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Insights into selenate removal mechanism of hydrogen-based membrane biofilm reactor for nitrate-polluted groundwater treatment based on anaerobic biofilm analysis

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ABSTRACT

The selenate removal mechanism of hydrogen-based membrane biofilm reactor (MBfR) for nitrate-polluted groundwater treatment was studied based on anaerobic biofilm analysis. A laboratory-scale MBfR was operated for over 60 days with electron balance, structural analysis, and bacterial community identification. Results showed that anaerobic biofilm had an excellent removal of both selenate (95%) and nitrate (100%). Reduction of Selenate → Selenite → Se^0 with hydrogen was the main pathway of anaerobic biofilm for selenate removal with amorphous Se^0 precipitate accumulating in the biofilm. The element selenium was observed to be evenly distributed along the cross-sectional thin biofilm. A part of selenate (3%) was also reduced into methyl-selenide by heterotrophic bacteria. Additionally, *Hydrogenophaga* bacteria of *β-Proteobacteria*, capable of both nitrate and selenate removal, worked as the dominant species (over 85%) in the biofilm and contributed to the stable removal of both nitrate and selenate. With the selenate input, bacteria with a capacity for both selenate and nitrate removal were also developed in the anaerobic biofilm community.

1. Introduction

Hydrogen-based membrane biofilm reactors (MBfR) have recently been developed as a novel groundwater treatment technology to simultaneously remove multiple oxidized contaminants, especially selenate (Se(VI)) and nitrate which are both considered as pollutants for groundwater (Tang et al., 2013; Zhang et al., 2009; Zhou et al., 2014a). As a clean and nontoxic gas, hydrogen is applied as an electron donor to reduce both selenite and nitrate simultaneously through an anaerobic biofilm in MBfR. Hydrogen gas is diffused through membrane pores, to provide an electron source to bacteria accumulating in the biofilm on the membrane surface to reduce oxidized contaminants. The technology of MBfR has been gradually promoted for the groundwater treatment market, and a pilot scale MBfR has been operated in the United States for almost a year (Zhao et al., 2014). Many studies have reported that MBfR could effectively remove selenate in nitrate-polluted groundwater with high nitrate removal rates, simultaneously (Chung et al., 2010; Ontiveros-Valencia et al., 2016; Van Ginkel et al., 2011). Previous studies (Chung et al., 2006a, 2007; Zhou et al., 2018b) were mostly focused on performance of selenate removal for nitrate-polluted groundwater treatment in MBfR, and pointed out that loading concentration, hydraulic retention time (HRT, which is groundwater retention time), hydrogen pressure, or flux showed a certain effect on the selenate removal in MBfR for nitrate-polluted groundwater treatment. Furthermore, recent literature considered that anaerobic biofilm, as the electron transformation media, would directly control the above factors and work as the key point in the MBfR operation (Li et al., 2018; Zhou et al., 2014a). The latest studies have started to work on identification of anaerobic biofilm characteristics to better understand the mechanism of MBfR for selenate removal in nitrate-polluted groundwater (Ontiveros-Valencia et al., 2016; Zhou et al., 2018a). Thus, as the donor transportation carrier, anaerobic biofilm is regarded as the main reason for selenate removal in MBfRs. Ontiveros-Valencia et al. (2016) tried to analyze anaerobic biofilm communication to understand the competition between selenate removal and sulfate removal. Wen et al. (2016) carried out real-time PCR to target the selenate reductase gene for quantitative detection of selenate-reducing bacteria in anaerobic biofilm. But, a series of questions for selenate removal in MBfR are still

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Table 1
Operational conditions of MBfR.

Phase	Time (d)	NO_3^- −N (mg N/L) [a]	Se(VI) (mg/L) [a]	Flux rate (mL/min)	H_2 Pressure (kPa)
I	1^{st} − 31st	10	0	5.2	40
II	32^{nd}-68th	10	2	5.2	40

[a] NO^3−N and Se(VI) in Table 1 represented the their concentration in the influent.

unclear..

Theoretically the mechanism of selenate removal in anaerobic biofilm of MBfR is "Selenate → Selenite → Se^0", and Se^0 precipitate would be produced in the anaerobic biofilm. This mechanism raises questions such as; where does the Se^0 precipitate, if all selenate is transferred into Se^0 in the anaerobic biofilm, and what/how the structure and distribution of selenium in the anaerobic biofilm is. It has also been reported that inorganic components have direct effects on biofilm formation, and its bacterial community (Zhou et al., 2017). Raising the question of what the relationship between selenate and bacterial community in anaerobic biofilm is. Consequently, the mechanism of selenate removal in MBfR with nitrate-polluted groundwater is still required for further study, especially based on an anaerobic biofilm assessment in MBfRs.

This study aimed to identify the selenate removal mechanism of MBfR for nitrate-polluted groundwater treatment based on anaerobic biofilm analysis. A laboratory-scale MBfR was operated for over 60 days with two stages (Stage I: only with nitrate; Stage II: with both nitrate and selenate). The structure and distribution of selenium were identified using SEM-EDX (scanning electron microscopy-energy dispersive X-ray analyzer) with map and line scanning, X-ray photoelectron spectroscopy (XPS), liquid chromatography (LC) inductively coupled plasma mass spectrometry (ICP-MS), transmission electron microscope (TEM) and bacterial community measurement.

2. Materials and methods

2.1. Reactor set-up and operation

As can be seen in Fig. S1 (Supporting Information, SI), a laboratory-scale MBfR with 600 mL working volume was set-up in this study for characteristic identification of anaerobic biofilm for selenate removal in groundwater. Two micro-filtration (0.4 μm) membrane modules, manufactured with polyvinylidene difluoride (PVDF) (Litree Company, Suzhou, China), were installed in the middle of the reactor. The membrane module contained 9.9 dm^2 membrane surface of 132 fibers with a 0.1 μm membrane pore size. Pure hydrogen (H_2) gas was supplied through the membrane pores of the membrane module, till biofilm developed on the whole membrane module surface (Biofilm was normally around and/or on the membrane pores. Detailed in the following section). HRT of the reactor was maintained at 5.2 h with a flux rate of 1 mL/min under operation of a single peristaltic pump (BT100-2 J, Longer, Shijiazhuang, China). A magnetic stirrer was used to achieve a complete mixture in the reactor to promote substance exchange and inhibit dead zone formation. The temperature of the reactor was maintained at around 24–26 °C with a heating blanket and air condition throughout the whole operation. The influent for the reactor was synthetic groundwater (pH = 7.5) with additives (Xu et al., 2015) of $NaHCO_3$ (0.663 mg/L), KH_2PO_4 (0.292 mg/L), $MgCl_2$ (0.010 mg/L), $FeSO_4{\cdot}7H_2O$ (0.001 mg/L), $CaCl_2{\cdot}2H_2O$ (0.001 mg/L), $ZnSO_4{\cdot}7H_2O$ (0.013 mg/L), H_3BO_3 (0.038 mg/L), $CuCl_2{\cdot}2H_2O$ (0.001 mg/L), $Na_2MoO_4{\cdot}2H_2O$ (0.004 mg/L), $MnCl_2{\cdot}4H_2O$ (0.004 mg/L), $CoCl_2{\cdot}6H_2O$ (0.025 mg/L) and $NiCl_2{\cdot}6H_2O$ (0.001 mg/L). $NaNO_3$ and Na_2SeO_4 were added into the influent as a source of nitrate and selenite, respectively. The chemical agents were purchased from Sigma-Aldrich (USA). The groundwater was firstly bubbled with pure N_2 gas to maintain an anaerobic condition before being sealed with a rubber stopper and stored in a 10 L glass bottle. MBfR was started up with 10 mg/L NO_3^--N at Stage I, and then was operated with both 10 mg/L NO_3^--N and 2 mg/L Se(VI) at Stage II. A stable MBfR performance was regarded to be achieved after approximately 5 cycles of HRT operation according to the effluent condition (Chung et al., 2008; Ziv-El and Rittmann, 2009), and thus each condition in this study was operated for over 65 HRTs for stable performance.

2.2. Anaerobic biofilm development

The biofilm was seeded from sludge in an anoxic tank at Quyang municipal wastewater treatment plant (WWTP). To enrich the bacteria for nitrate and selenate removal, the anaerobic sludge was cultured with synthetic groundwater that included hydrogen, 10 mg/L nitrate and 2 mg/L selenate for over 180 h in sealed bottles. 200 mL of enriched culture from the best performing bottle, was then injected into the MBfR. The influent and effluent pumps were then turned off, and magnetic stirring was maintained at approximately 300 rpm to promote the anaerobic biofilm development of enriched culture on the membrane surface. H_2 gas was also supplied to the reactor to enhance anaerobic biofilm development. After a 24 h cycle, the synthetic groundwater was supplied into MBfR at a rate of 0.2 mL/min. Anaerobic biofilm usually developed in around 3–4 days, the anaerobic biofilm was then maintained to achieve stable nitrate and selenite removal.

2.3. SEM-EDX, XPS and TEM analysis

To better identify the inner structure of biofilm, SEM (XL30, Philips, Netherlands)-EDX (Oxford Isis, UK) was carried out in this study. A short membrane fiber (around 8–10 mm) with a biofilm sample was cut from the middle of the membrane module (the gap was sealed with plastic stick later), the fiber was then air-dried and applied for SEM-EDX measurement. During the whole sampling process, where the reactor had to be momentarily opened, nitrogen gas was bubbled in the MBfR to maintain anaerobic conditions. XPS (PHI−5000C ESCA, PerkinElmer, USA) measurement and TEM (JEM, 2011; JEOL, Japan) was also applied with similar pretreatment as SEM-EDX. For statistical analysis, XPS measurement was applied twice with each of the 3 different samples.

2.4. LCICP-MS identification

Selenite and selenate was also detected by a liquid chromatograph (LC) equipped with a CRC8 reversed-phase column (Agilent (USA), 3.0 μm diam. particles, 3.0 mm i. d. × 1500 mm length) and ICP-MS (Agilent 7700 Series, USA) according to Wolf et al. (2011). The mobile phase was a mixture of 2.0 mM tetrabutyl ammonium phosphate, 0.5 mM ethylene diamine tetraacetic acid and 10% methanol, where its flow rate was maintained at 1 mL/min. The injection volume of the sample was 20 μL. Peaks of selenite and selenate were recorded at 1.409 min and 3.585 min respectively. The soluble selenite and selenate samples in the influent and effluent were measured with the LCICP-MS method after filtration with a 0.4 μm filter. The total selenium concentration in the influent and effluent was measure with ICP-MS (Agilent 7700 Series, USA) after HNO_3/HCl heating digestion. The total selenium concentration was subtracted by soluble selenium concentration to get insoluble selenium concentration based on mass balance.

2.5. Bacterial community measurement

The microbial community of biofilm was analyzed through a cloning library at the end of Stage I and Stage II. The seeded sludge was

also identified in this study. Biofilm samples (collected from 3 pieces 10 cm hollow fibers) were scraped from different locations on the membrane modules. Biofilm DNA was extracted with the Fast DNA Spin Kit (MP Biomedicals, LLC, France) following the manufacturer's instructions. Genomic DNA was then analyzed with complete cloning library according to reference (Zhou et al., 2016). Operational taxonomic units (OTUs) were defined as groups in which the sequence similarity was more than 97%. The phylogenetic tree was analyzed with software Mega 5, and functional profiles of biofilm microbial ecology was calculated according to 16 S rRNA data with software FAPROTAX (Asshauer et al., 2015).

2.6. Other analysis

All liquid samples were filtrated using a 0.2 μm filter (Anpel Company, Shanghai, China) and then kept at 4 °C. Nitrate, nitrite, sulfate, selenate and selenite were measured using an ion chromatograph (ICS-1000, Dionex, USA) containing an AS-20 column, an AG-20 precolumn and a 150 mg/L injection loop. Dissolved oxygen (DO) concentration and pH were measured via a DO-and-pH meter (HQ4d, Hach, USA). Based on the kinetic analysis for identifying the relationships among electron donor and acceptor (Ziv-El and Rittmann, 2009), consumed electrons were calculated by electron balance according to surface loadings of the electron acceptors (modulated simultaneously by varying the HRT or individually by changing the influent concentration), the effluent concentrations and removal fluxes.

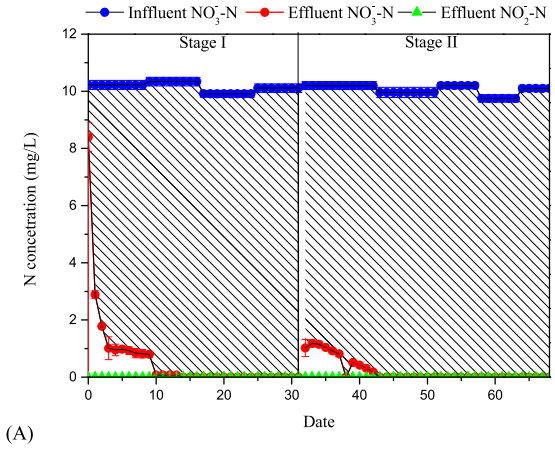
3. Results and discussion

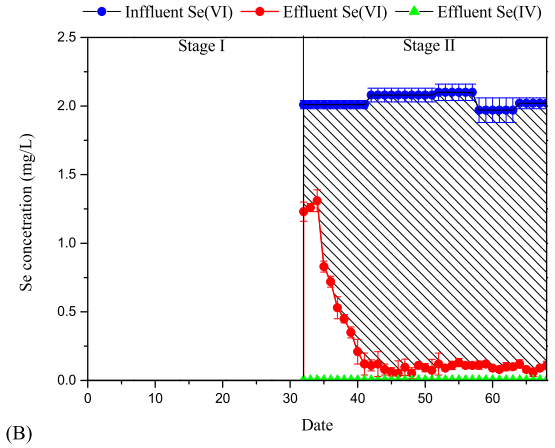
3.1. Removable capability of anaerobic biofilm

The nitrate and selenate removal capability from anaerobic biofilm over 60 days are shown in Fig. 1A and Fig. 1B, respectively. The influent nitrate concentration was maintained at around 10 mg N/L (Fig. 1A) during both Stage I (without selenate) and Stage II (with 2 mg/L selenate). For both Stage I and Stage II, anaerobic biofilm presented an adaptive phase with effluent nitrate decreasing from 8.40 to 0.08 mg/L nitrate and 1.10 to 0.18 mg/L nitrate, respectively. Finally, in both Stage I and Stage II, no nitrite was detected in the effluent, indicating that the whole reaction of nitrate removal (Nitrate→Nitrite→Nitrogen gas) occurred completely and the removal capability of anaerobic biofilm can be varied with influent concentrations. As Fig. 1B shows, selenate removal efficiency was approximately 75% at the beginning and reached around 95% during Stage II over 20 days. This result is similar as previous papers of Chung et al. (2006b) and Ontiveros-Valencia et al. (2016). Moreover, no selenite was detected in the effluent (Fig. 1B) during reactor operation, which confirmed that the reduction reaction of selenate removal (Selenate → Selenite → Se^0) was completed in this operation. At the beginning of Stage II, no detectable nitrite and selenite meant that selenate variation in the influent did not cause the inhibition of nitrate reduction. Moreover, the electron mass balance for nitrate and selenate removal was calculated based on the removal of nitrate and selenate (Fig. 1C). Unconsumed electrons of nitrate (0.52 mol e^-/d) was close to the consumed electrons of selenate (0.48 mol e^-/d) at the beginning of Stage II, indicating that some electrons for nitrate reduction was possibly used for selenate removal. In addition, the biomass of anaerobic biofilm was maintained at approximately 3.6 μg/cm fiber at the beginning of Stage II, before being increased to around 4.0 μg/cm fiber (Fig. 1C).

3.2. Structure and distribution of selenium in anaerobic biofilm

As seen in Fig. 2, the color of anaerobic biofilm changed from white (Stage I) into red (Stage II), indicating that selenate was reduced into Se^0 (Se^0 is normally observed as a red precipitate), which was similar to reactors from previous operations (Chung et al., 2006b; Li et al., 2017).





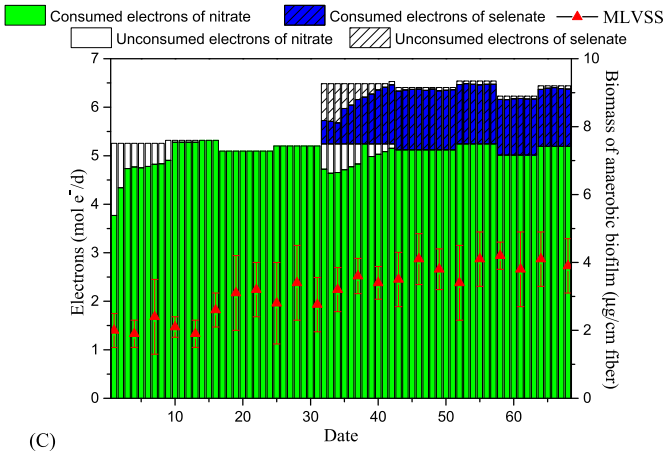


Fig. 1. Long-term performance of (A) nitrate removal (n = 3), (B) selenate removal (n = 3), and (C) consumed electron distribution & biomass variation (n = 4). (Consumed electrons of nitrate (or Se(VI)) was calculated based on the particle removal of nitrate (or Se(VI)); Total consumed electrons of nitrate (or Se(VI)) for removing all nitrate (or Se(VI)) subtracted consumed electrons of nitrate (or Se(VI)) to get unconsumed electrons of nitrate (or Se(VI)).).

TEM analysis was also carried out for anaerobic biofilm identification. Nano-size globular particles (Fig. S2A) were observed in the anaerobic biofilm with selenate removal and were identified to contain only the element selenium with EDX analysis, indicating this particle as the elementary substance of Se^0. This phenomenon proves that parts of Se^0 precipitate accumulated into anaerobic biofilm. In a previous study, the mechanism of selenate removal through Se^0 precipitation in MBfR was shown, but none has identified Se^0 precipitate, especially in biofilms

(A)

(B)

Fig. 2. Anaerobic biofilm (the middle area of membrane module) image during (a) Stage I (28th day) and (b) Stage II (65th day).

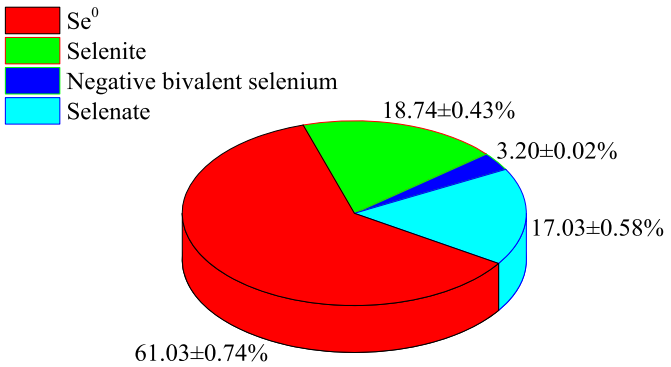
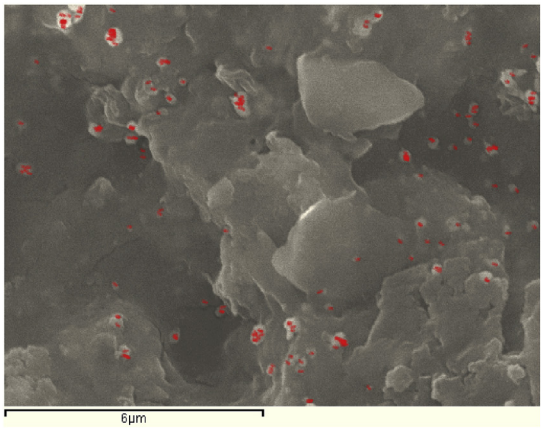
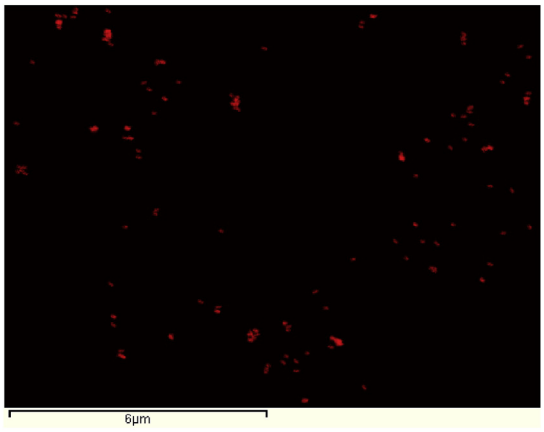


Fig. 3. Structure distribution of selenium in anaerobic biofilm based on XPS analysis. (3 different samples were analyzed twice, n = 6; selenite and selenate should be water-soluble in this study; total intensity of Peak 1–5 in Fig. S3 was considered as 100%.).

(Chung et al., 2006b). These particles transformed from globular to a rod-like shape after 7 days (Fig. S2B), indicating that the removal product of selenate was amorphous Se^0 precipitate. For further selenium structure identification in anaerobic biofilm, XPS was also carried out in this study (Fig. S3) and five peaks could be observed for Se^0 (Peak 1 and 3), selenite (Peak 2), negative bivalent selenium (Peak 4) and selenate (Peak 5). Based on XPS analysis (Fig. 3), Se^0 was the majority (61%) of selenium in the anaerobic biofilm, while selenite and selenate were both around 17%, which was close to the process of selenate reduction. Interestingly, there was approximately 3% negative bivalent selenium found in the anaerobic biofilm (Fig. 3), and negative bivalent selenium was also detected with LC-ICPMS (Fig. S4) and identified as methyl-selenide through GC-MS. Methyl-selenide has been reported as a



(A)



(B)

Fig. 4. Anaerobic biofilm (A) 4000 × SEM image and (B) its map scanning EDX analysis. (Anaerobic biofilm was on the short membrane fiber (around 8.8 mm) from the middle of the membrane module.).

product of selenate reduction for some heterotrophic bacteria (Burra et al., 2010; Lenz et al., 2011).

SEM-EDX was operated for the selenium distribution of anaerobic biofilm. Fig. 4A presents a relatively smooth surface of anaerobic biofilm but without any obvious inorganic particles (showed as angled flocs). This was because Se^0 production was in its amorphous form (Fig. S2), though the above results presented that selenate would be reduced into Se^0 precipitate in nano-size. Fig. 4B shows a map-scanning of EDX analysis for anaerobic biofilm presented in Fig. 4A. Generally, selenium was distributed evenly on the anaerobic biofilm surface, but occasionally selenium accumulated locally to form highly-reflective spots in the biofilm, indicating that bacteria for selenate reduction flocculated and grew together during MBfR operation. The cross-section of anaerobic biofilm was further revealed in Fig. 5. The biofilm was approximately 14 μm, which was much thinner than the biofilm in the membrane bioreactor (Zhou et al., 2015, 2017). The continuous hydrogen supplement indirectly offered a convection force to inhibit the substance accumulation and bacterial attachment onto the membrane surface. Moreover, Fig. 5D shows that selenium was uniformly distributed along the cross-section of the anaerobic biofilm. A previous study (Zhou et al., 2014b) reported that the selenium element in its ionic form was normally evenly distributed along the biofilm because of the high transportation of ions through biomass.

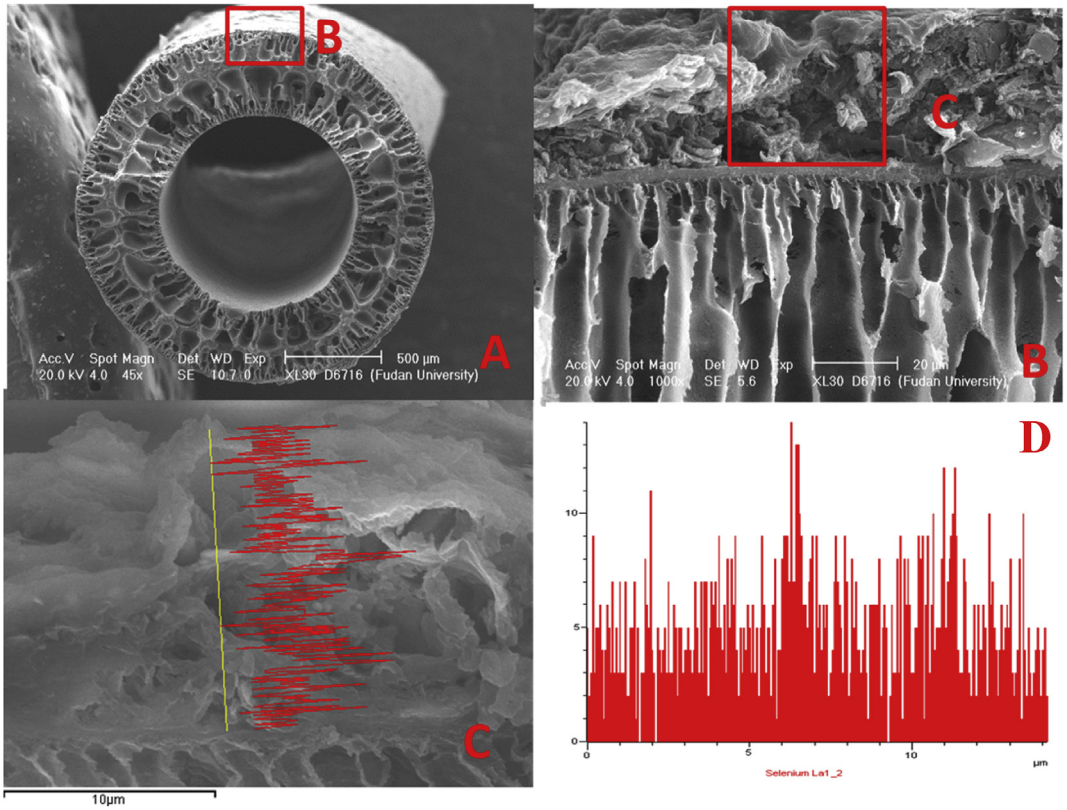
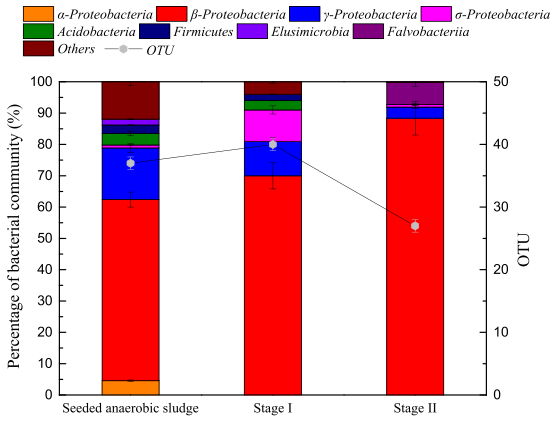


Fig. 5. (A) 45 × SEM image of anaerobic biofilm on whole membrane fiber (around 9.3 mm membrane fiber from the middle of the membrane module); (B) 1000 × SEM image of anaerobic biofilm on area B in Fig. 5A; (C) line scanning EDX analysis of anaerobic biofilm on area C in Fig. 5B; and (D) Se result of line scanning EDX analysis.

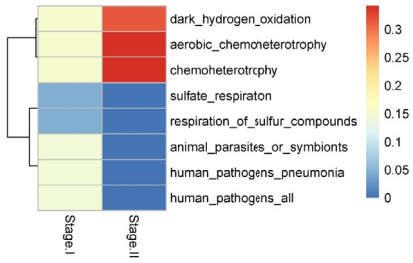
3.3. Bacterial community of anaerobic biofilm

As Fig. 1C reveals, organic mass of anaerobic biofilm slowly increased from 2.0 ± 0.1 to 4.0 ± 0.3 μg MLVSS/cm fiber during the operation of the MBfR, partly indicating the slowly increasing total bacterial count in the anaerobic biofilm. This was due to the long doubling time of anaerobic bacteria, which corresponded to the bacterial community results of previous studies (Ontiveros-Valencia et al., 2016; Van Ginkel et al., 2010; Wen et al., 2016; Zhou et al., 2014a). Thus, it could be regarded that the total bacterial amount of anaerobic biofilm was relatively stable during the short-term phase, such as at the beginning of Stage II (Shin et al., 2015; Tang et al., 2013). Bacterial community was further identified with a cloning library, and a phylogenetic tree of anaerobic biofilm at the end of Stage I and Stage II, shown in Fig. S5 (Community of seeded anaerobic sludge was also analyzed). As Fig. 6A shows, OTU of bacterial community in anaerobic biofilm decreased with MBfR operation, indicating that anaerobic biofilm species dropped with selenate input, and bacterial community was probably optimized to adapt to the requirement for simultaneous nitrate and selenate removal. Li et al. (2018) reported that bacterial communities have shown an optimization process and species decrease during MBfR operation. Furthermore, seeded anaerobic sludge chiefly consisted of bacteria of *α-Proteobacteria*, *β-Proteobacteria*, γ-*Proteobacteria*, *δ-Proteobacteria*, *Acidobacteria*, *Firmicutes*, *Eliusimicrobia*, *Flavobacteriia*. With reactor operation, species of *α-Proteobacteria* and *δ-Proteobacteria* almost disappeared, and *β-Proteobacteria* increased its abundance in the anaerobic biofilm, indicating that *β-Proteobacteria* was the dominant species in the MBfR. Previous studies (Van Ginkel et al., 2010; Zhang et al., 2010; Zhou et al., 2014a) have reported that *Proteobacteria* species dominated the anaerobic biofilm community in MBfR for different oxidized contaminant reduction, as most denitrifying bacteria belong to *Proteobacteria*, especially *α*, *β and* γ subciasses (Philippot, 2002). Moreover, Zhao et al. (2013) also had a similar result of *β-Proteobacteria* as the dominant species in the anaerobic biofilm of MBfR for simultaneous nitrate and perchlorate removal. Zhang et al. (2009) also observed a significant percentage increase of *β-Proteobacteria* in the biofilm community of MBfR with only nitrate removal. Therefore, *β-Proteobacteria* as the dominant species implied that denitrifying bacteria contributed to the majority of anaerobic biofilm for nitrate removal.

As the bacterial community revealed, bacteria of *Hydrogenophaga*, belonging to *β-Proteobacteria*, were found in the community of the anaerobic biofilm, especially during Stage I, contributing to over 85.0% of the bacterial community. *Hydrogenophaga* bacteria were considered as the dominant species for nitrate reduction with hydrogen (Zhang et al., 2009). Moreover, as can be seen by the functional profile analysis (Fig. 6B), dark hydrogen oxidation was the major function of anaerobic biofilm for both Stage I and Stage II, indicating that hydrogen utilization was the main pathway in this removal system. Consequently, *Hydrogenophaga* bacteria in the anaerobic biofilm was one of the main contributors to maintaining a stable performance of hydrogen utilization and nitrate removal. Additionally, in previous research (Zhao et al., 2013) and as observed in this study, revealed that no special bacteria was identified for any particular oxidized contaminant reduced during nitrate removal. Therefore, no significant variation of bacteria for selenate removal in this study implied that bacteria can contain the removable pathways for nitrate and selenate. Sabaty et al. (2001) reported that non-specific selenate and selenite reduction were carried out by some denitrifying microorganisms through the nitrate and nitrite reduction pathways. Zhao et al. (2013) also identified denitrifying bacteria with multiple oxidized contaminant reduction pathways. Consequently, it can be concluded, that the denitrifying bacteria mainly contributed to the selenate removal of anaerobic biofilm, which could further explain why *Hydrogenophaga* bacteria of *β-Proteobacteria*



(A)



(B)

Fig. 6. (A) Bacterial distribution of seeded anaerobic sludge and anaerobic biofilm during Stage I & Stage II (n = 3); (B) Functional profiles of anaerobic biofilm during Stage I and Stage II. (Functional profiles was analyzed based on the bacterial community.)

increased as the dominant species in biofilm with selenate input.

As Section 3.1 shows, at the beginning of Stage II, effluent nitrate was increased with selenate input and decreased as selenate removed, but without the detection of nitrite and selenite in the effluent. This phenomenon could be further explained with bacterial community analysis. At the beginning of Stage II, the total bacterial count of anaerobic biofilm was relatively stable, this phenomenon was because of the anaerobic biofilm community. Within the community, *Hydrogenophaga* bacteria contributed to both nitrate and selenate removal, and both removals were parts of the energy supply for *Hydrogenophaga* bacteria. Therefore, this phenomenon could be regarded as a switch of energy supply. With selenate input, bacteria with the capacity of both selenate and nitrate removal developed in the anaerobic biofilm community.

4. Conclusion

The selenate removal mechanism of MBfR for nitrate-polluted groundwater treatment was studied based on anaerobic biofilm. Anaerobic biofilm showed an excellent performance for both nitrate and selenate removal in MBfR operation. The anaerobic biofilm had the main pathway of selenate reduction, which progressed as Selenate → Selenite → Se^0. However, some amorphous Se^0 precipitate accumulation in biofilm, and parts of selenate was reduced into methyl-selenide by heterotrophic bacteria during selenate reduction in anaerobic biofilm. The element of selenium was evenly distributed along the cross-sectionally thin biofilm, additionally, *Hydrogenophaga* bacteria of *β-Proteobacteria*, capable of both nitrate and selenate removal, worked as the dominant species in biofilm and contributed to stable removal of nitrate and selenate. With selenate input, bacteria with the capacity of both selenate and nitrate removal developed in the anaerobic biofilm community.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.04.005.

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