Simultaneous removal of nitrate and sulfate using an up-flow three-dimensional biofilm electrode reactor: Performance and microbial response

Qi Tang^{a,b}, Yanqing Sheng^{a,*}, Changyu Li^{a,b}, Wenjing Wang^a, Xiaozhu Liu^{a,b}

^a Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Nitrate removal from low carbon water is a problem in the water treatment, especially in the presence of high sulfate. In this work, an up-flow three-dimensional biofilm electrode reactor (3D-BER) was established to remove nitrate and sulfate from low organic carbon water. Results indicated that sulfate negatively affected nitrate removal. Moreover, high electric current and short hydraulic retention time deteriorated the performance of nitrate and sulfate removal. When the influent of SO_4^{2-} was 150 mg/L, the removal efficiency of NO_3^- -N and SO_4^{2-} was $88.49 \pm 4.5\%$ and $29.35 \pm 5.5\%$, respectively. The high-throughput sequencing revealed that denitrifying bacteria dominated in the lower part of the reactor while sulfate reducing bacteria dominated in the upper part of the reactor. It was speculated that oxidation products of sulfide could serve as supplementary electron donors to enhance nitrate removal in the 3D-BER.

1. Introduction

Due to overuse of nitrogen fertilizer, sewage discharge and other anthropic activities, nitrate pollution in water bodies has become a global severe issue (Ogrinc et al., 2019). A huge quantity of nitrate has negative effects on human health and aquatic ecosystems. For human health, ingested nitrate under conditions that result in endogenous nitrosation is classified into Group 2A carcinogen by The International Agency for Research on Cancer of the World Health Organization. For

aquatic ecosystems, increasing nitrate in surface water is responsible for the eutrophication. Sulfate is a common oxygen-containing anion which coexists with nitrate in wastewater or groundwater (Nguyen et al., 2016). Normally, the sulfate concentration is limited to 250 mg/L in drinking water and ranges from 30 to 300 mg/L SO_4^{2-} -S in coastal wastewater (Wu et al., 2018). Although sulfate itself is not a serious pollutant, its reduction product, sulfide, has raised concern because of adverse health and environment effects such as toxic to humans, odour nuisance and sewer corrosion (Xu et al., 2017). Consequently, the water

* Corresponding author.

E-mail address: yqsheng@yic.ac.cn (Y. Sheng).

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contaminated by nitrate and sulfate requires applicable treatment before use or discharge.

Generally, nitrate and sulfate contaminated water contains low organic carbon, such as groundwater and the secondary effluent of wastewater treatment plants (Chang et al., 2016; Xu et al., 2018). Therefore, additional electron donors are essential to drive denitrification and sulfate reduction. Straws have been added as carbon source in the treatment of nitrate and sulfate contaminated wastewater, and high total organic carbon and colority in the outflow were detected after the addition of straws (Chang et al., 2016). Biofilm electrode reactors (BERs) combine microbial physiology with electrode, can treat wastewater lacking electron donors and realize autotrophic removal of targeted contaminants (Blázquez et al., 2017; Xu et al., 2018). In BERs, hydrogen produced through electrolysis of water is a kind of clean electron donor. In addition, it is found that some microorganisms can receive electrons directly at the cathode surface for biological reduction without intermediate agent such as hydrogen (Chen et al., 2017; Cheng et al., 2017).

BERs are usually designed with two chambers separated by proton/cation/anion/exchange membrane. This structure hinders the diffusion of proton and hydroxide ion between cathode and anode, resulting in pH gradient between the two chambers (pH decreases in anode chamber while increases in cathode chamber). Subsequently, the internal resistance increases and current density decreases (Call and Logan, 2008). Therefore, phosphate buffer solution is essential in the two-chamber BERs. In contrast, the single-chamber BERs eliminate the internal resistance caused by exchange membrane and improve current density, thereby it is conducive to reducing construction and operating cost (Miller et al., 2019). Moreover, to enhance the performance of single-chamber BERs, a three-dimensional biofilm electrode reactor (3D-BER) is designed by inserting conductive particles such as anthracite, granular activated carbon, sulfur and iron between anode and cathode, and these particles are usually called third bipolar electrodes (Feng et al., 2018; Huang et al., 2020). 3D-BER can not only improve surface area for biomass attachment but also enhance current efficiency by forming numerous charged microelectrodes (Wu et al., 2019).

Up to now, the chemolithotrophic denitrification and autotrophic sulfate removal in BERs have been widely investigated. Studies of bioelectrochemical autotrophic denitrification are focused on electrode material (Hao et al., 2018), optimization of operating conditions and microbial communities (Nguyen et al., 2015), and accumulation of intermediates (Tian and Yu, 2020). In terms of autotrophic sulfate removal, researchers have found that a sulfur cycle, including reduction of sulfate to sulfide and oxidation of sulfide to elemental sulfur, could be achieved in BERs (Blázquez et al., 2016), hence the sulfur cycle can achieve detoxification of sulfide and elemental sulfur recovery. Nonetheless, there are few reports on simultaneous removal of nitrate and sulfate in low organic carbon water in BERs, especially in single-chamber BERs, which may be caused by the complicated competition between biological denitrification and sulfate reduction. Therefore, the objectives of this study are to (1) investigate nitrate removal efficiency in the presence of sulfate in a single-chamber 3D-BER; (2) evaluate the effects of electric current and hydraulic retention time (HRT) on the performance of simultaneous nitrate and sulfate removal; (3) identify the microbial responses at different heights of the 3D-BER; (4) recognize the potential mechanism of nitrate and sulfate removal in 3D-BER; (5) assess the feasibility in engineering application.

2. Materials and methods

2.1. Experimental apparatus

Fig. 1 was the schematic diagram of the experimental apparatus. The main body of the 3D-BER was a cylindrical plexiglass with a diameter of 10.5 cm and 50 cm high. The height of two biofilm sampling ports was 13.75 cm and 31.25 cm from the outlet port, respectively. The

Fig. 1. Schematic diagram of 3D-BER (1) influent tank; (2) peristaltic pump; (3) backwashing pipe; (4) supporting layer; (5) water distribution plate; (6) biofilm sampling port; (7) activated carbon; (8) cathode; (9) outlet port; (10) anode; (11) DC power supply.

cathode was stainless steel mesh, which was arranged along the inner wall of the reactor. A graphite rod (diameter of 2 cm and height of 43 cm) setting in the center was used as anode. Granular activated carbon (4–8 mm) was selected as the third electrode, which was filled between the anode and cathode after pretreated according to previous methods (Hao et al., 2013). Under the action of electric field, activated carbon could not only generate CO_2 to stabilize pH in the solution and provide inorganic carbon source for autotrophic microbes, but also be induced into complex bipolar microelectrodes to promote current efficiency (Huang et al., 2020). Furthermore, it would provide large surface area for microorganism attachment. The direct current (DC) power supply (MS-303D, Dongguan Maihao Electronic Technology Co., Ltd, Dongguan, China) provided a controlled constant current. Electrical heating tape was wrapped around outside of the reactor to maintain the temperature $\sim 30^\circ\text{C}$. The 3D-BER had an effective volume of 3.46 L.

2.2. Synthetic influent and reactor operation

Simulated wastewater was prepared by tap water added with KNO_3 (216.43 mg/L), Na_2SO_4 (added as desired), KH_2PO_4 (27.20 mg/L), CH_3COONa (15 mg/L) and NaHCO_3 (300 mg/L). The pH was adjusted to 7.3 ± 0.3 with HCl (1 mol/L) and NaOH (1 mol/L). N_2 was connected for more than 1 h to maintain the dissolved oxygen (DO) less than 0.2 mg/L.

Electrochemical reduction experiment without microorganisms was conducted after activated carbon was saturated with synthetic influent. Sterilized synthetic influent was prepared every two days. The effluent concentrations of NO_3^- -N, NO_2^- -N, NH_4^+ -N and SO_4^{2-} were measured 3 days after each current change. Then, the reactor was

Table 1
Detailed operating conditions.

Time (d)	1–29	30–66	67–94
SO ₄ ²⁻ (mg/L)	50–350	250	250
HRT (h)	18	18	6–24
I (mA)	240	40–320	240
T (°C)	30	30	30

inoculated with anoxic sludge which was taken from a local wastewater treatment plant (Yantai, China). Characteristics of seed sludge were below: SV 59%, MLSS 2624 mg/L, MLVSS 1631 mg/L, SVI 22.48 ml/g, MLVSS/MLSS 0.62 (SV: Settling Velocity, MLSS: Mixed Liquid Suspended Solids, MLVSS: Mixed Liquor Volatile Suspended Solids, SVI: Sludge Volume Index). Nutrient substances were added into the tank regularly. Driven by a peristaltic pump, the sludge circulated continuously between the reactor and plastic tank containing anoxic sludge for 6 days with an electric current of 40 mA and a HRT of 24 h. After biofilm immobilization, the reactor was operated using simulated wastewater (with the sulfate concentration of 250 mg/L) to achieve biofilm acclimatization. During this phase, HRT was set at 18 h and current was increased from 80 to 160 and then to 240 mA with a change period of 10 days. The NO₃⁻-N and SO₄²⁻ in influent and effluent were monitored every 2 days. The biofilm was considered to acclimate successfully until the removal efficiency of NO₃⁻-N and SO₄²⁻ reached over 80% and 20%, respectively. After biofilm was well developed, the 3D-BER was performed with simulated wastewater, the effects of sulfate concentration, current intensity and HRT were investigated as well. The detailed operating conditions were listed in Table 1.

2.3. Sampling and analysis

The influent and effluent concentrations of NO₃⁻-N, NO₂⁻-N, NH₄⁺-N, SO₄²⁻, S²⁻ and pH were measured each day. On a UV-Visible spectrophotometer (TU-1810, Beijing Pgeneral, China), NO₃⁻-N, NO₂⁻-N, NH₄⁺-N and SO₄²⁻ were measured according to standard method (APHA, 1999). Sulfide was analyzed by iodometric method (APHA, 1999) and the pH was determined using a pH meter (FE28, Mettler Toledo, Switzerland). The surface morphology of activated carbon after pretreatment and biofilm acclimatization were observed by scanning electron microscopy (SEM, Hitachi S-4800), respectively.

The samples for microbial community analysis were taken from the two sampling ports in Fig. 1 under the operating condition of I 240 mA, HRT 18 h and influent SO₄²⁻ 250 mg/L. Samples from the upper and lower sampling port were named R1.U and R1.L respectively. The

extracted genomic DNA was amplified with primers 515F and 806R PCR in the V4 region of 16S rRNA gene. The PCR amplification and microbial analysis were based on the procedures described in previous study (Liu et al., 2020).

The removal efficiency (*E*) showing the percentage of target contaminants removed from influent was calculated as Eq. (1):

$$E = ((C_{in} - C_{eff})/C_{in}) \times 100\% \quad (1)$$

where *C_{in}* and *C_{eff}* are the concentrations (mg/L) of target contaminants in the influent and effluent, respectively.

The reducing rate (*R*, mg/L/d) of NO₃⁻-N (NRR) and SO₄²⁻-S (SRR) was calculated as Eq. (2):

$$R = (C_{in} - C_{eff})/HRT \quad (2)$$

where unit of HRT is day.

The current efficiency (*CE*) in 3D-BER was calculated according to Eq. (3) as follows:

$$CE = \frac{[5 \times (\Delta C_{NO_3^- - N} - C_{NO_2^- - N}^{eff} - C_{NH_4^+ - N}^{eff}) + 2C_{NO_2^- - N}^{eff} + 8C_{NH_4^+ - N}^{eff} + 8 \times \Delta C_{SO_4^{2-} - N} - 8C_{CH_3COO^-}^{in}] \times F \times Q}{I} \quad (3)$$

where $\Delta C_{NO_3^- - N}$ is the difference of NO₃⁻-N (mmol/L) between influent and effluent; $C_{NO_2^- - N}^{eff}$ and $C_{NH_4^+ - N}^{eff}$ are the concentrations (mmol/L) of NO₂⁻-N and NH₄⁺-N in the effluent, respectively; $\Delta C_{SO_4^{2-} - N}$ is the difference of SO₄²⁻ (mmol/L) between influent and effluent; $C_{CH_3COO^-}^{in}$ is the influent concentration (mmol/L) of acetate; *F* is Faraday's constant (96485 C/mol); *Q* is influent flow rate, L/s; *I* is the applied current, mA. The electron transfer numbers of NO₃⁻-N to N₂, NO₂⁻-N and NH₄⁺-N are 5, 2 and 8, respectively. Per mole of sulfate transforms to sulfide assuming 8 electrons. Mineralization of acetate produces 8 electrons/mol of acetate.

Energy consumption (*EC*, kWh/m³) of 3D-BER was calculated on the basis of Eq. (4) (Wu et al., 2019):

$$EC = UIt/1000/V \quad (4)$$

where *U* is the average voltage, V; *I* is the applied current, A; *V* is the volume of treated water, m³; *t* is the treatment time, h.

3. Results and discussion

3.1. Electrochemical reduction of nitrate and sulfate

The electrochemical reduction capacity of the 3D-BER without the addition of microorganisms was shown in Fig. 2. In nitrate electro-reduction process, it was generally believed that NO₃⁻-N was directly

Fig. 2. Performances of electrochemical reduction of nitrate and sulfate (a) and removal efficiency (b) at different currents (operating condition: influent NO₃⁻-N 40 mg/L, influent SO₄²⁻ 250 mg/L, HRT 18 h).

reduced by electrons transferred from the cathode or/and indirectly reduced by hydrogen produced by electrolysis of water at the cathode (de Vooy et al., 2000). Similarly, the electrocatalytic reduction of sulfate may take place at the cathode using electrons or/and hydrogen. In this work, when the applied current increased from 20 to 240 mA, the NO_3^- -N removal efficiency increased from $13.62 \pm 1.1\%$ to $37.27 \pm 1.1\%$ (Fig. 2(b)). Synchronously, the SO_4^{2-} removal efficiency was enhanced from $1.48 \pm 1.5\%$ to $12.94 \pm 1.8\%$ as the current intensity increased. The results indicated that more electrons and hydrogen would be available in the reactor to reduce nitrate and sulfate along with greater current applied. The main process of nitrate electroreduction could be described as: NO_3^- -N adsorbed on the surface of cathode was reduced to NO_2^- -N and further reduced to N_2 or NH_4^+ -N (Peel et al., 2003). It can be seen in Fig. 2(a) that the concentration of NO_2^- -N stabilized at ~ 3 mg/L with the increase of current. In contrast, the effluent NH_4^+ -N gradually increased as current increased, which was consistent with previous study that N_2 was produced at low current densities while NH_4^+ -N was formed at high current densities (Xing et al., 1990). In this work, such phenomenon could be explained that the amount of H atom generated in system increased with the increase of current, which was conducive to the formation of N-H bond with low bond energy instead of $\text{N}\equiv\text{N}$ bond with high bond energy. Although NO_3^- -N and SO_4^{2-} were partially removed by electrochemical reduction, it led to the formation of NH_4^+ -N and high NO_2^- -N in effluent, producing undesired by-product and intermediate product in reduction.

3.2. Effects of sulfate on nitrate removal in 3D-BER

In order to reveal the effect mechanism of SO_4^{2-} on nitrate removal in waters with insufficient organic carbon in 3D-BER, reactor was run from day 1 to day 29 at different influent SO_4^{2-} concentrations under a HRT of 18 h and current of 240 mA. Generally, biological denitrification involves four stepwise reduction: $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ (Xu et al., 2018), and this process is driven by a series of reductase in denitrifying bacteria (DB). Similarly, sulfate reducing bacteria (SRB) can reduce sulfate to sulfide via three steps. Sulfate is firstly activated by ATP sulfurylase to generate adenosine phosphosulfate (APS) and pyrophosphate, then sulfite is formed by APS under the effect of APS reductase, and finally sulfite is reduced to sulfide by the complex sulfite reductase enzyme system (Ontiveros-Valencia et al., 2012). In this work, NO_3^- -N removal efficiency in treated water gradually decreased with influent SO_4^{2-} increase (Fig. 3(a)). When SO_4^{2-} increased from 50 to 350 mg/L, the NO_3^- -N removal efficiency diminished from $92.58 \pm 2.9\%$ to $72.96 \pm 4.6\%$ (Fig. 3(c)), indicating that SO_4^{2-} presented a negative effect on NO_3^- -N removal in the 3D-BER. Sulfate was shown to affect the activity of SRB (Moosa et al., 2002). The increased SO_4^{2-} could contribute to improve the activity of SRB, and further enhance the competitiveness of SRB for electron donors.

However, the SRR began to decline when the influent SO_4^{2-} reached 350 mg/L. Actually, the addition of nitrate would be an effective method to inhibit sulfate reduction by the competition of electron donors between DB and SRB and inhibition of denitrification intermediates (nitrite and nitric oxide) on SRB (Mohanakrishnan et al., 2011; Ontiveros-Valencia et al., 2012). In addition to nitrate, sulfide also presented inhibitory effect on the growth of SRB by reacting with cytochrome to inactivate the enzyme (Okabe et al., 1995). Sulfide production would increase with the increase of sulfate, and high concentration of sulfate might reduce the activity of SRB by the feedback inhibition of sulfide. Whereas, sulfide was not observed in the effluent throughout the experiment, because it can be readily oxidized via abiotic and biotic (Sabuda et al., 2020). Since there was no obvious variation on influent NO_3^- -N and effluent NO_2^- -N (Fig. 3(a)), a reasonable explanation was that the increased sulfide generated by sulfate reduction restrained the activity of SRB when the influent sulfate was 350 mg/L, leading to the decrease of SRR. As a result, high SO_4^{2-}

(≥ 350 mg/L) not only impeded the reduction of NO_3^- -N but also inhibited the SO_4^{2-} reduction in 3D-BER. The concentration of NO_2^- -N was below 3 mg/L in the effluent, which was lower than the NO_2^- -N (6.8 mg/L) accumulated in a bioelectrochemical denitrification system with SO_4^{2-} of 50 mg/L (Nguyen et al., 2016).

3.3. Effect of current on nitrate and sulfate removal in 3D-BER

The effect of current on performance of 3D-BER was investigated from day 30 to 66 by increasing current from 40 to 320 mA under the conditions of influent SO_4^{2-} 250 mg/L and HRT 18 h. In Fig. 3, the NO_3^- -N in the effluent gradually decreased, and the NO_3^- -N removal efficiency improved from $27.56 \pm 6.9\%$ to $79.95 \pm 2.7\%$ with current from 40 to 240 mA. This result was in agreement with previous study, in which the denitrification efficiency in bioelectrochemical reactor could increase linearly with the increase of current (Ghafari et al., 2009). Increasing current promoted bacteria to gain more energy for cell growth (Xu et al., 2018). And the activity of denitrifying enzymes fixed on the cathode surface was higher than that in the solution under the stimulation of electric current (Mellor et al., 1992). However, NO_3^- -N in the effluent presented a sudden increase with an obvious decline in NO_3^- -N removal efficiency when the current further increased to 320 mA (Fig. 3(c)). Previous studies pointed out that the increase of hydrogen in biofilm at high electric current might lead to the detachment of biofilm, forming hydrogen inhibition (Flora et al., 1994; Sakakibara et al., 1994). Therefore, the reduced NO_3^- -N removal efficiency might be caused by the loss of biomass. For sulfate removal, the effluent SO_4^{2-} was higher than influent SO_4^{2-} when the current was 40, 80 and 320 mA (Fig. 3(b)). It was observed that SO_4^{2-} removal efficiency rose from $4.3 \pm 2.8\%$ to $16.97 \pm 6.5\%$ when current increased from 160 to 240 mA (Fig. 3(c)). This phenomenon could be explained that greater current applied would produce more electrons and hydrogen at the cathode for sulfate reduction by SRB (Luo et al., 2014). Similar to the NO_3^- -N removal, high current (320 mA) resulted in negative effect on SO_4^{2-} reduction. In addition, effluent pH dropped sharply to the lowest value of 4.7 (Fig. 3(b)), which was below the optimal pH of DB and SRB. Thus, it was necessary to apply appropriate current when removing nitrate and sulfate in BER since excessive current inhibited both denitrification and sulfate reduction. The optimal electric current of 3D-BER in this work was 240 mA under the conditions of influent NO_3^- -N and SO_4^{2-} were 40 and 250 mg/L, respectively.

As shown in Fig. 3(a), when current increased from 40 to 240 mA, the effluent NO_2^- -N and NH_4^+ -N gradually decreased. And the NO_2^- -N and NH_4^+ -N in the effluent were less than 0.9 mg/L and 0.5 mg/L respectively when the current was over 80 mA, suggesting the denitrification process was relatively complete with few intermediate product and byproduct. Whereas, effluent NH_4^+ -N illustrated an ascending trend to the highest value of 4.82 mg/L when the current increased to 320 mA, which would attribute to NH_4^+ -N accumulation occurred at high current density due to dissimilatory nitrate reduction to ammonia (DNRA) (Nguyen et al., 2016). When the current was 240 mA, the effluent NO_3^- -N, NO_2^- -N and NH_4^+ -N were less than 11 mg/L, 0.9 mg/L and 0.5 mg/L, respectively, and all were lower than the WHO guideline values for drinking water quality.

3.4. Effect of HRT on nitrate and sulfate removal in 3D-BER

From day 67 to day 94, the 3D-BER was performed under the current of 240 mA and influent SO_4^{2-} of 250 mg/L to examine the influence of HRT from 24 h to 6 h. As shown in Fig. 3(c), the average removal efficiency of NO_3^- -N declined from $90.29 \pm 2.2\%$ to $50.60 \pm 3.7\%$ as HRT shortened from 24 h to 6 h. The result was consistent with previous report that adequate HRT contributed to maintain high denitrification efficiency due to the slow growth of autotrophic bacteria (Hao et al., 2018). Similarly, short HRT (≤ 12 h) led

Fig. 3. Profiles of NO_3^- -N, NO_2^- -N and NH_4^+ -N (a), SO_4^{2-} and pH (b) over time, the average reducing rate and removal efficiency of NO_3^- -N and SO_4^{2-} (c) at different operating conditions.

to rapid decrease in SO_4^{2-} removal. Constant amount of electron donors was supplied by DC power supply in the 3D-BER and shortening HRT substantially would increase NO_3^- -N and SO_4^{2-} load in the influent. So, the distinct decrease in NO_3^- -N and SO_4^{2-} removal efficiency owing to insufficient electron donors was observed at short HRT. As shown in Fig. 3(a), the effluent NO_2^- -N presented upward tendency with the decreasing HRT. And the highest concentration of NO_2^- -N (6.56 ± 0.95 mg/L) were observed at the HRT of 6 h, which were 10 times higher than that when HRT was 18 h. The result indicated that short HRT slowed down both the first step (reduction of nitrate) and second step (reduction of nitrite) of biological denitrification. In

addition, the effluent NH_4^+ -N also increased as the reduced HRT, which demonstrated that shortening HRT could accelerate the process of DNRA. Therefore, an adequate HRT (e.g. 18 h) should be taken to achieve satisfactory denitrification and sulfate removal efficiency as well as the cost-effective goal in the bioelectrochemistry system.

3.5. Microbial community analysis

Illumina high throughput sequencing technology was used to identify microbial communities in the different parts of the 3D-BER. A series of indexes such as Shannon, Simpson, Chao 1 and ACE indexes

Fig. 4. Microbial community analysis of samples taken from the lower (R1.L) and upper (R1.U) part of the 3D-BER at phylum level (a) and genus level (b).

were detected to evaluate the richness and diversity of the samples. It was found that R1.L presented higher Simpson index and lower Shannon index than R1.U, which indicated that community diversity in R1.L was lower than that in R1.U. The Chao 1 and ACE indexes in R1.U were higher than those in R1.L, which demonstrated that community richness in R1.U was higher than that in R1.L.

The Fig. 4 showed the microbial abundance under influent sulfate 250 mg/L, current 240 mA and HRT 18 h. At the phylum level, *Proteobacteria* was the predominant phylum in R1.L and R1.U, which played an important role in carbon, nitrogen, sulfur and phosphorus cycles in wastewater treatment (Hamilton et al., 2016; Liu et al., 2020). *Firmicutes*, *Bacteroidetes* and *Euryarchaeota* were the following dominant phyla in R1.L and R1.U. *Bacteroidetes* was also commonly observed like *Proteobacteria* in activated sludge wastewater treatment (Liu et al., 2020). *Firmicutes* and *Euryarchaeota* were belonged to SRB and archaea, respectively, and they could reduce sulfate to sulfide using organic matter or hydrogen (Hamilton et al., 2016).

Detailed analysis of the microbial community at genus level (without others) was exhibited in Fig. 4(b). In R1.L, the dominant genera were *Dechloromonas* (21.57%), *Dethiobacter* (18.20%), *Thauera* (17.20%) and *Azonexus* (10.51%), followed by *Desulfovibrio*, *Sulfurospirillum*, *Thermomonas* and *Dechlorobacter*. *Dechloromonas* played the key role of denitrifying phosphorus removal in the anoxic condition (Liu et al., 2020). The genus *Thauera* was widely found in denitrifying reactor because it could use various electron donors (hydrogen, organic matter or sulfide) to perform denitrification (Mao et al., 2013). *Azonexus* was recognized as a known facultative autotrophic denitrifier (Zhao et al., 2011). *Sulfurospirillum* was reported to have the ability of cell synthesis by utilizing the energy of sulfide oxidation to sulfate under the autotrophic nitrate reduction (Wen et al., 2019). *Thermomonas* and *Dechlorobacter* were the contributors to nitrate reduction and *Thermomonas* was often found in autotrophic bioelectrochemical denitrification (Van Doan et al., 2013). Microorganisms in the 8 genera reached 84.56% relative abundance and most of them were closely related to nitrate reduction except *Dethiobacter* and *Desulfovibrio*. According to the metabolic function of detected microorganisms, heterotrophic DB, sulfur and hydrogen autotrophic DB coexisted in the 3D-BER. This result further indicated the existence of various electron donors such as hydrogen, organic carbon and sulfide in the 3D-BER.

As for R1.U, the first 7 dominant genera were *Desulfovibrio* (10.72%), *Acetobacterium* (10.56%), *Dethiobacter* (9.83%), *Desulfomicrobium* (8.75%), *Thauera*, *Microbacter* and *Paludibacter*, accounting for 61.43% relative abundance of the total. *Desulfovibrio* was capable of reducing sulfate to sulfide. *Desulfovibrio-desulfuricans* and *Desulfovibrio-vulgaris* were the two *Desulfovibrio* species identified in

R1.U, which were typical electroactive SRB that could accept electrons directly from electrodes (Luo et al., 2017). In addition, some species of *Desulfovibrio* could reduce nitrate to nitrite and ammonia in turn under the action of periplasmic nitrate reductase and cytochrome c nitrite reductase (Ontiveros-Valencia et al., 2012). *Acetobacterium* was reported that it could use H_2 and CO_2 as growth substrate to produce acetate or formate (Schuchmann and Mueller, 2013), thus it was able to provide organic matter for heterotrophic microorganisms. *Dethiobacter* was facultatively autotrophic bacteria which could use short chain fatty and hydrogen as electron donors for sulfur reduction (Sorokin et al., 2008). *Desulfomicrobium* had been reported that it preferred to use hydrogen as electron donor for sulfate reduction (Luo et al., 2020). Besides, it was observed in a heterotrophic SRB biofilm which exposed to nitrate (Mohanakrishnan et al., 2011). As can be seen, the dominant genera in the upper zone were focused on SRB such as electroactive SRB, heterotrophic and hydrogen autotrophic SRB. These microorganisms were most likely responsible for sulfate removal in 3D-BER.

3.6. Performance of simultaneous nitrate and sulfate removal

3.6.1. Interactions between nitrate and sulfate reductions

Compared to pretreated activated carbon without inoculation of microorganisms, the surface morphology of activated carbon had changed greatly after biofilm acclimatization. And it displayed an interwoven porous structure might due to the attachment of microorganisms and extracellular polymeric substances. In this work, both electrochemical reduction and biological reduction contributed to the co-reduction of nitrate and sulfate in 3D-BER. For example, on the part of NO_3^- -N removal efficiency, it was $37.27 \pm 1.1\%$ in the absence of microbes while it was $81.14 \pm 3.0\%$ in the presence of biofilms at an applied current of 240 mA (Fig. 2(b) and (c)). The results illustrated that microorganisms could serve as the catalytic or driving factor for nitrate reduction and lower the energy input in 3D-BER. Furthermore, in contrast to electrochemical reduction, there were low NO_2^- -N and NH_4^+ -N in the effluent by bioelectrochemical reduction in the effluent.

Nitrate reduction was thermodynamically more favorable than sulfate reduction and DB was more competitive with electron donors than SRB (Ontiveros-Valencia et al., 2012). Therefore, the SRR was much lower than NRR regardless of sulfate concentrations as displayed in Fig. 3(c). In a hydrogen-fed biofilm, DB outcompeted SRB when hydrogen was the limiting factor, and SRB started sulfate reduction only after almost complete nitrate reduction (Ontiveros-Valencia et al., 2012). According to the analysis of microbial community distribution in Fig. 4, it showed that DB dominated in the lower zone while SRB dominated in the upper zone. In view of the up-flow water pattern of

the 3D-BER, it suggested that nitrate reduction mainly took place at the lower part of the reactor and sulfate reduction principally occurred at the upper part.

In this study, sulfate reduction could occur in the influent NO_3^- -N of 40 mg/L, and the highest SRR reached 21.78 ± 4.76 mg/L/d (Fig. 3(c)). However, nitrate was generally believed to preclude sulfate reduction. This result might be attributed to the type of microorganisms in the 3D-BER. *Desulfovibrio* and *Desulfomicrobium* were the first and fourth dominant genera in R1.U (Fig. 4(b)), respectively. It was reported that *Desulfovibrio* and *Desulfomicrobium* probably possessed the ability to tolerate nitrite, thus nitrate was unlikely to inhibit the sulfate reduction process of these populations. (Mohanakrishnan et al., 2011). So, their presence likely led to the sulfate reduction in the presence of high influent NO_3^- -N in this study.

3.6.2. Potential mechanism of nitrate and sulfate removal in 3D-BER

Sulfide in the effluent was not detected. Generally, sulfide could be oxidized to elemental sulfur (S^0), thiosulfate and sulfate at the anode by oxygen, and the main electrochemical oxidation product was elemental sulfur at low current density (~ 11.9 A/m²) (Dutta et al., 2009). Blázquez et al. (2016) used an autotrophic biocathode to treat sulfate wastewater, and accumulation of S^0 in the biocathode was detected by energy dispersive X-ray spectroscopy (Blázquez et al., 2016). In a sulfate and nitrate co-reduction system with high organic input, thiosulfate was not observed, and S^0 recovery, which calculated by mass balance, was up to 70% (Xu et al., 2014). In this work, the highest anode current density was 11.58 A/m², the electrochemical oxidation product of sulfide might be mainly elemental sulfur.

In Fig. 3(b) and (c), from day 30 to 45 (with the current of 40 and 80 mA, respectively), the effluent SO_4^{2-} was mostly higher than influent SO_4^{2-} . And same situation occurred at day 60 to 66 (with the current of 320 mA) and day 81 to 94 (with the HRT of 12 and 6 h, respectively), indicated that reduced sulfur compounds accumulated in the reactor were oxidized to sulfate in these stages. Based on these results, a hypothesis about the fate of sulfide in the 3D-BER was proposed. On the one hand, sulfide was immediately oxidized to sulfate for denitrification by sulfur-oxidizing bacteria (SOB) such as *Sulfurospirillum* detected in the reactor (Eq. (5)). On the other hand, sulfide was oxidized to S^0 or thiosulfate by SOB and electrochemical oxidation (Eqs. (6) and (7)). The oxidation products of sulfide could accumulate and serve as the supplementary electron donors for autotrophic denitrification when electron donors were insufficient in the 3D-BER (Eqs. (8) and (9)). Low electric current (day 30 to 45) or short HRT (day 81 to 94) resulted in the deficiency of electron donors in 3D-BER. At these stages, sulfide oxidation products could be used as supplementary electron donors to enhance nitrate removal. As can be seen from Eqs. (8) and (9), sulfur and thiosulfate-based autotrophic denitrification produced H^+ and sulfate, which could result in pH decrease and sulfate increase in the effluent. Coincidentally, in addition to sulfate increase, pH decrease in the effluent was observed at these stages (Fig. 3(b)), which confirmed the above hypothesis.

Based on the abundance of microbial community as well as effluent concentrations of sulfur and nitrogen compounds, the potential mechanism of nitrate and sulfate removal in 3D-BER was deduced in Fig. 5. Organic carbon in the influent or produced by microbes such as

Fig. 5. Potential mechanism of nitrate and sulfate removal in the 3D-BER.

Acetobacterium was quickly utilized by heterotrophic microorganisms or oxidized at the anode, which could promote the coupling of heterotrophic microorganisms and autotrophic microorganisms for contaminants removal. In the lower part of the 3D-BER, autotrophic DB either used hydrogen or obtained electrons directly from the cathode for nitrate reduction. In the upper part of the 3D-BER, sulfate reduction mainly occurred by SRB. The produced sulfide could further be oxidized to thiosulfate or elemental sulfur, which could be used as electron donors for sulfur autotrophic denitrification.

3.7. Perspectives and limitations

The 3D-BER realized simultaneous nitrate and sulfate removal in single-chamber bioelectrochemical reactor, which would provide a promising method in the remediation of low organic carbon wastewater. It took advantage of electron donors provided by DC power supply to reduce nitrate and sulfate and thus avoided the possible secondary pollution due to the addition of organic compounds. No phosphate buffer solution was added due to the neutralization of anode reaction, and effluent pH was kept at ~ 8 . Moreover, compared to the two-chamber BERs, exchange membrane fouling was avoided during long-time operation. The main operating cost of 3D-BER was electric energy consumption. When the current increased from 160 to 240 mA, energy consumption increased from 3.07 to 3.33 kWh/m³ to 5.88–7.18 kWh/m³ as well as the improved removal efficiency in nitrate and sulfate. In engineering application, reducing energy consumption is the key step to cost saving. If renewable energy such as solar energy can be used to produce electricity, this process could have considerable economic competitiveness.

However, several challenges are needed to address for further application. First of all, the sulfate removal efficiency was far below nitrate removal efficiency though influent SO_4^{2-} -S was higher than NO_3^- -N. Further efforts are needed to improve the sulfate removal efficiency, for example, adding trace elements that are conducive to the growth of SRB in the influent. Second, removal efficiency could be improved by adjusting the current properly, but the increase of current led to the dissolve of graphite rod. This kind of small particle carbon affected the effluent water quality (e.g. color). Therefore, it is necessary to explore suitable anode materials to ensure a stable long-time operation. Third, the current efficiency was in the range of 25% – 40%, which was low compared with the metallic catalyzed denitrification (21.75% – 77.43%) (Zhang et al., 2016). And 29% – 60% current efficiency was obtained in a BER for sulfate removal (Wang et al., 2017). Current efficiency is a considerable parameter that determines the feasibility of BERs for scaling up and practical application (Zhang et al., 2016). In the case of ensuring treatment capacity, current efficiency of BER can be improved by reducing internal resistance and shortening HRT.

4. Conclusions

The capacity of 3D-BER for simultaneous removal of nitrate and sulfate in wastewater with low organic carbon was investigated under different influent sulfate concentration, current and HRT. High influent sulfate, excessive electric current and short HRT decreased nitrate removal efficiency as well as sulfate removal efficiency. There were numerous kinds of denitrifying bacteria and sulfate reducing bacteria in the 3D-BER, which led to the performance of nitrate and sulfate removal through denitrification and sulfate reduction. Further efforts are needed to improve the sulfate removal efficiency and look for suitable anode material.

CRedit authorship contribution statement

Qi Tang: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization. **Yanqing Sheng:** Conceptualization, Supervision. **Changyu Li:** Investigation. **Wenjing Wang:** Investigation. **Xiaozhu Liu:** Investigation.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2020.124096>.

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