane surface area in each stage (0.5–0.5) resulted in a lower effluent TIN concentration than having the first stage's membrane surface area greater than the second stage's (0.75–0.25), assuming that the combined biofilm surface areas in the two MBBRs were equivalent. However, increasing the total biofilm surface areas in the two MBBRs (0.75–0.5) resulted in a TIN concentration below 2 mg N/L, once carbon was added. In summary, staging the MBBRs in the 2-stage configuration prevents AMX outcompetition by OHO and allows for heterotrophic denitrification polishing to reach ultra-low TIN concentrations, which is not achievable by the 1-stage configuration.

Hybrid and 2-stage configurations are less likely to experience oxygen inhibition and ammonia diffusion limitation

Decreasing the biofilm thickness in the MABRs from 700 μm in the 1-stage configuration to 300 μm in the hybrid and 2-stage configurations changed the ideal niche for AMX from the biofilm in the MABR to the bulk liquid and the biofilm in the MBBR, respectively. This change in biofilm thickness decreased the likelihood that AMX is oxygen inhibited and made it less likely that the biofilm in the MABR was ammonia diffusion limited. From previous research studies in marine habitats, AMX was shown to be very sensitive to oxygen and experienced a half maximal inhibitory concentration for oxygen between 0.03 and 0.36 mg/L (Jensen *et al.* 2008; Kalvelage *et al.* 2011; Babbin *et al.* 2014). In wastewater and freshwater samples, complete inhibition of AMX was experienced at even lower oxygen concentrations, ranging from 0.02 to 0.12 mg/L (Strous *et al.* 1997; Egli *et al.* 2001; Oshiki *et al.* 2016; Seuntjens *et al.* 2018). This could become problematic for 1-stage PNA MABRs, as the AMX located in the biofilm could experience micro-aerobic conditions within this inhibitory range. Given the dynamic nature of wastewater flows, the air loading to the reactor will have to be modified with respect to the changing loads of nitrogen and COD, which could present occurrences of unintentionally inhibiting AMX performance. Using a thinner biofilm in the MABR and changing AMX's ideal niche from the biofilm to the bulk liquid or in a separate biofilm would move it further away from the source of oxygen transfer and could help minimize inhibition.

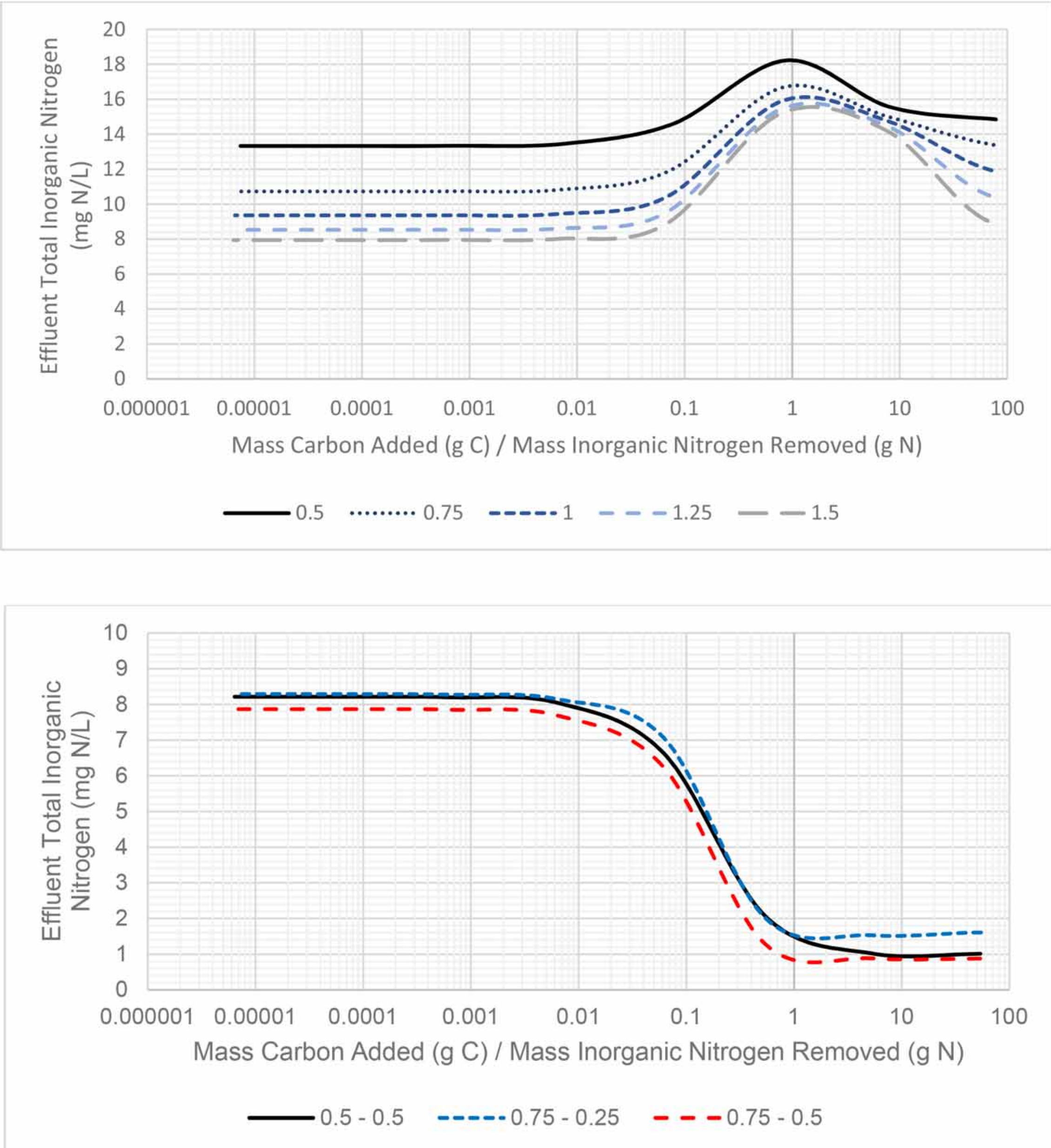


Figure 7 | (A-B): Comparison of the effluent TIN concentration for the 2-stage configuration with readily biodegradable carbon added to the MBBR after normalizing and changing the biofilm surface area in the MBBR. Each line represents a normalized biofilm surface area in the MBBR for an un-staged MBBR (top, A) and for a staged MBBR (bottom, B). The values in the legend of the bottom figure represent the normalized biofilm surface areas in the first and second MBBRs, respectively, with the second MBBR receiving the carbon addition. When calculating the mass of TIN removed in the staged MBBR configuration, the combined quantity of nitrogen removed from the first and second MBBRs was used.

Differences in biofilm thickness, and therefore diffusion limitation of ammonia, are also important in describing the variance in the configurations' performance. A modeling study has shown that diffusion resistance began limiting a PNA MABR's performance at biofilm thicknesses greater than 450 μm and was hypothesized to explain a decrease in an experimental PNA MABR's performance at influent nitrogen concentrations less than 70 mg N/L (Lin *et al.* 2015). Given the difference in biofilm thicknesses for the configurations and an influent nitrogen concentration of 50 mg N/L, diffusion resistance could have negatively impacted the 1-stage configuration's performance. The oxygen loading was also able to be increased in the hybrid and 2-stage configurations without impacting NOB suppression. Therefore, the hybrid and 2-stage configurations would be more effective in nitrogen removal in mainstream wastewater than the 1-stage configuration.

The 2-stage configuration is flexible and able to easily incorporate multiple nitrogen removal metabolisms

Scenario 3 showed the potential for a 2-stage PNA MABR to reach low TIN concentrations when modifying the air loading and membrane surface area. Normalized air loadings of 1.1–1.2 were able to achieve TIN concentrations below 10 mg N/L (Figure 6), while the 1-stage struggled to meet an effluent TIN concentration of 15 mg N/L. Since the optimal air loading to a PNA MABR is hypothesized to be impacted by the influent nitrogen loading, the 2-stage's wider operational range could result in easier transitions to variable nitrogen loadings in addition to improved performance (Terada *et al.* 2007; Pellicer-Nàcher *et al.* 2010; Zhao *et al.* 2010; Bunse *et al.* 2020). This conclusion has also been seen in a study by Veys *et al.* (2010) who compared 1 and 2-stage PNA reactors and found that the 2-stage reactor performed better over a wider range of optimal process conditions.

In scenario 5, the 2-stage's effluent TIN concentration was further decreased with the addition of readily biodegradable carbon in a staged MBBR. By using a 2-stage configuration, key microbial metabolisms were separated with aerobic AOOs located in the MABR, AMX located in the first MBBR, and heterotrophic denitrifying organisms located in the second MBBR. This separation of metabolisms was required to prevent AMX from being outcompeted for substrates or physical space in the biofilm, shown in Figure 7. These modeling results are encouraging and show that the performance of 2-stage PNA MABRs could be increased with heterotrophic denitrification polishing in WRRFs with strict effluent total nitrogen permits. Experimental validation will also be important in this polishing step, as carbon sources can vary in performance (Le *et al.* 2019).

Partial nitritation rates in an MABR can be increased in full-scale applications

When comparing the modeling results from this study to the literature base, it is important to note that SUMO 19.2 models oxygen transfer in MABRs as a fixed loading of oxygen supplied at the base of the biofilm which cannot be compared to a membrane pressure, which is typically used in practice. In experimental systems, however, the oxygen transfer through the membrane is balanced in relation to the oxygen demand of the biofilm. Lab-scale reactors can easily increase the oxygen transfer rate beyond this balanced equilibrium by changing the inlet gas feed pressure, similar to increasing the membrane air loading in this study. At full-scale, the inlet gas feed pressure can also be increased in the same manner. Thus, the ability to increase the oxygen transfer rate can be applied directly to practice.

The ability to increase the partial nitritation rate in an MABR is not well understood, but the results from this study do support its feasibility. Partial nitritation rates in MABRs have been hypothesized to be impacted by a variety of factors including the membrane air pressure, wastewater temperature, concentrations of influent nitrogen and oxygen, gradients of nitrogen and oxygen within the biofilm, pH, and biokinetics of AOO and NOB (Terada *et al.* 2004; Downing & Nerenberg 2008a; Lackner *et al.* 2010; Landes *et al.* 2011; Lackner & Smets 2012; Wang *et al.* 2016b; Ma *et al.* 2017; Li *et al.* 2018). Given the assortment of hypotheses, it remains unclear what conditions govern partial nitritation rates in MABRs.

All the configurations were able to achieve low levels of NOB accumulation, but the hybrid and 2-stage MABRs had larger partial nitritation rates and TIN removal rates (Figure 5). This could be explained by the change in biofilm thickness and thus the ammonia diffusion into the biofilm. Biokinetics could also support this theory, since the modified air loading and biofilm thickness impacted the growth rates of AOO, NOB, and AMX. Surprisingly, the dissolved oxygen gradient in the configurations' biofilms did not seem to impact the ability to suppress NOB. The gradient in the 2-stage and hybrid MABRs was approximately 7 times larger than the 1-stage's gradient, but both configurations were able to limit NOB growth. Further research should be conducted to elucidate when and how partial nitritation rates can be increased, but given that partial nitritation has been reliably achieved in this study, other models, and experimental reactors, 2-stage PNA MABRs appear to be the most promising configuration for mainstream nitrogen removal (Downing & Nerenberg 2008a; Lackner *et al.* 2010).

CONCLUSIONS

This is the first study that has evaluated the impact of the reactor configuration (1-stage, hybrid, or 2-stage) on the location of the preferred niche for anammox bacteria and the overall nitrogen removal performance for PNA MABRs. The main findings from this study are:

- A 1-stage PNA MABR required a lower air loading of 4.1 g O_2/m^2-d to prevent oxygen inhibition of AMX and NOB growth. It produced less nitrite and was less efficient than the MABR found in the hybrid and 2-stage configurations.
- AMX were most abundant in the bulk liquid and in the MBBR for the hybrid and 2-stage configurations respectively, which decreased AMX's potential to be inhibited by oxygen from the membrane. The oxygen flux in the MABR could therefore be

increased to 11.5 g O_2/m^2-d without impacting NOB suppression, resulting in better TIN removal rates and more cost effective membranes.
- The 2-stage configuration achieved a max TIN removal rate of 3 g N/m^2-d. It attained an effluent total nitrogen concentration below 10 mg N/L, and when additional carbon was added to a staged MBBR, the effluent TIN concentration was decreased to less than 3 mg N/L.

We therefore conclude that:

- All PNA MABR configurations can be implemented in mainstream nitrogen removal, but the 2-stage may be the most viable configuration in facilities with strict effluent total nitrogen permits. The 2-stage configuration had increased flexibility in its nitrogen removing metabolisms and can support anammox and heterotrophic denitrification when correctly staged.
- As a result of the increased oxygen flux between the 1 and 2-stage configurations, fewer 2-stage MABR modules would be needed to produce the same quantity of nitrite as the MABR modules in the 1-stage. In full-scale treatment, it will likely be more cost-effective to use less expensive MBBR media for anaerobic metabolisms (anammox and heterotrophic denitrification) and to focus on maximizing oxidation rates with the more costly MABRs. However, the optimal configuration will likely be facility specific, as they differ in operating costs, construction costs, footprint, and effluent limits.
- Practitioners and researchers may shift away from single-sludge systems and increase their focus on 2-stage and hybrid MABRs, which are easier to control and can result in better performance.

Experimental validation will be important to confirm these modeling results. Specifically, a 2-stage PNA MABR with a thin biofilm should be compared to a 1-stage PNA MABR with a thick biofilm. The mechanisms governing partial nitritation rates and NOB suppression in PNA MABRs should also be further studied. Doing this will help increase the likelihood of PNA MABR adoption in mainstream nitrogen removal which would generate significant carbon and energy saving opportunities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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