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Nitrogen Removal by Coupling PN-Anammox-DAMO in Membrane Biofilm Reactor (MBfR) Systems

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Abstract Because of its economic benefits, the combined partial nitritation anaerobic ammonium oxidation (PN-Anammox) process is increasingly adopted/recommended for efficient nitrogen removal, despite its application limitations such as the concomitant nitrate production. The discovery of denitrifying anaerobic methane oxidation (DAMO) processes offers a potential solution to overcome the limitations of the PN-Anammox process and enables complete/high-level nitrogen removal through coupling PN-Anammox-DAMO, which could be favorably supported in membrane biofilm reactor (MBfR) systems. This paper aims to understand / evaluate the novel MBfR-based complete/high-level nitrogen removal technology with focus on the system performance and microbial interactions under different conditions of operations and reactor configurations.

Keywords MBfR**,** DAMO**,** PN-Anammox**,** Nitrite, Nitrate

Introduction

The anaerobic ammonium oxidation (Anammox) process is an energy-efficient and economical nitrogen removal process, as it does not require organic carbon or aeration, and produces much less sludge in comparison to the conventional processes [1-3]. Recently, the combined nitritation and Anammox process has been regarded as one of the most efficient ways for autotrophic nitrogen removal. In this process, ammonium is aerobically oxidized to nitrite partially by ammonia-oxidizing bacteria (AOB), which is then an aerobically reduced to dinitrogen gas with the remaining ammonium as electron donor by Anammox bacteria. However, this process is not able to remove nitrate present in the wastewater, and in fact converts part (20%) of the nitrite to nitrate (see Equation 1) [4]. Thus, high-level nitrogen removal may not be achieved although the produced nitrate load would be relatively small compared to the overall nitrogen load to the plant.

$$1.32NO_2^- + 1NH^+_4 + 0.066HCO^-_3 + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O + 0.066CH_2O_{0.5}N_{0.15} \quad (1)$$

The discovery of denitrifying anaerobic methane oxidation (DAMO) processes, in which methane is oxidized an aerobically to provide electrons for denitrification [5], provides a potential solution to this problem. Previous work has confirmed that DAMO archaea can reduce nitrate to nitrite using electrons derived from methane oxidation (Equation 2) [6] while DAMO bacteria can convert nitrite to dinitrogen gas also with methane as the electron donor (Equation 3) [8]. Methane is an inexpensive, widely available carbon source as compared to other electron donors such as methanol and ethanol. It can be generated on site with proper security measures implemented at a wastewater treatment plant (WWTP) through an aerobic sludge digestion [8-11]. Thus, the DAMO processes could be potentially coupled to the previous nitritation-Anammox to form a new nitrogen removal process [2]. The partial

nitritation would produce a mixture of ammonium and nitrite as the feed for the Anammox reactor. Methane can be produced through an aerobic digestion in the treatment plant, and a small fraction could be fed to the Anammox reactor to support the growth of DAMO micro-organisms thus achieving the removal of nitrate produced by Anammox (see Equation 2,3).

$NO^-_3 + 2/8CH_4 \rightarrow NO^-_2 + 2/8CO_2 + 4/8H_2O$ (2)

$NO^-_2 + 3/8CH_4 + H^+ \rightarrow 1/2N_2 + 3/8CO_2 + 10/8H_2O$ (3)

In our previous work [12]**,** a biofilm model integrating Anammox and DAMO processes was developed, calibrated and validated using experimental data from a lab-scale MBfR fed with nitrate and ammonium (without nitrite accumulation in the system). The effects of NO_2/NH_4þ ratio and CH_4 surface loading on the system performance were preliminarily investigated individually using the model. However, the capability of the model to describe the system with the presence of nitrite and hence possible nitrite inhibition remained not assessed. Through adjusting the feeding NO_2 concentration whilst keeping a constant NH_4þ concentration in Chen *et al* [2]**,** the obtained results in terms of the effect of influent NO_2/NH_4þ ratio were not completely separated from that of total nitrogen (sum of $NO_2$and NH_4þ) loading. The influence of CH_4 surface loading was also not explore data NO_2/NH_4þ ratio of 1.32, which was not able to clearly reveal the contribution of DAMO microorganisms other than Anammox bacteria to the overall nitrogen removal. Moreover, the combined effects of NO_2/NH_4þ ratio and CH_4 surface loading on the system performance haven't been investigated. The impacts of other important system parameters (e.g., total nitrogen loading and biofilm thickness) were also not clear. Therefore, the mathematical model was further extended and applied in this work to investigate the development of microbial community consisting of Anammox bacteria, DAMO archaea and DAMO bacteria in the MBfR under different operational conditions.

The validity of the model describing the system with the presence of nitrite and hence possible nitrite inhibition was evaluated by using batch experimental data from a complex MBfR system containing an Anammox-DAMO biofilm with different feeding nitrogen compositions. Through changing one parameter whilst keeping others fixed in the model, the separate effects of nitritation produced NO_2/NH_4þ ratio, methane supply and biofilm thickness were investigated. The combined effects of nitritation produced NO_2/NH_4þ ratio, methane supply and total nitrogen (TN) surface loading on the system performance and microbial community structure were then studied with the model. Finally, the proof-of-concept feasibility of a single-stage MBfR coupling nitritation-Anammox-DAMO for complete nitrogen removal was tested through integrating the model with AOB and nitrite-oxidizing bacteria (NOB) processes whilst controlling the dissolved oxygen (DO) concentration in the simulated system.

Material and Methods

MBfR Operation and Batch Experimental Data for Model Evaluation

The total volume of the experimental MBfR was 1150 mL, including 400 mL of hollow fiber membranes, 300 mL interior space for gas supply, and 450 mL external space outside the membranes for completely mixed liquid. In order to provide methane, a feeding gas cylinder was connected to the interior of the hollow fiber membranes, which had a total surface area of 1 m^2. The liquid was continuous lyre circulated through an overflow bottle with 150 mL in liquid volume and 180 mL as head space, which was also used for liquid and gas sampling. The MBfR was operated in a temperature-monitored laboratory at 22-2 ºC, with pH controlled at 7.0-0.2. The feeding synthetic wastewater of the MBfR contained nitrate and ammonium as substrates at 200–600 mg N L^1 and 200–300 mgNL^1, respectively. The methane flux into the MBfR was maintained at around 1105 m^3h^1 through adjusting the gas pressure on a gas regulator. More information of the reactor setup and wastewater composition can be found in reported literature [6]. After setup, the MBfR was operated as a sequential batch reactor (SBR) with a cycle time of 1 day. At the beginning of each cycle, 150 mL fresh medium was fed within 5 min, thus leading to a hydraulic retention time (HRT) of 3 days. During the SBR operation of the MBfR, the inner hollow fiber membranes were constantly connected to the gas cylinder, with the pressure maintained at 1.3 atm. The feeding ammonium and nitrate concentrations were step wise increased with the improvement of the MBfR performance. After over 400-day SBR operation of the MBfR, fluorescent in sit hybridization (FISH) results and reactor performance confirmed the

coexistence and joint dominance of DAMO archaea, DAMO bacteria and Anammox bacteria in the biofilm [6]. Six Batch tests A, B, C, D, E, and F were then conducted to investigate the capability of this Anammox-DAMO biofilm system for treating wastewaters with different nitrogen compositions, that is, at different initial ratios between ammonium, nitrate and/or nitrite. At the start of each test, fresh medium was fed into the reactor. Concentrated stock solutions were then added, giving rise to an initial ammonium, nitrate and/or nitrite concentration between 30–200 mg N L^1. Methane was supplied through the hollow fibres in all tests by maintaining a pressure of 1.3 atm. During each test, liquid samples were taken to determine the consumption rates of the added nitrogen substrates. The ammonium, nitrate and nitrite concentrations were measured using a Quik Chem 8000 Flow injection Analyzer**. T**he model has been tested using the long-term (over 400 days) dynamic experimental data from the MBfR fed with nitrate and ammonium as substrates (without detectable nitrite accumulation in the system) [2]. In this work, we further tested the capability of the model extended with the nitrite inhibition terms to describe the system under different feeding conditions, particularly with the presence of nitrite.

Simulation Strategies

Steady-state model simulations were then performed under different operational conditions to evaluate the implementation of the partial nitritation followed by the Anammox-DAMO biofilm system (MBfR). The effect/control of the partial nitritation was investigated through changing the feeding nitrogen composition to the MBfR in terms of NO_2/NH_4þ ratio produced from the pre-nitritation. Simulations were run to reach steady-state conditions indicated by constant (less than 0.05% change in 200 days) effluent concentrations, biomass compositions in biofilm and biofilm thickness. Six different scenarios are considered in this work. The first simulation scenario was performed under conditions of an influent TN concentration of 500 g m^3 and a nitritation produced NO_2/NH_4þ ratio of 1.32 which is generally applied for the Anammox process [13]. The applied TN surface loading (LTN), methane surface loading (LCH_4) and biofilm thickness (Lf) were 0.68 g N m^2d^1, 0.062 g $m^2d^1$and 1,000 mm, respectively. The depth profiles of substrate sand microbial community distribution in the MBfR biofilm were generated to provide insights into the mechanisms behind the process performance. The parameter combinations were chosen systematically over wide ranges of NO_2/NH_4þ ratio (0–4), LCH_4 (0–0.250 g m2d1), Lf (200–1,400mm) and LTN (0.41–0.95 g Nm^2d^1). Finally, the proof-of-concept feasibility of a single-stage MBfR coupling nitritation-Anammox-DAMO in one biofilm system for efficient nitrogen removal was tested through controlling the DO concentration of the system in the model. The effect of the DO concentration in the bulk liquid phase of the MBfR on the microbial interactions between AOB, NOB, Anammox bacteria, DAMO archaea, and DAMO bacteria in the biofilm was then analyzed.

Results and Discussion

Model Evaluation with Experimental Data

Model and parameters evaluation was based on the comparison between the model predictions using the parameter values and the six sets of independent experimental data collected from batch tests A–F under different initial wastewater conditions using the MBfR system containing an Anammox-DAMO biofilm. The model evaluation results of the six different batch experiments are shown in Fig. 1, as well as the concentrations measured in the experimental MBfR. Conversion rates measured in the experiments were compared to the ammonium and nitrite consumption and to the ammonium and nitrate utilization rates obtained from the model. The nitrogen conversion rates resulting from the model in the six batch tests did not differ with more than 10% from the values found in the experiments. The good agreement between simulations and the measured results in this work supported the validity of the extended model structure and parameters (especially the newly-added nitrite inhibition terms for Anammox and DAMO microorganisms) to describe the nitrogen conversion processes in the MBfR with the coexistence of DAMO archaea, DAMO bacteria and Anammox bacteria in the biofilm, which also confirmed that the addition of the nitrite inhibition terms would not affect the established parameters in Chen et al [2]. The good model predictions were likely due to the previously well-calibrated parameter values of DAMO processes and the well-structured model processes as well as the accurate initial conditions used. The model evaluation results in Fig 1 also confirmed

that the model is capable of describing the Anammox-DAMO biofilm systems under different influent nitrogen composition conditions.

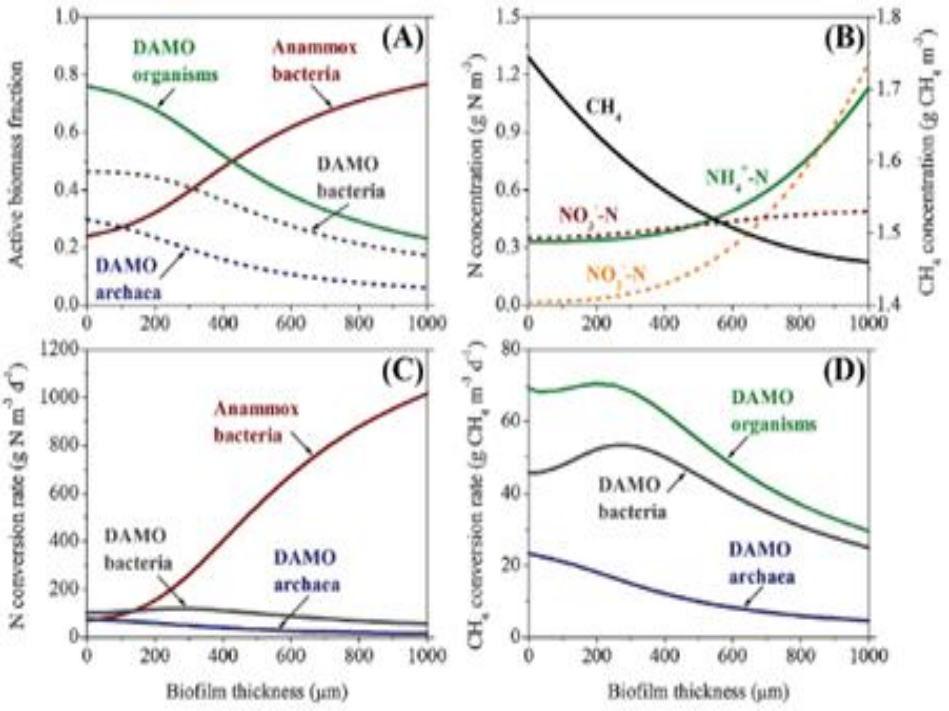

Figure 1: Model simulation results of the Anammox – DAMO system (depth zero represents the membrane surface at the base of the biofilm): (A) Microbial population distribution, (B) substrate profiles, (C) species – specific nitrogen conversion rates, and (D) species – specific methane conversion rates. The applied TN surface loading (LCH_4) and biofilm thickness (L_F) were 0.68 g Nm^{-2} d^{-1}, 1.32, 0.062 g m^{-2} d^{-1} and 1.000 μm respectively

Key Factors Affecting the Anammox-DAMO Biofilm System

Steady-state microbial population distribution and the concentration profiles of NH_4þ, NO_2, NO_3, and CH_4 as well as the species-specific nitrogen turn over rates within the Anammox-DAMO biofilm in the MBfR under the operational as shown in Fig. 2. The abundance of Anammox organisms reached up to 77% at the biofilm surface and decreased gradually to 24% at the base of biofilm. In contrast, the abundance of DAMO organisms was around 23% (including 6% for DAMO archaea and 17% for DAMO bacteria) at the biofilm surface and increased to 76% (including 30% for DAMO archaea and 46%for DAMO bacteria) at the base of biofilm . Both NH_4þ and NO_2 concentrations decreased from the surface to the base of the biofilm. However, NO_2 showed a higher decreasing rate due to the NO_2 consumption by both DAMO bacteria and Anammox bacteria while ammonium was mainly utilized by Anammox bacteria. Nitrate produced by Anammoxs lightly decreased from the surface to the base of the biofilm due to its reduction by DAMO archaea. In contrast, CH_4 gradually decreased from the base to the surface of the biofilm as the result of the consumption by DAMO microorganisms. The concentration gradients of methane, ammonium, nitrite and nitrate resulted in the stratification of the Anammox-DAMO biofilm in the MBfR. DAMO archaea and DAMO bacteria attach close to the membrane surface (dominant at the biofilm base from 0mm to 400mm), where methane, nitrite and nitrate are available with the nitrate produced by Anammox bacteria, while Anammox bacteria mainly grow in the biofilm layer close to the bulk liquid (dominant at the outer layer from 400mm to 1,000mm) where ammonium and nitrite are available. The steady state TN removal efficiency was up to 99.4%. Anammox bacteria, DAMO archaea, and DAMO bacteria approximately accounted for around 80, 6, and 14% of the total nitrogen turn

over, respectively. Methane utilization prevailed over the entire biofilm range, with DAMO archaea and DAMO bacteria accounting for about 22 and 78% of the methane converted, respectively. Therefore, Anammox bacteria are the key contributor to the overall nitrogen turnover, while DAMO archaea and DAMO bacteria also play significant roles in achieving complete nitrogen removal in such systems.

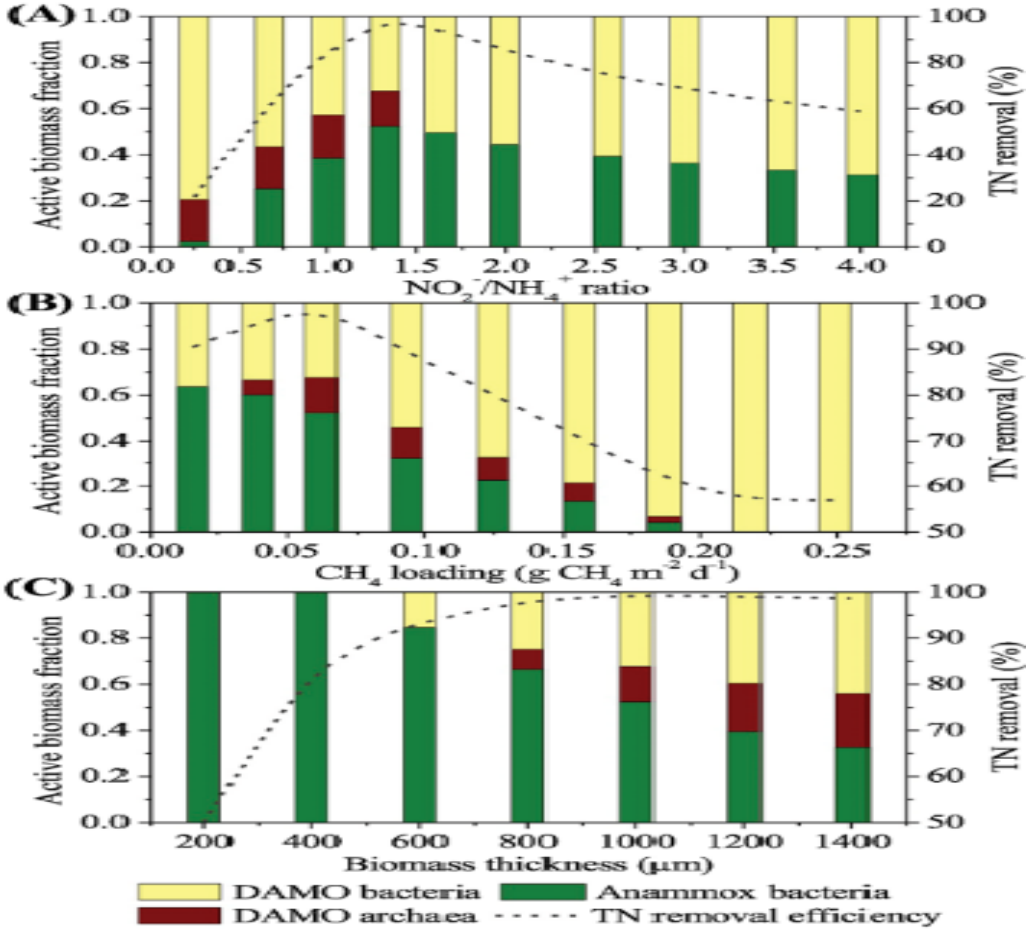

Figure 2: Model simulation results of the Anammox – DAMO biofilm system from Scenarias 1-3 in Table 1 : (A) Effect of the NO_2^- / NH_4^+ ratio (B) Effect of CH_4 loading , and (C) Effect of biofilm thickness on TN removal efficiency and microbial community structure of the biofilm

The impact of the potential NO_2/NH_4þ ratio produced from partial nitritation on the TN removal and the microbial abundance in the following MBfR with Anammox-DAMO biofilm is shown in Fig. 3. The TN removal efficiency was only 21.7% at the nitritation produced NO_2/NH_4þ ratio of 0.25, where as it increased to the maximum of 99.4% at the NO_2/NH_4þ ratio of 1.32. Further increasing the NO_2/NH_4þ ratio would result in the decrease of the TN removal efficiency, reaching 58.8% at the NO_2/NH_4þ ratio of 4. The variation of TN removal performance was mainly due to the changing microbial structure under different NO_2/NH_4þ ratio conditions. With the increase of NO_2/NH_4þ ratio to 1.32, the fraction of Anammox bacteria increased while that of DAMO bacteria decreased due to the increasing NO_2 availability. In contrast, the fraction of DAMO archaea kept at around 18%. At the NO_2/NH_4þ ratio of 1.32 with the highest TN removal efficiency, Anammox bacteria and DAMO microorganisms would coexist and indeed jointly dominate the biofilm, with the active biomass fractions of 53, 15, and 32% for Anammox bacteria, DAMO archaea, and DAMO bacteria, respectively. Further increase of the NO_2/NH_4þ ratio would largely decrease the fraction of DAMO archaea and increase the fraction of DAMO bacteria due to the competition between DAMO archaea and bacteria for methane. The increase of LCH_4 to 0.062 g m^2d^1 would increase the abundance of

DAMO archaea from 0% to 15% while that of DAMO bacteria would slightly decrease from 36% to 32% and that of Anammox bacteria would drop from 64% to 53%. Correspondingly, the TN removal efficiency increased from 90.4% to the maximum of 99.4% at LCH_4 of 0.062 g m^2d^1. Further increase in LCH_4 would favor the growth of DAMO bacteria to a great extent, and thus Anammox bacteria were gradually out competed due to the low availability of nitrite. The fraction of DAMO bacteria would therefore increase continuously while those of Anammox bacteria and DAMO archaea would decrease significantly at LCH_4 of 0.218 g m^2d^1, resulting in a decreased TN removal efficiency down to 56.9%. These results indicated the significance of the methane supply in regulating the performance and microbial structure of the Anammox-DAMO biofilm. Although an energy source that can be harvested on site of the treatment plants, methane is a potent green house gas with a warming potential more than 25 times stronger than carbon dioxide (CO_2) [14]. In general, the increasing of Lf would increase TN removal efficiency with the optimal TN removal of over 99.0% at a steady-state thickness of more than 1,000mm under the simulated conditions.

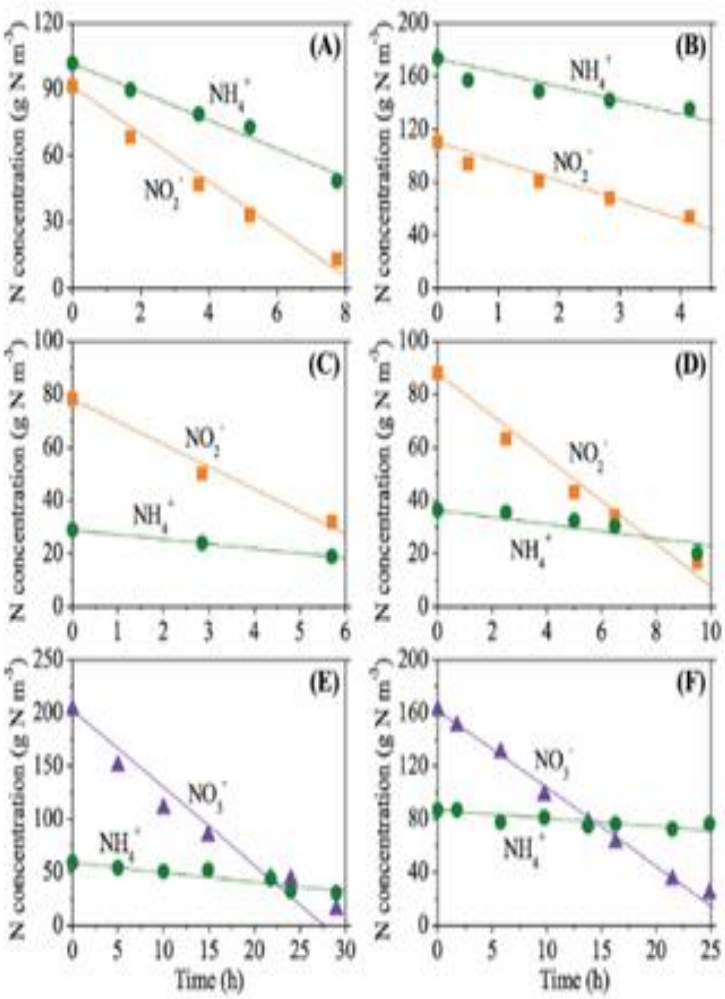

Figure 3: Model evaluation results based on the six batch tests with different compositions (experimental data, symbols, model predictions, solid lines): (A-D) Batch Test A-D with different initial concentrations of NO_2 and NH_4^+ as substrates, and (E-F) Batch Teat E-F with different initial concentrations of NO_2^- and NH_4^+ as substract

Testing the Feasibility of Coupling Nitritation-Anammox-DAMO Processes in one Single-Stage MBfR by Controlling DO Concentration

Different from the separate system, nitritation by AOB would also be included in the stratified biofilm to aerobically convert ammonium to nitrite partially. DO would be introduced in the bulk liquid at a certain level to support the growth of AOB but wash out NOB, and also to produce a suitable NO_2/NH_4þ ratio for both Anammox and DAMO microorganisms at a given TN surface loading (as indicated in Fig. 4), without further inhibition on Anammox [15-16] and DAMO microorganisms [17]. Therefore, the DO control would be essential for successfully coupling nitritation-Anammox-DAMO processes in one single-stage MBfR for efficient nitrogen removal. Figure 5 shows the shifts of the microbial structure and the resulting TN removal efficiency of the single-stage MBfR coupling nitritation-Anammox-DAMO at steady state under different bulk liquid DO concentration conditions. The active fractions of both DAMO archaea and DAMO bacteria decreased while that of Anammox bacteria increased with increasing DO concentration to 0.17 g m^3 with the maximum TN removal efficiency of 90.7%. Further increase of DO would decrease Anammox growth and eliminate DAMO archaea from the biofilm. The corresponding TN removal efficiency would drop substantially. The abundance of AOB changed slightly within the DO range studied, while NOB growth remained in an unfavorable position without any presence in the biofilm until DO was higher than 0.27 g m^3. In contrast to the Anammox-DAMO biofilm system where the cooperation between Anammox bacteria and DAMO archaea determined the optimal nitrogen removal performance, DAMO bacteria played an important role in the TN removal of the single-stage MBfR coupling nitritation-Anammox-DAMO. In contrast, Anammox and DAMO bacteria dominated the inner layer of the biofilm (from 0 to 800 mm) where DO was relatively low and methane, ammonium and nitrite were available, with the last produced by AOB. It should be noted that the aerobic methane oxidation was not included in the presented model due to its negligible effect on the steady-state microbial community structure and the system performance, which has been confirmed in our previous work [12]. Never the less, the model could be easily expanded to consider the aerobic methane oxidation process if necessary. These simulation results clearly demonstrated the proof-of-concept feasibility of successfully coupling nitritation-Anammox-DAMO processes in one single-stage MBfR by controlling bulk liquid DO concentration, e.g., DO concentration at 0.17 g m^3 under the simulated conditions, although the real implementation of such a coupling nitritation-Anammox-DAMO biofilm system still warrants further experimental verification. However, the finding soft his work would be useful for the development of this promising process for efficient nitrogen removal.

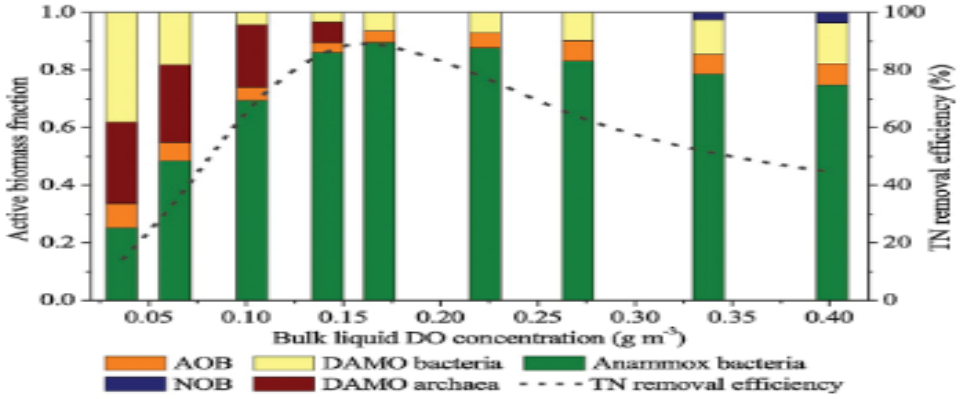

Figure 4: Model simulation results for the shifts of the microbial structure and the resulting TN removal efficiency of the single – stage MBfR coupling nitritation – Anammox – DAMO at steady state under different bulk liquid DO concentration conditions. The optimal methane surface loading of0.009 gm^{-2} d^{-1} with the influent TN concentration of 300 g N m^{-3}

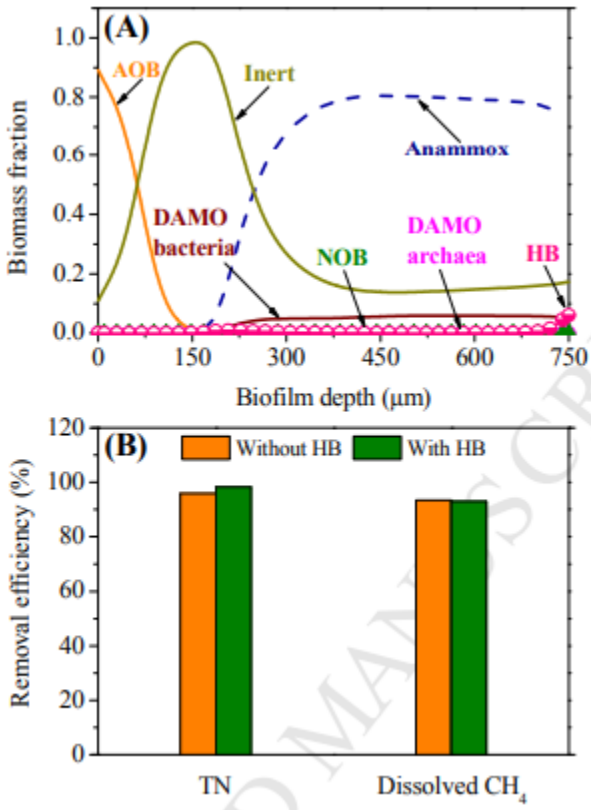

Figure 5: Model simulation results of the partial nitritation – Anammox DAMO biofilm system in consideration of the potential existence of heterotrophic bacteria (HB): (A) Microbial population distribution ;(B) TN and dissolved methane removal efficiencies with / without considering HB. The additionally applied influent organic carbon concentration is 100 g COD m^{-3}. The related stoichiometrics and kinetics of HB are directly taken from literature [15]

Conclusions

In this work, the mechanisms and operational window for achieving complete nitrogen removal by coupling nitritation-Anammox and methane-dependent denitrification in the MBfR were explicitly investigated using mathematical modeling. The validity of the extended model structure and parameters related to nitrite inhibition was verified by the batch experimental data from an MBfR containing an Anammox-DAMO biofilm with different feeding nitrogen compositions. The optimum NO_2/NH_4þ ratio produced from nitritation for the Anammox-DAMO biofilm system was found to be 1.0 in order to achieve the maximum TN removal of over 99.0%, which was irrespective of the applied TN surface loading, while the corresponding optimal methane supply increased with the increase of TN surface loading, accompanied by the decreasing methane utilization efficiency. The feasibility of one single-stage MBfR coupling nitritation-Anammox-DAMO for complete nitrogen removal was tested through controlling the bulk liquid DO concentration in the system. The maximum TN removal was found to be achieved at the bulk DO concentration of around 0.17 g m^3 under the simulation conditions (depending on process parameters), with the AOB, Anammox bacteria and DAMO organisms coexisting in the biofilm successfully.

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