Model Evaluation of the Microbial Metabolic Processes in a Hydrogen-Based Membrane Biofilm Reactor for Simultaneous Bromate and Nitrate Reduction

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Abstract: The H2-based membrane biofilm reactor (H2-MBfR) has been acknowledged as a cost-effective microbial reduction technology for oxyanion removal from drinking water sources, but it remains unknown how the evolution of biofilm characteristics responds to the changing critical operating parameters of the H2-MBfR for simultaneous bromate (BrO3−) and nitrate (NO3−) elimination. Therefore, an expanded multispecies model, applicable to mechanistically interpret the bromate-reducing bacteria (BRB)- and denitrifying bacteria (DNB)-dominated metabolic processes in the biofilm of the H2-MBfR, was developed in this study. The model outputs indicate that (1) increased BrO3− loading facilitated the metabolism of BRB by increasing BRB fraction and BrO3− gradients in the biofilm, but had a marginal influence on NO3− reduction; (2) H2 pressure of 0.04 MPa enabled the minimal loss of H2 and the extension of the active region of BRB and DNB in the biofilm; (3) once the influent NO3− concentration was beyond 10 mg N/L, the fraction and activity of BRB significantly declined; (4) BRB was more tolerant than DNB for the acidic aquatic environment incurred by the CO2 pressure over 0.02 MPa. The results corroborate that the degree of microbial competition for substrates and space in the biofilm was dependent on system operating parameters.

Keywords: BrO3−; NO3−; bioreactor; microbial reduction; multispecies model; biofilm characteristics

1. Introduction

Bromate (BrO3−), a common disinfection byproduct formed during the advanced oxidation processes (especially ozonation) of drinking water, has been assigned as a Group 2B carcinogen with a maximum contaminant level of 10 µg/L in potable water by the World Health Organization [1–3]. The BrO3− concentration varies typically from below 30 µg/L in the ozonated surface water to 0.8–1.4 mg/L in the groundwater intruded by industrial discharges [2,4]. Current physicochemical approaches for aquatic BrO3− elimination, involving adsorption, separation, chemical reduction, and photo-/electro-catalysis, exhibit limited application potential due to their disadvantages in terms of high energy consumption, stringent optional conditions, and requirements of post-treatment steps [5–7]. Comparably, autotrophic microbial reduction has received increasing interest in the past decade, owing to its proven capacity of cost-efficiently converting BrO3− to innocuous Br− under mild circumstances and the organic-deficient nature of most drinking water sources [6,8].

Nitrate (NO3−) is a pervasive contaminant which coexists with BrO3− at a typical concentration range of 10–30 mg N/L in surface water and groundwater, and which can
result in methemoglobinemia in infants [8–11]. This accentuates the necessity to develop robust technology capable of simultaneously eliminating BrO$_3^-$ and NO$_3^-$ from polluted drinking water sources. As an advanced autotrophic microbial reduction technology, the H$_2$-based membrane biofilm reactor (H$_2$-MBfR) has been successfully practiced at a pilot and/or commercial scale for eradicating a variety of oxidized pollutants, e.g., BrO$_3^-$, NO$_3^-$, sulfate, perchlorate, selenate, and chromate [9,12]. In this system, electron donor H$_2$ diffuses through the wall of gas-permeable hollow fiber membranes (HFM), and supports the functional microorganisms in the HFM-attached biofilm to respire the electron acceptors (i.e., oxidized pollutants); such a scheme of passive gas delivery enables the full utilization of H$_2$, as well as the efficient removal of targeted contaminants [9,13]. Several case studies have shown that, when the influent concentrations of BrO$_3^-$ and NO$_3^-$ were in the range of 0.1–1.5 mg/L and 5–20 mg N/L, respectively, which were approachable to those in real drinking water sources, the appreciable removal (beyond 95%) of both oxyanions could be realized in H$_2$-MBfRs [1,8].

In H$_2$-MBfRs, the biofilm microorganisms, which can dominate the reduction of BrO$_3^-$ and NO$_3^-$, are defined as bromate-reducing bacteria (BRB) and denitrifying bacteria (DNB), respectively [1,11]. Since H$_2$ is shared by functional bacteria (e.g., BRB and DNB) in the biofilm as the sole electron donor to drive the reduction of oxidized pollutants, the microbial competition for H$_2$ availability (controlled by H$_2$ supplying pressure) inevitably occurs in H$_2$-MBfRs [14]. The results of empirical studies corroborated that BrO$_3^-$ and NO$_3^-$ concentrations were critical to the efficiencies of microbial reduction, and the BrO$_3^-$ reduction processes could be inhibited in the case of exorbitant NO$_3^-$ loading [11,15]. Microbial community investigations have revealed the variation trends in the species and abundance of BRB and DNB in the biofilm systems under diverse BrO$_3^-$ and NO$_3^-$ loadings [6,11]. Despite the above progress, it remains understudied the extent to which the critical operating parameters drive the evolution of biofilm microenvironment, structure, and activity of the H$_2$-MBfR for simultaneous BrO$_3^-$ and NO$_3^-$ elimination. This constitutes a major hindrance to our understanding of the interaction mechanism between BRB and DNB in the biofilm. The development of a systematic and quantitative framework might be a solution to this concern, given the difficulty in the use of present experimental means for elucidating the stratification features of biofilm.

The one-dimensional, multispecies biofilm model, developed on the AQUASIM platform, is a versatile tool applicable to quantitatively assess the steady-state characteristics of the biofilm, e.g., the substrate profiles, as well as microbial distribution and activities [16]. To date, a model framework, which can be implemented to simulate the simultaneous removal processes of BrO$_3^-$ and NO$_3^-$ in the H$_2$-MBfR, is still unavailable in the literature. In this work, a sophisticated biofilm model was established on the AQUASIM platform to mechanistically interpret the BrO$_3^-$ and NO$_3^-$ reduction processes of H$_2$-MBfR. On the basis of the previous model frameworks for NO$_3^-$ removal [14,16], we proposed an expanded model framework through incorporation into the algorithm for the metabolic process kinetics of BRB, as well as the influences of CO$_2$ addition (as carbon source and pH regulator) on metabolic process kinetics of BRB and DNB. Long- and short-term experimental data regarding BrO$_3^-$ and NO$_3^-$ removal, obtained from a lab-scale H$_2$-MBfR, were used for the calibration of key model parameters and the validation of the model outputs, respectively. The validated model was then applied to evaluate the gradients of substrates (H$_2$, BrO$_3^-$, and NO$_3^-$), as well as the distribution and activities of BRB and DNB in the biofilm, in the case of changing system operating parameters including the H$_2$ pressure, CO$_2$ addition, and influent BrO$_3^-$ and NO$_3^-$ concentrations.

2. Materials and Methods

2.1. Reactor Setup and Start-Up

The schematic of the H$_2$-MBfR used in this study is shown in Figure 1. The reactor consisted of a module containing 96 HFMs (Litree Company, Suzhou, China) and a microporous tube placed in a plexiglass cylinder (height = 22 cm, inner diameter = 6 cm,
The average outer diameter and pore size of HFM was 1.5 mm and 0.02 µm, respectively, and the total membrane surface area of HFM module was 633 cm². The module with a sealed lower end was connected to a H₂ tank for delivering pressurized H₂. Pure CO₂ was supplied by the microporous tube with its bottom end linked to a CO₂ tank to serve as carbon source and pH regulator. Synthetic influent was introduced into the reactor via a peristaltic pump (BT101L-DG-1, Lead Fluid, Baoding, China), and the effluent was collected from the outlet of the reactor. A recirculation pump (BT101L-YZ15/25, Lead Fluid, Baoding, China) was sustained at a high flowrate of 100 mL/min to keep the bulk liquid completely mixed. The reactor was inoculated with 50 mL of biomass collected from a denitrifying H₂-MBF in our lab. The synthetic influent, containing 10 mg N/L NaNO₃ with an identical composition to that described in Li et al. [17], was used in the start-up period of the reactor. During the start-up period, the influent flowrate was maintained at 1.6 mL/min (resulting in an HRT of 5.8 h), and the H₂ and CO₂ pressure was kept at 0.04 and 0.012 MPa, respectively. After 30 days of operation, the influent flowrate was changed to 2.0 mL/min (corresponding to an HRT of 4.7 h) until the biofilm was well formed, and the performance of the reactor reached a steady state with stable NO₃⁻ removal.

**Figure 1.** Schematic of the H₂-MBF.

### 2.2. Short- and Long-Term Experiments and Sample Analysis

On days 31–40 of stage 1, short-term experiments were performed to assess the effects of H₂ pressure (0.01–0.08 MPa), influent NO₃⁻ (1–20 mg N/L), and CO₂ pressure (0.004–0.036 MPa) on the NO₃⁻ and BrO₃⁻ removal of the H₂-MBF. The detailed information concerning the operational conditions of short-term experiments can be found in Table S1. For each experiment, one system operating parameter was changed, while keeping the others fixed. Once the experiment condition was changed, aqueous samples were collected after three HRT operations of the system, in order to allow the stabilization of effluent contaminant concentrations [18]. Following the short-term experiments, long-term experiments (days 41–140) were carried out to investigate how the oxyanion removal performance of the system responded to the changing BrO₃⁻ loadings. As summarized in Table S2, in long-term experiments, the influent concentrations of BrO₃⁻ were set at 0.1, 0.5, and 1.0 mg/L in stage 3, stage 4, and stage 5, respectively, and the H₂ and CO₂ pressure were maintained at 0.04 and 0.012 MPa, respectively.

The collected aqueous samples were filtered immediately through a 0.22 µm pore size polycarbonate membrane filter (Anpel Co., Ltd., Shanghai, China) and stored in a refrigerator (4 °C) until analyzed. The concentrations of NO₃⁻, NO₂⁻, and BrO₃⁻ were determined by ion chromatography (ICS-1000, Dionex, Sunnyvale, USA) equipped with an AS-19 column (4 × 250 mm, Dionex, Sunnyvale, USA). The dissolved H₂ was determined using a H₂ microsensor (H₂10, Unisense A/S Corp., Aarhus, Denmark). The pH value was
measured using a pH meter (pHS-3C model, Leici, Shanghai, China). The contaminant removal flux was calculated using Equation (1):

\[
J = \frac{Q}{A} (S_i - S_e),
\]

where \(J\) is the NO\(_3^–\) and BrO\(_3^–\) removal flux in units of g N/(m\(^2\)-day) and g BrO\(_3^–\)/(m\(^2\)-day), respectively, \(Q\) signifies the influent flowrate (m\(^3\)/day), \(A\) refers to the total membrane surface area (m\(^2\)), and \(S_i\) and \(S_e\) denote the influent concentration of NO\(_3^–\) or BrO\(_3^–\) and the effluent concentration of NO\(_3^–\) or BrO\(_3^–\), respectively.

2.3. Model Development and Evaluation

2.3.1. Model Framework Development

Unlike the classical biofilm models established by Tang et al. \[14,16\], the framework of the developed mathematical model involves the biochemical process of hydrogenotrophic BrO\(_3^–\) reduction, and it considers the effects on the metabolic process kinetics of BRB and DNB in the case of CO\(_2\) addition (as carbon source and pH regulator). Figure 2 shows the schematic of the involved biochemical processes and the correlated interactions between the model components in the biofilm. The model components include five solid components (i.e., BRB, DNB, heterotrophic bacteria (HB), extracellular polymeric substances (EPS), and inert organics (IO)) and five dissolved components (i.e., H\(_2\), CO\(_2\), NO\(_3^–\), BrO\(_3^–\), and soluble microbial products (SMP)). H\(_2\) is exploited by BRB and DNB as the electron donors, BrO\(_3^–\) and NO\(_3^–\) as the electron acceptors, and CO\(_2\) as the carbon source. In the conversion processes of BrO\(_3^–\) and NO\(_3^–\) to Br\(^–\) and N\(_2\), BRB and DNB obtain energy to sustain their growth and metabolism. H\(_2\) was the sole energy source of the hydrogenotrophic bacteria, since no organic carbon was introduced to the influent; thus, BRB and DNB could merely abstract electrons from H\(_2\) to drive the BrO\(_3^–\) and NO\(_3^–\) reduction. HB grows either on the SMP directly produced by BRB and DNB or indirectly on the hydrolyzed EPS (i.e., SMP) originated from BRB and DNB. The decay of BRB, DNB, and HB generates nonbiodegradable IO. The electrons provided from H\(_2\) can be classified into three portions during the BRB and DNB metabolic processes: bacterial cell synthesis (\(k_1\)), SMP formation (\(k_2\)), and EPS formation (\(k_3\)); thus, \(k_1 + k_2 + k_3 = 1\). The electrons for cell synthesis are subdivided into the fraction for energy-providing reaction (\(f^0_s\)) and the other for synthesis reaction of true yield of biomass (\(f_s^0\)); therefore, \(f_s^0 + f^0_s = 1\). Here, \(f^0_s\) represents the fraction of biodegradable biomass; hence, \(1 - f^0_s\) refers to the remaining nonbiodegradable biomass.

Figure 2. Schematic of the interactions between model components involved in the biochemical processes of the biofilm.

2.3.2. Model Solution and Calibration

The numerical solution of the model was implemented on software AQUASIM 2.1g, by inputting the process matrix (Table 1), stoichiometric and kinetic parameters (Table S3), process kinetic rate equations (Table 2), and experiment operational parameters of the
H$_2$-MBfR (Table S4). In particular, the calculation procedure for stoichiometric coefficients of model components (shown in Table 1) in the BRB metabolic process is exhibited in Section S1. Table S3 merely lists the calculated and recalibrated stoichiometric and kinetic parameters of the BRB metabolic process in this work, while other pertinent parameters can be found in Table S5. Bulk liquid pH is known to be a key factor correlated to the activities of the hydrogenotrophic microorganisms [13,19,20]. In order to quantitatively assess how bulk liquid pH affects the microbial metabolic activities, the inhibition factor of bulk liquid pH ($f_{pH}$) was introduced to the kinetic rate expressions of the model framework, as shown in Table 2. According to the normalized Michaelis pH function [21], $f_{pH}$ can be computed using Equation (2).

$$ f_{pH} = \frac{1 + 2 \times 10^{0.5(pK_h-pK_i)} + 10^{(pK_i-pH)} + 10^{(pK_h-pH)}}{1 + 10^{(pH-pK_h)} + 10^{(pK_i-pH)}} $$

where pH values are the experimental measurements in this study; $pK_h$ and $pK_i$ denote the upper and lower pH values at which the metabolic rates are equal to 50% of the maximum rate at the optimum pH, respectively, and their values of 9.04 and 6.27 were adopted from previous studies [21–23].

Table 1. Process matrix for the developed model of components.

| Component (i) | Process (j) | $S_{H_2}$ | $S_{NO_3}$ | $S_{BO_3}$ | $S_{SMF}$ | $S_{CO_2}$ | $X_{DNB}$ | $X_{BRB}$ | $X_{HB}$ | $X_{O}$ | $X_{EPS}$ |
|---------------|-------------|----------|----------|----------|----------|----------|---------|---------|---------|-------|---------|
| DNB growth    | $-\frac{14}{S_{DNB}}$ | $-k_1 \frac{S_{DNB}}{S_{DNB}^{0.5}}$ | $\frac{X_{DNB}}{S_{DNB}^{0.5}}$ | $1$ | $k_1$ | $k_2$ | $k_3$ |
| BRB growth    | $-\frac{14}{S_{BRB}}$ | $-\frac{1}{k_1} \frac{S_{BRB}}{S_{BRB}^{0.5}}$ | $\frac{X_{BRB}}{S_{BRB}^{0.5}}$ | $1$ | $k_1$ | $k_2$ | $k_3$ |
| HB growth     | $-\frac{1}{S_{HB}}$ | $-\frac{X_{HB}}{S_{HB}^{0.5}}$ | $1$ | $k_1$ | $k_2$ | $k_3$ |
| DNB decay     | $1$ | $1$ | $-1$ | $1$ | $-1$ | $1$ | $-1$ | $-1$ | $-1$ | $-1$ | $-1$ |

Table 2. Process kinetic rate equations for the developed model.

| Process (j) | Kinetic Rate Expressions |
|-------------|--------------------------|
| DNB growth  | $\mu_{DNB} \frac{S_{H_2}}{S_{H_2} + K_{m_{H_2}}} \frac{S_{NO_3}}{S_{NO_3} + K_{m_{NO_3}}} \frac{S_{BO_3}}{S_{BO_3} + K_{m_{BO_3}}} \frac{S_{SMF}}{S_{SMF} + K_{m_{SMF}}} \frac{S_{CO_2}}{S_{CO_2} + K_{m_{CO_2}}} X_{DNB} f_{pH}$ |
| BRB growth  | $\mu_{BRB} \frac{S_{NO_3} + K_{m_{NO_3}}}{S_{NO_3}} \frac{S_{BO_3} + K_{m_{BO_3}}}{S_{BO_3}} \frac{S_{SMF} + K_{m_{SMF}}}{S_{SMF}} \frac{S_{CO_2} + K_{m_{CO_2}}}{S_{CO_2}} \frac{X_{BRB}}{X_{BRB} f_{pH}}$ |
| HB growth   | $\mu_{HB} \frac{S_{NO_3} + K_{m_{NO_3}}}{S_{NO_3}} \frac{S_{SMF} + K_{m_{SMF}}}{S_{SMF}} \frac{X_{HB}}{X_{HB} f_{pH}}$ |
| DNB decay   | $b_{DNB} X_{DNB}$ |
| BRB decay   | $b_{BRB} X_{BRB}$ |
| HB Decay    | $b_{HB} X_{HB}$ |
| Hydrolysis  | $k_{hyd} X_{EPS}$ |

Disparate reactor configurations, operating conditions, and HFM patterns commonly result in the varying compositions and metabolic kinetics of biofilms [24]. For the aim of matching the specific simulation scenarios, several parameters, i.e., $H_2$ transfer coefficient of HFM ($K_m$), maximum specific growth rate of DNB and BRB ($\mu_{DNB}$ and $\mu_{BRB}$), and half-maximum-rate concentration of BrO$_3^-$ for BRB ($K_{BrO_3}$), were recalibrated by fitting the measured effluent NO$_3^-$ and BrO$_3^-$ concentrations of the long-term operated system to the modeled results using the AQUASIM built-in iterative algorithms (Equation (3)).
By minimizing the square error sums between the model predictions and experimental measurements, the selected parameters were calibrated to be the best-fit values.

\[ \chi^2(p) = \sum_{i=1}^{n} \left( \frac{y_{\text{meas},i} - y_i(p)}{\sigma_{\text{meas},i}} \right)^2, \]  

where \( y_{\text{meas},i} \) and \( y_i(p) \) represent the experimental results and model predictions at time \( i \), and \( \sigma_{\text{meas},i} \) and \( n \) signify the standard deviation and number of data points, respectively.

2.3.3. Model Validation and Evaluation

The calibrated model was further validated by comparing the simulated results with experimentally measured \( \text{NO}_3^- \) and \( \text{BrO}_3^- \) removal fluxes in the short-term experiments. The validated model was then used to simulate the profiles of substrates, microbial distribution, and metabolic activities in the biofilm of the H\(_2\)-MBfR operated in a series of system conditions, including influent \( \text{BrO}_3^- \) concentrations (0.1–1.0 mg/L), H\(_2\) pressure (0.01–0.08 MPa), influent \( \text{NO}_3^- \) concentration (1–20 mg N/L), and CO\(_2\) pressure (0.004–0.036 MPa), in order to investigate the evolution laws of biofilm microenvironment, structure, and activity in the H\(_2\)-MBfR for \( \text{NO}_3^- \) and \( \text{BrO}_3^- \) removal, in the case of changing system conditions. A schematic of the research methodology is shown in Figure 3.

**Figure 3.** Schematic of the research methodology.

3. Results and Discussion

3.1. Long-Term H\(_2\)-MBfR Performance and Model Calibration

The long-term experimental data obtained during the 140 days of operation of H\(_2\)-MBfR were used for the recalibration of the selected parameters by fitting the effluent \( \text{NO}_3^- \) and \( \text{BrO}_3^- \) concentrations to those of simulated results. As shown in Figure 4, the measured data and simulated results were finely matched with the corresponding coefficients of determination (R\(^2\)) up to 0.93 and 0.91 for the effluent \( \text{NO}_3^- \) and \( \text{BrO}_3^- \) concentrations, respectively. The analysis results concerning the sensitivity of the selected parameters to the effluent \( \text{BrO}_3^- \) and \( \text{NO}_3^- \) concentrations in the long-term operated H\(_2\)-MBfR are shown in Figure 5. It can be found that the effluent \( \text{BrO}_3^- \) concentration of the H\(_2\)-MBfR was most sensitive to \( \mu_{\text{BRB}} \) and relatively sensitive to \( \mu_{\text{DNB}} \) and \( K_m \) (Figure 5a), while the effluent \( \text{NO}_3^- \) concentration was sensitive to \( K_m \) and \( \mu_{\text{DNB}} \) (Figure 5b). These results are in line with the findings of previous studies, i.e., the maximum specific growth rate of microorganisms and the \( H_2 \) transfer coefficient of HFM were sensitive to the H\(_2\)-MBIR performance [9,24,25]. The best-fit values of \( K_m \), \( \mu_{\text{BRB}} \), \( \mu_{\text{DNB}} \), and \( K_{\text{BrO}_3} \) were estimated to be 0.189 m/day, 0.85 day\(^{-1}\), 0.57 day\(^{-1}\), and 0.014 mg/L, respectively.
It can be also seen from Figure 4 that, after 7 days of start-up at stage 1, the denitrification flux of the H2-MBfR stabilized at around 0.45 g N/(m²·day), corresponding to a NO$_3^-$ removal efficiency of over 99%. The slightly decreased denitrification flux of the system at stage 2 can be ascribed to the augment in the influent flowrate from 1.6 mL/min at stage 1 to 2.0 mL/min at this stage. The introduction of 0.1–1.0 mg/L BrO$_3^-$ into the influent at stages 3–5 did not further decrease the denitrification performance of the system, given the identical average denitrification flux (0.42 g N/(m²·day)) at days 50–60 of stage 2 (without BrO$_3^-$ addition) and stages 3–5. This observation is in agreement with the results of previous studies that a BrO$_3^-$ concentration below 50 mg/L had no inhibitory effect on denitrification [15,26]. Empirical studies suggest that it usually takes 11–40 days to enable the H$_2$-MBfRs to reach steady-state characteristics following the adjustment of operation conditions such as substrate loadings and HRT [17,27]. Similarly, our results
indicate that, in the case of BrO$_3^-$ addition, it took 15–25 days at stages 3–5 to enable the BrO$_3^-$ removal of the H$_2$-MBfR to reach a steady state. The stabilized BrO$_3^-$ removal flux was increased from 0.0044 g/(m$^2$·day) at stage 3 to 0.041 g/(m$^2$·day) at stage 5. This is presumably attributed to the augment in the abundance and/or reduction rate of BRB with increasing influent BrO$_3^-$ concentration. Furthermore, Downing and Nerenberg [15] found that a near 11-fold increase in the BrO$_3^-$ removal flux of H$_2$-MBfR was achieved when the influent BrO$_3^-$ concentration was increased from 0.1 to 10 mg/L.

3.2. Short-Term H$_2$-MBfR Performance and Model Validation

Short-term experiments were performed to evaluate the effects of the key influencing factors, i.e., H$_2$ pressure, influent NO$_3^-$ concentration, and CO$_2$ pressure, on the NO$_3^-$ and BrO$_3^-$ reduction of the H$_2$-MBfR. As shown in Figure 6a,b the determined BrO$_3^-$ and NO$_3^-$ removal fluxes were increased from 0.036 and 0.36 to 0.041 g/(m$^2$·day) and 0.42 g N/(m$^2$·day), respectively, as the H$_2$ pressure was increased from 0.01 to 0.04 MPa. This is likely owing to the increased availability of electron donor, which enhanced the activities of BRB and DNB. A higher H$_2$ pressure (0.06 and 0.08 MPa) did not ameliorate the BrO$_3^-$ and NO$_3^-$ removal, but led to the consumption of the remaining H$_2$ in the effluent. Figure 6c,d delineate that, although the BrO$_3^-$ and NO$_3^-$ were all accumulated in the effluent, the NO$_3^-$ removal flux was increased from 0.42 to 0.51 g N/(m$^2$·day), accompanied by the significant decrease in BrO$_3^-$ removal flux from 0.041 to 0.011 g/(m$^2$·day), when the influent NO$_3^-$ concentrations were augmented from 10 to 20 mg N/L. In accordance with this, Zhong et al. [6] observed that an influent NO$_3^-$ concentration of 11.3 mg N/L was able to inhibit the BrO$_3^-$ reduction in the H$_2$-MBfR, which gave rise to the advantage of DNB over BRB when competing for electron donors. CO$_2$ is arguably one of the most effective pH regulators in H$_2$-MBfRs, and it can serve as the supplemental inorganic carbon source for hydrogenotrophic bacteria [13,28]. As depicted in Figure 6e,f, the shift of CO$_2$ pressure from 0.012 to 0.036 MPa led to an apparent decline in the NO$_3^-$ removal flux from 0.42 g N/(m$^2$·day) at 0.012 MPa to 0.32 g N/(m$^2$·day) at 0.036 MPa, but had a negligible influence on BrO$_3^-$ removal flux. A plausible explanation is that, as the CO$_2$ pressure was in the range of 0.020–0.036 MPa, the resultant bulk liquid pH of 6.0–6.9 was obviously lower than the reported favorable pH range (7.0–9.0) of DNB, according to existing studies [19,20], and some specific BRB was possibly able to endure more acidic conditions. Additionally, the measured removal fluxes of BrO$_3^-$ and NO$_3^-$ in the short-term experiments were employed to further validate the calibrated model. It can be observed from Figure 6b–f that, in all cases, the modeled results were quite approximate to the experimental data, indicating the good accuracy and reliability of the developed model for prediction of the simultaneous BrO$_3^-$ and NO$_3^-$ reduction processes in the H$_2$-MBfR.

3.3. Model Evaluation of the Effects of BrO$_3^-$ Loading

Figure 7 shows the simulated distribution of substrates, DNB and BRB fractions, as well as their metabolic activities, in the steady-state biofilms at stages 3–5 using the validated model. As shown in Figure 7a–c, in line with the counter-diffusional characteristics of dissolved substrate in H$_2$-MBfR biofilm [29], the H$_2$ concentration gradually decreased from HFM surface (i.e., biofilm thickness of zero point) toward to the bulk liquid side, while the other substrates (including CO$_2$, NO$_3^-$, and BrO$_3^-$) originated from bulk liquid presented the contrary tendency. H$_2$ and CO$_2$ concentrations across the biofilm exhibited negligible difference when the influent concentration varied in the range of 0.1–1.0 mg/L (Figure 7a), attributed to the limited electron donor and carbon source consumption by BRB at low BrO$_3^-$ loadings. It can also be seen from Figure 7a that CO$_2$ was not a limiting factor as a carbon source for BRB and DNB anabolism, since its concentrations within the whole biofilm were much higher than the half-maximum-rate concentration (0.004 mg/L) adopted in the model. Moreover, the resultant bulk liquid pH 7.5 at the CO$_2$ pressure of 0.012 MPa would not adversely affect the metabolic processes of BRB and DNB, since the maximum NO$_3^-$ and BrO$_3^-$ removal fluxes were attained at this CO$_2$ pressure, as shown
in Figure 6f. In line with this, the optimal pH reported for hydrogenotrophic denitrification and bromate reduction has been documented at neutral or weakly alkaline values [13,15].

**Figure 6.** The system performance of the H2-MBR, as well as the compassion of the measured and simulated removal fluxes of NO3\(^{-}\) and BrO3\(^{-}\), in the case of changing H2 pressure (a,b), influent NO3\(^{-}\) concentration (c,d), and CO2 pressure (e,f).

**Figure 7. Cont.**
Figure 7. Model-simulated profiles for (a) H$_2$ and CO$_2$, (b) NO$_3^-$, (c) BrO$_3^-$ concentrations, and (d) DNB and BRB fractions, as well as (e) DNB and (f) BRB metabolic activities, in the biofilm of H$_2$-MBfR as a function of influent BrO$_3^-$ concentration ranging from 0.1 to 1.0 mg/L.

Figure 7d plots the simulated profiles of DNB and BRB fractions in the biofilm. DNB dominated in the biofilm compared to BRB at all stages, indicating that DNB outcompeted DNB for space, likely associated with the much higher concentration of NO$_3^-$ in the biofilm than BrO$_3^-$ . In particular, in response to the increase of the influent BrO$_3^-$ concentration from stage 3 to stage 5, the BRB fraction (proportion) obviously increased in the biofilm. Figure 7e shows the DNB activity in the biofilm as a function of influent BrO$_3^-$ concentration. The declined DNB activity in the biofilm range of 0–210 µm was mainly due to the inhibition of electron acceptors for DNB metabolism, given that the NO$_3^-$ concentrations within this range were below the half-maximum-rate concentration of NO$_3^-$ for DNB (0.2 mg N/L) [14]. The observed decrease in DNB activity from stage 3 to stage 5 at the outer layer biofilm was the result of the lower H$_2$ concentrations. As shown in Figure 7f, the BRB activity in the biofilm increased from stage 3 to stage 5, owing to the augmented BRB fraction and BrO$_3^-$ gradients in the biofilm, which facilitated the metabolism of BRB. In addition, the increased BRB fraction and activity, as exhibited in Figure 7d,f, helps explain the augmented BrO$_3^-$ removal flux from stage 3 to stage 5 (Figure 4).

3.4. Model Evaluation of the Effects of H$_2$ Pressure

Empirical studies [13,20] have collectively shown that excessive H$_2$ supply pressure results in the H$_2$ off-gassing problem, while an insufficient supply leads to unsatisfactory pollutant removal due to the electron donor scarcity in biofilms. Figure 8a plots the simulated H$_2$ and CO$_2$ concentration profiles in the biofilm at diverse H$_2$ pressure (0.01–0.08 MPa). The H$_2$ concentration in the biofilm increased with the increase in H$_2$ pressure. At the H$_2$ pressure of 0.01 MPa, more than half of the outer biofilm region (biofilm thickness > 200 µm) could not deliver sufficient H$_2$. H$_2$ constitutes a limiting factor for microbial metabolic processes when its content is below the half-maximum-rate concentration. When H$_2$ pressure was increased to 0.04 MPa, the H$_2$ contents across the biofilm were all higher than the half-maximum-rate concentrations of H$_2$ for DNB and BRB metabolism (0.002 mg/L) [14]; thus, it can be inferred that the H$_2$ concentration within biofilm was not a decisive factor limiting NO$_3^-$ and BrO$_3^-$ removal at relatively high H$_2$ pressure. Note that, at this H$_2$ pressure, the simulated H$_2$ concentration in the bulk liquid was 0.0074 mg/L, which is quite close to the measured result (0.008 mg/L) during the short-term experiments (Figure 6a) and is also comparable to the recommended aquatic H$_2$ concentration (0.009 mg/L) in H$_2$-MBfR [18]. Upon further increasing the H$_2$ pressure, the H$_2$ off-gassing phenomenon occurred. It can also be seen from Figure 8a that the CO$_2$ concentration gradients in the biofilm hardly changed when the H$_2$ pressure was higher than 0.04 MPa. This implies that the excessive H$_2$ supply would not accelerate the carbon source utilization of BRB and DNB.
Figure 8. Model simulated profiles for (a) H$_2$ and CO$_2$, (b) NO$_3^-$, (c) BrO$_3^-$ concentrations, and (d) DNB and BRB fractions, as well as (e) DNB and (f) BRB metabolic activities, in the biofilm of H$_2$‐MBIR as a function of H$_2$ pressure ranging from 0.01 to 0.08 MPa.

As depicted in Figure 8b,c, with the increase in H$_2$ pressure from 0.01 to 0.04 MPa, the NO$_3^-$ and BrO$_3^-$ concentrations in the biofilm were found to markedly decline. This can be explained by the increased H$_2$ availability for DNB and BRB compared to the biofilm exterior, which allowed a greater reduction in NO$_3^-$ and BrO$_3^-$ in the exterior rather than the interior of biofilm. However, NO$_3^-$ and BrO$_3^-$ concentration gradients hardly varied once the H$_2$ pressure exceeded 0.04 MPa, which is in accordance with the experimental findings of the short-term experiments that the NO$_3^-$ and BrO$_3^-$ removal marginally changed at the excessive H$_2$ pressure (shown in Figure 6b). According to the simulated BrO$_3^-$ concentration profiles, BrO$_3^-$ concentrations in the biofilm were all greater than its calibrated half-maximum-rate concentration (0.014 mg/L) at the simulated H$_2$ pressure range, indicating that BrO$_3^-$ loading was also not a limiting factor to BRB activity. The increased H$_2$ availability in the outer layer of the biofilm resulted in the extension of the active region of DNB and BRB from the biofilm interior to the whole biofilm, as revealed in Figure 8e,f. In particular, the decreased activities of DNB and BRB in the inner layer of biofilm with increasing H$_2$ pressure were mainly the consequence of the concurrently
decreased concentrations of NO$_3^-$ and BrO$_3^-$ (Figure 8b,c) and the fractions of DNB and BRB (Figure 8d). In the case of low H$_2$ pressure (0.01 and 0.02 MPa), the biofilm thicknesses of 200 and 260 µm were the locations where the H$_2$ concentrations were all below the half-maximum-rate concentrations of DNB and BRB (Figure 8a); beyond these thicknesses, the metabolic activities of DNB and BRB began to dramatically decrease, as delineated in Figure 8e,f.

3.5. Model Evaluation of the Effects of NO$_3^-$ Loading

The experimental results, as shown in Figure 6c, corroborate that the NO$_3^-$ loading significantly impacted the BrO$_3^-$ removal of the H$_2$-MBiR. To understand the underlying mechanism, we employed the validated model to simulate the substrate profiles and microbial activities in the biofilm at the influent NO$_3^-$ concentrations of 1–20 mg N/L. As shown in Figure 9a, the H$_2$ and CO$_2$ concentration gradients in the biofilm sharply decreased with increasing influent NO$_3^-$ concentration, implying that high NO$_3^-$ loadings might significantly enhance the activity of DNB, leading to greater consumption of these two substrates. In particular, H$_2$ off-gassing was found at the influent NO$_3^-$ concentration lower than 10 mg N/L, owing to the lack of an electron acceptor for H$_2$ consumption. Once the influent NO$_3^-$ concentration was greater than 10 mg N/L, NO$_3^-$ was found to accumulate in the biofilm with high concentrations (Figure 9b), presumably due to the limitation of overall DNB population, while over 90% of the inputted BrO$_3^-$ could not be consumed by BRB in the biofilm (Figure 9c), suggesting the severe inhibition of BRB activity by high NO$_3^-$ loadings. As exhibited in Figure 9d, when the influent NO$_3^-$ concentration was increased from 1 to 20 mg N/L, the BRB fractions across the biofilm decreased from beyond 20% to approximately 1%, which was accompanied by the surge of the DNB fraction across the biofilm, suggesting the competitive advantage of DNB over BRB in the case of the high NO$_3^-$ loadings.

Figure 9e,f show the simulated DNB and BRB metabolic activities in the biofilm of the system operated under various influent NO$_3^-$ concentrations. As the influent NO$_3^-$ concentration was less than 10 mg N/L, the high DNB and BRB activities appeared at the outer layer of the biofilm, and a closer distance from the HFM side led to lower activities of DNB and BRB. This trend coincides with that of NO$_3^-$ and BrO$_3^-$ concentration gradients in the biofilm, as shown in Figure 9b,c. When the influent NO$_3^-$ concentration was higher than 10 mg N/L, the DNB activities sharply dropped at the biofilm thickness of beyond 210 µm. This is because the H$_2$ concentrations in the locations beyond this thickness were lower than the half-maximum-rate concentration of DNB (0.002 mg/L). In addition, the BRB activities were strongly inhibited at the higher influent NO$_3^-$ concentration (especially 20 mg N/L), indicating that BRB was overwhelmed by DNB for electron donor competition, when NO$_3^-$ concentration in the influent was much higher than that of BrO$_3^-$. This is consistent with the previous findings that an excessive influent NO$_3^-$ concentration could inhibit BrO$_3^-$ reduction [6,30].

Figure 9. Cont.
were, therefore, conducted to investigate the effects of CO$_2$.

As shown in Figure 10a–c, the increase in CO$_2$ activities of DNB and BRB in the biofilm interior. When the CO$_2$ pressure was increased from 0.004 to 0.036 MPa, the accumulation of H$_2$ and NO$_3^-$ was increased in the biofilm. The BrO$_3^-$ concentrations in the biofilm were slightly increased at the CO$_2$ pressure of 0.036 MPa. This implies that DNB was more sensitive to the acidic conditions (pH 6.0–6.2 at CO$_2$ pressure 0.028–0.036 MPa) than BRB.

As depicted in Figure 10d, as the CO$_2$ pressure was increased from 0.004 to 0.036 MPa, no apparent difference was found in the DNB fractions, while a slight increase in the BRB fractions was observed. It can be seen from Figure 10e,f that, when the CO$_2$ pressure was less than 0.02 MPa, the DNB and BRB activities in the biofilm all gradually augmented with the increase in distance from the HFM side. This can be attributed to the relatively higher NO$_3^-$ and BrO$_3^-$ concentrations in the biofilm exterior. It is interesting to note that, compared to the activities of DNB and BRB at the CO$_2$ pressure below 0.02 MPa, a CO$_2$ pressure beyond this value gave rise to decreased and increased microbial activities in the exterior and interior of the biofilm, respectively. This can be explained by the fact that DNB and BRB in the biofilm exterior were in the vicinity of the bulk liquid, and the acidification of bulk liquid severely inhibited their metabolic activities; subsequently, the declined activities of microorganisms in the biofilm exterior led to more diffusion of NO$_3^-$ and BrO$_3^-$ toward the HFM side (Figure 10b,c), which consequently led to increased activities of DNB and BRB in the biofilm interior. When the CO$_2$ pressure was increased from 0.012 to 0.036 MPa, DNB activity in the majority of the biofilm significantly declined, while the decreased BRB activity in the biofilm exterior was accompanied by an increase in

3.6. Model Evaluation of the Effects of CO$_2$ Pressure

CO$_2$ has dual functions, i.e., as a carbon source to support microorganism growth and for pH control to regulate the microbial activities in H$_2$-MBRs [19,31]. Simulations were, therefore, conducted to investigate the effects of CO$_2$ addition on the evolution of stratification characteristics of the biofilm at the supply pressure of 0.004–0.036 MPa. As shown in Figure 10a–c, the increase in CO$_2$ pressure from 0.004 to 0.02 MPa led to insignificant changes in the H$_2$, NO$_3^-$, and BrO$_3^-$ concentration gradients of the biofilm. Once the CO$_2$ pressure was higher than 0.02 MPa, the accumulation of H$_2$ and NO$_3^-$ was increased in the biofilm. The BrO$_3^-$ concentrations in the biofilm were slightly increased at the CO$_2$ pressure of 0.036 MPa. This implies that DNB was more sensitive to the acidic conditions (pH 6.0–6.2 at CO$_2$ pressure 0.028–0.036 MPa) than BRB.
BRB activity in the biofilm interior (Figure 10e,f). This also indicates that BRB was more tolerant of the acidic aquatic environment than DNB.

![Figure 10](image.png)

**Figure 10.** Model simulated profiles for (a) H₂ and CO₂, (b) NO₃⁻, (c) BrO₃⁻ concentrations, and (d) DNB and BRB fractions, as well as (e) DNB and (f) BRB metabolic activities, in the biofilm of H₂-MBfR as a function of CO₂ pressure ranging from 0.004 to 0.036 MPa.

4. Conclusions

An expanded multispecies model was developed to provide key information regarding the microbial BrO₃⁻ and NO₃⁻ reduction processes in the H₂-MBfR in diverse operating conditions. The selected model parameters, K_B, μ_B, and K_Br, were calibrated to the best-fit values of 0.189 m/day, 0.85 day⁻¹, 0.57 day⁻¹, and 0.014 mg/L, respectively. The good agreement between experimental measurements and modeled results indicates the accuracy and reliability of the calibrated model. The evolution of the substrate gradients, as
well as microbial fraction and activity, was driven by the changing system operating parameters involving H₂ pressure, BrO₃⁻ and NO₃⁻ loading, and CO₂ pressure. Increasing H₂ pressure led to more efficient BrO₃⁻ and NO₃⁻ reduction in the biofilm, but an exorbitant pressure (beyond 0.04 MPa) gave rise to off-gassing of H₂. The augment in BrO₃⁻ loading had no significant influence on NO₃⁻ reduction, while an influent NO₃⁻ concentration higher than 10 mg N/L resulted in the apparent inhibition of BrO₃⁻ reduction. A CO₂ pressure over 0.02 MPa had a distinct negative influence on NO₃⁻ reduction, but minorly impacted BrO₃⁻ reduction. The simulation results of the developed model offer important mechanistic insights into the BrO₃⁻ and NO₃⁻ reduction processes of H₂-MBF₂R.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/membranes12080774/s1: Table S1. Short-term operational conditions of influencing factor experiment of the H₂-MBF₂R; Table S2. Startup and long-term operational conditions of the H₂-MBF₂R; Table S3. Stoichiometric and kinetic parameters of BRB related metabolic process for the developed model; Table S4. Experiments operational parameters for the model development; Section S1. Calculation of the stoichiometric coefficients for BRB metabolic process. Refs. [32,33] are cited on Supplementary Materials.

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