Combined bioelectrochemical and sulfur autotrophic denitrification for drinking water treatment

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Abstract

A combined bioelectrochemical and sulfur autotrophic denitrification process for drinking water treatment was put forward and investigated extensively in this paper. In this new process, the bioelectrochemical denitrification was carried out in the upper part of the reactor while sulfur denitrification in the lower part. The H^+ produced in Sulfur Part could be consumed by hydrogen denitrification in Bioelectrochemical Part. Therefore, the limestone for pH adjustment in Sulfur Part was not necessary in this combined process, which avoided the problem of hardness increase. The sulfate accumulation in this combined reactor was less than that of the sulfur limestone autotrophic denitrification system. The effluent from two parts was kept neutral at optimum operation conditions. When the influent nitrate was 30 mg-N/L, the reactor could be operated efficiently at the hydraulic retention time ranged from 1.9 to 5 h (corresponding minimum current was 16–3 mA), i.e. the effluent NO_3^- -N removal ranged from 90% to 100% without nitrite accumulation and the effluent sulfate concentration was lower than 170 mg/L. The maximum volume-loading rate of the reactor was 0.381 kg NO_3^- -N/(m³ d). The biomass and scanning electron microscope micrographs of Sulfur Part were also analyzed.

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1. Introduction

Nitrate contamination of groundwater is becoming more and more serious in many countries [1]. In China, the nitrate contamination of drinking water increases rapidly, and the nitrate concentration in some drinking water source is higher than 30 mg-N/L [2]. It is reported by World Health Organization that the nitrate concentration in drinking water should be lower than 10 mg-N/L [3]. Nitrate can cause methemoglobinemia when ingested by infants [4–6], and they can even cause carcinoma, malformation and mutation when transformed into nitrosoamines [7].

Biological denitrification process, which comprises autotrophic denitrification and heterotrophic denitrifica-

tion, is considered an efficient technology in the treatment of nitrate-contaminated water. Heterotrophic denitrification is very efficient in nitrate removal if adequate amounts of organic carbon are available. However, in drinking water treatment, insufficient organic carbon may limit the application of heterotrophic denitrification unless external organic carbon sources are supplied [8]. Autotrophic denitrification has been booming recently due to two major advantages compared with heterotrophic denitrification: (1) no external carbon is needed, which reduces the cost and risk of operation and (2) less sludge is produced, which minimize the bacteria in the effluent [9]. Both of hydrogen gas and elemental sulfur can be used as ideal electron donor for autotrophic denitrification because they are completely harmless to human health, and no further steps are needed to remove either excess substrate or its derivatives [10,11].

Hydrogen gas is a kind of clean electron donor for autotrophic denitrification, however, its transportation

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and generation is dangerous and expensive. In a new electrode-biofilm reactor [12,13], denitrifying microorganisms immobilized on the cathode will utilize H_2 , which is produced by the electrolysis of water, to convert nitrate into N_2 . Thus, the problems of hydrogen gas transportation and generation could be avoided. The feasibility of nitrate contaminated water treatment by electrode-biofilm reactor has been investigated experimentally and theoretically, and the nitrate removal efficiency could reach 100%. A study on the use of bipolar-particles-electrodes-biofilm in potable water denitrification showed that the electric utilization efficiency could get to 200% [2].

Sulfur limestone autotrophic denitrification (SLAD) systems have been studied widely in Europe and USA [8,9,14,15], and their high denitrification efficiency can be compared with that of the heterotrophic denitrification in the nitrate-contaminated water treatment. In such processes, elemental sulfur is used as electron donor while limestone is used to adjust the pH and 7.54 mg/L sulfate will be produced stoichiometrically when 1 mg-N/L nitrate is removed. There will be hardness increase in treated water because of Ca^{2+} produced by the limestone for pH adjustment.

The electron donor and pH values are the key influencing factors for autotrophic denitrification process. In order to take full advantage of bioelectrochemical hydrogen denitrification and elemental sulfur denitrification, a new combined bioelectrochemical and sulfur autotrophic denitrification (CBSAD) process was brought forward and studied. The advantages of the two processes were integrated in this CBSAD process. On one hand, the H^+ produced by sulfur denitrification was helpful to the bioelectrochemical hydrogen denitrification at the cathode, thus the hydrogen denitrification could be carried out easily. On the other hand, because the H^+ produced in sulfur denitrification was consumed by the bioelectrochemical hydrogen denitrification, the pH adjustment limestone was not necessary and the effluent hardness increase could be avoided. Furthermore, the CO_2 produced on anode can serve as the supplement of inorganic carbon (IC) source and pH buffer to the whole denitrification. The effluent sulfate concentration could be controlled through the hydraulic retention time (HRT) and current adjustment. The feasibility of this CBSAD process was verified and its optimum operation conditions were presented.

2. Materials and methods

2.1. Experimental set-up

As shown in Fig. 1, the reactor consisted of two parts, i.e., the bioelectrochemical hydrogen autotrophic denitrification part (hereinafter referred to as Bioelectro-

Fig. 1. The experimental set-up.

chemical Part) and the sulfur autotrophic denitrification part (hereinafter referred to as Sulfur Part). The effective volume of the reactor was 1.23 L. Bioelectrochemical Part, which was in the upper part of the reactor, was made of stainless steel column with 7.28 cm diameter and 16 cm length, and its effective volume was 0.52 L. The anode was carbon rod of 2.5 cm diameter and 14 cm length and the cathode was the cylindrical stainless steel wall with 321 cm² surface area. The distance between the electrodes was 2.4 cm. Sulfur Part was a cylindrical plexiglas in the lower part of the reactor with 7.28 cm diameter and 35 cm length. 746 g sulfur granules (Luoyang Sunrise Industrial Co., Ltd, Luoyang, China), which are of 3.0–4.0 mm diameter, were packed in Sulfur Part. The reactor was kept in the incubator at constant temperature of 30°C. Silicon Rectifier (Model GCA12/14, Beijing Chunshu Rectifier Instruments Plant, Beijing, China) supplied direct current to the reactor.

2.2. Experimental design

The whole experiment included three stages: pre-inoculation stage, inoculation-acclimation stage and operation stage. The influent was mixed by tap water (Beijing No. 9 Water Plant) and other ingredients as listed in Table 1, and the IC in the tap water was 8 mg-C/L. All chemicals used in this experiment were of analytical grade (Beijing Agent Plant, Beijing, China).

The variation of the oxidation–reduction potential (ORP), pH, TOC, NO_3^- -N, and NO_2^- -N in the CBSAD reactor were monitored at different HRT and current at pre-inoculation stage.

At inoculation-acclimation stage, the CBSAD reactor was inoculated with the effluent from a heterotrophic denitrification system. Under continuous-flow, biofilm was formed on the cathode and sulfur granule surface in

Table 1
Influent ingredients and applied current at different stages

Ingredients	Time		
	Pre-inoculation stage (14 d)	Inoculation acclimation stage (100 d)	Operation stage (400 d)
NaNO ₃ (mg-N/L)	30 (adding 20 mg-N/L NaNO ₂)	30	30
Phosphate	7.7 ml-Buffer/L (Buffer:0.25 M Na ₂ HPO ₄ · 12H ₂ O & 0.025 M KH ₂ PO ₄)	7.7 ml-Buffer/L (Buffer:0.25 M Na ₂ HPO ₄ · 12H ₂ O & 0.025 M KH ₂ PO ₄)	2 mg-P/L KH ₂ PO ₄
CH ₃ OH (mg-C/L) & according electric current (mA)	0&:2–20 HRT 1.5 h (1 d)		
	0&:2–20 HRT 2 h (2 d)	90&0 (30 d)	0&:2–20 HRT 1.9 h (72 d)
	0&:2–20 HRT 4 h (2 d)	80&2 (14 d)	0&:2–20 HRT 2.5 h (72 d)
	0&:2–20 HRT 8 h (2 d)	60&4 (14 d)	0&:2–20 HRT 3.0 h (72 d)
	0&:2–20 HRT 10 h (2 d)	30&6 (14 d)	0&:2–20 HRT 4.0 h (72 d)
	0&:2–20 HRT 12 h (2 d)	15&8 (14 d)	0&:2–20 HRT 5.0 h (72 d)
	0&:2–20 HRT 14 h (3 d)	0&10 (14 d)	

about 1 month. The influent, in which the ratio of carbon to NO₃⁻-N was 3:1, was delivered to the reactor by a pump (Model EH-B310-220R4, Iwaki Co., Ltd., Tokyo, Japan). The pH was kept neutral by buffering solution of K₂HPO₄ and KH₂PO₄ with the ratio of 1:1. When the biofilm was formed on the cathode and sulfur granule surface, direct current was applied gradually from 2 to 10 mA to acclimatize the bacteria. Meanwhile, the ratio of carbon to nitrogen in the influent was decreased to zero gradually, and then the reactor was operated under complete autotrophic denitrification. When this CBSAD system was acclimated to complete autotrophic denitrification, the pH buffering system was removed and only 2 mg-P/L K₂HPO₄ was added to the influent as nutrition.

Optimum operation conditions were determined at the operation stage. The system was tested at HRT of 1.9, 2.5, 3.0, 4.0, 5.0 and 6.0 h. At each HRT, direct current of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 mA were applied to the reactor sequentially. The quasi-steady-state experiment started at the highest HRT (6 h) and then run sequentially. Under different HRT and current, the system run and stabilized over 2 days. The system was not considered in quasi-steady-state condition until the sample data (NO₃⁻, NO₂⁻, SO₄²⁻, biomass concentration and pH in the effluent) variation of three sequential retention times was less than 5%. Under quasi-steady-state condition, the corresponding experiment or measurements were conducted. All the data in this paper were the average value of the three sequential samples within 5% variation.

2.3. Analytical methods

The NO₃⁻-N, NO₂⁻-N, SO₄²⁻, pH, biomass, total organic carbon (TOC) and total chemical oxygen demand (TCOD) of the influent, Sulfur Part effluent and Bioelectrochemical Part effluent were measured at each quasi-steady-state condition. Samples were filtered through 0.45 µm membrane for NO₃⁻-N, NO₂⁻-N, SO₄²⁻ and TOC analysis. The NO₃⁻-N, NO₂⁻-N, SO₄²⁻ were measured by ion chromatograph (Dionex-4500i, Dionex Inc., Sunnyvale, CA, USA). TOC was measured by TOC Analyzer (Model Applo9000, Tekmar Dohrmann Inc., Mason, OH, USA). TCOd was determined with a standard microtechnique method [16]. The ORP was measured on line by 9778BN ORP electrode connected to an Orion-720A Analyzer (Orion Research Inc., Beverly, MA, USA). The pH was measured using a 9165BN pH electrode connected to Orion-828 pH Analyzer (Orion Research Inc., Beverly, MA, USA).

To measure the biomass in the effluent, phospholipids of the samples were extracted by chloroform-methanol. The treatment steps were detailed in the report of Islam and Suidan [12]. After pretreatment, the light absorbance of the samples was monitored using an ultraviolet raster spectrophotometer at 610 nm (Model 752, Shanghai Precision Science Instrument Inc.).

The fixed biomass of Sulfur Part was evaluated by determination of its COD [17], suspended solid (SS) and volatile suspended solid (VSS) [18]. The biofilm sample was taken out from Sulfur Part and the fixed biomass

was recovered from the packed granules following the steps reported by Liu [17]. Then, the disrupted fixed biomass was diluted to 100 mL by distilled water and the COD, SS and VSS were analyzed by the standard methods [16].

Sample preparation for scanning electron microscope (SEM) was carried out as follows. The granules were put into 50-mL serum bottle containing 4% glutaraldehyde in 0.05 mol/L cacodylate buffer, then the bottle was sealed. Fixation was carried out for 12 h at 4°C. The fixed granules were washed with phosphoric acid buffer (1:1) for three times, and then fixed with 1% osmium tetroxide. The granules were then dehydrated with a graded series of ethanol in distilled water from 50% to 100% (v/v) and replaced by tert.-amyl acetate. The specimens were dried in CO₂ critical point dryer (SPI Inc., PA, USA), mounted on brass discs, and sputter coated in Eiko Ion Coater (model IB-3, Hatachi Inc., Naka, Japan) with platinum/palladium target (60:40). The prepared specimens were examined (HITACHI S-570 SEM, Hatachi Inc., Naka, Japan) at an accelerating voltage of 12 kV.

Sulfur denitrificans were identified by the classical cultivation and isolation methods [19].

3. Results and discussion

3.1. Principle of the CBSAD system

During the CBSAD process, nitrate is reduced to nitrogen gas by the biofilm on sulfur granule surface in Sulfur Part [20], and at the same time H⁺ is produced:

H⁺ migrates to the upper Bioelectrochemical Part, and then it will be reduced into hydrogen gas through reaction (2) at the cathode:

Another hydrogen gas production reaction occurred at the cathode is

The electrode potentials for reactions (2) and (3) are about the same at pH = 7 according to the Nernst–Peters equation [21]. Therefore, from the point of view of energy consumption, both reactions (2) and (3) can take place simultaneously at the cathode. The hydrogen gas produced serves as the electron donor for further hydrogen denitrification by biofilm on the cathode [12], and the H⁺ produced in Sulfur Part was also consumed:

In Bioelectrochemical Part, CO₂ is formed prior to O₂ at the anode according to their electrode potential as given in reactions (5) and (6) [22]:

As a result, CO₂ is produced and anoxic condition is provided in Bioelectrochemical Part, and the produced IC is supplied to the denitrification as IC source supplement. The produced CO₂ is also dissolved and then dissociates into bicarbonate and carbonate ions to adjust pH. Thus, favorable circumstance for the denitrification process in the whole reactor could be afforded.

The overall electrochemical reaction in Bioelectrochemical Part can be obtained from the combination of reactions (2), (3) and (5):

Therefore, it can be considered that the H⁺ produced by reaction (1) is not consumed by reactions (7) and (2) but by reaction (4).

3.2. Influence of current on ORP, TOC, TCOD, NO₃⁻, NO₂⁻ and pH before inoculation

The reactor was operated under 2–20 mA current for 2 weeks to test its electrochemical changes under continuous feeding. The HRT was 0.5–14 h and the influent nitrate concentration was 30 mg-N/L. The ORP decreased sharply from +200 to –200 mV after the current applied for 90 min, and a favorable condition for denitrification could be easily formed in this system. The results were consistent with the research presented by Sakakibara et al. [23].

The concentration of TOC, TCOD, NO₃⁻ and NO₂⁻ in the reactor did not change during operation, which indicated that no organic pollutants were produced in the electrochemical reaction, and the current had no influence on the nitrate and nitrite variation without bacteria. Because CO₂ was formed at the anode as reaction (7), the pH was keeping neutral under 2–20 mA currents. This pH condition was favorable to the denitrificans whose optimum pH was 6.5–8.0 [24]. It can be concluded from the results mentioned above that the system was suitable for denitrification process.

3.3. pH variation and H⁺ balance in the denitrification process

It can be concluded from Section 3.1 that neutral condition in the whole reaction could be provided when the H⁺ produced from reaction (1) is balanced by reaction (4). Assuming that the bacteria yield coefficient

is 0.08, reaction (4) can be expressed as follows:

It can be seen from reaction (8) that 2.52 mol H^+ would be consumed by hydrogen denitrification when 1 mol NO_3^- -N was reduced into nitrogen gas. The H^+ consumed in reaction (8) should be afforded by sulfur denitrification, in which 1.97 mol NO_3^- -N was reduced to nitrogen gas according to reaction (1). Therefore, when the nitrate removal ratio of Bioelectrochemical part to Sulfur Part is 1:1.97, the H^+ will be balanced by sulfur and hydrogen denitrification and the whole reactor kept neutral. Furthermore, the CO_2 produced on anode could also serve as pH buffer for the whole reactor.

Henze et al. reported that the optimum pH range is 7–9 [25] and Wang [24] reported it is 6.5–8.0 for denitrification. The influence of current on effluent pH of both Bioelectrochemical Part and Sulfur Part at 2.5 h HRT was shown in Fig. 2. It was shown in Fig. 2 that the effluent pH of both parts increased slightly with the increase of current, and the effluent was kept neutral when the current was near 12 mA. If the current was lower than 12 mA, some of the H^+ produced in sulfur denitrification (reaction (1)) could not be consumed by hydrogen denitrification (reaction (8)), which resulted in lower effluent pH. When the current was less than 12 mA, more H_2 would be supplied from reaction (7) for reaction (8) with the increase of applied current, and more H^+ produced by reaction (1) will be consumed accordingly. Therefore, the effluent pH increases with the increase of current when the applied current is less than 12 mA. When the applied current increased to 12 mA, the nitrate removal ratio of Bioelectrochemical Part (124.3 mg-N) to Sulfur Part (244.7 mg-N) was 1:1.97, thus the H^+ produced (23.4 mmol) from reaction (1) was totally consumed by reaction (8) (23.4 mmol H^+ consumed) and neutral effluent from both parts could be obtained. At 12 mA current, the pH of Sulfur Part effluent was almost the same as that of SLAD system [26]. If the current was higher than 12 mA, the H^+ needed by reaction (8) was more than that produced from reaction (1), however, the produced CO_2 could buffer pH to keep neutral as seen in Fig. 2. The neutral effluent of CBSAD reactor showed that the CBSAD reactor could adjust pH effectively without limestone, and the effluent hardness increase could be avoided. Because the pH adjustment in Sulfur Part was provided by reaction (8) in Bioelectrochemical Part and certain time was needed for H^+ transportation to the cathode, the effluent pH of Sulfur Part was about 0.2 lower than that of Bioelectrochemical Part under the same current.

Fig. 2. pH of influent and effluent.

3.4. Influence of current on denitrification and current efficiency

In this CBSAD reactor, nitrate was firstly reduced in Sulfur Part, and then the residual nitrate was further reduced in Bioelectrochemical Part. Under the condition of 2.5 h HRT and 30 mg NO_3^- -N/L influent, the effect of current on both denitrification efficiency and electric current efficiency was illustrated in Fig. 3. Voltage of 1.8–4.0 V was applied to the CBSAD reactor and the corresponding current were 2–20 mA. As shown in Fig. 3, the nitrate removal in Sulfur Part increased slightly with the applied current from 70% to 78.9% because of slight pH increase, and about 2.6 mg-N/L nitrite was accumulated at 2.5 h HRT. The residual nitrate and accumulated nitrite would flow into the upper Bioelectrochemical Part for further removal. It can be seen from Fig. 3 that the current intensity had great influence on the denitrification in Bioelectrochemical Part, which is in accordance with the research of Isam and Suidan [12]. Both the nitrate and nitrite removal were increased steadily with the increase of current. About 87% nitrate could be removed when the current went up to 10 mA, and the residual nitrate and nitrite in the effluent were about 5.0 and 0.09 mg-N/L, respectively. And the higher the current, the better the removal rate. Under 12 mA current, no nitrite was detected in the effluent and the nitrate removal was increased to 90%. Nitrate removal was up to 100% when 18 mA current was applied. These results suggested that the applied current be the key influencing factor on the CBSAD process under the voltage from 1.8 to 4.0 V.

During hydrogen denitrification, both reaction (4) and (9) were taken place in biofilm on cathode:

Fig. 3. Nitrate removal and electric current efficiency at 2.5 h HRT.

i.e. 5 mol electron in reaction (4) and 3 mol electron in reaction (9) are consumed in the reduction of 1 mol of nitrate and nitrite, respectively. The effective current (I_E) of Bioelectrochemical Part, which is determined by the nitrate loading, was defined as

$$I_E = [(C_{in} - C_{eff})5 + (C_{2in} - C_{2eff})3] \times VF/HRT,$$

where C_{in} and C_{eff} is influent and effluent nitrate concentration of Bioelectrochemical Part (mol-N/L), C_{2in} and C_{2eff} are influent and effluent nitrite concentration of Bioelectrochemical Part (mol-N/L), F is Faraday constant (C/mol), V is the effective volume (L). So the electric current efficiency (EC_e) was defined as the ratio of I_E to the applied current (I), i.e., $EC_e = I_E/I$. It can be concluded from Fig. 3 that the EC_e of Bioelectrochemical Part kept 98% until the current was increased to 16 mA. Then the efficiency decreased with the increase of applied current, and it would be decreased to 92% at 18 mA, which was resulted from the incomplete consumption of hydrogen gas by hydrogen denitrification. During denitrification process, the total IC was sufficient for the growth of autotrophic denitrifying microorganisms, which can be calculated by reactions (1) and (8) stoichiometrically.

3.5. The minimum current at different HRT

To determine the optimum operation conditions, the experiment was carried out at different current and HRT, and the influent nitrate concentration was kept at 30 mg-N/L. The minimum current was defined as that which yield 90% nitrate removal, i.e. the effluent nitrate and sulfate concentration was lower than 3.0 mg-N/L and 170 mg/L while no nitrite was detected. The minimum current of different HRT were shown in

Fig. 4. Minimum current at different HRT.

Fig. 4. It can be seen from Fig. 4 that the minimum current must be increased with the decrease of HRT to maintain 90% denitrification efficiency. For example, the minimum current of the reactor must be increased to 16 mA to obtain 90% denitrification removal under 1.9 h HRT. The nitrate and nitrite concentration in Sulfur Part effluent decreased with the increase of HRT, so 90% nitrate removal could be attained at lower current intensity in Bioelectrochemical Part. If the HRT increased to 5 h, 3 mA current was enough to obtain 90% denitrification efficiency. When the HRT was higher than 5 h, 90% denitrification efficiency without nitrite accumulation was produced in Sulfur Part at 3 mA current, and the sulfate concentration was higher than 170 mg/L. Therefore, Bioelectrochemical Part played no role in the nitrate removal under such conditions, and the operation HRT of the combined reactor should not be higher than 5 h. When the current was lower than 3 mA, pH in Sulfur Part was lower than 6.5 (Fig. 2), and nitrate removal could not reach 90% at any HRT. So 3 mA was the minimum current for CBSAD reactor to work effectively. During the operation, the maximum volume-loading rate (VLR) was 0.381 kg NO₃-N/(m³ d), and the maximum electrode surface loading rate (ESLR) of the cathode was 0.043 mg NO₃-N/(cm² d).

3.6. SO₄²⁻ in the effluent

When the nitrate concentration of the influent was 30 mg-N/L, the variation of SO₄²⁻ in the effluent was given in Fig. 5. The effluent sulfate concentration could be controlled by HRT and current adjustment. It can be concluded from Fig. 5 that effluent sulfate concentration remained lower than 170 mg/L during the whole operation period (400 d), which met the secondary

Fig. 5. Sulfate and $\text{SO}_4^{2-}/\text{NO}_3^-$ -N ratio change in effluent.

maximum contaminant level of sulfate from the US EPA, i.e. 250 mg/L. The effluent sulfate of this CBSAD reactor was also lower than that of conventional sulfur denitrification reactor and SLAD system under the same influent concentration [10, 26]. The concentration ratio of sulfate to consumed NO_3^- -N was between 5.20 and 5.80, which was lower than the reported values of 6.4, 7.89, 9.9, 11.1 [27–29,15] and the stoichiometric value of 7.45. The residual nitrate removal in Bioelectrochemical Part led to lower nitrate loading of Sulfur Part, thus decreased sulfate concentration in the effluent.

3.7. Biomass analysis

The biomass in the two parts effluent was very low and did not change obviously with operation time. The phospholipid concentration was about 0.88×10^{-6} mol/L in Sulfur Part effluent and 0.64×10^{-6} mol/L in Bioelectrochemical Part effluent. TCOD in the effluent of both parts was lower than 10 mg/L.

The variation of the COD, SS and VSS of the fixed biomass in Sulfur Part was illustrated in Fig. 6. Under the condition of 12 mA current and 2.5 h HRT, the biomass decreased with the increase of the reactor height. At the height of 1.1 cm, the COD, SS, and VSS were 319, 17.8 and 17.1 mg/g-packed-granule, and they were decreased to 164, 4.6 and 3.1 mg/g-packed-granule at the top of Sulfur Part (35 cm height). The above-mentioned phenomena were resulted from nitrate decrease with the increase of the reactor height. As the electron donor, the nitrate decrease led to the decrease of denitrificans and the biomass.

Under 1.9 h HRT and quasi-steady-state condition, the COD, SS, and VSS were 150, 3.9 and 2.8 mg/g-packed-granule at the top of Sulfur Part under 12 mA current, and they were increased to 200, 6.1 and 4.2 mg/g-packed-granule at 6 mA current. Similar phenomena were observed at other HRTs, which showed that the applied current in Bioelectrochemical Part also influenced the biomass amount at the top of Sulfur Part.

Fig. 6. Biomass distribution of Sulfur Part.

Fig. 7. SEM micrographs of the granule surface at the height of 18 cm.

However, the biomass amount at the top of Sulfur Part could be neglected comparing with the whole biomass amount of Sulfur Part at certain conditions. The nitrate removal of the biomass at the top of Sulfur Part could be neglected comparing with the whole nitrate removal of Sulfur Part accordingly, i.e. the whole nitrate removal of Sulfur Part was not influenced by the biomass change at the top of Sulfur Part under certain HRT. Therefore,

the current influence on the biomass at the top of Sulfur Part was not conflict with the results in Section 3.4, i.e. the whole nitrate removal of Sulfur Part increased slightly with the increase of the applied current (Fig. 3) because of slight pH increase.

The SEM micrographs of the granule surface at the height of 18 cm in Sulfur Part under 12 mA current and 2.5 h HRT was illustrated in Fig. 7. The structure and diversity of the microorganisms on the granule surface were presented in the micrographs, from which it can be concluded that the packed granule surface have complex bacterial composition with some lacuna. *Thiobacillus denitrificans* were identified as the predominant denitrifiers on the granule surface. It could be deduced from the result that *Thiobacillus denitrificans* clustered with other denitrifiers, inorganic material and extracellular polymers to carry out the sulfur denitrification process.

4. Conclusions

It is clear from the results in this paper that combining the bioelectrochemical autotrophic denitrification and sulfur autotrophic denitrification into one process is possible for drinking water treatment. The two denitrification processes could realize in this integrated process, and high denitrification efficiency could be obtained. The H^+ produced in Sulfur Part could be consumed in Bioelectrochemical Part, which was helpful to the hydrogen denitrification and effluent neutralization of both parts. The limestone for pH adjustment in Sulfur Part was not necessary and therefore the hardness increase in the effluent could be avoided. This CBSAD reactor could provide a favorable pH environment for denitrification under optimum operation conditions.

Because bioelectrochemical hydrogen denitrification and sulfur denitrification proceeded in one reactor, only part of the nitrate removal carried out in Sulfur Part through HRT and current adjustment. Therefore, the sulfate concentration in the final effluent of the CBSAD reactor was lower than 170 mg/L, which was lower than that of the SLAD and conventional sulfur denitrification system.

The optimum HRT was 1.9–5 h and the corresponding minimum current was 3–16 mA. Under the optimum conditions mentioned above, the nitrate removal was higher than 90% and even up to 100% without nitrite accumulation. The maximum VLR of the reactor was 0.381 kg NO_3^- -N/(m^3 d), and the maximum ESLR of cathode was 0.043 mg NO_3^- -N/(cm^2 d).

Attached biomass in Sulfur Part decreased with the height increase of the reactor, and the predominant denitrifying bacteria in Sulfur Part were *Thiobacillus denitrificans*. The current in Bioelectro-

chemical Part also influenced the amount of denitrifiers at the top of Sulfur Part, and further studies should be carried out to investigate the mechanism of this phenomenon.

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