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Iron shavings supported biological denitrification in sequencing batch reactor

Zi Wang, Hongwu Wang*, Luming Ma

State Engineering Research Center for Urban Pollution Control, College of Environmental Science and Engineering, Tongji University, No. 588, Miyun Road, Yangpu District, Shanghai 200092, P.R. China
Tel./Fax: +86 21 65984569; email: wanghongwu@tongji.edu.cn

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ABSTRACT

Iron shavings, as the main substrate, were applied to support biological denitrification process in a sequencing batch biological reactor. Operation performances of 248 days were evaluated. The results indicate that stable nitrate removal process can be achieved with a denitrification rate of 15.49–52.32 g NO_3^-–N m^{-3} d^{-1} in different operation conditions. Hydrogen generated from iron corrosion contributed 66.5% of total needed electron and other 33.5% may be provided by both ferrous iron and endogenous carbon. Nitrate removal was accompanied by increase of iron concentration in suspended solid in reactor and average ratio of total removed N to consumed iron was 1:7.52. Effluent total iron concentration was as low as 2–4 mg L^{-1} and the consumed iron were mainly adsorbed over the microorganism's surface or in the form of settleable small particles. The sludge activity was seriously repressed when the percentage of iron in suspended solid was over 70% and can be recovered by highly reducing iron concentration in reactor.

Keywords: Activated sludge; Denitrification; Iron shavings; SBR

1. Introduction

Nitrate, as a widespread groundwater contaminant in the world, is a priority pollutant due to its contribution to eutrophication and its toxicity related to methemoglobinemia and to the possible formation in the gastric system of N-nitroso compounds [1]. A nitrate-contaminated water is commonly treated by chemical or biological reduction, by ion exchange, by adsorption with mineral-based and/or surface-modified adsorbents [2], or by reverse osmosis [3]. Among these methods, biological denitrification is generally used due to its end-product of nitrogen gas and relatively inexpensive cost [4,5]. It can be involved in heterotrophic ways (using organic carbon sources as electron donors) and autotrophic ways (using hydrogen or sulfur as electron donors). Compared to heterotrophic denitrification, autotrophic denitrification can produce less biomass and soluble microbial products, and so awakened considerable interest [6–10]. Although hydrogen [11–14] and sulfur [15–17] have been reported to support autotrophic denitrification for a long time, relatively high cost and some defects (explosive properties of hydrogen, for example) limited their large-scale use.

Corrosion of Fe(0) in the absence of oxygen can induce a polarizing hydrogen film forming at the metal surface [18]. This hydrogen film may be coupled with the reduction of nitrate and act as an electron donor for denitrification [19–21]. The main process can be described by reaction (1) [19], (2), and (3) [22,23] and many researchers have showed their interests on this process.

*Corresponding author.

$$Fe(0) + 2H_2O \rightarrow H_2 + Fe^{2+} + 2OH^- \quad (1)$$

$$NO_3^- + 1.13H_2 + 0.01H^+ + 0.05CO_2 \rightarrow 0.99NO_2^- + 0.01C_5H_7O_2N + 1.1H_2O \quad (2)$$

$$NO_2^- + 0.122CO_2 + H^+ + 1.78H_2 \rightarrow 0.488N_2 + 0.0244C_5H_7O_2N + 2.19H_2O \quad (3)$$

De Windt et al. [24] revealed that iron corrosion processes were mainly determined by H_2 removal and precipitation of iron mineral particles on the cell surface in the presence of *Shewanella oneidensis* MR-1. Shin et al. [25] reported a complete nitrate reduction within 3 days in a nanoscale Fe(0)-cell reactor, while only 50% of the nitrate was abiotically reduced over 7 days at 25°C. Neha Sunger and Purneudu Bose [20] found that a continuous flow reactor with nitrate loading of 28.9 mg m^{-3} d^{-1} and Hydraulic Retention Time (HRT) of 15.6 days can produce effluent with nitrate concentration of 0.025 mg N L^{-1} (95% nitrate removal) by using hydrogen generated from metallic iron corrosion. Based on previous researches, the key issue for iron-assisted autotrophic denitrification is to balance the competitive reactions between iron with nitrate (abiotic nitrogen removal) and iron with water (generating hydrogen for biotic reaction). Good balance can be attained by choosing iron materials with relatively low surface-area-to weight ratio, which can exhibit the least propensity to abiotically reduce nitrate [26,27]. Besides, accumulated precipitation in cell surface may decrease the activity of denitrification microbe [28], so it is important to recover the activity after long time operation.

In this research, iron shavings were used as iron material. Iron shavings used in this experiment were made from mechanical cutting of commercial purchased cast iron ingot and have a lower iron content (around 95%) and lower specific surface area (0.002634 m^2 g^{-1}) than iron powder or nanoiron particles which have iron content of >98% and specific surface area of >1 m^2 g^{-1} iron [29]. They were suitable to support iron-assisted autotrophic denitrification for their relatively low surface area-to-weight ratio [27].

In previous work, our research group has used iron shavings for pretreatment of wastewater to improve the biodegradability and obtained satisfying results [30]. Based on it, the objective of this research was to evaluate the feasibility of iron shavings as main substrate supporting biological denitrification by directly adding it into the reactor and running in a relatively short HRT (<1 d). The long-term performance and the capacity of potential nitrate removal with different iron shavings adding and microbe concentration were researched, too. This research would provide a reference information on developing an easily applied iron-assisted biological denitrification method in the future.

2. Methods

2.1. Experimental design

The experimental set-up is shown in Fig. 1. The cylinder reactor was made of glass fibre-reinforced plastic with 400 mm in height and 120 mm in diameter. The available volume of each reactor was 2 L. Two reactors were both running a process of biological denitrification. Reactor 1 was used as control experiment. Twenty gram iron shavings were added in the bottom of reactor 2 and no iron was added in reactor 1. They had been operated for 248 days. Among this, the first 75 days were operated for acclimation and enrichment processes of the denitrifying biomass. Then formal experiments were begun. Firstly, in order to investigate the effect of iron shavings quantity on nitrate removal efficiency, extra 20 g iron shavings were added into reactor 2 at day 98. Secondly, to observe the effect of microbe concentration on nitrate removal efficiency, Mixing Liquor-suspended Solids (MLSS) concentration in reactor 2 was increased to two times of before by transferring all of suspended solids of another parallel operation reactor into reactor 2 at day 106. Thirdly, to evaluate the maintaining of long-time running, two main methods were applied. One is that old iron shavings in the reactor 2 were substituted wholly by 40 g new iron shavings at day 136, 176, and 234, respectively. The other is that half of total suspended solids, including biomass and different types of iron precipitate, were removed and then 2,000 mg fresh sludge which came from sewage plant was added in as replenish when total iron concentration in reactor 2 exceeded 4,800 mg L^{-1}. Because total iron concentration

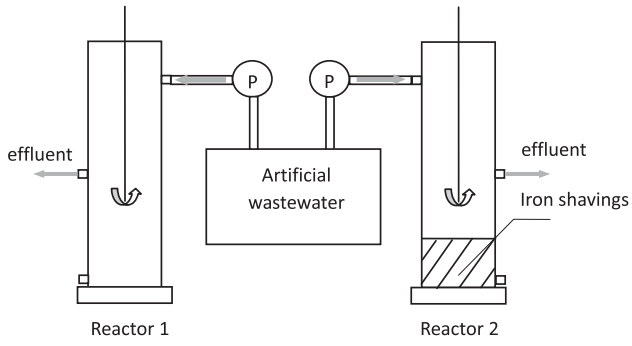

Fig. 1. Experimental set-up.

in fresh sludge was 1–2 mg L^{-1}, this method would make total iron concentration in reactor 2 be decreased to half. It was done at day 176 and 234 to alleviate the adverse effect of total iron accumulated in reactor 2 on microbial activity.

An electromagnetic valve was set at the end of the effluent pipe to control drainage. Operation cycle was controlled by three automatic clock switches. The cycle was divided into four phases: 0.5 h for filling, 6.5 h for anoxic reaction, 0.75 h for settling, and 0.25 h for decanting. For the filling time of one single cycle was 0.5 h, so the middle time point (0.25 h) of filling could be considered as the completely mixing point and the reaction starting point of each cycle. Decant time (0.25 h) was excluded from reaction time. As a result, the actual reaction time in one single cycle was 7.5 h (8−0.25−0.25 h). This value was used for calculation of denitrification rate.

The iron shavings were purchased from a machinery factory and its chemical composition is shown in Table 1. Before using, it was degreased by 1 M NaOH solution for 10 min and then soaked in 1 M HCl solution for 10 min. An agitator (120 rpm) was applied to ensure that microbes in reactor can contact fully with the iron shavings. To ensure that biofilm would not form on iron shavings surface to limit the release of iron, we artificially churned iron shavings in reactor 2 every 3 or 5 days.

The characteristics of the artificial wastewater are shown in Table 2. Influent nitrate concentration (as N) was 20.0 ± 1.0 mg L^{-1} during first 45 days and then was increased to 41.5 ± 1.5 mg L^{-1}. From day 76, no organic electron donor was supplied in influent. Mineral elements needed for microbe growth was supplied by diluting real domestic wastewater (with chemical oxygen demand [COD] 90–120 mg L^{-1}, total nitrogen [TN] 20 ± 1.0 mg L^{-1} and total phosphorus [TP] 1–2 mg L^{-1}), which came from quyang municipal sewage plant of Shanghai, China and was diluted 50 times for preparing artificial wastewater. The operation temperature was between 25 and 30°C. Inoculated sludge came from sludge thickener of the municipal sewage plant mentioned above and the initial MLSS concentration was about 10,000 mg L^{-1}, which gradually auto-reduced to 2,500 ± 100 mg L^{-1} in two reactors after 19 days because of microbial loss and death. Except for initial 45 days, sludge retention time was 80–100 days and was not strictly controlled because there was a very small fraction of MLSS flowing out in effluent every day. Dissolved oxygen concentrations in both reactors were 0 mg L^{-1}.

2.2. Sampling and analysis

Influent and effluent samples were taken at fixed time each day and pH, nitrate, nitrite, ammonia, and total iron concentration in samples were tested immediately. MLSS, Mixed Liquor Volatile Suspended Solids (MLVSS), and total iron concentration in reactor were tested every 5–6 days. All chemicals used in analysis were of analytical grade. Purkinje General UV spectrophotometer (TU1810) was used for measurements of light absorption. The test items and processes were carried out following standard methods [31].

For testing MLSS, MLVSS, and total iron concentration in reactors, 10 mL of culture was taken from reactor after short time of intense stirring which ensure all solid except for iron shavings suspending homogeneously in reactor, and then it was diluted to 100 mL. Fifty milliliter of the sample diluent was filtered through dried qualitative filter paper and then drying in 105°C for 2 h, weighing and getting result W_1 (mg L^{-1}). W_1 represents suspended solids

Table 1
Chemical composition of iron shavings used in study (%)

Fe	C	Si	Mn	Mo	Cr	Al
94.20–96.95	0.35–0.42	0.20–0.45	0.30–0.60	0.15–0.25	1.35–1.65	0.70–1.10

Table 2
Characteristics of artificial wastewater during study period (mg L^{-1})

Date	NO_3^-–N	NO_2^-–N	NH_4^+–N	$NaHCO_3$	TP	COD	pH
1–45d	20.0 ± 1.0	<0.1	<0.1	200 ± 2	1.00 ± 0.05	100 ± 5*	7.9 ± 0.1
46–75d	41.5 ± 1.5	<0.1	<0.1	200 ± 2	1.00 ± 0.05	100 ± 5*	7.9 ± 0.1
76–248d	41.5 ± 1.5	<0.1	<0.1	200 ± 2	1.00 ± 0.05	<5	7.9 ± 0.1

*100 mg L^{-1} COD consists of 50 mg glucose COD L^{-1} and 50 mg sodium acetate COD L^{-1}.

concentration in reactor. The other 50 mL of the sample diluent was diluted 10–10,000 times again for normal total iron testing and the result W_2 (mg L^{-1}) was got. W_2 represents total released iron concentration in reactor. In this paper, the difference between W_2 and W_1 was used as value of MLSS concentration in the reactor. After testing MLSS, the sample was burned in 600℃ for 2 h and the weight loss before and after burning was used for calculating MLVSS.

3. Results and discussion

3.1. Reactor performance

The total operating performances of the two reactors among 248 days are shown in Fig. 2. As can be seen, during the first 20 days there was no significant difference between two reactors in effluent nitrate concentration when influent nitrate concentration was 20 ± 1.0 mg L^{-1}. Then the difference gradually became significant and gradually attained around 8 mg L^{-1} when the influent nitrate concentration increased to around 41.5 ± 1.5 mg L^{-1}. And after stopping organic electron donor supply from day 75, effluent nitrate concentration in both two reactors increased immediately. Effluent nitrate concentration in reactor 1 gradually increased to average 40.60 mg L^{-1} and remained largely unchanged during the time left. At the same time, nitrate removal in reactor 2 gradually became stable at about 32.51 mg L^{-1}. Then as a result of adding 20 g extra iron shavings into reactor 2 at day 98 and doubling MLSS concentration at day 106, the average effluent nitrate concentration decreased to 21.59 and 8.33 mg L^{-1}, respectively. Then operation condition kept stable after that. The effluent nitrate concentration was stable at 20–24 mg L^{-1} (from day 115–248) when iron concentration was lower than 4,800 mg L^{-1}.

No biofilm was observed forming on iron shavings surface and the surface was turning black after long time running. At the same time, a thin biofilm began to grow on the inner surface of reactor after about 7-days operation and its thickness gradually increased. This biofilm had been swept away from reactor at day 45, 104, 135, 175 and 233 and total iron concentration adsorbed in film was tested. This result indicates that iron released by corrosion would partly suspend in reactor 2 and partly absorb to biofilm. It was observed that total iron firstly almost all suspended in reactor 2 and would rapidly adsorb to biofilm when film began to form on reactor's inner surface. Ratio of MLVSS to MLSS concentration in different operation stages was 0.8–0.85 and kept stable, so only MLSS was used to describe microbe concentration in reactor in this paper.

3.2. TN removal and total iron accumulation

TN removal profiles in reactor 2 between day 115 and 248 are shown in Fig. 3. As can be seen, TN removal efficiency in reactor 2 had a close relationship with total iron concentration of the mixed liquid. When total iron concentration was below 4,800 mg L^{-1}

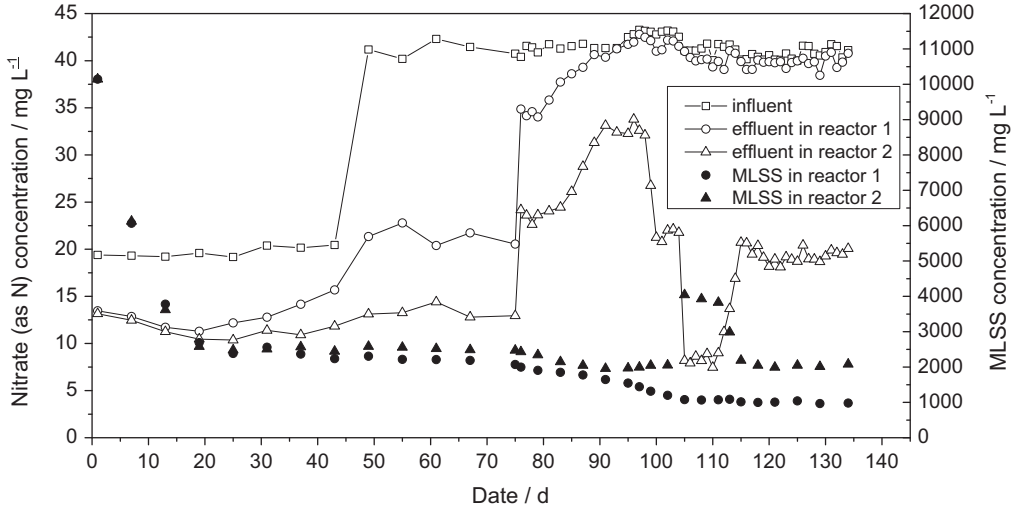

Fig. 2. The concentration changes of nitrate in influent or effluent in different operation condition in reactors with or without iron during first 135 days. Influent nitrate concentration (as N) was 20.0 ± 1.0 mg L^{-1} during first 45 days and then was increased to 41.5 ± 1.5 mg L^{-1}. From day 76 no organic electron donor was supplied in influent. Extra 20 g iron shavings were added into reactor 2 at day 98; MLSS concentration in reactor 2 was increased to two times in day 106. The operation temperature was 25–30℃. HRT was 8 h.

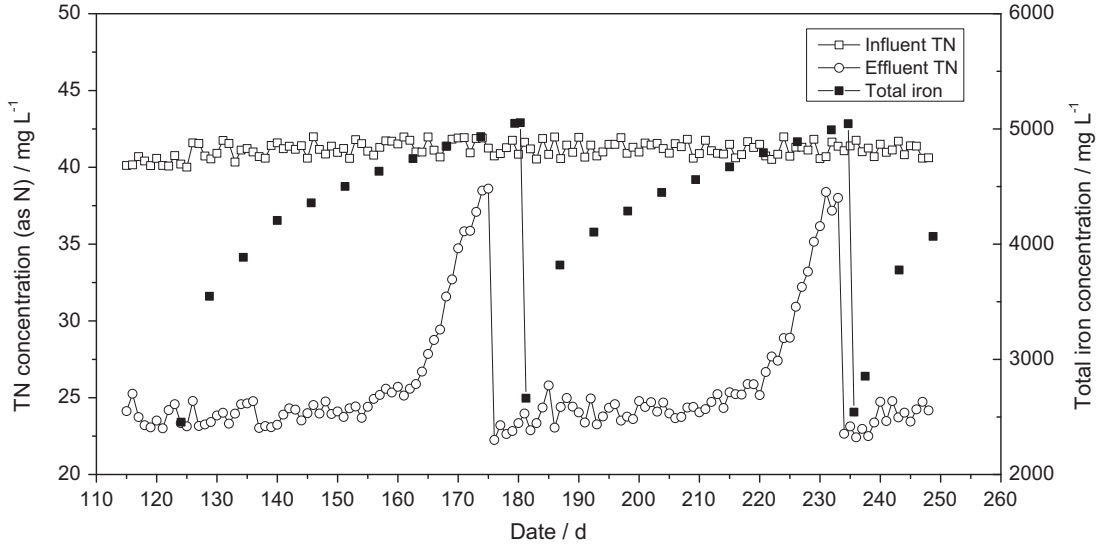

Fig. 3. The concentration changes of TN and total iron in different operation conditions between day 115 and day 248 in reactor 2. Iron shavings were got out wholly from reactor 2 and 40 g new iron shavings were added in as substituent at day 136, 176 and 234, respectively; Total iron concentration was decreased to half in day 176 and 234.

and influent TN concentration was $41.5 \pm 1.5\,mg\,L^{-1}$, effluent TN concentration was stable at $24.00 \pm 1.97\,mg\,L^{-1}$ for about 45 days. When total iron concentration was higher than $4{,}800\,mg\,L^{-1}$, effluent TN concentration increased to $38–39\,mg\,L^{-1}$ within about 10 days and effluent nitrate concentration began to increase rapidly from about 24 to $31.25\,mg\,L^{-1}$ within 8 days. As soon as total iron concentration in reactor 2 was decreased to $2{,}662\,mg\,L^{-1}$, effluent TN and nitrate concentration decreased. And when total iron concentration in reactor 2 exceeded $4{,}800\,mg\,L^{-1}$ again and be decreased to half in day 234, the changes of effluent TN and nitrate concentration were similar as before. Total iron concentration in effluent was as low as $2–4\,mg\,L^{-1}$ when total iron concentration in reactor 2 was lower than $4{,}800\,mg\,L^{-1}$ and its maximum value did not exceed $9\,mg\,L^{-1}$ in effluent after total iron concentration in reactor 2 was higher than $4{,}800\,mg\,L^{-1}$. Effluent total organic carbon (TOC) concentration was stable at $2–5\,mg\,L^{-1}$. Because pH value was 8–9 in reactor 2 during operation, ferrous or ferric ions in reactor would mainly exist as colloidal state [32]. And since MLSS concentration did not change during this period, the quick decrease of TN removal efficiency with total iron concentration over $4{,}800\,mg\,L^{-1}$ may be due to that the high concentration of iron ions and colloids adsorbed to cell surface that were in general negatively charged and hindered the diffusion of substrate and nutrient to cells [28]. So it was inferred that $4{,}800\,mg\,L^{-1}$ total iron concentration in reactor with $2{,}000\,mg\,L^{-1}$ MLSS in reactor 2 (that means iron in suspended solid accounts for more than 70%) may be a threshold only below which the microbe can maintain their normal activity.

Recovery of microbial activity is very important when total iron concentration is too high. Removing half of total suspended solid (including biomass and different types of iron precipitate) and adding about 2,000 mg new fresh sludge with very low total iron concentration ($1–2\,mg\,L^{-1}$) into reactor at day 176 and 234 were proved to be successful. By doing this, total iron concentration was reduced to half of before and TN removal efficiency was recovered completely. The fresh added MLSS did not have the same denitrifying population as functioning in reactor 2 because the microbe from sludge thickener of traditional sewage plant have not gotten acclimatized yet, but it did not significantly affect the recovery of TN removal efficiency. New added MLSS alleviated adverse effect of high concentration total iron on previously existing nitrate-reducing bacterium. This method is very simple, convenient and easy operation.

Another important question is that how many irons will be consumed in this denitrification process. The total quantity of removed N and consumed iron in different operation period was estimated according to TN, total iron concentration, and effluent water volume of each day (Table 3). As can be seen, ratio of iron to N was from 7.06:1 to 8.09:1 with an average ratio of 7.52:1. According to the stoichiometric relationship of reaction (1)–(3), 11.6 mg iron was needed to remove 1 mg NO_3^-–N completely if ferrous iron is

Table 3
Relationship between N removing and iron accumulation

Period (days)	N removing (mg)	Iron consumed (mg)		Ratio of iron to N (mg iron mg^{-1} N)
		Suspended in reactor	Absorbed to biofilm	
1–45	159	297	989	8.09
46–104	1,957	3,247	10,574	7.06
105–111	741	4,493	1,502	7.42
114–175	3,087	5,250	17,148	7.26
176–233	2,692	4,766	15,951	7.70
234–248	804	2,428	3,795	7.61

the main product. But in reactor 2, the factual iron consumption was much lower. This result indicates that hydrogen generated from reaction (1) was not the sole electron donor and there must be other electron donors.

In this denitrification system, iron, ferrous iron which released from corrosion of iron and endogenous carbon which came from decomposition of microbial cells were all possible electron donors [33,34]. One batch experiment was conducted to give a glimpse of the extent to which different substances involved in this process affect nitrate removal in stable sub-stage. In this batch experiment, three independent systems including hydrogen with microbe, iron with microbe, and microbe alone were established. Active sludge was from reactor 2 at day 140 and the initial nitrate concentration was $30.00 \pm 0.50\,mg\,L^{-1}$, which is the same as the nitrate concentration in reactor 2 after influent mixing completely with remained water in last running period. Experiment was carried out in 250-mL wild-mouthed bottles at 30°C with triple repeats. Microbe and iron concentration were similar to reactor 2. Hydrogen was supplied all along the experiment. The results were shown in Table 4. As can be seen, hydrogen with microbe showed the highest nitrate removal and was 27.2% higher than iron with microbe. It verifies that iron promoting biological denitrification is mainly attributed to hydrogen generated from corrosion of iron. Microbe alone attributed to 33.5% of total nitrate removal compared to iron with microbe. Because it was probable that ferrous iron and endogenous carbon co-existed in active sludge used here, it cannot be decided which one played a major role. If the other main electron donor was ferrous iron, which would supply electrons for denitrification as reaction (4) in the presence of nitrate-reducing Fe(II)-oxidizing, end product of iron corrosion would be ferric iron and reducing 1 mg NO_3^-–N would consume 6.67 mg iron and theoretical ratio of iron to N was 6.67. This value is very close to factual ratio. At the same time, if endogenous carbon was another main electron donor, iron would afford the other 66.5% and the ratio of iron to N will be 7.71 (11.6 mg iron mg^{-1} N multiply by 66.5%). The value was consistent with the factual ratio, too. Considering that 2–5 $mg\,L^{-1}$ TOC and 2–4 $mg\,L^{-1}$ total iron were both monitored in effluent and both of them were by-products in iron-based autotrophic denitrification process [35], ferrous iron and endogenous carbon maybe both function in this process. Besides, denitrification bacterium in this experiment might obtain electron directly from iron shavings surface [36]. In addition, data in Table 4 indicate that biofilm formed on inner surface of reactor may contribute little to nitrate removal because there was almost the same nitrate concentration after 8 h reaction in iron with microbe condition (without biofilm) as the effluent nitrate concentration of reactor 2 with biofilm. At last, as low as 2–4 $mg\,L^{-1}$ effluent total iron concentration indicate that the risk of having high total iron concentration in effluent need not be concerned.

$$10Fe^{2+} + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18H^+ \quad (4)$$

Table 4
Effect of different substances on nitrate removal with microbe in stable sub-stage

Condition	Iron with microbe	Hydrogen with microbe	Microbe alone
Initial nitrate concentration	30.00 ± 0.50	30.00 ± 0.50	30.00 ± 0.50
Nitrate concentration after 8 h	21.53 ± 0.95	19.23 ± 1.38	27.16 ± 0.42

3.3. Effect of iron shavings quantity and MLSS concentration

Based on the above discussion, the electron donors used for denitrification were mainly supplied by hydrogen, ferrous iron, and endogenous carbon. So it can be inferred that iron shavings quantity and MLSS concentration were closely related with nitrate removal, which was verified by experimental result. After adding 20 g extra iron shavings in reactor 2 at day 98, the average effluent nitrate concentration decreased from 32.72 to 21.59 mg L^{-1}, and after doubling MLSS concentration at day 105, effluent nitrate concentration decreased further to 8.33 mg L^{-1}. Additional iron can generate more hydrogen to support denitrification and so bring speedier nitrate removal rate. Since the decrease of effluent nitrate concentration was observed almost immediately after iron quantity was increased in reactor 2, it can be inferred that there were more hydrogen-dependent autotrophic denitrifiers in reactor 2 than actual functioning population. On the other side, this deduction indicates that the promotion effect of adding iron shavings on nitrate removal may be limited in condition of invariable denitrifier population.

Increasing MLSS concentration may function from two aspects: more hydrogen-dependent denitrifiers and nitrate-reducing Fe(II)-oxidizing bacteria utilized more electrons for denitrification and higher ferrous iron and endogenous carbon concentration supplied more electrons. Besides this, it should be noticed that no obvious changes of effluent nitrate concentration were observed in the condition that iron shavings were continuously consumed and total iron concentration in reactor had been increasing. It indicates that there may be a dynamic balance between increasing of hydrogen-dependent denitrifier population and decreasing of iron shavings quantity. In fact, because iron shavings used in this experiment had a thickness of 0.2–0.4 mm which made new surface emerge after old surface gradually diminished as corrosion, the total surface areas that iron shavings supplied would not decrease too much.

Nitrite and ammonia concentration changing profiles in two reactors from day 75 to 135 are given in Fig. 4(a) and (b) in condition of no organic electron donor supplying. As can be seen, the effluent nitrite and ammonia concentration in reactor 1 was stable and just slightly higher than influent. By contrast, effluent from reactor 2 was more fluctuant. When iron shavings concentration increased from 10 to 20 g L^{-1} at day 98 in reactor 2, the effluent nitrite and ammonia concentration increased a little. Finally the concentration became stable and it is less than 2.0 mg L^{-1}.

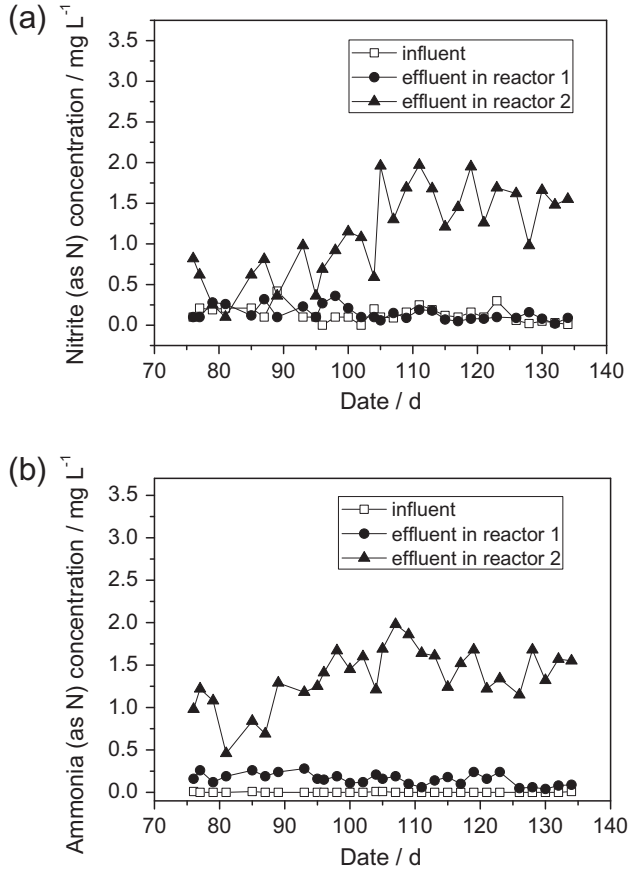

Fig. 4. The concentration changes of (a) nitrite, (b) ammonium in different operation conditions from day 75 to 135 in reactors with or without iron. No organic electron donor was supplied in influent. Extra 20 g iron shavings were added into reactor 2 at day 98; MLSS concentration in reactor 2 was increased to two times in day 106.

No nitrite accumulation was observed. The results indicate that the main products of nitrate reduced in this process were not nitrite and ammonia. In fact, bubbles had produced and observed during the anoxic reaction. So it can be concluded that N_2 or N_2O may be the most possible main reduction products in this process [37–39].

Considering that no other electron donor was supplied in influent and hydrogen generated from iron corrosion was the main electron donor of denitrification, it may be concluded that autotrophic denitrification was mainly functioning in reactor 2. The highest denitrification efficiency can attain 79.75% with the conditions of 8 h HRT, 20 g L^{-1} iron shavings and 3,930 ± 100 mg L^{-1} MLSS.

It should be noticed that synergism effect between iron and microbe concentration was not discussed in this study. It was possible that there was an optimum

ratio of iron shavings (iron surface area) to microbe concentration to provide a maximum limit of denitrification, which could be studied in a deep-going way in future research.

3.4. Denitrification process in one single cycle

When iron shavings were added into biological reactor with nitrate in influent, abiotic and biotic nitrate reduction would happen at the same time [40]. Mixotrophism, including organic matter in influent and iron shavings in reactor, were supplied as main denitrifying electron donors during the first 75 days and only iron shavings were as main denitrification electron sources from day 76. The changes of nitrate concentration with time in mixotrophic and autotrophic cultivation in one single cycle during stabilization sub-stage are shown in Fig. 5. The electron donors were 100 mg L^{-1} COD and 10 g L^{-1} iron shavings in mixotrophic cultivation sub-stage and were 20 g L^{-1} iron shavings in autotrophic cultivation sub-stage. Filling was over in 0.5 h. As can be seen, mixotrophism sustained higher nitrate removal efficiency than iron shavings alone during first 1 h. Nitrate removing rate in mixotrophic cultivation was high in the first 1 h and gradually slowed down. But the nitrate-removing rate in autotrophic cultivation kept almost constant at removing 1.70 mg L^{-1} in average each hour. Organic carbon source was easily be utilized by heterotrophic denitrifies [9], which may explain the rapid decrease of concentration in the first 1 h in mixotrophic cultivation. When the organic carbon source was almost exhausted, iron shavings began to undertake the main role of supplying electrons and therefore nitrate removal rate became slow. Nitrate concentration changes in alkalescency anoxic condition are shown in Fig. 6 when only iron shavings were reducer. Initial nitrate concentration was 40 mg L^{-1} and reaction temperature was 28°C. As can be seen, abiotic nitrate reduction rate was very slow in this condition. The main by-product was ammonia (result was not shown), as observed in previous researches [39,41]. So it can be inferred that HRT of 8 h is very short for abiotic nitrate reduction and abiotic nitrate reduction in mixotrophic cultivation of this process can be ignored.

Comparing nitrate concentration changes in two different sub-stages from 3 h after one single cycle beginning to the end of the cycle, it can be found that 20 g L^{-1} iron shavings exhibited higher nitrate removal rate than 10 g L^{-1} iron shavings. In mixotrophic cultivation sub-stage the concentration difference of effluent nitrate between reactor 1 and reactor 2 attained around 8 mg L^{-1} when there was 41 ± 1.5 mg L^{-1} nitrate in influent. This difference should be mainly attributed to the addition of iron shavings.

Biswas and Bose [26] observed a reduction in nitrate concentration from 40 to <1 mg L^{-1} with a retention time of 13 days by using steel wool as H_2 supplier. In this study, the HRT was rather short, but would obtain the same removal efficiency at current rate if reaction time was prolonged from 8 to 16 h (data was not shown). It may be attributed to that the microbes in reactor 2 had been acclimated for 75 days before terminating organic electron donor supply in influent, resulting in a better microbial adaptation and metabolism activity. pH rose by 0.9 unit with the degradation of nitrate, suggesting that it may require pH control if influent nitrate concentration or the reaction time (HRT) had been increased because the optimum pH for autotrophic denitrification was in the range 7.7–8.6 [42].

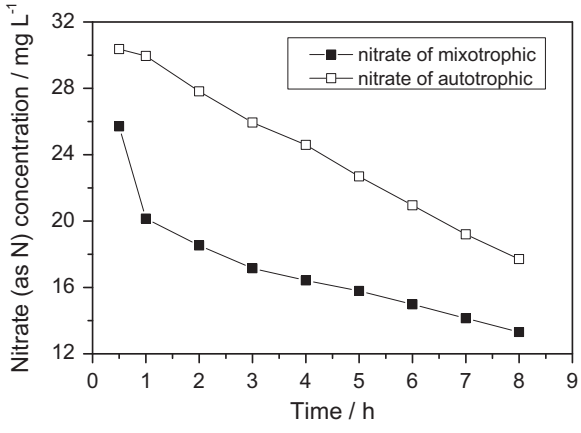

Fig. 5. Changes of nitrate concentration and pH in one single cycle during mixotrophic and autotrophic operation condition, repectively.

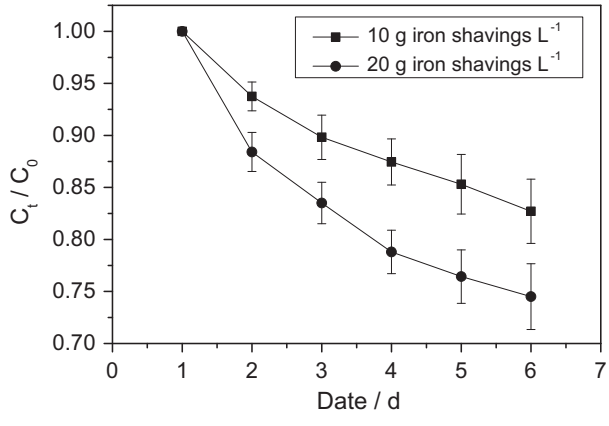

Fig. 6. Nitrate concentration changes in anoxic condition when iron shavings were reducers. Initial nitrate concentration was 40 mg L^{-1} and reaction temperature was 28°C.

Table 5
Denitrification rate in reactor 2 during different stable sub-stage

Period (d)	Iron ($g\ L^{-1}$)	MLSS ($mg\ L^{-1}$)	Nitrate(as N) concentration($mg\ L^{-1}$)		Removing quantity ($mg\ L^{-1}$)	Reaction time (h)	Rate as iron ($mg\ NO_3^-$–$N\ g^{-1}$ iron h^{-1})	Rate as area ($mg\ NO_3^-$–$N\ m^{-2}$ iron h^{-1})
			Influent	Effluent				
89–96	10	1,950 ± 50	42.25	32.58	4.84	7.5	0.06453	24.49
100–104	20	2,000 ± 50	42.78	21.60	10.59	7.5	0.07060	26.80
106–111	20	3,930 ± 100	41.13	8.33	16.40	7.5	0.1093	41.50
115–134	20	2,050 ± 50	40.55	19.38	10.59	7.5	0.07060	26.79
136–164	20	2,050 ± 100	41.50	22.01	9.75	7.5	0.06497	24.66
176–223	20	2,050 ± 100	41.50	20.64	10.43	7.5	0.06953	26.40
235–248	20	2,050 ± 100	41.50	19.35	11.08	7.5	0.07383	28.03

3.5. Denitrification rate

Autotrophic denitrification rate in reactor 2 was calculated by using data of average influent and effluent concentration during stable sub-stage in different operation conditions (Table 5). As can be seen, the denitrification rate was 0.065–0.109 mg NO_3^-–$N\ g^{-1}$ iron h^{-1} in different sub-stages. Since the reaction between iron shavings and microbes had some connection with the surface area of iron shavings and the specific surface area of iron shavings used in this experiment was 26.34 $cm^2\ g^{-1}$ iron, it is meaningful to normalize the reaction rate to specific surface area, which was 24.49–41.50 mg NO_3^-–$N\ m^{-2}$ iron h^{-1}. Besides, the removing capacity rate was 15.49–52.32 g NO_3^-–$N\ m^{-3}\ d^{-1}$ and was much higher than the rate of autotrophic denitrification using hydrogen generated from metallic iron corrosion [20].

The results indicate that iron shavings can support denitrification process as main substrate in a relatively steady rate under the stable operation condition. Although new iron shavings had replaced the old three times in day 136, 176, and 234, respectively, the effluent nitrate removal rate did not change distinctly. It reveals that this process was easily maintained without affecting nitrate removal. In addition, iron shaving used in the experiment has an iron content of 94.20–96.95% (w/w). Compared to pure iron, the impurities in iron shavings may affect iron corrosion and so have an effect on denitrification. The effect is complicated according to some research results [43,44] and may be considered in future work.

4. Conclusions

The results provided a lot of meaningful information on iron shavings (with specific area of 26.34 $cm^2\ g^{-1}$) as main substrate supporting denitrification process as following:

First, iron shavings can be used as main substrate to support denitrification process with SBR operation. Nitrate removal efficiency could reach as high as 79.8% with the condition of 8 h HRT, 3,930±100 $mg\ L^{-1}$ MLSS and 20 $g\ L^{-1}$ iron shavings. Effluent nitrite and ammonia concentrations were below 2.0 $mg\ L^{-1}$ and kept stable. Effluent total iron concentration was as low as 2–4 $mg\ L^{-1}$. The nitrate removal rate was 0.065–0.109 mg NO_3^-–$N\ g^{-1}$ iron h^{-1} and the capacity removing rate was 15.49–52.32 g NO_3^-–$N\ m^{-3}\ d^{-1}$ in different stable sub-stages.

Second, TN removal efficiency was closely related with total iron concentration in reactor. Sludge activity was seriously repressed when total iron concentration was over 4,800 $mg\ L^{-1}$ in reactor (that means iron in suspended solid accounts for more than 70%), which may be the endurance threshold for activated sludge. Removing half of total suspended solid (including MLSS and different types of iron precipitate) and adding new MLSS with very low total iron concentration (1–2 $mg\ L^{-1}$) into reactor was proved to be a successful method to recover microbial activity.

Third, the average ratio of total removed N to consumed iron was 1:7.52. The main electron donors for denitrification were hydrogen generated from iron corrosion, which contributed 66.5% of total electrons needed for nitrate removal in this process. The other 33.5% may be contributed by ferrous iron and endogenous carbon together. This process was easily maintained by replacing iron shavings periodically.

At the same time, it should be noticed that the biggest disadvantage of this process maybe is the low denitrification rate compared to sulfide-driven autotrophic denitrification [45] or traditional heterotrophic denitrification [9]. So the feasible application area of this process may be nitrate removal from groundwater or some industrial wastewater when the rate was not essential considerations. The process needs to improve

denitrification rate further and engineering adaptability for different application environments in future research.

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