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Model-Based Evaluation of Selenate and Nitrate Reduction in Hydrogen-Based Membrane Biofilm Reactor

Xueming Chen¹, Chun-Yu Lai², Fang Fang³, He-Ping Zhao², Xiaohu Dai⁴, Bing-Jie Ni^{4,*}

¹Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

²Department of Environmental Engineering, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China

³Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Hohai University, Nanjing 210098, China

⁴State Key Laboratory of Pollution Control and Resources Reuse, Shanghai institute of pollution control and ecological security, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

*Correspondence to

Dr. Bing-Jie Ni, P: +86 21 65986849; F: +86 21 65983602; E-mail: bjni@tongji.edu.cn

ABSTRACT

A biofilm model was developed to describe the simultaneous NO_3^- and SeO_4^{2-} reduction in a H_2 -based membrane biofilm reactor (MBfR). Model calibration and validation was conducted using the experimental data of a reported H_2 -based MBfR. With a good level of identifiability, the SeO_4^{2-} affinity constant and the SeO_3^{2-} affinity constant were estimated at $9.80 \pm 0.51 \text{ g Se m}^{-3}$ and $1.83 \pm 0.38 \text{ g Se m}^{-3}$, respectively. The model was then applied to evaluate the effects of key operating conditions on the single-stage H_2 -based MBfR and the role of reactor configuration through comparing two-stage to single-stage MBfR systems. The results

showed that i) high SeO_4^{2-} or low NO_3^- concentration in the influent favored the growth of selenate-reducing bacteria (SeRB) and therefore benefited the Se removal, ii) the influent dissolved oxygen slightly inhibited the Se removal through enhancing the aerobic microbial respiration on H_2 , iii) the H_2 supply should be controlled at a proper level to avoid SeRB suppression and H_2 wastage, iv) thin biofilm should be avoided to ensure a protected niche for SeRB and therefore a promising Se removal, and v) the two-stage MBfR configuration offered relatively higher efficiency in removing Se and NO_3^- simultaneously under the same loading condition.

Keywords: Hydrogen-based membrane biofilm reactor; selenate; nitrate; mathematical modelling; model calibration

INTRODUCTION

Despite its role as an essential trace element for life, selenium (Se) can also pose severe threats on organisms. Selenium pollution of aquatic environment is a worldwide phenomenon and is mainly caused by a broad spectrum of human activities ranging from agricultural practices to industrial manufacturing operations (Lemly, 2002). Human intake of high concentrations of selenium produces definite toxic symptoms (WHO, 2011). Although the level of selenium in groundwater and surface water is typically below 1 g Se m^{-3} (WHO, 2011), it has been reported to reach up to 6 g Se m^{-3} in certain areas such as the Yellow Cat Area, Grand County, Utah (Cannon, 1964). To regulate the water quality for the sake of health and wellbeing, the US EPA has set the maximum contaminant level (MCL) of 0.05 g Se m^{-3} in drinking water (EPA, 2009) while the European Union and World Health Organization have recommended 0.01 and 0.04 g Se m^{-3} , respectively, for drinking water. The US EPA has also set the effluent Se limit for the steam electric power generating sector at 12 mg Se m^{-3} for existing sources and 5 mg Se m^{-3} for new sources (EPA, 2015).

The presence of selenium contamination is mainly in the form of oxyanions, such as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}). In contrast, the most reduced form of selenium, selenide (Se^{2-}), is rather unstable and can be readily oxidized to elemental selenium (Se^0), which is highly insoluble and of lower toxicity (Lai et al., 2014). Therefore, the common practice to remediate selenium-contaminated water is to reduce $\text{SeO}_4^{2-}/\text{SeO}_3^{2-}$ to Se^0 which can then be recovered by filtration or centrifugation (Chung et al., 2006; Fellowes et al., 2011; Fesharaki et al., 2010). To achieve the reduction of $\text{SeO}_4^{2-}/\text{SeO}_3^{2-}$ to Se^0 , biological processes are usually preferred to physical-chemical techniques due to their advantages in terms of adaptability to different wastewater types and treatment costs as well as their ability to work at low Se concentrations and efficiency in removing selenite (Chung et al., 2006); most physical-chemical methods have been documented to be suitable for selenite removal but less effective in removing selenate (Chung et al., 2006; Lee, 1989). Carbon-based reduction of selenate and selenite has been proposed (Cantafio et al., 1996; Gerhardt et al., 1991; Macy et al., 1993; Mal et al., 2017; Squires et al., 1989), however the use of organic carbon source as the electron donor typically necessitates post-treatment to get rid of excessive organic carbon addition to stabilize treated water.

Autotrophic reduction of selenate and selenite using inorganic electron donors such as hydrogen gas (H_2) represents a good alternative. H_2 is not toxic to humans, and its low water solubility ensures negligible residual electron donor in the treated effluent. The membrane biofilm reactor (MBfR) is particularly suitable for delivery of H_2 to microorganisms in spite of the explosive nature of H_2 which necessitates proper measures to reduce the risk of explosions (Di Capua et al., 2015) and has been successfully applied to drive the respiratory reduction of selenate and selenite (Chung et al., 2006; Lai et al., 2014). In such a H_2 -based MBfR, H_2 as the electron donor was supplied through gas-permeable bubbleless membranes,

while SeO_4^{2-} was provided in the bulk liquid and diffused into biofilm where SeO_4^{2-} was subject to sequential reduction to Se^0 via SeO_3^{2-} by a group of selenate-reducing bacteria (SeRB).

Nevertheless, SeO_4^{2-} reduction is significantly affected by the introduction of nitrate (NO_3^-) in the H_2 -based MBfR. With the MCL of 10 g N m^{-3} in drinking water, nitrate is often concomitant in water contaminated by selenium (Zhang et al., 2003) and can induce methemoglobinemia in infants (Knobeloch et al., 2000). Chung et al. (2006) reported improved SeO_4^{2-} reduction in the presence of a small amount of NO_3^- in the MBfR when H_2 was not limiting. By comparison, another study by Lai et al. (2014) found that a high NO_3^- loading would cause H_2 limitation which suppressed SeO_4^{2-} reduction. Different interactions between SeO_4^{2-} and NO_3^- reduction are highly related to the microbial community structure in the biofilm. With the cooccurrence of SeO_4^{2-} and NO_3^- as the electron acceptors in the H_2 -based MBfR, microbial competitions arise between SeRB and denitrifying bacteria (DB) for the electron donor (i.e., H_2). On top of the intrinsic differences in physiological and biochemical properties between microorganisms, the extent of microbial competitions and the resulting reduction performance are highly dependent on the operating conditions of the H_2 -based MBfR. So far, a number of uncertainties regarding the microbial competitions and the associated performance of the H_2 -based MBfR under different operating conditions remain undisclosed.

The aim of this work is therefore to investigate the impacts of operating conditions on the microbial community structure as well as the system performance of the H_2 -based MBfR treating SeO_4^{2-} and NO_3^- simultaneously. To this end, a biofilm model integrating the key biological mechanisms mediating reduction of SeO_4^{2-} , SeO_3^{2-} and NO_3^- as well as the

potential aerobic growth of Se reducers (Lai et al., 2014) and H_2 -utilizing denitrifiers (Tang et al., 2012) in the H_2 -based MBfR was developed. The developed model was calibrated and validated using the long-term operating data collected from a single-stage H_2 -based MBfR reported in Lai et al. (2014), which was fed with different NO_3^- concentrations (i.e., 0, 1.0, 5.0 and 10.0 g N m^{-3}) together with constant SeO_4^{2-} and dissolved oxygen (DO) concentrations in the influent at different operating stages. The model was then applied to systematically quantify the effects of key operating factors including influent SeO_4^{2-} , NO_3^- and DO concentrations, H_2 loading and biofilm thickness on the system performance as well as the steady-state microbial community structure of the single-stage H_2 -based MBfR treating SeO_4^{2-} and NO_3^- simultaneously. A brief comparison between two-stage (i.e., two MBfRs connected in series) and single-stage MBfR systems under same loading condition was also presented to shed light on the role of reactor configuration as an additional factor.

MATERIALS AND METHODS

Model Development

The multi-species process model was developed to describe both anoxic and aerobic mechanisms of SeRB, hydrogen-based autotrophic denitrifying bacteria (ADB) and heterotrophic bacteria (HB). In total, as detailed in Table S1 in the Supporting Information (SI), the model describes the relationships among seven dissolved components, i.e., hydrogen (S_{H_2}), nitrate (S_{NO_3}), selenate (S_{SeO_4}), selenite (S_{SeO_3}), dissolved oxygen (S_{O_2}), utilization-associated products (UAP, S_{UAP}) and biomass-associated products (BAP, S_{BAP}), and six particulate components, i.e., ADB (X_{ADB}), SeRB (X_{SeRB}), HB (X_{HB}), elemental selenium (X_{Se}), inert organics (X_I) and extracellular polymeric substances (EPS, X_{EPS}). In the model as presented in Table 1, H_2 serves as the electron donor and energy source driving the microbial reduction of O_2 , SeO_4^{2-} , SeO_3^{2-} and NO_3^- . Electrons are fractionized based on mass balance

for the synthesis of new biomass as well as the accompanying production of UAP and EPS. Biomass growth and UAP and EPS formation are linked to substrate consumption via yield coefficient (Y). EPS are hydrolyzed to BAP, which together with UAP are oxidized by HB to reduce O_2 and NO_3^- . As listed in Table S2 in the SI, dual-substrate Monod equations were applied to describe the species-specific interactions between electron acceptors (O_2 , SeO_4^{2-} , SeO_3^{2-} and NO_3^-) and electron donors (H_2 , UAP and BAP). As aerobic growth was included in addition to anoxic growth for all three microorganisms in the model, non-competitive oxygen inhibition functions were incorporated into the corresponding kinetic rate expressions. It should be noted that the intermediate NO_2^- was not specifically included in the model considering its higher H_2 -utilization priority as well as the fact that it was not detected in related H_2 -based MBfR systems (Chen et al., 2017; Tang et al., 2013; Tang et al., 2012). The definitions, values, units and sources of all parameters used in the developed model are listed in Table S3 in the SI.

The one-dimensional biofilm model was then constructed on the software AQUASIM 2.1d (Reichert, 1998) to simulate the H_2 -based MBfR as a two-compartment structure: a completely mixed gas compartment representing the membrane lumen and a biofilm compartment containing the biofilm and bulk liquid. The H_2 inflow in the gas compartment was determined by the applied gas pressure along with the gas flow rate. The H_2 supply to the biofilm was simulated according to Henry's law and using a diffusive link, which connected the gas compartment to the base of the biofilm and modeled diffusive mass exchange of substances between compartments through membranes or boundary layers. Same with Terada et al. (2007), the boundary layer thickness was set at 100 μm , irrespective of biofilm thickness. The transport of dissolved components through the diffusion boundary layer and into or out of the biofilm was described with the resistance approach using Fick's first law.

Diffusion coefficients for dissolved components in the biofilm liquid phase were set at 0.8-fold of the values in water. The specifications as well as the influent conditions in the model were set according to the operating conditions of experiments, the data of which were used for the subsequent model evaluation. More details related to the biofilm model setup can be found in Chen et al. (2017).

Experimental Data

Experimental data from the single-stage H_2 -based MBfR reported in (Lai et al., 2014) were used to calibrate and validate the developed model. The single-stage MBfR with a total volume of 65 mL contained a bundle of 32 composite hollow fiber membranes fixed at the bottom and another bundle of 10 coupon hollow fiber membranes in a separate tube which were used for microbial community analysis. The MBfR was initially inoculated with diluted activated sludge obtained from a wastewater treatment plant, and the microbial community was enriched by circulating $10 \text{ g m}^{-3} \text{ SeO}_4^{2-}$ for 48 hours. Upon the attainment of the enrichment as evidenced by the significant decrease in the bulk SeO_4^{2-} concentration as well as the visually observed formation of biofilm outside the membrane surface, a medium containing SeO_4^{2-} was fed to the MBfR and the concentration was maintained around 1 g Se m^{-3} throughout the experiment. On top of SeO_4^{2-} , NO_3^- was also provided in the medium but at varied concentrations based on which the experiments were categorized into different operating stages: 0, 10, 0, 1 and 5 g N m^{-3} at Stages 1, 2, 3, 4 and 5, respectively. The next operating stage only commenced after the current operating stage reached steady state, which was indicated by stable effluent concentrations of all chemical indexes. The DO concentration in the influent was between 7.7 and 8.0 g m^{-3} for all stages. The liquid was completely mixed using a peristaltic pump at 100 mL min^{-1} . The influent feeding was maintained at 0.5 mL min^{-1} , the H_2 pressure at 1.17 atm , and the temperature at $25 \text{ }^\circ\text{C}$ for all

stages. More details related to the system configuration, operation and analysis of the single-stage H_2 -based MBfR can be found in Lai et al. (2014).

Model Calibration and Validation

Slightly different from the framework proposed by Rittmann et al. (2018) that was in least favor of adjustments in kinetic and stoichiometric parameters, priority of this work was indeed placed in the tuning and determination of parameters associated with the anoxic processes of H_2 -based reduction of SeO_4^{2-} and SeO_3^{2-} . This was in view of the fact that only parameters related to the anoxic mechanisms of ADB and HB and the aerobic bioconversion of H_2 have been well established in the previous work (Chen et al., 2017). These parameter values were therefore directly adopted in this work. Of the remaining six parameters of interest, the yield in H_2 -based SeO_4^{2-} reduction (Y_2), the yield in H_2 -based SeO_3^{2-} reduction (Y_3), the maximum growth rate in H_2 -based SeO_4^{2-} reduction (μ_2) and the maximum growth rate in H_2 -based SeO_3^{2-} reduction (μ_3) were obtained at 0.13 g COD g⁻¹ COD, 0.11 g COD g⁻¹ COD, 0.037 h⁻¹ and 0.032 h⁻¹, respectively, by thermodynamic state calculations assuming an energy-transfer efficiency of 0.6 (Kleerebezem and Van Loosdrecht, 2010). This approach as detailed in the SI has been well received and proved suitable for modelling similar H_2 -based MBfR systems (Chen et al., 2017; Tang et al., 2012). Hence, in the end, only the SeO_4^{2-} affinity constant for SeRB (K_{SeO4}^{SeRB}) and the SeO_3^{2-} affinity constant for SeRB (K_{SeO3}^{SeRB}) were evaluated using the long-term experimental data.

The experimental data collected during Stages 1, 2 and 3 of the single-stage H_2 -based MBfR system (Lai et al., 2014) were used for model calibration, which was conducted through minimizing the sum of squares of the deviations between the experimental measurements and the model predictions. Parameter estimation and uncertainty evaluation were conducted

according to Batstone et al. (2003) with a 95% confidence level for significance testing and parameter uncertainty analysis. A modified AQUASIM 2.1d was used to obtain the parameter confidence region (Ge et al., 2010). Model validation was performed with the calibrated model parameters using the experimental data obtained during Stages 4 and 5 of the single-stage H_2 -based MBfR system (Lai et al., 2014). With an initial value set at $1\ \mu\text{m}$ in the beginning of Stage 1, the thickness of biofilm, which was assumed to have a biomass density of $79300\ \text{g COD m}^{-3}$ (Tang et al., 2012), was not specifically compared but was controlled with the limit of $100\ \mu\text{m}$ in the model evaluation process. The profiles of SeO_4^{2-} , SeO_3^{2-} and NO_3^- were used simultaneously for the model calibration and validation processes. Se^0 was not specifically considered in the model evaluation process because it was not measured experimentally.

Assessment on Key Operating Factors

The evaluated model was then applied to simulate the implementation of the H_2 -based MBfR treating SeO_4^{2-} and NO_3^- simultaneously under different operating conditions. As shown in Table 2, six different scenarios are considered in total in this work. The first simulation scenario (i.e., Scenario 0 of Table 2) investigated the spatial distribution characteristics of the single-stage H_2 -based MBfR through generating depth-wise profiles of microbial community and substrates distribution in the biofilm. The SeO_4^{2-} , NO_3^- and DO concentrations for Scenario 0 were set at $1.0\ \text{g Se m}^{-3}$, $10.0\ \text{g N m}^{-3}$ and $8.0\ \text{g m}^{-3}$, respectively, which were comparable to Lai et al. (2014). H_2 surface loading (L_{H_2}), influent surface loading (L_{In}) and biofilm thickness (L_f) were controlled at $0.128\ \text{g COD m}^{-2}\ \text{h}^{-1}$, $0.00236\ \text{m h}^{-1}$ and $100\ \mu\text{m}$, respectively. As SeO_4^{2-} , NO_3^- and DO all act as electron acceptors, their varied concentrations in the real contaminated water would to great extent affect the H_2 -based MBfR. Therefore, Scenarios 1, 2 and 3 of Table 2 were designed to unveil the impacts of the influent SeO_4^{2-} ,

NO_3^- and DO concentrations, respectively, on the single-stage H_2 -based MBfR. The influent SeO_4^{2-} concentration was varied from 0.1 to 5.0 g Se m^{-3} while the NO_3^- concentration was adjusted between 0.1 and 15.0 g N m^{-3} . To account for all oxygenous conditions that might exist in reality, the DO concentration studied covered both anoxic condition (i.e., 0) to nearly saturated condition (i.e., 8.0 g m^{-3}). Scenarios 4 and 5 of Table 2 explored the effects of L_{H_2} (0.063 – 0.140 g COD $\text{m}^{-2} \text{h}^{-1}$) and L_f (40 – 180 μm), respectively, on the steady-state system performance and the related microbial community structure of the single-stage H_2 -based MBfR. Different L_f affected the quantity of biomass in the biofilm for different scenarios, which all assumed a biomass density of 79300 g COD m^{-3} (Tang et al., 2012). The last scenario (Scenario 6 of Table 2) compared two MBfR configurations: two-stage MBfR system (consisting of two 65 mL MBfRs which were connected in series) and one-stage MBfR system (consisting of one 65 mL MBfR). Despite the slight difference in the steady-state biofilm thickness setting as shown in Table 2, the two systems were subject to the same influent concentrations of SeO_4^{2-} , NO_3^- and DO, the same influent surface loading and the same H_2 supply.

The initial concentrations of all soluble components in the biofilm and the bulk liquid for each simulation scenario were assumed to be zero. An average biofilm thickness was applied in the model without consideration of its variation with locations. The steady-state biofilm thickness (L_f) was controlled by the surface detachment velocity equation (u_{de}) reported in Ni and Yuan (2013) as $u_{\text{de}} = u_F \times (L_f / L_{f,\text{mean}})^2$, where u_F represented the biofilm growth velocity while $L_{f,\text{mean}}$ stood for the desired mean biofilm thickness. No re-attachment of detached particulates was considered in the model. All simulations assumed an initial biofilm thickness of 10 μm and were run for up to 1000 days to reach steady-state conditions indicated by constant effluent concentrations, biofilm thickness and microbial compositions

in biofilm. To systematically evaluate the MBfR's capacity of removing SeO_4^{2-} and SeO_3^{2-} , the removal efficiency of soluble Se (referred to as Se removal efficiency hereinafter) was used in addition to the NO_3^- removal efficiency and the H_2 utilization efficiency to represent the steady-state system performance.

RESULTS AND DISCUSSION

Model Calibration and Validation

$K_{\text{SeO}_4}^{\text{SeRB}}$ and $K_{\text{SeO}_3}^{\text{SeRB}}$ were estimated using the experimental data collected during Stages 1, 2 and 3 of the single-stage H_2 -based MBfR system, with the best fits obtained at 9.80 g Se m^{-3} and 1.83 g Se m^{-3} , respectively. Figure 1A illustrates the model calibration results including the predicted and measured dynamic profiles of SeO_4^{2-} , SeO_3^{2-} and NO_3^- in the influent and effluent fluxes, while Figure 1B directly compares the predicted and measured Se and NO_3^- removal efficiencies. During Stage 1 (days 1 – 67) when SeO_4^{2-} was the only input electron acceptor, SeO_4^{2-} was reduced to SeO_3^{2-} , which was further reduced to Se^0 . With the influent SeO_4^{2-} maintained around 1.0 g Se m^{-3} , the effluent SeO_4^{2-} concentration gradually decreased, resulting from the increased biomass and hence enhanced reduction capacity of SeRB in the biofilm. SeO_3^{2-} accumulated in the MBfR due to the higher rate of SeO_4^{2-} reduction than SeO_3^{2-} reduction (Lai et al., 2014). As the difference between SeO_4^{2-} removed and SeO_3^{2-} produced represented the total Se removed, the Se removal of the MBfR increased slightly with time. At Stage 2 (days 67 – 106), the introduction of about $10.0 \text{ g NO}_3^- \text{ N m}^{-3}$ to the MBfR suppressed the Se removal through microbial competitions for the electron donor (i.e., H_2). As a result, the effluent SeO_4^{2-} increased significantly. In contrast, around 80% of the feeding NO_3^- was removed in the MBfR, leading to the steady effluent NO_3^- concentration of about 2.0 g N m^{-3} . When the feeding NO_3^- was removed at Stage 3 (days 106 – 138), SeO_4^{2-} reduction recovered. The noted discrepancies between the model predicted and

experimentally measured effluent SeO_4^{2-} levels and Se removal efficiencies at Stages 2 and 3 in Figure 1 could be ascribed to the possible overlook of i) the capability of some SeRB to degrade nitrate (Lai et al., 2014) or ii) the impact of the changing influent composition on the biofilm structure in the model of this work. Nevertheless, as evidenced by the acceptably high correlation (0.59) between model predicted and experimentally measured profiles of effluent Se oxyanions (i.e., $\text{SeO}_4^{2-} + \text{SeO}_3^{2-}$) as well as the comparable levels of model predicted and experimentally measured effluent Se oxyanions ($0.84 \pm 0.23 \text{ g Se m}^{-3}$ versus $0.89 \pm 0.26 \text{ g Se m}^{-3}$) and NO_3^- ($1.63 \pm 0.02 \text{ g NO}_3^- \text{-N m}^{-3}$ versus $1.87 \pm 0.38 \text{ g NO}_3^- \text{-N m}^{-3}$), the model was able to capture the general variation trends of SeO_4^{2-} , SeO_3^{2-} and NO_3^- to a satisfactory extent, which supported the validity of the calibrated model.

The 95% confidence region for $K_{\text{SeO}_4}^{\text{SeRB}}$ and $K_{\text{SeO}_3}^{\text{SeRB}}$ in combination with their uncorrelated confidence intervals obtained during the model calibration process is delineated in Figure S1 in the SI. The uncorrelated confidence intervals of both parameters were relatively small and fully covered by the correlated confidence region, indicating a good level of reliability and identifiability of the estimated values.

Model validation was conducted by comparing the experimental data at Stages 4 and 5 with the model predictions obtained using the calibrated model, the results of which are shown in Figure 2. At Stage 4 (days 138 – 183), the reappearance of $1.0 \text{ g NO}_3^- \text{-N m}^{-3}$ in the influent of the MBfR didn't significantly affect the Se removal. With the influent SeO_4^{2-} concentration of about 1.3 g Se m^{-3} , the effluent SeO_4^{2-} and SeO_3^{2-} concentrations fluctuated but stayed relatively stable ($0.4 - 0.5 \text{ g Se m}^{-3}$ and $0.1 - 0.2 \text{ g Se m}^{-3}$, respectively). In the meanwhile, the influent NO_3^- was completely removed, leaving trace amount in the effluent. Further increase in the influent NO_3^- concentration to 5.0 g N m^{-3} at Stage 5 brought insignificant

changes to the Se removal, which was in the form of Se^0 and was proved by the visual observation of reddish-brown color in the MBfR. The good match between the model predicted and measured trends, supported by the satisfactory correlation between model predictions and experimental measurements for effluent Se oxyanions (0.69) and NO_3^- (0.56) as well as their comparable concentration levels and removal efficiencies as shown in Figure 2, further verified the validity of the developed model.

Characteristics of the H_2 -Based MBfR Biofilm

Scenario 0 of Table 2 investigated the spatial distribution characteristics of the biofilm which reflected the acting mechanisms behind the system performance of the single-stage H_2 -based MBfR treating SeO_4^{2-} and NO_3^- simultaneously. With 99.9% of the H_2 supplied being utilized as electron donor, the steady-state Se and NO_3^- removal efficiencies were 40.5% and 95.7%, respectively. The steady-state distribution profiles of solid and dissolved species in the biofilm under the operating conditions of Scenario 0 are shown in Figure 3. As shown in Figure 3A, SeRB were most abundant (6%) at the base of the biofilm where H_2 was supplied. In contrast, HB, the abundance of which was independent of H_2 supply, were densest (12%) at the biofilm surface. ADB were the most dominant microbial species, with an abundance of over 40% across the biofilm. Considering the counter diffusion of H_2 and NO_3^- as the substrates for ADB from the two sides of the biofilm, the abundance of ADB reached the peak (55%) in the middle of the biofilm (See Figure 3A). Inert biomass was higher on the membrane side and decreased significantly towards the bulk liquid, which was in contrast with a similar H_2 -based MBfR system fed with NO_3^- and ClO_4^- (Tang et al., 2012) as well as another H_2 -based MBfR system treating NO_3^- and SO_4^{2-} (Tang et al., 2013). More active biomass was therefore present at the biofilm surface, hence the higher fraction of EPS on the

bulk liquid side. Despite the different trend observed, the overall fraction of EPS in the biofilm was still comparable with Tang et al. (2012) and Tang et al. (2013).

Figure 3B demonstrates the associated profiles of dissolved species within the biofilm of Scenario 0. H_2 decreased from the membrane surface where it was supplied towards the bulk liquid. On the contrary, the SeO_4^{2-} and NO_3^- concentrations both decreased from the bulk liquid where they were provided to the base of the biofilm. The SeO_3^{2-} concentration almost remained unchanged across the biofilm thickness. UAP and BAP were produced in the biofilm and then diffused into the liquid, therefore, their concentrations were higher in the inner layer of the biofilm. The DO supplied in the influent was quickly consumed within the top biofilm layer, creating a nearly anoxic environment in the biofilm under the simulation conditions of Scenario 0.

As presented in Figure 3, the counter-diffusional supply of gas and liquid substrates resulted in the stratification in the biofilm, which determined the system performance of the single-stage H_2 -based MBfR. This heterogeneous, stratified characteristic of biofilm has been shown to vary among systems and be controlled by operating conditions (Chen et al., 2017; Tang et al., 2013; Tang et al., 2012). Therefore, a systematic study on the impacts of key operating factors should be conducted to comprehensively evaluate the feasibility of applying the H_2 -based MBfR technology to treat SeO_4^{2-} and NO_3^- simultaneously.

Key Operating Factors Affecting the Single-Stage H_2 -Based MBfR

The impact of the influent SeO_4^{2-} concentration on the steady-state system performance and microbial community structure of the single-stage H_2 -based MBfR (Scenario 1 of Table 2) is shown in Figure 4A. There was no SeO_4^{2-} reduction and hence no Se removal when the

influent SeO_4^{2-} concentration was less than 0.75 g Se m^{-3} . The H_2 supply was solely used for NO_3^- reduction, thus rendering the NO_3^- removal efficiency of 95.8% and the H_2 utilisation efficiency of 99.8%. SeO_4^{2-} reduction arose with the Se removal efficiency of 23.4% at the influent SeO_4^{2-} concentration of 0.75 g Se m^{-3} . Further increase in the influent SeO_4^{2-} concentration led to consistently increased Se removal efficiency, reaching 68.6% at the highest influent SeO_4^{2-} concentration of 5 g Se m^{-3} studied in this work. The corresponding NO_3^- removal efficiency slightly decreased to 94.7% while the complete H_2 utilisation (100%) was achieved. The varying system performance was attributed to the changing microbial community structure in the biofilm under different influent SeO_4^{2-} concentration conditions, as delineated in Figure 4A. A low influent SeO_4^{2-} concentration of below 0.75 g Se m^{-3} led to the washout of SeRB from the biofilm. As a result, ADB and HB dominated the biofilm, each accounting for 93% and 7% of the active biomass. With the increasing influent SeO_4^{2-} concentration, SeRB started to gain advantage in competing with ADB for H_2 . Consequently, SeRB (2%) appeared in the biofilm at the influent SeO_4^{2-} concentration of 0.75 g Se m^{-3} . Thereafter, the active fraction of SeRB increased while that of ADB decreased. The decreased active fraction of ADB benefited the growth of HB which competed with ADB for NO_3^- , thus leading to the increased active fraction of HB in the biofilm. Therefore, at the influent SeO_4^{2-} concentration of 5 g Se m^{-3} , SeRB, ADB and HB coexisted in the biofilm, each taking up 11%, 77% and 12% of the total active biomass, respectively.

The relationship between the influent NO_3^- concentration and the steady-state system performance as well as microbial community structure of the single-stage H_2 -based MBfR (Scenario 2 of Table 2) is shown in Figure 4B. When the influent NO_3^- concentration was lower than 1.0 g N m^{-3} , HB were outcompeted by ADB, resulting in the joint dominance of ADB and SeRB in the biofilm. However, with the increased NO_3^- concentration in the

influent, the abundance of ADB increased while that of SeRB decreased, hence the decreased Se removal efficiency. The excessive H_2 supply at relatively low influent NO_3^- concentration led to stable and high-level NO_3^- removal ($\sim 95.0\%$), and the H_2 utilisation efficiency increased with the increased influent NO_3^- concentration. At the influent NO_3^- concentration of 2.5 g N m^{-3} , HB appeared in the biofilm with the abundance of 3% while ADB and SeRB each accounted for 89% and 8% of the active biomass. The corresponding NO_3^- removal efficiency, Se removal efficiency and H_2 utilisation efficiency were 95.7%, 53.2% and 77.8%, respectively. Further increase in the influent NO_3^- concentration induced more HB, which negatively affected the existence of SeRB in the biofilm and hence the Se removal efficiency. When the influent NO_3^- concentration reached over 10.0 g N m^{-3} , H_2 supply became limited, indicated by the 100% H_2 utilisation efficiency and the decreased NO_3^- removal efficiency. Consequently, SeRB were completely washed out of biofilm and ADB (90%) and HB (10%) dominated the biofilm. In that case, no Se removal was observed in the MBfR.

The role of the influent DO concentration on the steady-state microbial community structure and system performance of the single-stage H_2 -based MBfR (Scenario 3 of Table 2) is depicted in Figure 4C. When there was no DO in the influent, SeRB, ADB and HB coexisted in the biofilm, each occupying 4%, 89% and 7% of the active biomass. The resulting NO_3^- removal efficiency, Se removal efficiency and H_2 utilisation efficiency were 96.0%, 44.6% and 94.6%, respectively. The increase in the influent DO concentration slightly decreased the active biomass fraction of SeRB, which reached 3% at the maximum DO concentration of 8.0 g m^{-3} studied in this work. Consequently, the Se removal efficiency decreased gradually to 40.5%. The increased introduction of DO also enhanced the aerobic microbial respiration on H_2 , hence the increased H_2 utilisation efficiency which reached 99.9% at the maximum DO concentration of 8.0 g m^{-3} . No significant change was observed for the NO_3^- removal

efficiency which remained around 96.0%, due to the almost unchanged active biomass fraction of ADB in the biofilm.

The dependence of the steady-state microbial community structure and system performance of the single-stage H_2 -based MBfR on the H_2 loading (L_{H_2}) (Scenario 4 of Table 2) is depicted in Figure 4D. When L_{H_2} was relatively low ($<0.115 \text{ g COD m}^{-2} \text{ h}^{-2}$), ADB (90%) dominated the biofilm with the coexistence of a low fraction of HB (10%), due to their competitive advantage over SeRB for H_2 . The H_2 supplied was completely consumed. As a result, there was no Se removal while the NO_3^- removal efficiency kept increasing from 39.5% at L_{H_2} of $0.063 \text{ g COD m}^{-2} \text{ h}^{-2}$. The increase of L_{H_2} to $0.125 \text{ g COD m}^{-2} \text{ h}^{-2}$ increased the availability of H_2 for SeRB. Consequently, SeRB (1%) appeared and coexisted with ADB (91%) and HB (8%) in the biofilm, rendering the Se and NO_3^- removal efficiencies of 20.1% and 94.9%, respectively, at L_{H_2} of $0.125 \text{ g COD m}^{-2} \text{ h}^{-2}$. Further increase in L_{H_2} to $0.128 \text{ g COD m}^{-2} \text{ h}^{-2}$ stimulated the growth and enhanced the fraction of SeRB but slightly depressed that of ADB, giving rise to the increased Se removal efficiency of 40.5%. However, excessive H_2 supply of over $0.132 \text{ g COD m}^{-2} \text{ h}^{-2}$ did not make further significant change to the microbial community structure, with SeRB, ADB and HB taking up 4%, 89% and 7% of the total active biomass, respectively. The resulting Se and NO_3^- removal efficiencies maintained at 44.2% and 95.9%, respectively. The accompanying H_2 utilization efficiency dropped consistently from 100% to 91.5% at L_{H_2} of $0.140 \text{ g COD m}^{-2} \text{ h}^{-2}$.

The sensitivity of the steady-state system performance and microbial community structure of the single-stage H_2 -based MBfR to the biofilm thickness (L_f) (Scenario 5 of Table 2) is illustrated in Figure 4E. Due to the loss in the competition for H_2 , SeRB were absent from the biofilm at L_f of $<80 \text{ }\mu\text{m}$, hence zero Se removal in the system. However, the Se removal

efficiency quickly increased to 40.5% at L_f of 100 μm due to the appearance of SeRB (3%) in the biofilm. Further increase in L_f slightly favored the growth of SeRB but their abundance was confined to 4% due to the finite H_2 supply, as evidenced by the complete (100%) H_2 utilization. Therefore, the maximum Se removal efficiency was limited to around 50% (See Figure 4E). The NO_3^- removal efficiency was not significantly impacted by the enhanced abundance of SeRB in the thicker biofilm but remained high (94.2%) at the maximum L_f of 180 μm studied in this work.

Overall, the single-stage H_2 -based MBfR was particularly suitable for treating wastewater with high-level SeO_4^{2-} (e.g., $>2 \text{ g Se m}^{-3}$) but low-level NO_3^- (e.g., $<5 \text{ g N m}^{-3}$) (i.e., a relatively high influent $\text{SeO}_4^{2-}/\text{NO}_3^-$ ratio) which favored the anoxic growth of SeRB on H_2 in the presence of ADB and HB (Figures 4A and 4B). The influent DO slightly affected the Se removal through enhancing the aerobic microbial respiration on H_2 (Figure 4C). When operating such a single-stage H_2 -based MBfR, the H_2 supply should be controlled carefully. As shown in Figure 4D, a too low L_{H_2} would suppress the proliferation of SeRB in the biofilm and thus compromise the Se removal, while a too high L_{H_2} would bring about energy wastage. Moreover, a thin biofilm which would fail in providing a protected niche for SeRB (Figure 4E) should be avoided. This work doesn't focus on the process optimization of the H_2 -based MBfR in consideration of various operating conditions, but it is feasible via modeling and warrants further work. From Figure 4 it seems that high-level (e.g., $>90\%$) Se removal is hard to achieve only with the single-stage H_2 -based MBfR unless the influent concomitant NO_3^- concentration is extremely low. Therefore, downstream refining process (such as those aforementioned physical-chemical methods) should be included to meet the direct discharge limit of 0.05 g Se m^{-3} .

Comparison between Single-Stage and Two-Stage MBfR Systems

Figure 5 compares the two-stage and single-stage MBfR systems under the same loading condition set in Scenario 6 of Table 2. As shown in Figure 5A, different microbial compositions were observed in the two MBfRs of the two-stage system: ADB (89%), SeRB (3%) and HB (8%) were present in the first-stage MBfR while ADB (89%) and SeRB (11%) dominated the biofilm of the second-stage MBfR without HB. The reason for the different microbial community structure lied in the different influent compositions for the two MBfRs. According to Figure 5B, around 40.5% of Se removal and 95.7% of NO_3^- removal were achieved in the first-stage MBfR. The high-level NO_3^- removal in the first-stage MBfR gave rise to a low NO_3^- (compared to SeO_4^{2-}) concentration in the influent for the second-stage MBfR. The low influent NO_3^- concentration condition favored the growth of ADB over HB and therefore washed HB out of the biofilm in the long term, analogous to the low influent NO_3^- case in Scenario 2 (See Figure 4B). The higher abundance of SeRB in the second-stage MBfR contributed to 34.6% of Se removal while the NO_3^- removal was further enhanced with 96.9% removal efficiency (Figure 5B). Overall, as indicated in Figure 5C, the two-stage MBfR system rendered the Se and NO_3^- removal efficiencies of 61.1% and 99.9%, respectively, around 5% and 2% higher than those of the single-stage counterpart under the same loading condition.

The same H_2 utilisation efficiency of 83.5% for the two systems suggested that the two-stage MBfR configuration was more efficient in removing Se and NO_3^- simultaneously. Despite other benefits such as better control flexibility, the two-stage MBfR system might necessitate higher construction and operation cost which affects its applicability. The trade-off is highly case-specific and therefore warrants further research. In fact, the two-stage H_2 -based MBfR system has been tested in the lab by Zhao et al. (2013) aiming to remove NO_3^- and

perchlorate (ClO_4^-) while retaining sulfate (SO_4^{2-}). Despite the agreement that NO_3^- was mainly removed in the first-stage MBfR, this work differed from Zhao et al. (2013) in terms of the efficacy of the second-stage MBfR. Zhao et al. (2013) reported almost complete ClO_4^- removal in the second-stage MBfR even with the interference of SO_4^{2-} . However, in this work, the Se removal was less than 40% in the second-stage MBfR. The discrepancy was due to the lower kinetic properties (including growth rate and affinity for substrate) of SeRB (See the SI) compared to perchlorate reducing bacteria (See Chen et al. (2017)) as well as the aided growth of perchlorate reducing bacteria on NO_3^- (Tang et al., 2012).

CONCLUSIONS

A biofilm model integrating the simultaneous NO_3^- and SeO_4^{2-} reduction processes in the H_2 -based MBfR was calibrated and validated using the experimental data of a reported H_2 -based MBfR. The model was then applied to evaluate the effects of key operating conditions on the single-stage H_2 -based MBfR and the role of reactor configuration through comparing two-stage to single-stage MBfR systems. The results showed that the influent SeO_4^{2-} and NO_3^- concentrations, H_2 loading and biofilm thickness significantly influenced the steady-state microbial community structure and the resulting Se and NO_3^- removal in the single-stage H_2 -based MBfR while the influent DO concentration only played a minor role. A high SeO_4^{2-} or low NO_3^- concentration in the influent (i.e., a relatively high influent $\text{SeO}_4^{2-}/\text{NO}_3^-$ ratio such as $>0.4 \text{ g Se g}^{-1} \text{ N}$) favored the growth of SeRB and therefore benefited the Se removal. When operating the single-stage H_2 -based MBfR to treat SeO_4^{2-} and NO_3^- simultaneously, the H_2 supply should be controlled at a proper level to avoid SeRB suppression at a too low H_2 loading while minimizing H_2 wastage at a too high H_2 loading. A thin biofilm should be avoided to ensure a protected niche for SeRB and therefore a promising Se removal. The two-stage MBfR configuration offered relatively higher efficiency in removing Se and NO_3^- .

simultaneously under the same loading condition.

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Supplementary data of this work can be found in online version of the paper.

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Table and Figure Legends

Table 1. Stoichiometric Matrix for the Developed Model

Table 2. Overview of the Simulation Scenarios for the Reported Results

Figure 1. Model calibration results based on the experimental data of the MBfR reported in Lai et al. (2014): (A) concentration profiles and (B) removal efficiencies.

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Table 1. Stoichiometric Matrix for the Developed Model

<i>i</i>	Process	S_{H_2} COD	S_{NO_3} N	S_{O_2} O	S_{SeO_4} Se	S_{SeO_3} Se	X_{Se} Se	S_{UAP} COD	S_{BAP} COD	X_{ADB} COD	X_{SeRB} COD	X_{HB} COD	X_I COD	X_{EPS} COD
<i>H₂-based autotrophic denitrifying bacteria (ADB)</i>														
1	Aerobic growth	$-\frac{1}{Y_0}$		$-\frac{(1-Y_0)k_1}{Y_0}$				$\frac{k_2}{Y_0}$		k_1				$\frac{k_3}{Y_0}$
2	Anoxic growth	$-\frac{1}{Y_1}$	$-\frac{(1-Y_1)k_1}{2.86Y_1}$					$\frac{k_2}{Y_1}$		k_1				$\frac{k_3}{Y_1}$
3	Decay									-1			$1-f_d$	
<i>Selenate reducing bacteria (SeRB)</i>														
4	Aerobic growth	$-\frac{1}{Y_0}$		$-\frac{(1-Y_0)k_1}{Y_0}$				$\frac{k_2}{Y_0}$			k_1			$\frac{k_3}{Y_0}$
5	Anoxic growth on SeO_4^{2-} reduction	$-\frac{1}{Y_2}$			$-\frac{4.94(1-Y_2)k_1}{Y_2}$	$\frac{4.94(1-Y_2)k_1}{Y_2}$		$\frac{k_2}{Y_2}$			k_1			$\frac{k_3}{Y_2}$
6	Anoxic growth on SeO_3^{2-} reduction	$-\frac{1}{Y_3}$				$-\frac{2.47(1-Y_3)k_1}{Y_3}$	$\frac{2.47(1-Y_3)k_1}{Y_3}$	$\frac{k_2}{Y_3}$			k_1			$\frac{k_3}{Y_3}$
7	Decay										-1		$1-f_d$	
<i>Heterotrophic bacteria (HB)</i>														
8	Hydrolysis of EPS								1					-1
9	Aerobic growth on UAP			$-\frac{1-Y_4}{Y_4}$				$-\frac{1}{Y_4}$				1		
10	Aerobic growth on BAP			$-\frac{1-Y_5}{Y_5}$					$-\frac{1}{Y_5}$			1		
11	Anoxic growth on UAP		$-\frac{1-Y_4}{2.86Y_4}$					$-\frac{1}{Y_4}$				1		
12	Anoxic growth on BAP		$-\frac{1-Y_5}{2.86Y_5}$						$-\frac{1}{Y_5}$			1		
13	Decay											-1	$1-f_d$	

Table 2. Overview of the Simulation Scenarios for the Reported Results

Scenario	Simulation condition	Variable condition
Scenario 0 Standard simulation of the MBfR with NO_3^- and SeO_4^{2-} in the influent	$S_{\text{SeO}_4} = 1.0 \text{ g Se m}^{-3}$ $S_{\text{NO}_3} = 10.0 \text{ g N m}^{-3}$ $S_{\text{O}_2} = 8.0 \text{ g m}^{-3}$ $L_{\text{In}} = 0.00236 \text{ m h}^{-1}$ (i.e., HRT = 3.0 h) $L_{\text{H}_2} = 0.128 \text{ g COD m}^{-2} \text{ h}^{-1}$ $L_f = 100 \text{ }\mu\text{m}$	–
Scenario 1 Effect of influent SeO_4^{2-} concentration on the MBfR	$S_{\text{NO}_3} = 10.0 \text{ g N m}^{-3}$ $S_{\text{O}_2} = 8.0 \text{ g m}^{-3}$ $L_{\text{In}} = 0.00236 \text{ m h}^{-1}$ (i.e., HRT = 3.0 h) $L_{\text{H}_2} = 0.128 \text{ g COD m}^{-2} \text{ h}^{-1}$ $L_f = 100 \text{ }\mu\text{m}$	$S_{\text{SeO}_4} = 0.1 - 5.0 \text{ g Se m}^{-3}$
Scenario 2 Effect of influent NO_3^- concentration on the MBfR	$S_{\text{SeO}_4} = 1.0 \text{ g Se m}^{-3}$ $S_{\text{O}_2} = 8.0 \text{ g m}^{-3}$ $L_{\text{In}} = 0.00236 \text{ m h}^{-1}$ (i.e., HRT = 3.0 h) $L_f = 100 \text{ }\mu\text{m}$	$S_{\text{NO}_3} = 0.1 - 15.0 \text{ g N m}^{-3}$
Scenario 3 Effect of influent DO concentration on the MBfR	$S_{\text{SeO}_4} = 1.0 \text{ g Se m}^{-3}$ $S_{\text{NO}_3} = 10.0 \text{ g N m}^{-3}$ $L_{\text{In}} = 0.00236 \text{ m h}^{-1}$ (i.e., HRT = 3.0 h) $L_{\text{H}_2} = 0.128 \text{ g COD m}^{-2} \text{ h}^{-1}$ $L_f = 100 \text{ }\mu\text{m}$	$S_{\text{O}_2} = 0 - 8.0 \text{ g m}^{-3}$
Scenario 4 Effect of L_{H_2} on the MBfR	$S_{\text{SeO}_4} = 1.0 \text{ g Se m}^{-3}$ $S_{\text{NO}_3} = 10.0 \text{ g N m}^{-3}$ $S_{\text{O}_2} = 8.0 \text{ g m}^{-3}$ $L_{\text{In}} = 0.00236 \text{ m h}^{-1}$ (i.e., HRT = 3.0 h) $L_f = 100 \text{ }\mu\text{m}$	$L_{\text{H}_2} = 0.063 - 0.140 \text{ g COD m}^{-2} \text{ h}^{-1}$
Scenario 5 Effect of L_f on the MBfR	$S_{\text{SeO}_4} = 1.0 \text{ g Se m}^{-3}$ $S_{\text{NO}_3} = 10.0 \text{ g N m}^{-3}$ $S_{\text{O}_2} = 8.0 \text{ g m}^{-3}$ $L_{\text{In}} = 0.00236 \text{ m h}^{-1}$ (i.e., HRT = 3.0 h) $L_{\text{H}_2} = 0.128 \text{ g COD m}^{-2} \text{ h}^{-1}$	$L_f = 40 - 180 \text{ }\mu\text{m}$
Scenario 6 Comparison between two-stage and single-stage MBfR systems under same loading condition	$S_{\text{SeO}_4} = 1.0 \text{ g Se m}^{-3}$ $S_{\text{NO}_3} = 10.0 \text{ g N m}^{-3}$ $S_{\text{O}_2} = 8.0 \text{ g m}^{-3}$ $L_{\text{In}} = 0.00118 \text{ m h}^{-1}$ (i.e., HRT = 6.0 h)	$L_f = 100 \text{ }\mu\text{m}$ for single-stage MBfR system $L_f = 100/40 \text{ }\mu\text{m}$ for first/second-stage MBfR of the two-stage system

SeO_4^{2-} : ■ influent — model □ effluent SeO_3^{2-} : — model △ effluent
 NO_3^- : ● influent — model ○ effluent

Se removal: — model □ experiment NO_3^- removal: — model ○ experiment

Figure 1. Model calibration results based on the experimental data of the MBfR reported in Lai et al. (2014): (A) concentration profiles and (B) removal efficiencies.

SeO_4^{2-} : ■ influent — model □ effluent SeO_3^{2-} : — model △ effluent
 NO_3^- : ● influent — model ○ effluent

Se removal: — model □ experiment NO_3^- removal: — model ○ experiment

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Highlights

- A model was developed to describe SeO_4^{2-} and NO_3^- reduction in H_2 -based MBfR.
- A high influent $\text{SeO}_4^{2-}/\text{NO}_3^-$ ratio and a thick biofilm thickness favored Se removal.
- Moderate H_2 supply avoided SeRB suppression whilst minimizing H_2 wastage.
- Two-stage MBfR had a slightly better removal performance than single-stage MBfR.

Graphical Abstract