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Coupling of palladium nanoparticles with biofilm improved co-reduction of chromium(VI) and nitrate

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ABSTRACT

Chromium (Cr) contamination in water poses significant health risks, yet advanced remediation methods remain limited. Cr(VI) reduction catalyzed by palladium nanoparticles (PdNPs) on hydrogen-transfer membranes has shown potential but requires further optimization. This study investigated the simultaneous microbial-driven and Pd-catalyzed Cr(VI) reduction, focusing on reduction efficiency and optimal conditions. Two hydrogen-based membrane reactors were compared: a Pd-biofilm reactor incorporating PdNPs associated with a biofilm, and a control biofilm reactor. Continuous experiments demonstrated the superior performance of the Pd-biofilm reactor, achieving immediate Cr(VI) reduction and effluent Cr(III) concentrations below $0.040 \text{ mg} \cdot \text{L}^{-1}$, compared to $0.3 \text{ mg} \cdot \text{L}^{-1}$ in the control biofilm reactor. High-throughput sequencing identified *Dechloromonas* as the dominant microbial species within Pd-biofilm, which plays a critical role in metal ion reduction. The Pd-biofilm reactor maintained high Cr(VI) reduction flux across varying conditions. When the influent Cr(VI) loading reached up to $10 \text{ mg} \cdot \text{L}^{-1}$, where the control biofilm reactor experienced inhibition, the Pd-biofilm reactor achieved a Cr removal of 99%. Increased nitrate loading and hydrogen pressure further enhanced Pd-biofilm reactor performance without compromising Cr(VI) reduction since Cr(VI) is the preferential electron acceptor, whereas the biofilm reactor required hydrogen pressures $\geq 15 \text{ psig}$ ($1 \text{ psig} = 6.895 \text{ kPa}$) for similar results. The optimal pH range for Cr(VI) reduction was 5.0–8.0 in the Pd-biofilm reactor and 7.0 in the biofilm reactor, with alkaline conditions being more inhibitory than acidic ones in both systems. The Pd-biofilm reactor effectively reduced Cr(VI) concentrations from 1 to $10 \text{ mg} \cdot \text{L}^{-1}$ to below the maximum contaminant level of $0.1 \text{ mg} \cdot \text{L}^{-1}$, thus appearing as an efficient technique to treat Cr-contaminated waters.

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1. Introduction

Chromium (Cr) is a heavy metal widely used in various industrial processes such as petroleum refining, electroplating, leather tanning, steel manufacturing, wood processing, and automobile production [1]. Discharges from these industries often lead to chromate contamination in groundwater, surface water, and drinking water [2]. The presence of Cr in water is particularly concerning due to its detrimental effects on the cardiovascular system and digestive tract [3]. In the United States, the

Environmental Protection Agency (EPA) has set a maximum contaminant level (MCL) of $100 \mu\text{g} \cdot \text{L}^{-1}$ for Cr in drinking water [4], while the European Union has adopted an even more stringent limit of $50 \mu\text{g} \cdot \text{L}^{-1}$, with German reducing it further to as low as $5 \mu\text{g} \cdot \text{L}^{-1}$ [5].

Cr exists in hexavalent (Cr(VI)) and trivalent (Cr(III)) forms in water [3]. While Cr(III) is less toxic and can be removed from water via precipitation, Cr(VI) is highly toxic and not easily removed by conventional adsorption or precipitation methods. Therefore, reducing Cr(VI) to Cr(III) is a key strategy for its effective removal from water. Biological processes for removing Cr(VI) are common due to their lower cost and better sustainability [6]. However, bioreduction of Cr(VI) faces several technical challenges: a long start-up period, a limited Cr(VI) loading, and inhibition by oxygen

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and/or nitrate in water [7]. Catalytic hydrogenation, particularly when using platinum-group metals (*i.e.*, platinum, palladium, rhodium, ruthenium, and iridium) can reduce Cr(III) with fast kinetics that lead to more precipitation [8]. It only requires hydrogen gas (H_2) as the chemical input and provides fast kinetics, mild operating conditions, and no secondary waste stream. Among the catalysts, palladium (Pd) is the most studied catalyst due to its great H_2 -absorption capacity and ability to form highly reductive atomic hydrogen on the catalyst's surface, which is activated to cleave the Cr=O bond in chromate and form H_2O and reduced Cr(III) [9]. However, conventional Pd-based catalytic approaches for Cr(VI) reduction have encountered challenges: inefficiencies in hydrogen supply and catalyst deactivation due to the loss of palladium [10].

The palladized membrane biofilm reactor (Pd-MBfR) has emerged as a promising solution to overcome these limitations [8,11,12]. The Pd-MBfR ensures complete H_2 utilization and the formation of a robust Pd-catalyst by efficient bubble-free delivery of H_2 through nonporous membrane fibers, which is precisely controlled by the H_2 pressure. The Pd-MBfR also facilitates microbially driven Pd recovery to form palladium nanoparticles (PdNPs) associated with the biofilm (Pd-biofilm), thereby efficiently immobilizing and stabilizing them to promote the reduction and removal of Cr.

Nitrate (NO_3^-) often accompanies chromate as a co-contaminant in water, and it poses environmental and health risks, including methemoglobinemia and an increased risk of stomach cancer [13]. In a Pd-biofilm system, nitrate reduction can have dual effects on Cr(VI) reduction. On the one hand, effective Cr(VI) removal requires cooperation between H_2 -based denitrification, microbial Cr(VI) export, and Cr(VI) reduction, as Cr(VI) transport and microbial resistance to Cr(VI) toxicity rely on H_2 -based denitrification for energy supply [14]. On the other hand, NO_3^- reduction can competitively inhibit the catalytic reduction of Cr(VI) [15]. Additionally, factors such as the influent surface loading, pH, and electron donor supplying flux can influence the Cr(VI) reduction. Despite these interactions, the impact of these factors on the synergistic removal of Cr(VI) in the Pd-MBfR system remains unexplored.

In a previous study, we developed a Pd-MBfR by coupling a biofilm with *in-situ* generated PdNPs and demonstrated that Cr(VI) reduction and precipitation of $Cr(OH)_3$ occurred in the Pd-MBfR [8]. However, the operational conditions that optimize Cr(VI) reduction in the presence of NO_3^- remain incompletely understood, and the overall performance of the Pd-MBfR needs enhancement. This study aims to evaluate the cooperative and competitive dynamics between Cr(VI) and NO_3^- reduction, using a comparative analysis of two membrane reactor systems: a biofilm setup and a Pd-biofilm setup. Over 55 days of continuous operation, we investigated the effects of varying surface loadings of Cr(VI) and NO_3^- , aiming to identify the optimal conditions for Cr(VI) reduction in the Pd-MBfR. Additionally, short-term tests were conducted to assess the influence of key operational parameters, such as Cr(VI) and NO_3^- loadings, hydrogen pressure, and pH, on the performance of the Pd-MBfR. The aim of this study is to discover the mechanisms underlying the improved performance of Cr(VI) reduction in the Pd-MBfR system and to explore the potential benefits of a synergistic platform for simultaneous Cr(VI) and nitrate removal.

2. Materials and Methods

2.1. Reactor configuration and setup

Experiments were carried out with two reactors operating in parallel. The reactors were configured similarly to the previous work, as depicted in Fig. S1 in the Supplementary Material [16].

Briefly, each reactor comprised two interconnected tubes joined by Viton tubing (Saint-Gobain, USA). One tube contained a bundle of 50 nonporous polypropylene hollow-fiber membranes (Teijin, Japan), with both ends linked to the H_2 gas pipeline. The other tube contained a bundle of 10 identical fibers serving as coupons, and its top end was connected to the H_2 pipeline. The reactors had a working volume of 65 ml and a total membrane surface area of 95 cm. An influent medium was introduced into the MBfR by one peristaltic pump (Longer, China), and the liquid in the reactor was thoroughly mixed by another recirculation pump. During the entire experiment, the temperature was maintained at $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

2.2. Inoculation and biogenic palladium deposition

The inoculation process of the two reactors was initiated by introducing anoxic sludge. Subsequently, a continuous supply of a medium, which contained $10\text{ mg}\cdot\text{L}^{-1}\text{ NO}_3^-$ -N, was provided to the reactors. The detailed composition of the basic medium is presented in Table S1. After a period of two months, mature biofilms were successfully formed within the MBfRs. The comprehensive procedures for inoculation and feeding have been described in a prior study [10].

For the deposition of PdNPs within the biofilm (Pd-biofilm), a batch-mode introduction of Pd(II) medium (containing $160\text{ mg}\cdot\text{L}^{-1}$ Pd(II) in the form of Na_2PdCl_4) was carried out in one of the MBfRs. This process was conducted under a H_2 pressure of 15 psig (1 psig = 6.895 kPa). The composition of the Pd(II)-containing medium has been detailed in a previous research [17]. The Pd-deposition process lasted 48 h until a dark Pd-biofilm formed on the fibers and more than 99% of the input Pd(II) was recovered after each addition. The created Pd-MBfR retained 11 mg total Pd (1.16 g Pd per m^2 membrane).

2.3. Continuous-flow operation the Pd- and Non-Pd MBfRs

The continuous operation of the Pd-MBfR and the non-Pd MBfR was partitioned into two distinct stages. In Stage I (ranging from Day 1 to Day 26), the reactors were fed with the basic medium supplemented with $5\text{ mg}\cdot\text{L}^{-1}\text{ NO}_3^-$ -N along with $0.5\text{ mg}\cdot\text{L}^{-1}$ Cr(VI). Subsequently, the concentration of Cr(VI) was adjusted to $1.0\text{ mg}\cdot\text{L}^{-1}$ in Stage II (spanning from Day 27 to Day 55). For both stages, the influent flow rate was maintained at $0.53\text{ ml}\cdot\text{min}^{-1}$, and the H_2 pressure was set at 15 psig. $2\text{-mmol}\cdot\text{L}^{-1}$ phosphate buffer was added to the influent, and the pH values of the influent were maintained within the narrow range of 7.2 ± 0.2 throughout the experiments. Prior to utilization, the influent medium was degassed with N_2 for 15 min, and this led to a dissolved oxygen (DO) concentration in the influent medium $<0.15\text{ mg}\cdot\text{L}^{-1}$.

2.4. Optimal conditions tests in the MBfRs

Following the 55-day operation of the two reactors, short-term experiments were implemented to systematically explore the impacts of various factors, namely Cr(VI) loading, NO_3^- loading, H_2 availability, and pH, on Cr(VI) reduction. These experiments were organized into five series (as detailed in Table S2), with hydraulic retention time (HRT) maintained at 2.1 h. Prior to each test, the two reactors were restored to a steady-state condition with an influent of $1\text{ mg}\cdot\text{L}^{-1}$ Cr(VI), $5\text{ mg}\cdot\text{L}^{-1}\text{ NO}_3^-$ -N, 15 psig H_2 pressure, and pH 7.0. For each short-term test, the alterations of the system conditions were sustained for a period equivalent to 5 HRTs to reach steady state, before samples were taken. To ensure the reliability and representativeness of the data, replicate samples were obtained at 6, 7, and 8 HRTs, and the results are presented as the average values.

2.5. Sampling and analysis

Liquid samples within the MBfRs were obtained using 3-ml syringes and subsequently filtered through 0.22- μm PVDF membrane filters. The Cr(VI) concentration was determined by the diphenyl carbazide method (Method 3500-Cr D, APHA, 1998). The concentrations of Pd(II) and soluble Cr (Cr(III) + Cr(VI)) were quantified by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700, Agilent Technologies, USA). NO_3^- and NO_2^- were analyzed by an ion chromatograph (IC, Dionex Aquion, USA) with an AS-19 column. pH and DO were measured using a multi-Parameter Meter (HACH HQ40d, USA).

Solid samples were collected from the two reactors by cutting off ~5-cm-long fiber pieces from the coupon bundles, and the remaining fiber was sealed by tying the end into a knot. After undergoing chemical fixation and ultra-microtome slicing, these fibers were examined and characterized using transmission electron microscopy (TEM, FEI Tecnai G2 F20, USA) coupled with energy-dispersive X-ray spectroscopy (EDX). In addition, solids were scraped off the fibers and promptly freeze-dried for subsequent analysis by X-ray diffraction (XRD, Bruker D8 advance, Germany) and X-ray photoelectron spectroscopy (XPS, Thermo ESCALAB 250, USA). Detailed protocols for sample fixation/slicing and information on the instruments are available in previous works [10,17].

2.6. Microbial community analyses

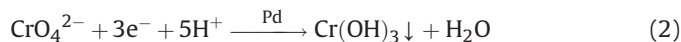
At the end of Stages II, a 10-cm segment was excised from a coupon fiber within each reactor. We used the barcoded primer set 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') to amplify the conserved V3-V4 regions of the bacterial 16S rRNA gene and purified the PCR products using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA). We sent all purified DNA samples to Majorbio Technology (Shanghai, China) to process Illumina MiSeq sequencing with standard protocols. We processed the raw data using QIIME (1.9.1) platform [18] and removed sequences shorter than 250 bps, homopolymers of 6 bps and primer mismatches, or a quality score lower than 25. We picked the operational taxonomic unit (OTUs) using the Greengenes database with uclust [19] based on $\geq 97\%$ identity, and randomly picked 5500 sequences per sample. Then we removed OTUs containing fewer than 2 sequences from our analysis and aligned representative sequences of OTUs to the Greengenes Database using PyNast. [20,21]. The potential chimeric sequences identified by CHimeraSlayer [22] were removed by a python script in QIIME. To assign taxonomy to OTUs, we assigned taxonomy to the OTUs using the Greengenes database using confidence threshold of 0.8 [23]. Newick-formatted phylogenetic trees were constructed using FastTree [24].

2.7. Flux calculations

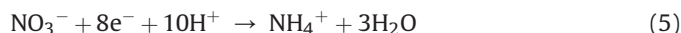
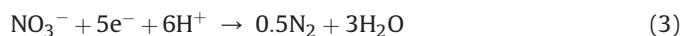
The flux of a substrate (J) was calculated by

$$J = Q \cdot (C_i - C_e) / A \quad (1)$$

where J is the flux, $\text{g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$; Q is the influent flow rate, $\text{m}^3 \cdot \text{d}^{-1}$; C_i and C_e are influent and effluent concentrations of each substrate (Cr and NO_3^-), $\text{g} \cdot \text{m}^{-3}$; and A is the effective membrane surface area, m^2 . The dechlorination flux in electron equivalents ($\text{e}^- \cdot \text{meq} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, $\text{e}^- \cdot \text{meq}$ means mmol electron equivalents) was calculated based on released Cl^- concentrations according to reaction stoichiometry:



The NO_3^- flux in electron equivalents ($\text{e}^- \cdot \text{meq} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) was calculated from Eqs. (3)–(5):



To indicate if the H_2 supply was limited, the maximum H_2 -supply flux at applied H_2 pressure was calculated according to Tang's method [25].

3. Results and Discussion

3.1. Characterization of the membrane-supported Pd-biofilm

The size, morphology and distribution of biogenic Pd nanoparticles were investigated by HRTEM and TEM-EDX analyses. Fig. 1(a), a typically section including the fiber and the biofilm in full depth, gives a comprehensive visualization of the heterogeneous “sandwich” structure perpendicular to the fiber surface. Black particles cross the biofilm were confirmed as elemental palladium (Pd^0) via EDX spectrum analysis (Fig. 1(d)). Larger Pd aggregates tended to gather toward the fiber surface and interspace (lighter area) edge of the biofilm, while the majority of Pd^0 crystallites layers were well-dispersed in the extra-cellular polymeric substances (EPS) matrix or less associated with the cell surfaces. A higher magnification of a microbial cell further reflects that PdNPs associated with cell mainly located on cell walls and in periplasmic spaces (Fig. 1(b)). The HRTEM image of individual crystallites on cell (Fig. 1(c)) confirmed the crystalline structure of the PdNPs, with measured lattice spacing of $(0.25 \pm 0.02) \text{ nm}$ corresponding to the (1 1 1) lattice fringe of Pd. The average sizes of Pd^0 crystallites were determined as $(6.7 \pm 1.6) \text{ nm}$ on the EPS outer layer, $(4.9 \pm 0.9) \text{ nm}$ within the EPS matrix, and $(5.7 \pm 1.0) \text{ nm}$ on microbial cell surfaces. The EPS contributed to maintaining smaller PdNP sizes by electrostatically stabilizing or complexing with the particles, aided by its three-dimensional reticular structure [26]. This heterogeneous distribution of PdNPs enhanced the total Pd^0 surface area and incorporated low-coordinated crystallites into the biofilm, potentially improving the catalytic reactivity of the PdNPs.

To further elucidate the chemical state and structural features of Pd within the biofilm, XRD and XPS analyses were conducted. The XRD pattern of the dried Pd-biofilm (Fig. 1(e)) displayed prominent diffraction peaks at 40.1° (1 1 1), 46.7° (2 0 0), 68.1° (2 2 0), and 8.1° (3 1 1), consistent with the crystalline Pd^0 structure dominated by the (1 1 1) facet. The estimated average crystallite size was 6.2 nm, aligning closely with TEM observations. Notably, no diffraction peaks corresponding to palladium oxide (PdO) were detected, indicating that Pd^0 was the predominant form. The high-resolution Pd 3d XPS spectrum (Fig. 1(f)) further confirmed the metallic nature of the Pd in the biofilm. The binding energy peaks at 340.7 eV and 335.5 eV were attributed to $\text{Pd}^0 3\text{d}_{3/2}$ and $\text{Pd}^0 3\text{d}_{5/2}$, respectively, consistent with metallic palladium. These findings corroborate that metallic Pd was successfully integrated as the active catalytic component within the biofilm. This combination of morphological, structural, and chemical analyses highlights the successful deposition and distribution of biogenic PdNPs within the membrane-supported biofilm, which is essential for enhanced catalytic reduction.

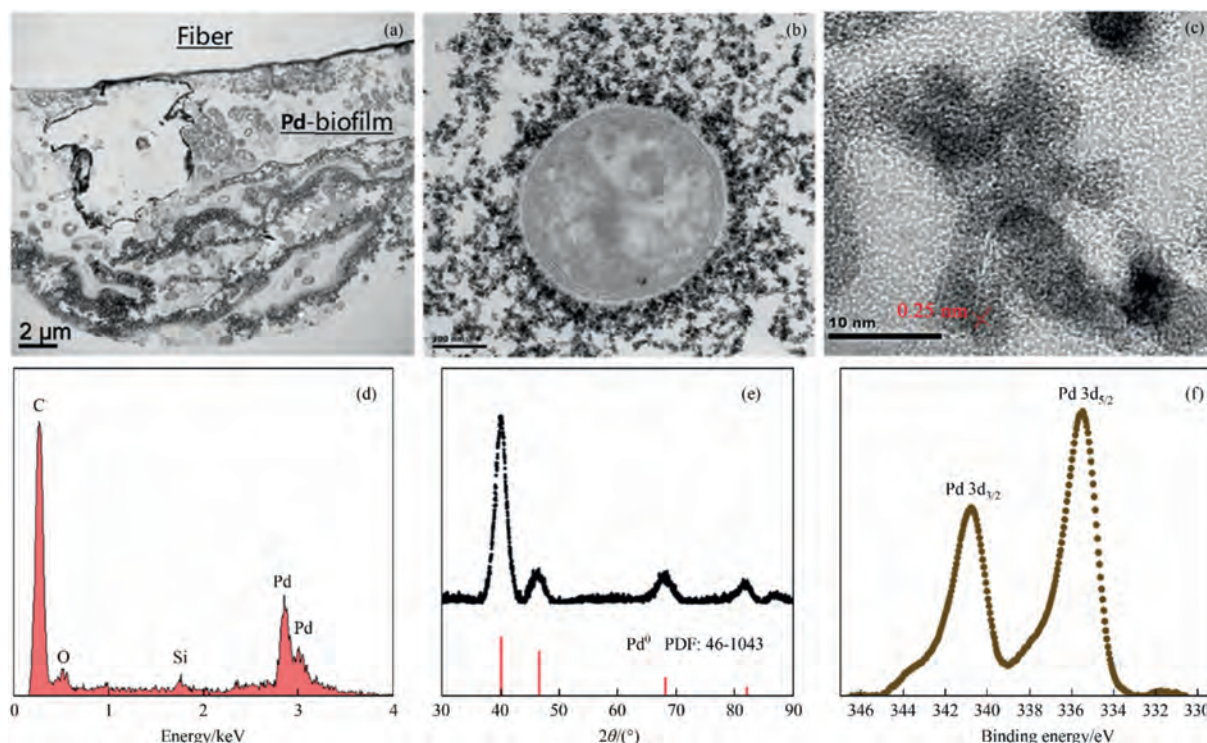


Fig. 1. TEM images of an ultrathin cross section of a fiber with Pd-biofilm (a). Boundary of the fiber and the biofilm (The grey shapes are bacterial cells, and the dark spots are PdNPs) (b). A representative microbial cell loaded on PdNPs (c). The lattice fringes of Pd⁰ crystallites associated with cell (d). And XRD pattern (e), deconvoluted Pd 3d XPS spectra (f) of the biogenetic Pd⁰ powders.

3.2. Continuous removal of Cr(VI) reduction in the parallel MBfRs

Continuous Cr(VI)-reduction experiments were conducted to investigate performances of the Pd-MBfR and in the non-Pd MBfR. H₂ availability was not a rate-limiting factor, since the actual H₂ fluxes ($\sim 150 \text{ e}^- \cdot \text{meq} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) were much lower than the maximum theoretical H₂ flux ($420 \text{ e}^- \cdot \text{meq} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) in each stage [25]. Each stage lasted more than 25 days, providing sufficient time to reach a steady state for all chemical species.

In the non-Pd MBfR, an influent Cr(VI) concentration of $0.5 \text{ mg} \cdot \text{L}^{-1}$ was introduced in Stage I (days 1–26). Effluent Cr(VI) concentrations gradually decreased, reaching below $0.05 \text{ mg} \cdot \text{L}^{-1}$ by the end of the stage (Fig. 2). In Stage II, the influent Cr(VI) concentration was increased to $1.0 \text{ mg} \cdot \text{L}^{-1}$, corresponding to a loading rate of $80 \text{ mg Cr} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. Initially, effluent Cr(VI) concentration spiked to $0.8 \text{ mg} \cdot \text{L}^{-1}$ within 4 days but decreased to $0.01 \text{ mg} \cdot \text{L}^{-1}$ by day 50. At this point, total Cr (TCr, Cr(VI) + soluble Cr(III)) concentrations in effluent stabilized around $0.29 \text{ mg} \cdot \text{L}^{-1}$, yielding a Cr(VI) reduction ratio of 99% and a Cr removal ratio of $\sim 70\%$. In contrast, the Pd-MBfR consistently outperformed the non-Pd MBfR. During Stages I and II, Cr(VI) in the Pd-MBfR effluent remained below the detection limit ($0.05 \text{ } \mu\text{g} \cdot \text{L}^{-1}$), while TCr concentrations at the end of Stage II were $0.043 \text{ mg} \cdot \text{L}^{-1}$, achieving a Cr removal of up to 96%. The Pd-MBfR required no extended acclimation period and exhibited immediate Cr(VI) reduction upon exposure to influent Cr(VI).

The concentrations of removed Cr were calculated as the difference between influent Cr(VI) and effluent TCr concentrations (Fig. 2). The Pd-MBfR removed over $0.95 \text{ mg} \cdot \text{L}^{-1}$ of Cr(III) via reduction and precipitation, compared to $\sim 0.7 \text{ mg} \cdot \text{L}^{-1}$ in the non-Pd MBfR, which manifests the Pd-MBfR's superior performance for both Cr(VI) reduction and Cr removal. In the Pd-MBfR, dissolved Cr(III) concentrations of $0.04\text{--}0.05 \text{ mg} \cdot \text{L}^{-1}$ matched the solubility

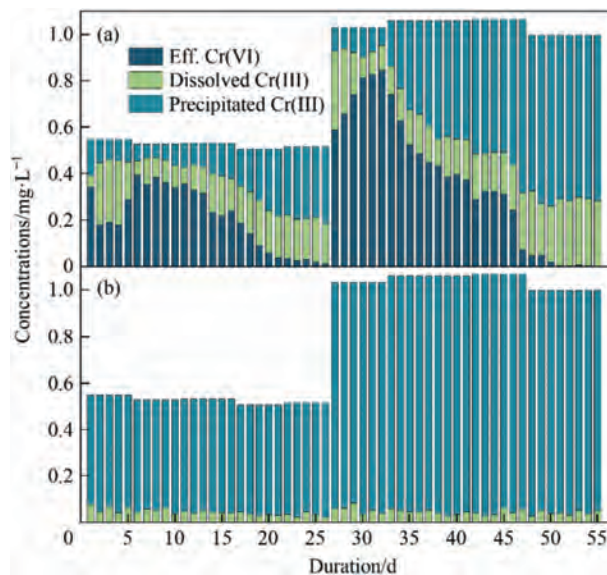


Fig. 2. The variation in influent Cr(VI), effluent Cr(VI) and effluent dissolved Cr(III), and Cr precipitated in the non-Pd MBfR (a) and the Pd-MBfR (b). The concentration of dissolved Cr(III) was calculated as the difference between the concentration of effluent TCr and effluent Cr(VI), and the concentrations of precipitated Cr(III) was calculated as the difference between the concentration of influent Cr(VI) and effluent TCr.

limits of Cr(OH), indicating efficient precipitation. In contrast, the non-Pd MBfR effluent contained significantly higher dissolved Cr(III) levels ($0.28\text{--}0.30 \text{ mg} \cdot \text{L}^{-1}$), which exceeded the solubility limit. The reason for the existence of a higher dissolved Cr(III) concentration was that a significant amount of Cr(III) in the non-Pd MBfR formed complexes and thus could not precipitate as Cr(OH), which restricted the TCr removal [8].

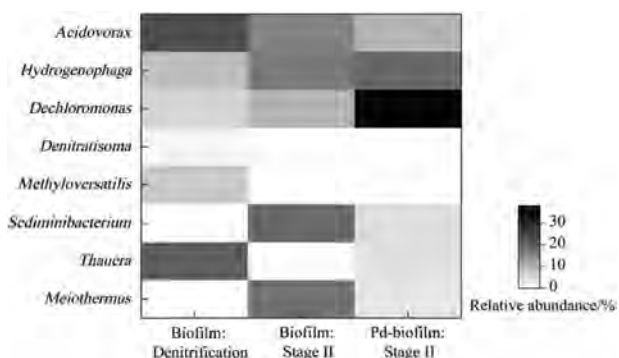


Fig. 3. Phylogenetic profiling of the Pd-biofilm and biofilm at the genus levels, <3% phylotypes in the biofilms are not shown.

Fig. 3 illustrates the microbial community composition of the biofilms during nitrate and Cr(VI) reduction in Stage II. In the Pd-biofilm, the predominant genus was *Dechloromonas*, known for its versatile respiration capabilities, including nitrate and metal reduction, such as U(VI) [27] and Fe(III) [28]. Two autotrophic denitrifying genera, *Acidovorax* and *Hydrogenophaga*, remained stable in Stages II, indicating that the microbial community was unaffected by Cr(VI) exposure. This stability highlights the resilience of Pd-biofilm, dominated by denitrifiers and Pd-reducing bacteria. In the non-Pd biofilm, the microbial community shifted significantly after 55 days of Cr(VI) exposure. Initially dominated by denitrifiers such as *Acidovorax* (23%), *Thauera* (21%), and *Hydrogenophaga* (7.8%), the community transitioned to include Cr-tolerant and reducing genera. *Sediminibacterium* became prominent, constituting 20% of the community, likely due to its metal resistance and Cr(VI)-reduction potential [29]. *Meiothermus* accounted for 19% of the total bacteria and has demonstrated Cr(VI) reduction via both membrane-bound and cytoplasmic enzymes in methane-based MBfRs [30]. This shift highlights the selective enrichment of Cr-tolerant microbes driven by Cr(VI) stress, in the absence of PdNPs.

3.3. Effects of typical conditions on Cr(VI) reduction

3.3.1. Enhanced Cr(VI) reduction and stability via Pd-biofilm synergy

The effect of influent Cr(VI) concentration on its reduction was evaluated under Series A, with influent concentrations of 1, 2, 5, and 10 mg·L⁻¹ (Table S2). In the Pd-MBfR, the effluent Cr(VI)

concentration slightly increased with the increase in influent Cr(VI) concentration, reaching a maximum concentration of 0.096 mg·L⁻¹—still below the water quality standard of 0.1 mg·L⁻¹ (Fig. 4(a)). The Cr(VI) removal efficiency consistently exceeded 99%. Moreover, NO₃⁻ reduction was unaffected by the influent Cr(VI) concentration, with effluent NO₃⁻-N concentrations remaining below 0.3 mg·L⁻¹, corresponding to removal efficiencies above 95%. Fig. 4(b) reveals that the Cr(VI) reduction flux increased dramatically from 80 mg·m⁻²·d⁻¹ to 771 mg·m⁻²·d⁻¹ as the influent Cr(VI) concentration rose, significantly surpassing the maximum Cr(VI) fluxes reported in conventional MBfRs (80–500 mg·m⁻²·d⁻¹) [30,31]. In the non-Pd MBfR, influent Cr(VI) concentrations below 2 mg·L⁻¹ showed minimal impact on microbial Cr(VI) and NO₃⁻ reduction, achieving Cr(VI) and NO₃⁻ removal efficiencies of 91% and 95%, respectively (Fig. 4(a)). However, at Cr(VI) concentration of 5 mg·L⁻¹, both processes were inhibited, with Cr(VI) and NO₃⁻ removal efficiencies dropping to 22% and 8%, respectively. High Cr(VI) concentrations were found to lower the redox potential of bacterial enzyme systems, inhibiting NO₃⁻ reduction and Cr(VI) transport and resistance [32].

The above results underscore that the Pd-biofilm significantly enhanced Cr(VI) reduction through the synergistic effect of Pd nanoparticles (PdNPs) and microorganisms. In previous studies, the prepared biogenic palladium typically required pre-treatment such as washing, dehydration, and grinding, thus losing its biological activity [33]. Moreover, suspended PdNPs are prone to loss and aggregation in the absence of suitable fixation materials, making it difficult to maintain catalytic activity over time [34]. No reduction ability decline was observed in the Pd-biofilm throughout the experimental tests, even after eight HRTs, indicating that the Pd-biofilm has a stable ability to remove Cr(VI).

To further evaluate the reduction kinetics of the two reactors, batch reduction experiments were conducted. The data from the Cr(VI) reduction processes in both reactors were fitted to reaction kinetic models, and the results showed that the Cr(VI) concentration changes in the reactors followed pseudo-first-order reduction kinetics. The Cr(VI) reduction rate constant in the Pd-MBfR was 0.76 h⁻¹, approximately 15 times higher than that in the non-Pd MBfR (0.03 h⁻¹) (Fig. S2).

3.3.2. Cr(VI) is the preferential electron acceptor over nitrate in the Pd-MBfR

As a common oxidized contaminant in water, NO₃⁻ has been widely reported to serve as a preferred electron acceptor in hydrogen-based biofilm, providing both an electron sink and a

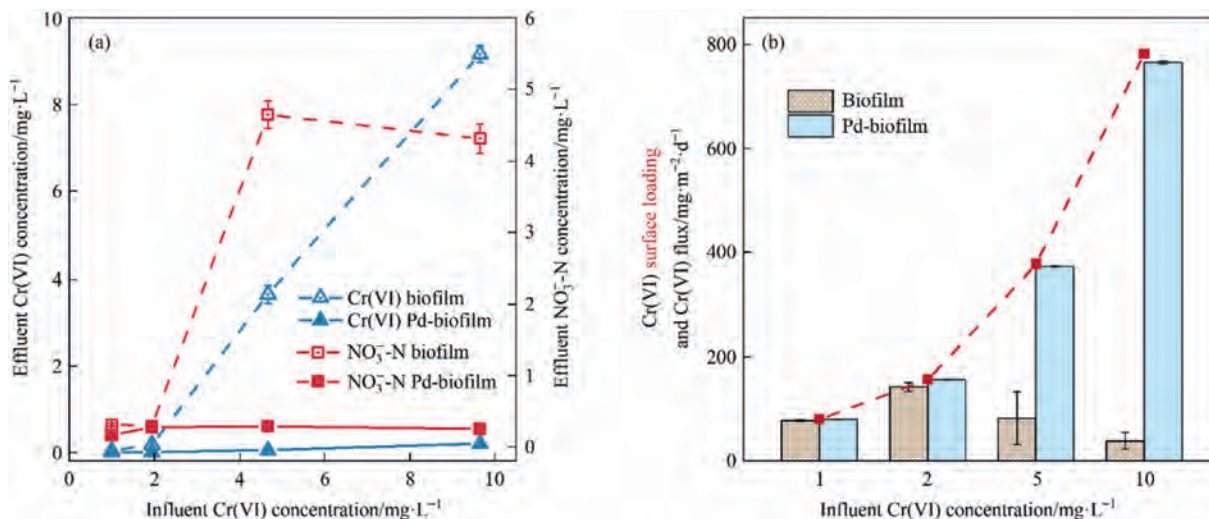


Fig. 4. The effects of Cr(VI) loading on Cr(VI) reduction in the Pd-MBfR and the non-Pd MBfR.

nitrogen source for microbial metabolism [35,36]. Series B explored the influence of NO_3^- loading on Cr(VI) reduction, with influent NO_3^- -N concentrations of 0, 5, 10, 20 and 40 $\text{mg}\cdot\text{L}^{-1}$. In the Pd-MBfR, the Cr(VI) removal remained consistently above 95% as influent NO_3^- -N concentrations increased up to 40 $\text{mg}\cdot\text{L}^{-1}$ (Fig. 5(a)), while NO_3^- removal slightly decreased to 85%. When the influent NO_3^- -N increased to 40 $\text{mg}\cdot\text{L}^{-1}$, the required H_2 -supply flux for reduction reached $580\text{ e}^-\cdot\text{meq}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, exceeding the theoretical maximum H_2 -transfer capacity of the hollow fiber membrane ($420\text{ e}^-\cdot\text{meq}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$). This hydrogen limitation resulted in competition between Cr(VI) and NO_3^- for the electron donor H_2 . Under H_2 -limited conditions, NO_3^- reduction was incomplete, whereas Cr(VI) reduction remained largely unaffected, suggesting that Cr(VI) was the preferential electron acceptor. This is attributed to the inability of metallic palladium to directly catalyze NO_3^- reduction. Zhou *et al.* [12] demonstrated that the reduction of nitrate to nitrite occurs *via* microbial activity in Pd-biofilm, while subsequent NO_3^- reduction was primarily catalyzed by Pd nanoparticles. Previous studies on Pd-based catalysts confirmed that the preferential order of electron acceptance by common oxyanions on Pd surfaces was $\text{CrO}_4^{2-} > \text{NO}_2^- > \text{BrO}_3^- \gg \text{ClO}_4^- > \text{NO}_3^-$ [37]. This explains why Cr(VI) reduction in Pd-MBfR was unaffected by competition from other electron acceptors. In contrast, the non-Pd MBfR exhibited a markedly different performance. In the absence of influent NO_3^- , Cr(VI) reduction was almost entirely inhibited, with a removal efficiency of only 10% (Fig. 5). This indicates that Cr(VI) reduction in non-Pd MBfR occurs via a cometabolic pathway, where nitrate reductase enzymes use NO_3^- as an essential electron acceptor to reduce Cr(VI) [38]. As the influent NO_3^- -N concentration increased, an inverse relationship was observed between Cr(VI) and NO_3^- reduction fluxes: higher NO_3^- loading significantly suppressed Cr(VI) reduction. At influent NO_3^- -N of 40 $\text{mg}\cdot\text{L}^{-1}$, the Cr(VI) removal dropped to 40%, likely due to competitive inhibition by NO_3^- at the enzymatic level. This suggests a dual effect of NO_3^- on Cr(VI) reduction in the non-Pd MBfR: low nitrate levels promote Cr(VI) reduction, while high nitrate levels inhibit it. Zhou *et al.* [14] found that effective Cr(VI) removal requires a synergistic process involving H_2 -based denitrification, Cr(VI) transport, and Cr(VI) reduction. This synergy depends on denitrification to supply the energy required for Cr(VI) transport and resistance to its toxicity, despite NO_3^- reduction having a competitive advantage over Cr(VI) reduction.

These results indicate that NO_3^- loading has minimal impact on Cr(VI) reduction in the Pd-MBfR, with Cr(VI) removal remaining >95%. However, strict control of NO_3^- loading is essential in the non-Pd MBfR to achieve effective Cr(VI) removal.

3.3.3. The Pd-MBfR exhibited higher hydrogen utilization for Cr(VI) reduction

Hydrogen gas, as the sole electron donor in reduction reactions, influences the electron competition in the reaction. H_2 has been controlled by adjusting hydrogen partial pressure in relevant studies [39,40]. Series C explored the effect of H_2 supply on Cr(VI) reduction, with H_2 pressures of 3, 6, 15, and 20 psig. In the Pd-MBfR system, when the H_2 pressure was 3 psig, the concentrations of Cr(VI) and NO_3^- in the effluent were 0.058 $\text{mg}\cdot\text{L}^{-1}$ and 0.88 $\text{mg}\cdot\text{L}^{-1}$, respectively (Fig. 6(a)). As the H_2 pressure increased from 3 to 6 psig, the concentrations of Cr(VI) and NO_3^- in the effluent decreased slightly. Consequently, the corresponding reduction fluxes increased marginally (Fig. 6(b)). When the H_2 pressure reached 15 psig, Cr(VI) was no longer detected in the effluent. Further increasing the H_2 pressure to 20 psig didn't result in further increase in reduction flux since Cr(VI) had been completely removed. In the non-Pd MBfR, as the H_2 pressure increased from 3 to 15 psig, the concentration of Cr(VI) in the effluent decreased significantly. Simultaneously, the Cr(VI) reduction flux increased noticeably, though the Cr(VI) removal is still lower than that of the Pd-MBfR. Continuing to increase to 20 psig, there was little change in the relevant indicator.

The above results demonstrate that increasing the H_2 pressure promotes the reduction of Cr(VI) and NO_3^- in both the Pd-MBfR and the non-Pd MBfR. Under the same H_2 pressure, the treatment effect of Cr(VI) by the Pd-MBfR is superior to that of the non-Pd MBfR. When the H_2 pressure reached 15 psig, the performance of the two reactors approached saturation since H_2 supply was no longer a limiting factor. Therefore, considering safety and economic costs, the optimal H_2 pressure should be 15 psig.

3.3.4. The Pd-MBfR had a wider optimal pH range for Cr(VI) reduction

pH significantly influences microbial activity [41] and the catalytic reduction of Cr(VI) [42]. The reactors were operated at pH levels of 5.0, 6.0, 7.0, 8.0 and 9.0. In the Pd-MBfR, Cr(VI) was completely removed at pH between 5.0 and 7.0, with stable Cr(VI)

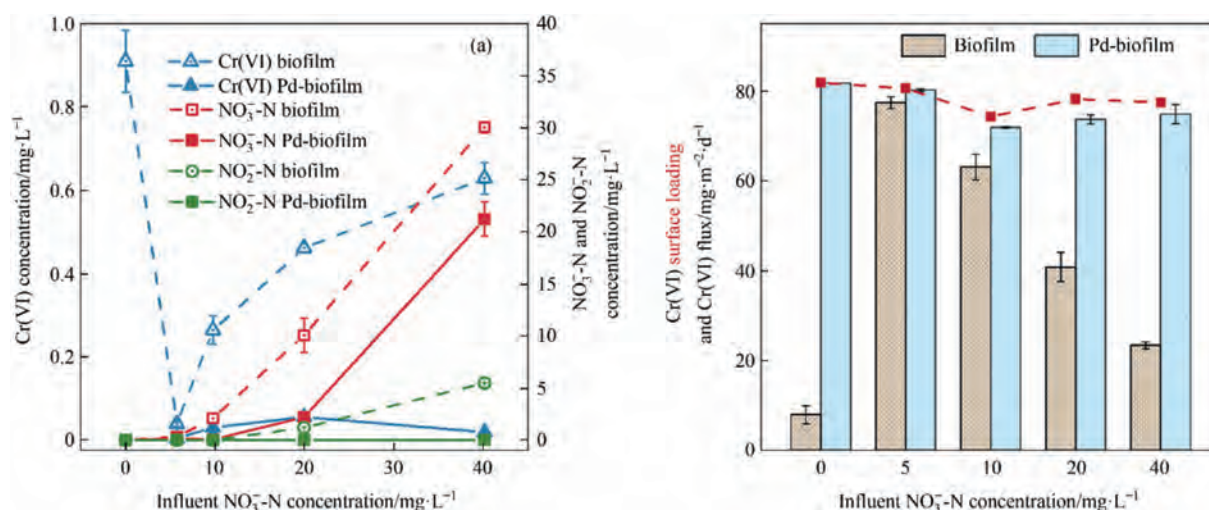


Fig. 5. The effects of nitrate loading on Cr(VI) reduction in the Pd-MBfR and the non-Pd MBfR.

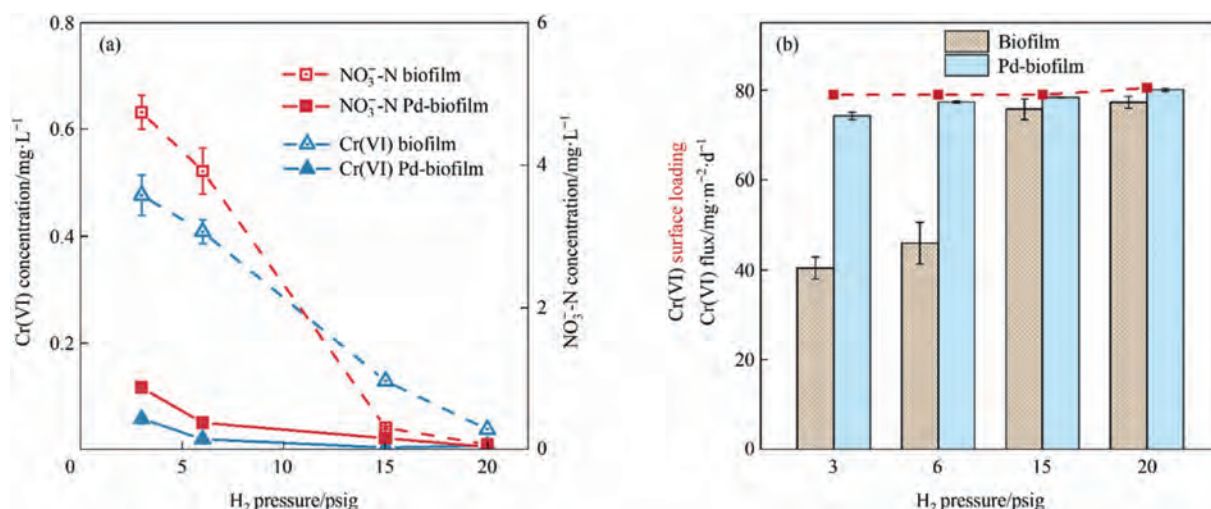


Fig. 6. The effects of hydrogen pressure on Cr(VI) reduction in the Pd-MBfR and the non-Pd MBfR.

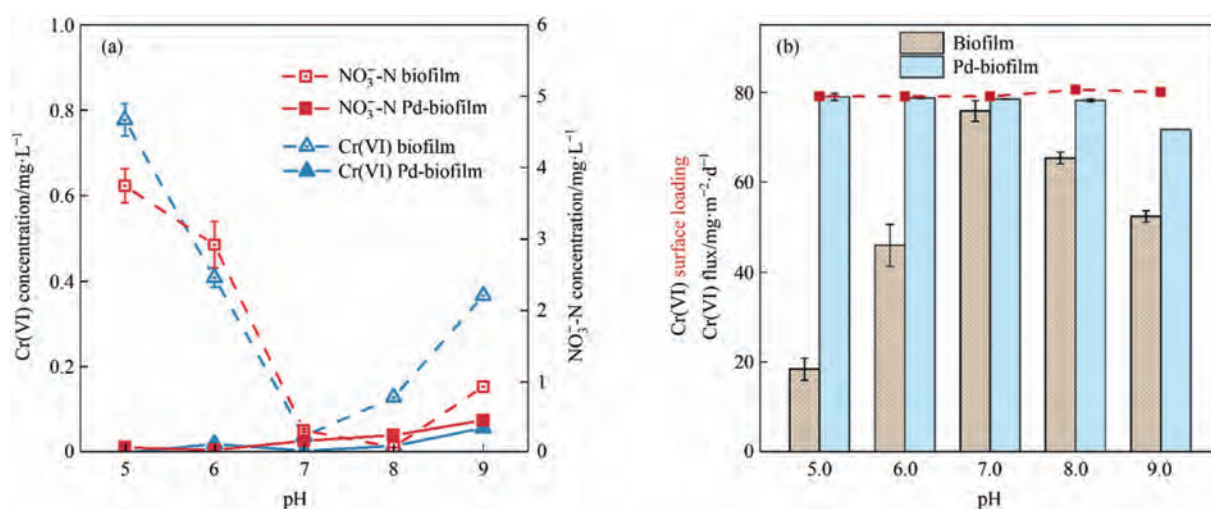


Fig. 7. The effects of pH on Cr(VI) reduction in the Pd-MBfR and the non-Pd MBfR.

reduction fluxes (Fig. 7(b)). However, the effluent Cr(VI) concentration slightly increased to $0.058 \text{ mg} \cdot \text{L}^{-1}$ at pH 9.0, and the removal and reduction flux decreased. In the non-Pd MBfR, Cr(VI) reduction improved as pH increased from 5.0 to 7.0, with lower effluent Cr(VI) concentrations and higher removal efficiency. At pH 9.0, the effluent Cr(VI) concentration rose to $0.36 \text{ mg} \cdot \text{L}^{-1}$, and both Cr(VI) removal and Cr(VI) flux declined.

Overall, the Pd-MBfR exhibited minimal sensitivity to pH variations, achieving optimal Cr(VI) reduction at pH 5.0–8.0, while Cr(VI) reduction was more inhibited under alkaline conditions due to the electrostatic repulsion between CrO_4^{2-} ions and the negatively charged biofilm [17]. The non-Pd MBfR showed optimal performance at pH 7.0, with acidic conditions (pH 5.0) severely inhibiting Cr(VI) reduction due to biofilm disruption. On the other hand, both too low and too high pH are not suitable for the growth and metabolism of microorganisms [41], which affects Cr(VI) reduction.

3.4. Equivalent electron flux analysis

To gain deeper insights into the competition among various pollutants (electron acceptors) for electrons provided by hydrogen (electron donor), effluent data from the Pd-MBfR and the non-Pd

MBfR under experimental Series A, B, C and D (influenced by influent Cr(VI) loading, influent NO_3^- loading, hydrogen pressure, and pH) were analyzed. Using calculations based on Eqs. (1)–(5), the electron distribution among pollutants under each condition was determined (summarized in Table 1).

In Series A, as the Cr(VI) loading increased, the total electron flux rose steadily in both reactors. In the Pd-MBfR, the electron flux allocated to Cr(VI) increased significantly, while the flux for NO_3^- decreased slightly, suggesting that Cr(VI) reduction had minimal inhibitory effects on NO_3^- reduction. However, in the non-Pd MBfR, the electron flux for Cr(VI) initially increased but declined at higher Cr(VI) loadings, while the electron flux for NO_3^- decreased significantly. This indicates that high Cr(VI) loadings suppressed both Cr(VI) and NO_3^- reduction in the non-Pd MBfR.

In Series B, increasing NO_3^- loading led to a rise in both the NO_3^- reduction flux and the total electron consumption flux in both reactors. In the Pd-MBfR, the Cr(VI) flux remained stable, indicating that Cr(VI) reduction was not affected by NO_3^- competition due to the preferential electron allocation to Cr(VI). In contrast, in the non-Pd MBfR, the Cr(VI) flux decreased significantly, showing that NO_3^- reduction gained competitive priority, suppressing Cr(VI) reduction.

Table 1
The electron flux and distribution of pollutants under different series of optimal conditions tests.

Series	Pd-MBfR				Non-Pd MBfR			
	Cr(VI)		NO ₃ ⁻ -N		Cr(VI)		NO ₃ ⁻ -N	
	Reduction flux /e ⁻ ·meq·m ⁻² ·d ⁻¹	Proportion/%	Reduction flux /e ⁻ ·meq·m ⁻² ·d ⁻¹	Proportion/%	Reduction flux /e ⁻ ·meq·m ⁻² ·d ⁻¹	Proportion/%	Reduction flux /e ⁻ ·meq·m ⁻² ·d ⁻¹	Proportion/%
A1	5.0	3.0	161	97.0	5	2.6	154	97.0
A2	9.0	5.5	154	94.5	9	4.9	156	94.7
A3	22.0	13.8	138	86.3	5	26.3	14	73.7
A4	44.0	23.9	140	76.1	2.4	8.5	26	91.5
B1	5.0	100.0	0	0.0	0.6	100.0	1	0.0
B2	5.0	3.0	161	97.0	5	2.6	158	97.1
B3	5.0	1.8	280	98.2	4	1.5	266	98.5
B4	5.0	1.0	490	99.0	2.6	0.9	300	99.1
B5	5.0	0.9	571	99.1	1.6	1.0	164	99.0
C1	4.0	3.1	124	96.9	2.6	6.3	39	93.8
C2	5.0	3.5	138	96.5	3	2.8	104	97.2
C3	5.0	3.2	152	96.8	5	2.6	184	97.4
C4	5.0	3.0	161	97.0	5	2.6	188	97.4
D1	5.0	3.0	161	97.0	1.5	3.9	37	96.1
D2	5.0	3.1	158	96.9	3.2	5.1	59	94.9
D3	5.0	2.9	166	97.1	5	2.7	179	97.3
D4	5.0	3.3	147	96.7	4	2.1	187	97.9
D5	4.0	3.0	128	97.0	3.4	2.1	157	97.9
								160.4

In Series C, as the hydrogen pressure increased, the total electron consumption flux increased in both reactors. In the Pd-MBfR, the Cr(VI) flux remained nearly constant, while the NO₃⁻ flux increased. This suggests that Cr(VI) reduction was prioritized under hydrogen-limited conditions, while NO₃⁻ reduction occurred only when sufficient hydrogen was available. In the non-Pd MBfR, the Cr(VI) flux decreased with increasing hydrogen supply, while the proportion for NO₃⁻ increased, indicating that NO₃⁻ became more competitive for electrons at higher hydrogen pressures.

In Series D, the Pd-MBfR demonstrated better adaptability to pH variations compared to the non-Pd MBfR. In the Pd-MBfR, Cr(VI) flux remained constant across the pH range, while NO₃⁻ flux gradually decreased under alkaline pH. In the non-Pd MBfR, the electron flux for Cr(VI) first increased and then decreased, while NO₃⁻ flux generally increased at alkaline pH. This shows that Cr(VI) reduction in the Pd-MBfR was less sensitive to pH changes, while in the non-Pd MBfR, both Cr(VI) and NO₃⁻ reductions were influenced by pH.

Overall, analysis of the equivalent electron flux under various influencing factors reveals that in both the Pd-MBfR and the non-Pd MBfR, the distribution of equivalent electron flux for NO₃⁻ is larger than that of Cr(VI). This means that NO₃⁻ reduction consumed more electron donor in both reactors, but since Cr(VI) is the preferred electron acceptor in the Pd-MBfR, its reduction was not affected by competition from NO₃⁻ reduction; whereas in the non-Pd MBfR, NO₃⁻ is the preferred electron acceptor, and once NO₃⁻ flux increased, Cr(VI) reduction was inevitably inhibited. Comparing the electron utilization of the two reactors, the Pd-MBfR always outperformed the non-Pd MBfR.

4. Conclusions

In-situ generated PdNPs associated with the biofilm to form a stable Pd-biofilm could enhance Cr(VI) reduction in the Pd-MBfR system. Continuous experiments showed that the Pd-MBfR outperformed the non-Pd MBfR in Cr(VI) reduction with a higher removal rate and lower effluent concentration. High-throughput sequencing revealed *Dechloromonas* as the dominant microbial species within the Pd-biofilm, playing a critical role in metal ion reduction. Short-term tests were conducted to evaluate the effects of typical factors such as Cr(VI) loading, NO₃⁻ loading, hydrogen pressure, and pH on Cr(VI) reduction. The results indicate that increasing the hydrogen pressure and maintaining an appropriate pH could enhance the reductions of Cr(VI) and NO₃⁻. Electron flux analysis revealed that in the Pd-MBfR, Cr(VI) was the preferred electron acceptor, and its reduction was less affected by NO₃⁻ reduction competition compared to the non-Pd MBfR. In summary, the Pd-MBfR demonstrated better performance and stability in Cr(VI) removal, providing valuable insights for the development of more efficient water treatment strategies.

CRedit Authorship Contribution Statement

Chengyang Wu: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Siqing Xia: Validation, Supervision, Methodology, Conceptualization. Qiyuan Pang: Investigation. Suyun Xu: Validation, Supervision. Hongbo Liu: Writing – review & editing, Validation, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cjche.2025.03.018>.

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