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Impacts of biofilm properties on start-up and performance of membrane biofilm reactor performing anammox and nitrate/nitrite-dependent anaerobic methane oxidation integrated processes: A model-based investigation

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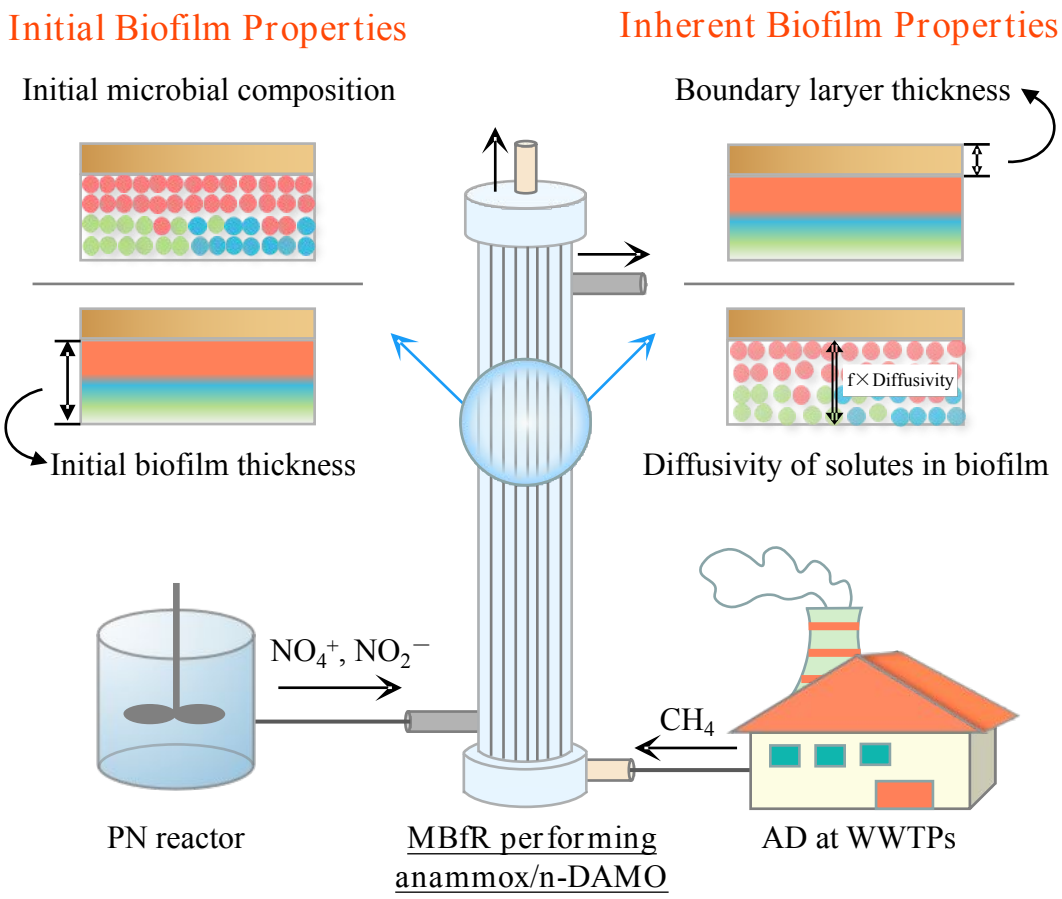
Synopsis: Impacts of key biofilm properties were investigated to obtain practical guidance for the rapid establishment of the MBfR with a high-level treatment capacity based on the anammox/n-DAMO integrated processes.

GRAPHICAL ART



ABSTRACT

Even though the membrane biofilm reactor (MBfR) performing the anammox and nitrate/nitrite-dependent anaerobic methane oxidation (n-DAMO) integrated processes has been known to enable complete nitrogen removal, the effects of key biofilm properties on such a promising MBfR are yet to be disclosed. In this work, a biofilm model was constructed to investigate the effects of the initial microbial composition of the biofilm, the initial biofilm thickness, the boundary layer thickness of the biofilm, and the diffusivity of solutes in the biofilm structure on the start-up process and the steady-state performance of the MBfR performing anammox/n-DAMO. The results showed that even though the four biofilm properties studied wouldn't affect the steady-state total nitrogen removal performance, they would significantly regulate the start-up time of the MBfR. Unless the MBfR was operated under undesired operational conditions (e.g., influent NH_4^+-N/NO_2^--N ratio of 2:3 in this work), inoculation of sludge comprised mainly of anammox bacteria or/and n-DAMO archaea to form a relatively thin initial biofilm would expectedly accelerate the start-up process of the MBfR. Moreover, measures could be taken during the operation to reduce the boundary layer thickness and the diffusivity of solutes in the biofilm structure, the latter of which would also enhance the methane utilization of the MBfR. This work would provide valuable practical guidance for the rapid establishment of the MBfR with a high-level treatment capacity based on the anammox/n-DAMO integrated processes.

Keywords: anammox; biofilm properties; membrane biofilm reactor (MBfR); modeling; nitrate/nitrite-dependent anaerobic methane oxidation (n-DAMO)

1. INTRODUCTION

Anammox is a sustainable biological nitrogen removal (BNR) technology without the need for organic carbon sources and aeration [1, 2]. Nevertheless, anammox will inevitably produce nitrate [3, 4] and thus ought to be coupled with other microbial processes to achieve complete nitrogen removal. The discovery of nitrate/nitrite-dependent anaerobic methane oxidation (n-DAMO) processes provides a crucial BNR alternative by adopting CH_4 as the electron donor [5, 6]. Therefore, the combination of anammox and n-DAMO processes would enable complete nitrogen removal by utilizing CH_4 produced directly on site of wastewater treatment plants [7, 8]. Considering the low solubility of methane (i.e., 22 mg CH_4 L^{-1} at 1 atm and 20.0 °C) [9] and the slow growth of both anammox and n-DAMO microorganisms [10, 11], the membrane biofilm reactor (MBfR) configuration was proposed and applied to take on this integrated BNR technology [12-20]. In such an MBfR, microorganisms attach onto the membrane surface and form a biofilm with methane diffusing from the membrane lumen to the biofilm and soluble substances provided (e.g., NH_4^+ and NO_2^-) entering the biofilm from the liquid bulk.

However, various factors could affect the performance of an MBfR and hence hinder its large-scale applications to the anammox/n-DAMO integrated processes. Therein lie the initial biofilm properties (e.g., the initial microbial composition of the biofilm and the initial biofilm thickness) and the inherent biofilm properties (e.g., the boundary layer thickness of the biofilm and the diffusivity of solutes in the biofilm structure)

which have been shown to influence the practical operation of biofilm-based reactors [21-24]. Firstly, changes in microbial composition during the operation have been demonstrated to exert a distinct influence on the performance of the MBfR [17], thus highlighting the potentially critical role of the initial microbial composition of the biofilm. Secondly, the initial biofilm thickness has been known as a key parameter regulating the mass transfer [25] and would therefore influence the substrates acquisition by microorganisms, the evolution of the microbial structure [26], and the start-up process of the MBfR. Thirdly, the boundary layer of the biofilm as an external resistance would influence bilateral solutes exchange, which might create discrepancies in both the removal of pollutants and the utilization of gas-phase substrates supplied to the MBfR [27, 28]. Lastly, the diffusivity of solutes in the biofilm structure would regulate the active transport of substrates inside the biofilm and hence the growth of functional microorganisms [29, 30]. Changes in the diffusivity of solutes in the biofilm structure might conduce to the varied performance of the MBfR reported by Khlebnikov, et al. [31]. However, the underlying impacts of the abovementioned biofilm properties on the MBfR performing anammox/n-DAMO in terms of the reactor start-up and the steady-state performance are yet to be disclosed and, without a doubt, require a significant amount of experimental input.

In this case, the application of model-based tools seems a reliable alternative. Similar efforts have been made to assess the feasibility of the MBfR performing anammox/n-DAMO [20, 32, 33] and the impacts of longitudinal heterogeneity [34] and operational

conditions [35] on such an MBfR. Through applying a previously-reported bioprocess model, this work further investigated the effects of biofilm properties (including the initial microbial composition of the biofilm, the initial biofilm thickness, the boundary layer thickness of the biofilm, and the diffusivity of solutes in the biofilm structure) on the MBfR performing the anammox/n-DAMO integrated processes. In addition to the total nitrogen (TN) removal and methane utilization efficiencies, the start-up time needed for the MBfR to reach the steady state was also included in the judging criteria since the typical operation of such an MBfR was featured with a long start-up process [12, 13, 16, 36]. The results obtained would expectedly provide important operational guidance for the rapid establishment of the MBfR with a high-level treatment capacity based on the coupling of anammox with n-DAMO.

2. MATERIALS AND METHODS

2.1. Bioprocess Model

The bioprocess model developed and validated experimentally by Chen, et al. [33] to describe microbial interactions between anammox bacteria and n-DAMO microorganisms was directly adopted in this work. As shown in **Tables S1** and **S2** in the supporting information (**SI**), the model involves four functional microorganisms (i.e., anammox bacteria (X_{An}), n-DAMO archaea (X_{Da}), n-DAMO bacteria (X_{Db}), and heterotrophic bacteria (X_{HB})) which are collectively responsible for the conversions of 6 soluble components (including ammonium (S_{NH4}), nitrite (S_{NO2}), nitrate (S_{NO3}), nitrogen gas (S_{N2}), methane (S_{CH4}), and readily biodegradable organics (S_S)) and 2 solid

components (i.e., slowly biodegradable biomass (X_S) and inert biomass (X_I)). Briefly, anammox bacteria can use ammonium as the electron donor to reduce nitrite and produce mainly nitrogen gas and some nitrate. With methane as the electron donor, n-DAMO archaea can reduce nitrate to nitrite while n-DAMO bacteria can further convert nitrite to nitrogen gas [5, 6]. In addition to conventional substrate-based Monod terms, the reported inhibition of NO_2^- on anammox bacteria and n-DAMO microorganisms are considered in the model, the details of which (i.e., values of stoichiometric and kinetic parameters) are summarized in **Table S3**.

2.2. Reactor Configuration

Referring to Chen, et al. [33], the total volume of the MBfR simulated in this work was 1.0 m^3, consisting of 0.04 m^3 membrane lumen and 0.96 m^3 biofilm reactor, while the total biofilm area was set at 235.0 m^2. A diffusive mode was used to link the biofilm reactor to the membrane lumen, which was assumed to be operated in an open-end mode to avoid the presence of gas back-diffusion. Variations in the methane supply were achieved by changing the methane flow rate at a constant gas pressure of 10 kPa. The counter-diffusional provision of the liquid-phase substrates (i.e., NH_4^+-N and NO_2^--N) and the gas-phase methane into the biofilm was driven by concentration gradients as described by Fick's law. As shown in **Table S4**, the mass transfer coefficient of methane from the membrane lumen to the biofilm and the substrate diffusion coefficients in water were adapted from Chen, et al. [33] and Haynes [37], respectively. The mass transfer resistance between the liquid phase and the biofilm

matrix was introduced by the boundary layer, while the mass transfer resistance from the membrane lumen to the biofilm was not specifically accounted for in this work [38]. Other operational parameters of the MBfR are listed in **Table S4**.

2.3. Simulation Scenarios

The model platform AQUASIM [39] with the implementation of the bioprocess mode introduced in **Section 2.1** was applied to study the effects of critical biofilm properties on the MBfR configured according to **Section 2.2**. The ideal operational conditions reported by Chen, et al. [33] and Chen, et al. [34] were applied to simulate the treatment of sidestream wastewater with an influent TN (i.e, the sum of NH_4^+-N and NO_2^--N) concentration of 500.0 g N m^{-3}, which included an influent ratio of NH_4^+-N to NO_2^--N of 1:1 and a CH_4 surface loading of 0.064 g COD m^{-2} d^{-1}. As shown in **Table 1**, scenarios 1 to 4 were designed to explore the effects of the initial microbial composition of the biofilm ($R_{An:Da:Db}$), the initial biofilm thickness (L_f), the boundary layer thickness of the biofilm (LBL), and the reduction factor for diffusivity of solutes in the biofilm structure (f, as compared with the diffusion coefficients of solutes in water), respectively, on the start-up time and the steady-state TN removal and methane utilization efficiencies of the MBfR performing anammox/n-DAMO. Scenarios 1 to 4 were also simulated under the operational conditions that deviated from the reported ideal ones to verify the general applicability of the results. As presented in **Table 1**, the influent ratio of NH_4^+-N to NO_2^--N was adjusted to 2:3 and 3:2 while the CH_4 surface loading was varied to 0.032 and 0.128 g COD m^{-2} d^{-1}. Each simulation scenario was

run to reach the steady state, and the time needed was recorded as the start-up time. The steady-state TN removal and CH_4 utilization efficiencies calculated by **Eqs. 1** and **2**, respectively, and the microbial composition of the biofilm together with the start-up time were used to comprehensively evaluate the impacts of the biofilm properties studied on the MBfR performing anammox/n-DAMO.

Table 1. Overview of simulation scenarios.

Scenarios	Biofilm properties	Substrates conditions
Scenario 1 Effect of initial microbial composition	$R_{An:Da:Db}$ = (0.05-20):(0.05-20):(0.05-20) L_f = 500 μm LBL = 40 μm f = 0.5	Influent NH_4^+-N:NO_2^--N = 1:1 (2:3 and 3:2) CH_4 surface loading = 0.064 g COD m^{-2} d^{-1} (0.032 and 0.128 g COD m^{-2} d^{-1})
Scenario 2 Effect of initial biofilm thickness	$R_{An:Da:Db}$ = 1:1:1 L_f = 50-500 μm LBL = 40 μm f = 0.5	
Scenario 3 Effect of boundary layer thickness	$R_{An:Da:Db}$ = 1:1:1 L_f = 500 μm LBL = 0-120 μm f = 0.5	
Scenario 4 Effect of reduction factor of diffusivity of solutes in biofilm structure	$R_{An:Da:Db}$ = 1:1:1 L_f = 500 μm LBL = 40 μm f = 0.2-0.8	

The substrates conditions inside brackets were used to verify the general applicability of the results obtained under ideal operational conditions outside brackets.

$$\text{TN removal efficiency} = \left(1 - \frac{S_{NH4,eff} + S_{NO2,eff} + S_{NO3,eff}}{S_{NH4,in} + S_{NO2,in}}\right) \times 100\% \quad \textbf{(Eq. 1)}$$

where $S_{NH4,in}$ and $S_{NO2,in}$ represent the influent concentrations of NH_4^+ and NO_2^- (g N m^{-3}), respectively; $S_{NH4,eff}$, $S_{NO2,eff}$ and $S_{NO3,eff}$ represent the effluent concentrations of

NH_4^+, NO_2^- and NO_3^- (g N m^{-3}), respectively.

$$\text{CH}_4 \text{ utilization efficiency} = \left(1 - \frac{\text{CH}_{4,\text{ out}}}{\text{CH}_{4,\text{ loading}} - \text{CH}_{4,\text{ gas}}}\right) \times 100\% \quad (\textbf{Eq. 2})$$

where $CH_{4,loading}$ is the CH_4 loading into the membrane lumen (g COD m^{-2} d^{-1}); $CH_{4,gas}$ is the CH_4 loading leaving the membrane lumen (g COD m^{-2} d^{-1}); $CH_{4,out}$ is the dissolved CH_4 loading in the effluent (g COD m^{-2} d^{-1}).

3. RESULTS AND DISCUSSION

3.1. Effects of Properties of Initial Biofilm

3.1.1 Initial microbial composition of biofilm (Scenario 1)

As shown in **Fig. 1** and **Table S5**, changes in the initial microbial composition of the biofilm were found to not affect the steady-state biomass composition (mainly with anammox bacteria (74.4%) and n-DAMO archaea (15.2%)), the TN removal efficiency (98.8%), and the CH_4 utilization efficiency (85.2%) under the reported ideal operational conditions, but exert a significant influence on the start-up time required for the MBfR to achieve the steady-state operation. To be specific, with the increasing initial proportion of anammox bacteria or n-DAMO archaea in the biofilm, the MBfR could reach the steady state faster which was due to the dominance of anammox bacteria and n-DAMO archaea at the steady state and their close cooperation for complete nitrogen removal. Comparatively, the initial proportion of n-DAMO archaea was found to play a more profound role in regulating the start-up process of the MBfR: the start-up time was significantly shortened from 789 to 67 days when the initial X_{Da}:X_{An}:X_{Db} ratio was

elevated from 0.05:1:1 to 20:1:1. This phenomenon resulted from the essentially inferior and slow growth of n-DAMO archaea which depended on the functioning of anammox bacteria to produce nitrate. Analogously, the increase in the initial proportion of n-DAMO bacteria in the biofilm prolonged the start-up time since it hindered the growth of anammox bacteria and n-DAMO archaea through substrate competition and therefore requested more time to wash n-DAMO bacteria out of the biofilm.

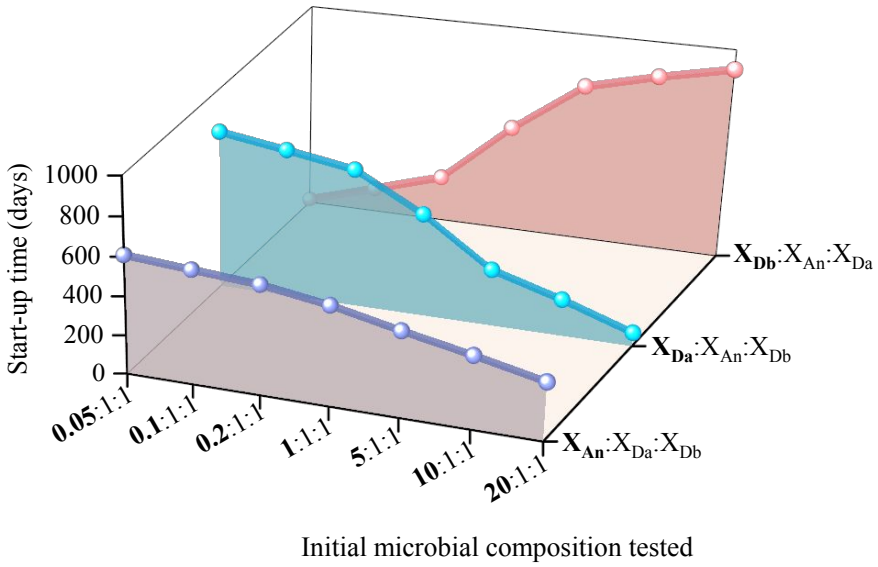


Figure 1. Effect of the initial microbial composition (X_{An}:X_{Da}:X_{Db}) of the biofilm on the time needed for the MBfR to reach the steady state at influent NH_4^+-N/NO_2^--N ratio of 1:1 and CH_4 surface loading of 0.064 g COD m^{-2} d^{-1}.

To verify the general applicability of the above findings, the simulations were repeated under different substrates conditions, i.e., influent NH_4^+-N/NO_2^--N ratios of 2:3 and 3:2 and CH_4 surface loadings of 0.032 and 0.128 g COD m^{-2} d^{-1}, as shown in **Table 1**. Despite the overall distinct TN removal and CH_4 utilization efficiencies obtained under

different operational conditions, the initial microbial composition of the biofilm did not affect the steady-state TN removal and methane utilization efficiencies (see **Table S5**) but posed a significant impact on the start-up time, as presented in **Fig. 2**. It could be observed that when the methane loading was halved (0.032 g COD m^{-2} d^{-1}) or doubled (0.128 g COD m^{-2} d^{-1}), increasing the initial proportion of anammox bacteria or n-DAMO archaea would generally facilitate the start-up process of the MBfR, while an opposite trend was observed for the initial proportion of n-DAMO bacteria in the biofilm (**Fig. 2A-C**). As shown in **Fig. 2D-F**, an inconsistent relationship was observed at the influent NH_4^+-N/NO_2^--N ratio of 2:3. This phenomenon was due to the completely different microbial composition (i.e., the coexistence of anammox bacteria and n-DAMO bacteria without n-DAMO archaea) for ultimately incomplete nitrogen removal in the MBfR at the steady state (see **Table S5**).

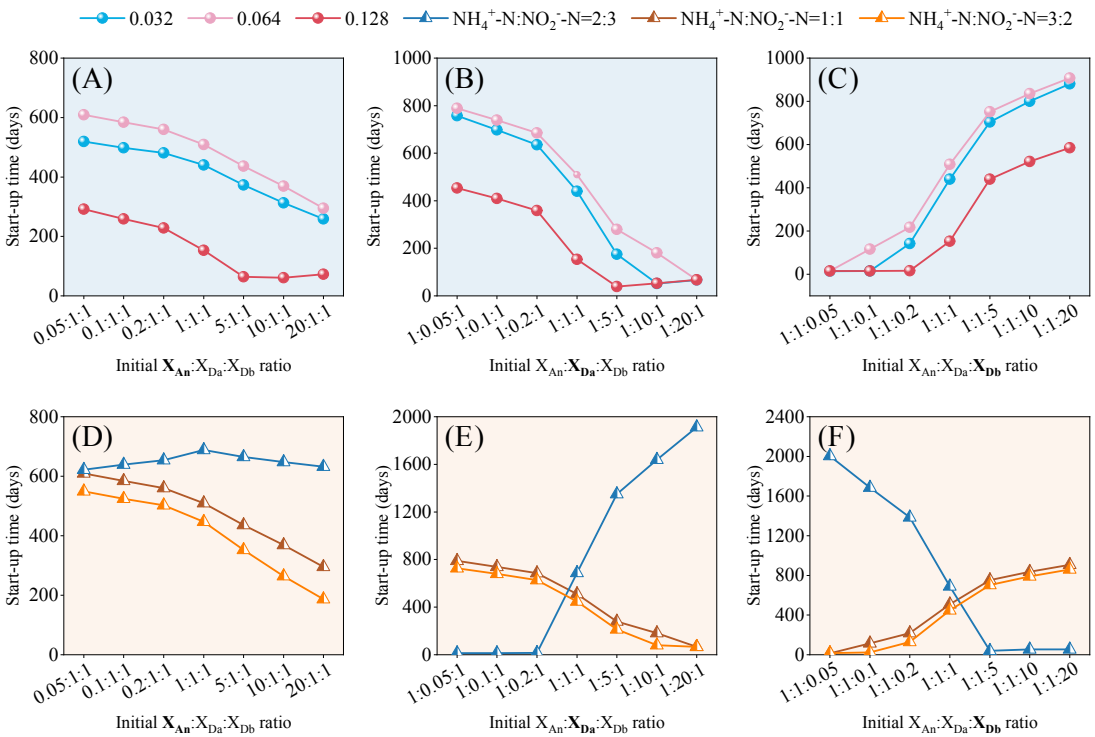


Figure 2. Effect of the initial microbial composition of the biofilm on the time needed for the MBfR to reach the steady state at CH_4 surface loadings of (**A**) 0.032, (**B**) 0.064 and (**C**) 0.128 g COD m^{-2} d^{-1}, and influent NH_4^+-N/NO_2^--N ratios of (**D**) 2:3, (**E**) 1:1 and (**F**) 3:2.

3.1.2. Initial biofilm thickness (Scenario 2)

Similarly, when the MBfR was operated under the reported ideal conditions, changes in the initial biofilm thickness did not affect the steady-state biomass composition, with anammox bacteria and n-DAMO archaea each accounting for 74.4% and 15.2% of the total biomass, respectively, thus leading to the fixed TN removal efficiency of 98.8% and CH_4 utilization efficiency of 85.2% in **Fig. 3A**. However, with the increase in the initial biofilm thickness from 50.0 to 500.0 μm, the start-up time needed for the MBfR to reach the stable coupling of anammox with n-DAMO was prolonged from 371 to 509 days (see **Fig. 3A**). Such an observation resulted from the intensified microbial competition for both the limited supply of substrates (i.e., NH_4^+-N, NO_2^--N and CH_4) and the compressed growth space brought about by the elevated initial biofilm thickness, thus extending the time needed for the biofilm to develop towards the steady state.

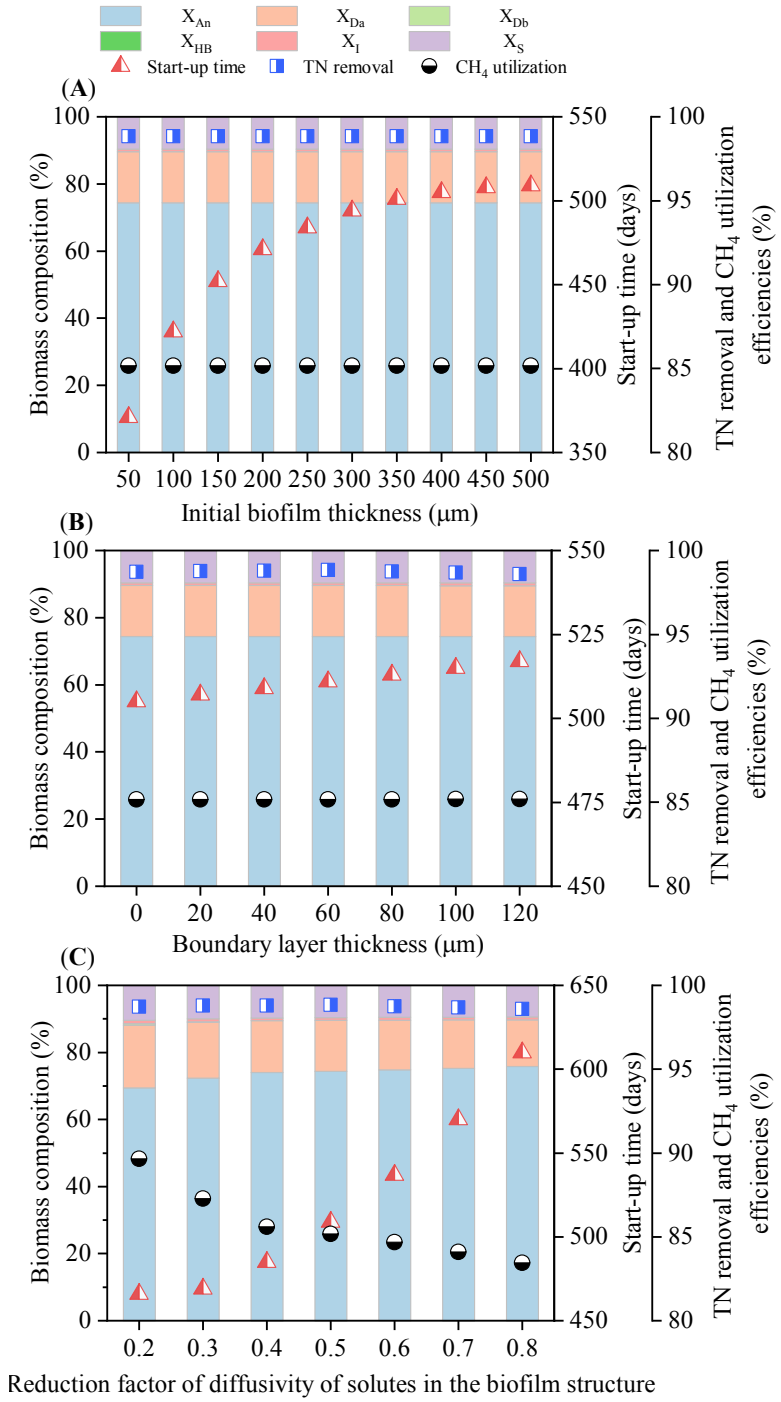


Figure 3. Effects of (**A**) the initial biofilm thickness, (**B**) boundary layer thickness and (**C**) reduction factor of diffusivity of solutes in the biofilm structure on the steady-state microbial composition, TN removal efficiency and CH_4 utilization efficiency, and the time needed for the MBfR to reach the steady state at influent NH_4^+-N/NO_2^--N ratio of 1:1 and CH_4 surface loading of 0.064 g COD m^{-2} d^{-1}.

To further test the impact of the initial biofilm thickness on the MBfR, different influent NH_4^+-N/NO_2^--N ratios of 2:3 and 3:2 and CH_4 surface loadings of 0.032 and 0.0128 g COD m^{-2} d^{-1} were simulated. Regardless of the distinct results obtained under different operational conditions, similar trends that the steady-state microbial composition, TN removal efficiency, and CH_4 utilization efficiency were not sensitive to changes in the initial biofilm thickness were obtained. While the positive relationship between the start-up time and the initial biofilm thickness was also noted at the influent NH_4^+-N/NO_2^--N ratios of 2:3 and 3:2 (see **Fig. 4A**), a different trend was observed at the excessive CH_4 surface loading of 0.128 g COD m^{-2} d^{-1} (see **Fig. 4B**), which led to the undesired coexistence of n-DAMO archaea and bacteria in the biofilm at the steady state (see **Table S6**).

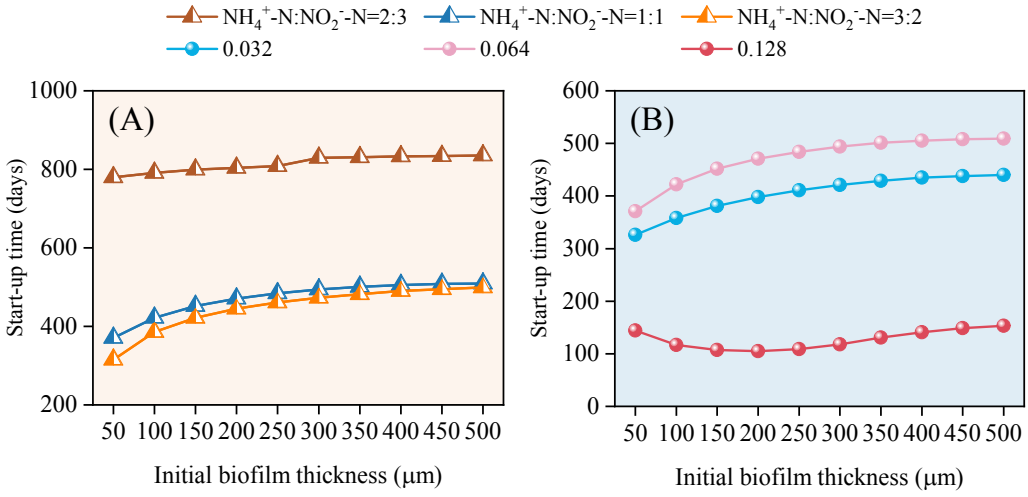


Figure 4. Effect of the initial biofilm thickness on the start-up time of the MBfR at (**A**) influent NH_4^+-N/NO_2^--N ratios of 2:3, 1:1 and 3:2, and (**B**) methane surface loadings of 0.032, 0.064 and 0.128 g COD m^{-2} d^{-1}.

3.2. Effects of Inherent Biofilm Properties

3.2.1 Boundary layer thickness (Scenario 3)

Based on the simulation results obtained under the reported ideal operational conditions, the boundary layer thickness was found to have no obvious effect on the biomass composition of the steady-state MBfR (**Fig. 3B**) with functional microorganisms being anammox bacteria (74.4%) and n-DAMO archaea (15.2%). In addition to the nearly unchanged TN removal efficiency of >98.0%, the resultant methane utilization efficiency stabilized at about 85.1%, which was different from the positive contribution of a boundary layer to gas utilization proposed by Martin and Nerenberg [38] and Nerenberg [40]. The same steady-state biomass composition contributing to the consumption of methane inside the biofilm minimized the role of the boundary layer in resisting the loss of methane to the bulk liquid. Comparatively, the time needed for the MBfR to reach the steady state slightly increased from 505 to 517 days with the boundary layer thickening from 0 to 120 μm. This might be due to the elevated resistance of soluble substrates diffusing from the bulk liquid to the biofilm [28], thus delaying the start-up process of the MBfR. Consistent results could be observed at varied substrate conditions that were tested in this work (see **Fig. S1**).

3.2.2 Diffusivity of solutes in biofilm structure (Scenario 4)

As exhibited in **Fig. 3C**, under the reported ideal operational conditions, changes in the diffusivity of solutes in the biofilm structure had no apparent effect on the TN removal

efficiency which stayed around 98.8% but played a noticeable role in the steady-state microbial composition and CH_4 utilization efficiency and the start-up time. With the rising diffusivity of solutes in the biofilm structure by increasing the reduction factor of diffusion coefficients from 0.2 to 0.8, the proportion of anammox bacteria increased gradually from 69.5% to 75.8% (**Fig. 3C**), due to the elevated fluxes of NH_4^+ and NO_2^- as the substrates for anammox bacteria from the bulk liquid into the biofilm. Meanwhile, the increase in diffusion coefficients accelerated the passing of methane and enhance its penetration through the biofilm, thereby reducing the methane utilization efficiency from 89.6% to 83.4% (**Fig. 3C**) and the associated proportion of n-DAMO archaea. In terms of the start-up time, a significant change from 466 to 610 days was observed with the increase in diffusion coefficients of solutes in the biofilm structure. This phenomenon was potentially attributed to the shortened effective time of contact between microorganisms and substrates caused by rapid diffusion, hence extending the time needed for microorganisms to grow towards the state steady.

The same operation under different substrate conditions rendered comparable results (see **Fig. 5** and **Fig. S2**). Although the steady-state microbial composition differed, the changing trend was the same: with the rise in the reduction factor of diffusivity in the biofilm structure, the proportion of anammox bacteria increased while those of n-DAMO microorganisms decreased (see **Fig. S2**). As exhibited in **Fig.** 5, the overall trends of the TN removal efficiency, CH_4 utilization efficiency, and start-up time were similar to those obtained under ideal conditions: in addition to the nearly flat TN

removal efficiency, the methane utilization efficiency dropped while the start-up time extended with the increasing reduction factor of diffusivity in the biofilm structure.

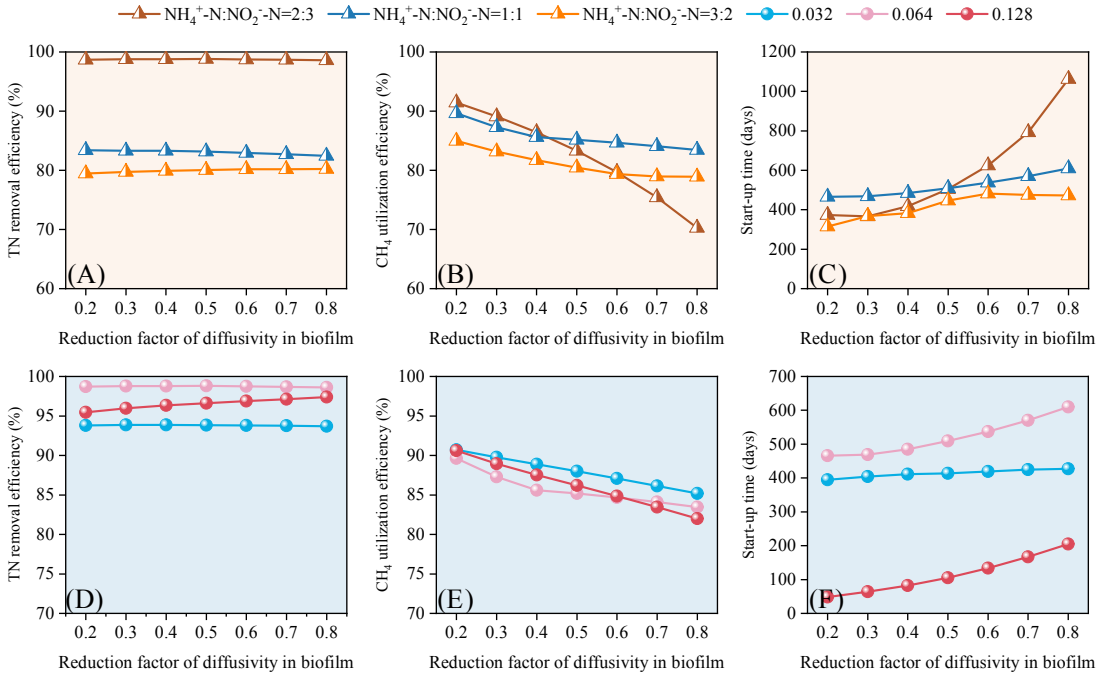


Figure 5. Effect of the reduction factor of diffusivity in the biofilm structure on the steady-state TN removal efficiency and CH_4 utilization efficiency, and the start-up time of the MBfR at influent ratios of NH_4^+-N to NO_2^--N of (**A**) 2:3, (**B**) 1:1 and (**C**) 3:2, and CH_4 surface loadings of (**D**) 0.032, (**E**) 0.064 and (**F**) 0.128 g COD m^{-2} d^{-1}.

3.3. Practical Implications of This Work

Although both experimental and modeling studies of the MBfR performing the anammox/n-DMO integrated processes have been reported [8, 20, 32, 34, 35], this work for the first time comprehensively explored the effects of key biofilm properties of on the start-up process and steady-state performance of such an MBfR. Through testing various operational conditions, the general applicability of the observations was

verified. The findings of this work would provide important guidance for improving the operation of the MBfR, although its validity might be improved by further experimentation.

The aforementioned simulations demonstrated that the properties of the biofilm to initiate the MBfR including the initial microbial composition and the initial biofilm thickness generally would not affect the steady-state microbial composition, TN removal, and CH_4 utilization of the MBfR performing the anammox/n-DAMO integrated processes. However, it has been observed from practical operations that MBfRs performing anammox/n-DAMO experienced different start-up times [16, 36, 41], where the contents of anammox bacteria and n-DAMO archaea in the inoculated sludge to form the initial biofilm would be an influencing factor. This work further demonstrated this point by clearly revealing the possibility of significantly accelerating the start-up process of the MBfR performing the anammox/n-DAMO integrated processes upon the availability of an initial biofilm comprised mainly of anammox bacteria or/and n-DAMO archaea, with the latter presenting a more profound advantage. Another important finding of this work when simulating the effects of initial biofilm properties was that more inoculated sludge (i.e., higher initial biofilm thickness) wasn't necessarily better for starting the MBfR. The mass transfer of substrates would drop with the increasing biofilm thickness [42, 43] and thus slow down the valid growth of microorganisms [44]. Therefore, selecting a relatively thin initial biofilm might be conducive to high substrate transport rates [43] and hence shorten the start-up time of the

MBfR performing anammox/n-DAMO.

The inherent properties of the biofilm including the boundary layer thickness and the diffusivity of solutes in the biofilm structure were noted to play minor roles in the steady-state results, which were consistent with a previous study on an autotrophic deammoniafication-based MBfR supplied with air [29]. Compared with the boundary layer thickness, the diffusivity of solutes in the biofilm structure was found to exert a more profound impact on the start-up time of the MBfR. Therefore, keeping a thin boundary thickness and low diffusivity of solutes in the biofilm structure would be conducive to achieving complete TN removal and high-level CH_4 utilization whilst shortening the start-up time process (see **Fig. 3B-C**). Previous studies proposed that internal circulation of the liquid phase at a proper flow rate could be set to weaken the boundary layer whilst compacting the biofilm structure [45, 46]. It needs to be mentioned that the diffusivity of solutes was assumed to be homogeneous inside the biofilm in this work, while it might change with the thickness/depth in practice [47]. Therefore, it warrants continuous research to explore the influence of heterogeneous diffusivity of solutes in the biofilm structure on the MBfR performing anammox/n-DAMO.

Noticeably, the abovementioned general findings regarding the critical role of proper selection of inoculated sludge as the initial biofilm in the start-up process of the MBfR performing anammox/n-DAMO might not be necessarily suitable for the MBfR operated under undesired operational conditions of i) undue upstream partial nitritation

process (e.g., producing influent NH_4^+-N/NO_2^--N ratio of 2:3 studied in this work) and ii) excessive methane supply (e.g., CH_4 surface loading of 0.128 g COD m^{-2} d^{-1} studied in this work). These operational conditions would promote the growth of n-DAMO bacteria as a major contributor to nitrogen transformation. Consistent with Chen, et al. [34], the such non-ideal microbial composition would not be in favor of realizing complete nitrogen removal in the MBfR performing anammox/n-DAMO processes.

4. CONCLUSIONS

A one-dimensional counter-diffusional multi-species and multi-substrates biofilm model was constructed to simulate the impacts of key biofilm properties on the MBfR performing anammox/n-DAMO. This work showed that even though the four biofilm properties studied wouldn't affect the steady-state total nitrogen removal performance, they would significantly regulate the start-up time of the MBfR. Unless the MBfR was operated under undesired operational conditions (e.g., influent NH_4^+-N/NO_2^--N ratio of 2:3 in this work), inoculation of sludge comprised mainly of anammox bacteria or/and n-DAMO archaea to form a relatively thin initial biofilm would expectedly accelerate the start-up process of the MBfR. Moreover, sufficient internal circulation in the liquid phase could be set during the operation to reduce the boundary layer thickness and the diffusivity of solutes in the biofilm structure, the latter of which would also enhance the methane utilization of the MBfR. This work would provide practical guidance for the rapid establishment of the MBfR with a high-level treatment capacity based on the anammox/n-DAMO integrated processes.

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