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Application of catalytic iron for microbial nitrate removal from wastewater

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ABSTRACT

The present study investigated the application of catalytic iron as electron donor for nitrogen removal from effluent of a wastewater treatment plant by biological denitrification in anoxic sequencing batch reactor (AnSBR).When iron is immersed in water, it produces hydrogen gas through the reduction of protons, which can be utilized by denitrifying populations. The results showed that the TN removal efficiency was around 30% in the AnSBR with catalytic iron (AnSBR-Fu-Cu), while it was only 12% in the control reactor (AnSBR-C). Heterotrophic denitrification and autotrophic denitrification were the main nitrogen removal pathways in AnSBR-Fe-Cu. Higher effluent COD was observed in AnSBR-Fe-Cu compared to AnSBR-C, which was consistent with the GC-MS analysis. GC-MS analysis also showed that not all the organic species were degraded. Furthermore, 3D-EEM and UHPLC-QTOF were used to characterize the organics in the wastewater, which showed that the addition of catalytic iron affected the degradation of organics during the biological denitrification process.

Keywords: Nitrate removal; Catalytic iron; Biological denitrification

1. Introduction

Nitrate is a common pollutant in wastewater, and it could lead to multidimensional problems, e.g., eutrophication, infant methemoglobinemia, and serious diseases such as cancer [1]. However, nitrate is difficult to be degraded if the wastewater contains very little biodegradable carbon [2]. The wastewater from dyeing and finishing process in the textile industry contains high amount of organic pollutants [3,4]. The effluent chemical oxygen demand (COD) can meet the latest discharge limitation for dyeing and finishing in the textile industry by a combination of biological and chemical technologies (e.g., hydrolysis and acidification, aeration tank and secondary sedimentation, and finally advanced oxidation) [4–7]. However, the concentration of effluent TN (generally >40 mg/L), which is mainly composed by nitrate, is higher than the discharge limitation (20 mg/L, GB 4287-2012 for dyeing and finishing in the textile industry), and it needs further treatment.

Biological denitrification has been widely applied for the removal of nitrate, which generally has low operational cost [8]. However, electron donor has to be provided for nitrate removal. For the treated dyeing and finishing wastewater (DFW), most of the organics have been removed by the combination of biological and chemical technologies, and only some refractory organics are left, which are difficult to be used for denitrification. Therefore, additional electron donor has to be added in order to remove nitrate from the treated DFW.

Waste iron shavings is abundant in China and has relatively low cost, and it mainly contains iron (>95%), carbon (0.35–0.42%), silica (0.2–0.45%) and a few other trace elements [9]. Waste iron shavings has been previously used for the enhancement of biological treatment of industrial wastewater in order to remove chlorinated aliphatic compounds, organic dyes, nitrobenzenes and chlorinated phenols et al. [9–12]. Recently, catalytic iron process has been developed in order to accelerate the corrosion of iron and improve the degradation of pollutants [13]. The planting of copper on iron can form bimetallic system, which result in the higher reductive ability of iron even under neutral and

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alkaline conditions [13]. Previously, nano zero-valent iron (NZVI) has been used as electron donor for denitrification in lab-scale experiments [14–16], however, it was generally limited by the relatively high cost and technical difficulties for practical application. Iron is the main component of waste iron shavings, and therefore catalytic iron has the potential to be applied for biological denitrification. The performances of biological denitrification based on catalytic iron remains to be investigated. In addition, the biological and catalytic ozonation treated DFW contains refractory organics, and the degradability and transformation of such refractory organics in the biological denitrification process based on catalytic iron need to be investigated.

The present study aimed to applycatalytic iron (Fe-Cu) for nitrogen removal from the biological and catalytic ozonation treated DFW by biological denitrification. The performances (The removal efficiency of nitrate and the changes of nitrogen species) of biological denitrification with and without catalytic iron were compared. In addition, the degradation and transformation of organics in the treated DFW were evaluated.

2. Material and methods

2.1. Wastewater and inoculum

The effluent of a full-scale wastewater treatment plant, located in a typical dyeing and finishing industry cluster in southeast China, was collected and used in the present study. The wastewater in the above wastewater treatment plant was treated by biological and catalytic ozonation methods [4]. The characteristics of the wastewater were as follows: TN 46.8–58.7 mg/L, nitrate 45.4–56.8 mg/L, nitrite 0.9–3.7 mg/L, ammonia 0.02–0.15 mg/L and COD 42–68 mg/L.

The inoculum used in the present study was the activated sludge, which was obtained from a wastewater treatment plant in Shanghai. The MLVSS and MLSS of the inoculum were 2100 mg/L and 2800 mg/L, respectively.

2.2. Preparation of catalytic iron

Waste iron shavings were collected from a metal machinery plant using 38CrMoAl steel. They were washed by commercial detergent to remove surface pollutants, especial oil stain, and then by dilute HCl to remove rust. Cu was deposited on the surface of waste iron shavings by a simple metal displacement reaction in aqueous solution [Eq. (1)]. Waste iron shavings were submerged in water, and a certain amount of $CuSO_4$ was added in order to obtain the catalytic iron with 0.3% Cu.

$$Fe^0 + Cu^{2+} = Fe^{2+} + Fe^0/Cu^0 \tag{1}$$

2.3. Continuous experiments

The reactors used in the present study were 6 L glass bottles with working volume of 4.8 L, and they were run as anoxic sequencing batch reactors (AnSBR) for biological denitrification. One reactor (AnSBR-Fe-Cu) was filled with catalytic iron with the concentration of 62.5 g/L. The other one (AnSBR-C) was used as control without the addition of catalytic iron. The activated sludge was inoculated to the two reactors with the final MLSS concentration of 2000 mg/L. Mechanical stirrers at 120 rpm was applied to both reactors. The HRT was controlled at 32 h in both reactors. One cycle of AnSBR lasted for 360 min, including 5 min feeding, 240 min reaction, 110 min settling and 5 min drainage.

2.4. Batch experiments

The nitrate removal pathways in reactor AnSBR-Fe-Cu were investigated by batch experiments. Nitrogen removal by the organics in the wastewater was studied. Mixture of 200 mL was obtained from AnSBR-Fe-Cu, and centrifuged. The centrifuged sludge and 200 mL wastewater were added to 500 mL serum bottles. In addition, the nitrogen removal by catalytic ironvia chemical reaction was investigated. Catalytic iron was added with the final concentration of 62.5 g/L, while sludge was not added. All the bottles were flushed with nitrogen and then incubated in a shaker with 120 rpm.

2.5 Analytical methods

The parameters including COD, MLSS, MLVSS, nitrate, nitrite, ammonia, and TN were measured periodically for the two continuous AnSBRs according to APHA [17]. TOC analyser (Shimadzu TOC-L, Japan) was used for the analysis of TOC and TN. During steady-states, the characterization of the residual organics in the two reactors was conducted. The 3D-EEM was determined using a fluorometer (HORIBA Jobin Yvon FluoroMax-4, France). GC-MS (Shimadzu GCMS-QP2010 SE, Japan with a HP5-MS column) and UHPLC-QTOF (Agilent 1290 UHPLC, Agilent 6540 QTOF, USA with Agilent ZORBAX SB-C18 HD column) were used to analyze the organic species according to our previous study [4]. The theoretical COD amounts required for the removal of nitrate and nitrite were calculated based on the values of 2.86 $gCOD/gNO_3$-N and 1.71 $gCOD/gNO_2$-N respectively [18].

3. Results and discussion

3.1. Process performances of the continuous reactors

3.1.1. Nitrogen removal

Fig. 1 shows the time courses of TN removal efficiencies in the two AnSBRs, and relatively stable TN removal efficiencies were achieved during the 100 d operation. The TN removal efficiency in AnSBR-C was around 12%, while it was increased to around 30% in AnSBR-Fe-Cu, which indicated that the addition of catalytic iron increased the TN removal efficiency. Fig. 2 shows the time courses of the concentrations of nitrate, nitrite and ammonia in the influent and effluent of both reactors. Higher concentration (30–60 mg/L) of nitrate was observed in both influent and effluent of the two reactors, while the concentrations of nitrite (<2 mg/L) and ammonia (<0.1 mg/L) were in very low levels. The nitrogen mass balances analysis for the influent and effluent also showed that nitrate was the main compo-

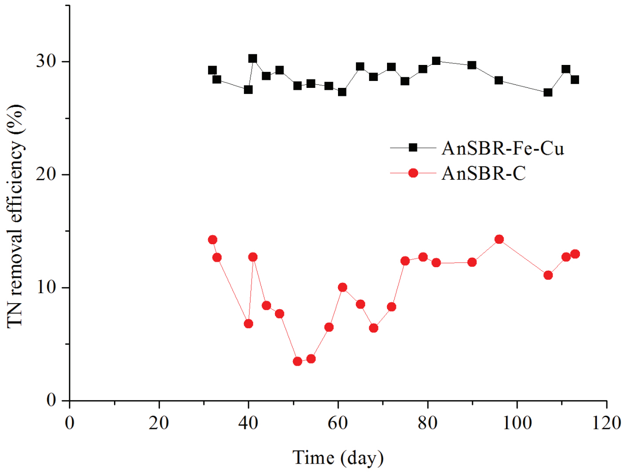


Fig. 1. TN removal efficiencies in AnSBR-Fe-Cu (with the addition of catalytic iron) and AnSBR-C. The two reactors were operated under HRT 32 h.

nent (>90%) in both influent and effluent (Fig. 3). Therefore, the removal of TN was mainly achieved by the removal of nitrate. Although the treated DFW undergone biological and chemical oxidation treatments, the organics in the treated DFW seems still can be used for biological nitrogen removal. The catalytic iron provided additional electrons, which therefore obviously increased the TN removal efficiency. The ammonia concentration in the effluent was very low, which indicated that nitrate was mainly removed by biological denitrification [15]. It was known that the abiotic reactions can produce ammonia [Eq. (2)] [19], which would decrease the total nitrogen removal efficiency. The above results clearly showed that iron mainly enhanced the nitrate removal by biological denitrification considering nitrate was the main nitrogen specie. The biological denitrification mechanism with catalytic iron could be as shown in Eqs. (3) and (4). It should be noted that the nitrate removal efficiencies (12% for AnSBRs and 30% for AnSBR-Fe-Cu) in the present study were not as high as previously reported, where H_2 and NZVI were used for denitrification and higher nitrate removal efficiencies (>90%) were achieved [8,20,21]. It might be due to that catalytic iron had low surface area compared to NZVI. Since catalytic iron is cheap and might be more practical to be utilized.

$$NO_3^- + 3Fe^0 + H_2O + 2H^+ \rightarrow NH_4^+ + Fe_3O_4 \tag{2}$$

$$Fe + 2H_2O \rightarrow H_2 + Fe^{2+} + 2OH^- \tag{3}$$

$$2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^- \tag{4}$$

3.1.2. COD removal

The concentrations of COD in the influent and effluent of the two reactors are shown in Fig. 4. The removal of COD was found in both reactors considering the lower concentration of COD in the effluent compared to that in the influent. During the steady-states, the concentration of COD in the effluent of AnSBR-C was around 20 mg/L, while it was around 40 mg/L

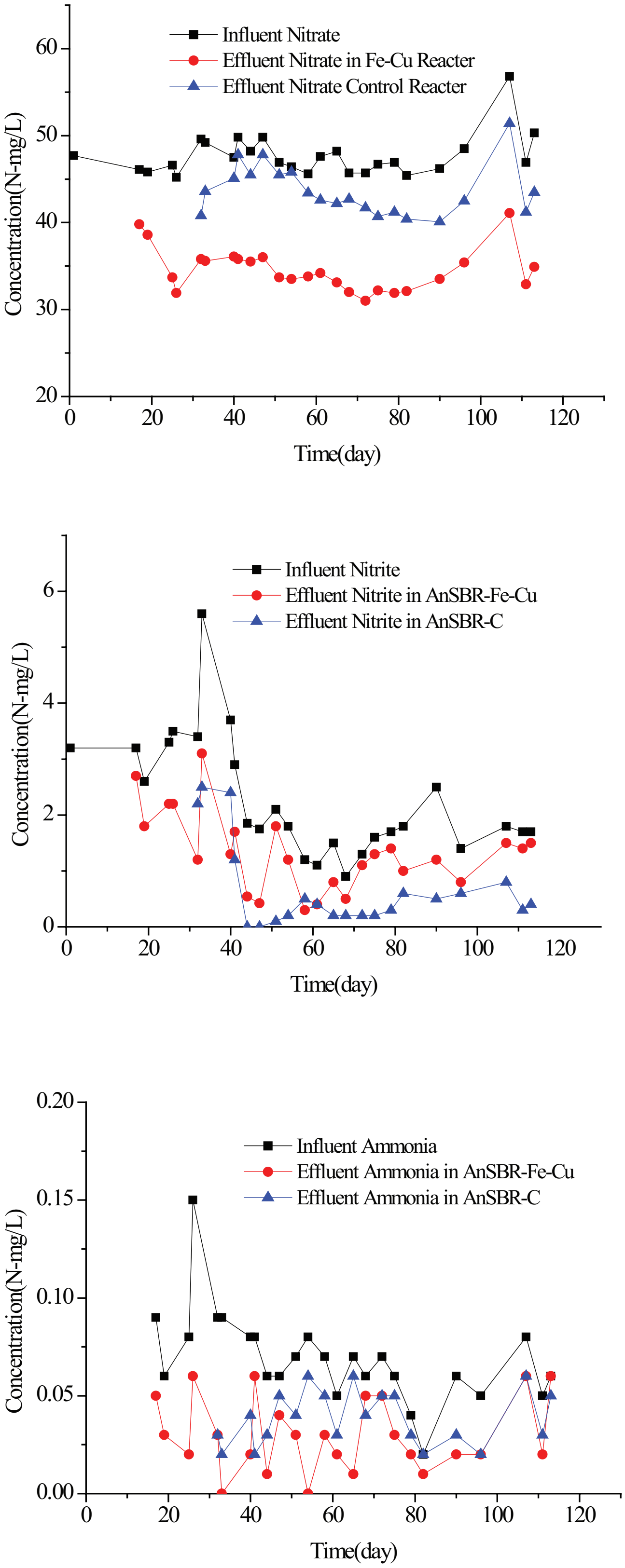


Fig. 2. The concentrations of nitrate, nitrite, and ammonia in the influent and effluent of AnSBR-Fe-Cu (with the addition of catalytic iron) and AnSBR-C. The two reactors were operated under HRT 32 h.

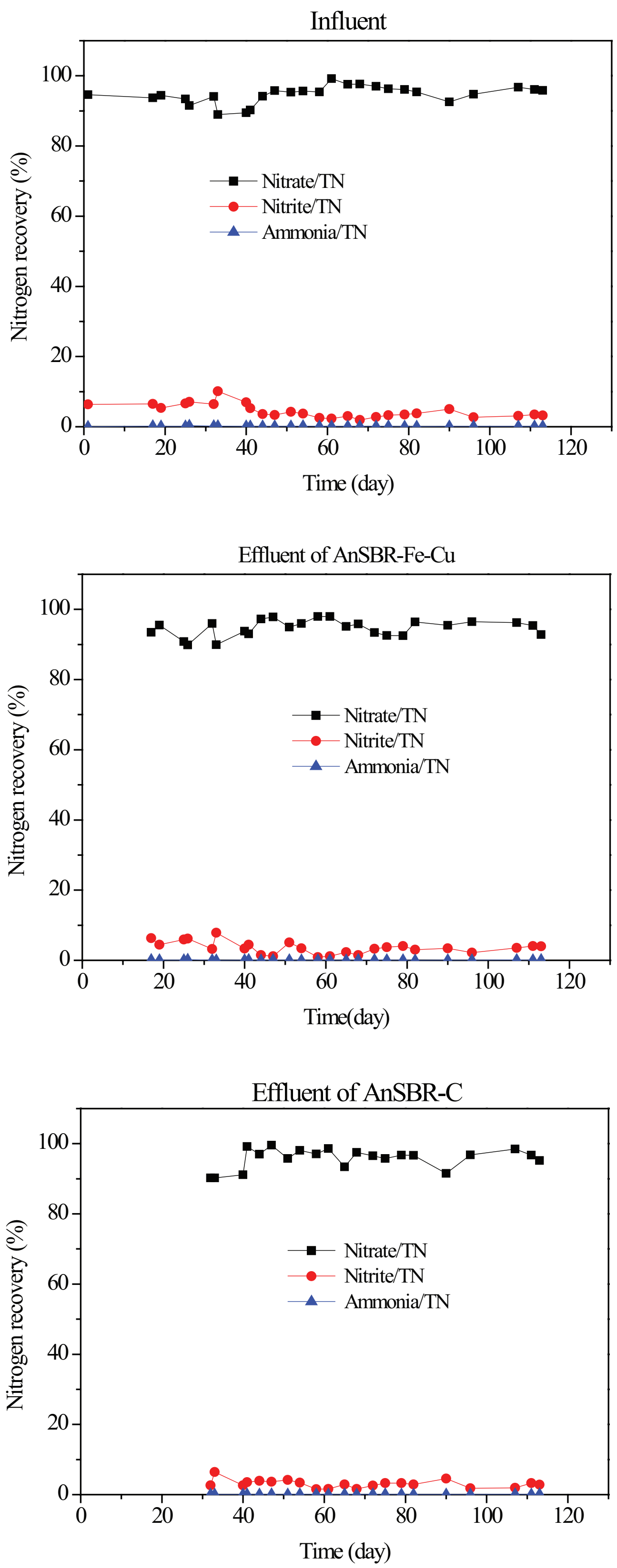


Fig. 3. Nitrogen mass balance for influent and effluent from AnSBR-Fe-Cu (with the addition of catalytic iron) and AnSBR-C. The two reactors were operated under HRT 32 h.

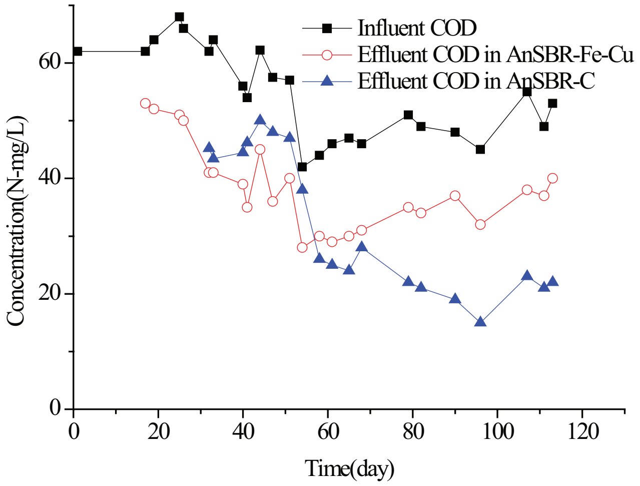


Fig. 4. The concentrations of COD in the influent and effluent of AnSBR-Fe-Cu (with the addition of catalytic iron) and AnSBR-C. The two reactors were operated under HRT 32 h.

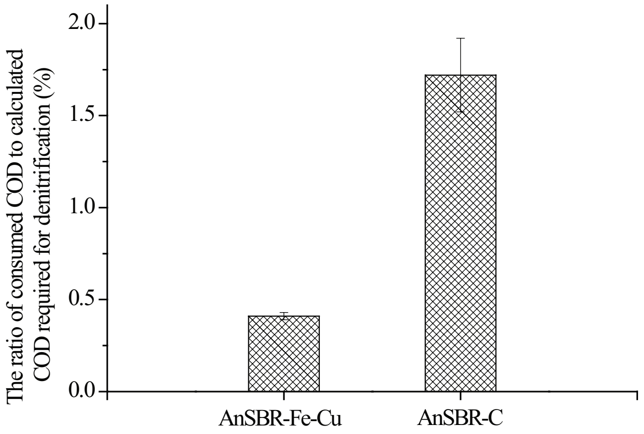


Fig. 5. The ratio of consumed COD to calculated COD required for denitrification in AnSBR-Fe-Cu (with the addition of catalytic iron) and AnSBR-C. The two reactors were operated under HRT 32 h.

for AnSBR-Fe-Cu. The higher concentration of COD in AnSBR-Fe-Cu could be due to that bacteria preferred to use catalytic iron instead of the organics in the threated DFW. Fig. 5 shows the ratio of removed COD to the theoretical COD needed for nitrate and nitrite removal. The ratio in AnSBR-Fe-Cu was close to 0.5, and it means the removed COD was not able to provide enough electrons for denitrification. The additional electrons in AnSBR-Fe-Cu should be mainly from the catalytic iron. For AnSBR-C, the ratio was around 1.7, which indicated the removed COD was not fully used for denitrification, and it could be due to that part of the removed COD was absorbed by the sludge and also part of the removed COD was used for the growth of microorganisms.

3.1.3. Concentrations of MLSS and MLVSS

Fig. 6 shows the concentrations of MLSS and MLVSS during the steady-states of the two reactors. The MLSS (4924

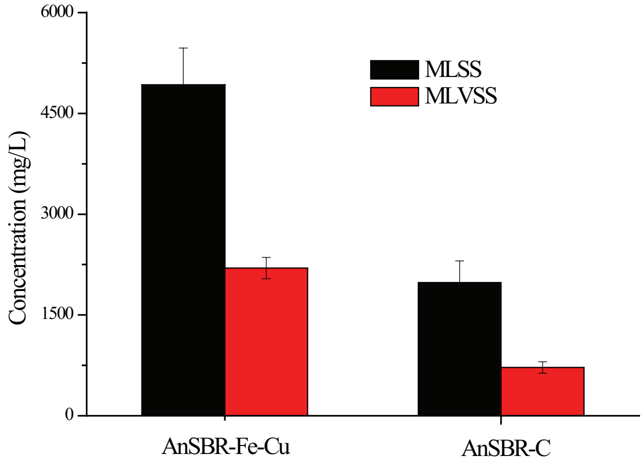
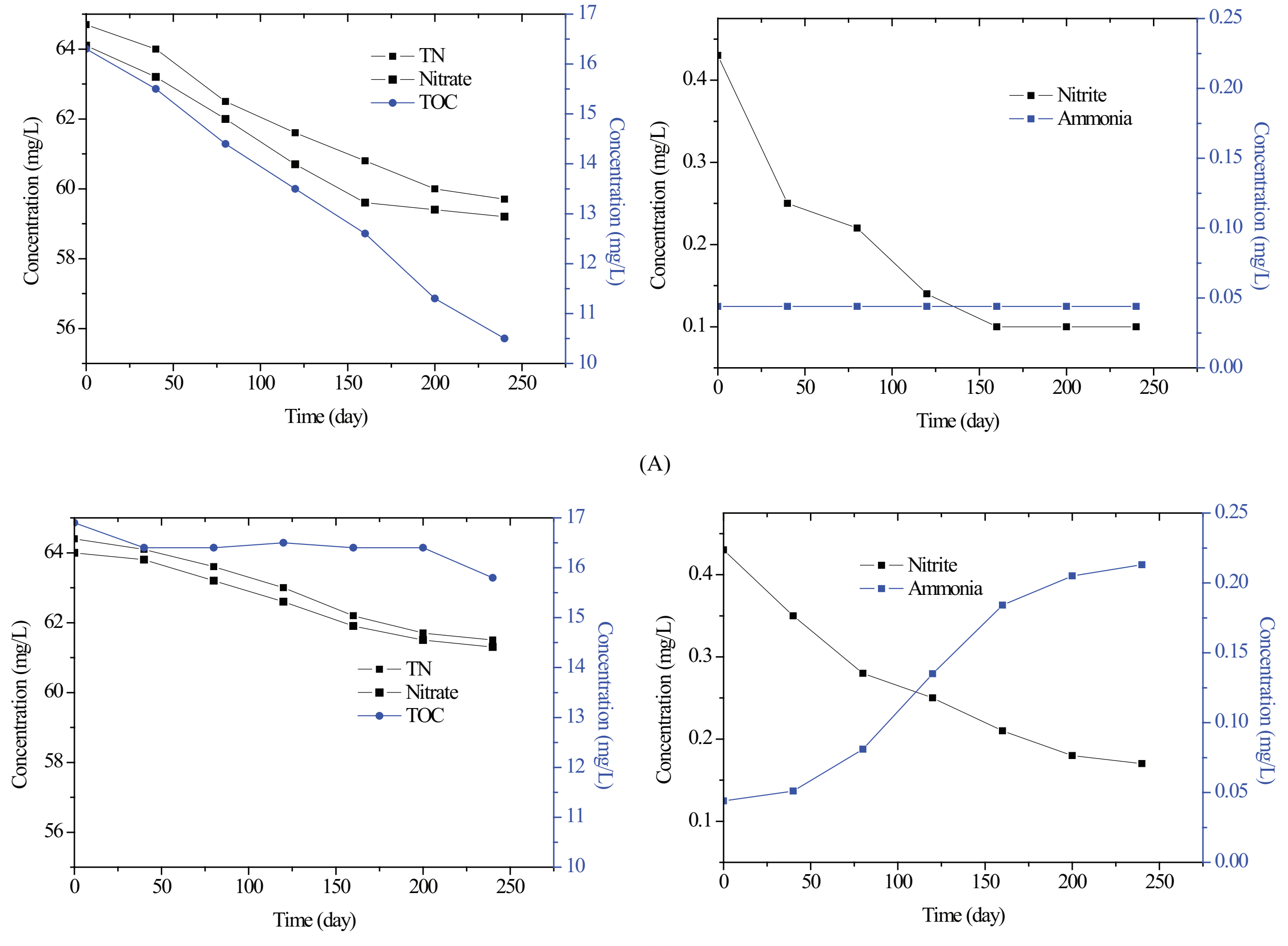


Fig. 6. The concentrations of MLSS and MLVSS in AnSBR-Fe-Cu (with the addition of catalytic iron) and AnSBR-C. The two reactors were operated under HRT 32 h.

mg/L) in AnSBR-Fe-Cu was around 2.5 times higher than that (1981 mg/L) in AnSBR-C, and the MLVSS (2198 mg/L) in AnSBR-Fe-Cu was around 3 times higher than that (718 mg/L) in AnSBR-C. MLVSS is a parameter representing the concentration of microorganisms in the system, and it was obvious the addition of catalytic iron increased the concentrations of microorganisms which further resulted in the higher removal efficiency of nitrogen. In the present study, samples in reactor AnSBR-Fe-Cu was only obtained from liquid phase, and it was possible that biofilm could be formed on the surface of catalytic iron which was not accounted in MLVSS [22]. Therefore, the actual microbial concentration should be higher than the obtained value.

3.2. Batch experiments

As shown in Fig. 7, there was no obvious decrease of nitrate when catalytic iron was used without the addition of sludge, and the concentrations of ammonia and nitrite were also not obviously changed, which indicated that denitrification via chemical reaction could be ignored in the present study. When



(A)

(B)

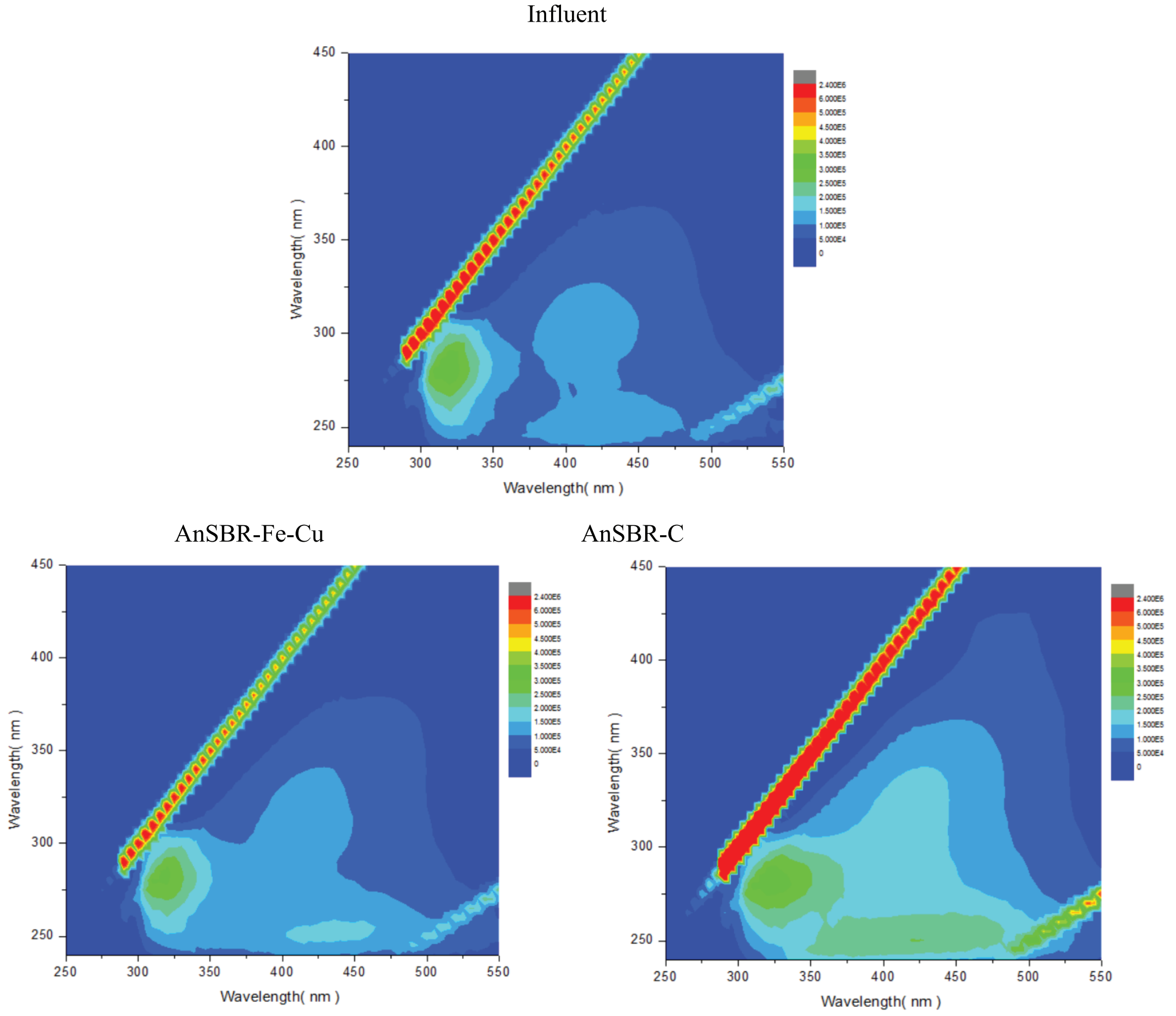
Fig. 7. The time courses of TN, nitrate, TOC, nitrite and ammonia in batch experiments. (A) Experiment with only sludge (B) Experiment with only catalytic iron.

the sludge was added without the addition of catalytic iron, obvious decrease of nitrate was found (Fig. 7), and it showed that biological denitrification by heterotrophic bacteria occurred in AnSBR-Fe-Cu. The above results suggested that the higher nitrogen removal efficiency in AnSBR-Fe-Cu compared to AnSBR-C was mainly attributed to biological denitrification by autotrophic bacteria, which utilized catalytic iron, since chemical reaction could be negligible.

3.3. Degradation of organics in the process

3D-EEM fluorescence analysis (Fig. 8) showed that one peak was present in the raw wastewater, and it corresponded to protein like compounds (*Em/Ex* = 275/325) [4,23]. However, the peak was still present in the wastewater even after treatment for both reactors, which indicated the protein like compounds were not degraded in the process. In addition, a peak at *Em/Ex* = 250/425 appeared in the effluent of both reactors. It corresponded to fulvic acid like compounds, which could be resulted from the biological transformation of some organic materials into biologically mature and stable and chemically complex organic compounds [24]. It should be noted that the intensity of peak at *Em/Ex* = 250/425 in AnSBR-C was much higher than that in AnSBR-Fe-Cu, which might be related with higher organic degradation efficiency in AnSBR-C (Fig. 4).

Organic species were determined by UHPLC-QTOF, and the raw wastewater contained a total of 574 species, most of which (92%) were strong polar species (Table 1). The effluents of both reactors contained relatively higher organic pollutant species compared to the raw wastewater,which could be related with the formation of new species in the reactor. The strong polar species were still dominant in the effluents. GC-MS was further used to detect the main organic species and the results are shown in Table 2. The total peak areas of main pollutants in raw wastewater, effluent of AnSBR-C,effluent of AnSBR-Fe-Cu were6080649, 3180570 and 3878034, respectively, and it indicated that the organics in the effluent of both reactors were lower than that in the raw wastewater, which was consistent with the decrease of the concentrations of COD after treatment. In addition, the higher peak area of effluent of AnSBR-Fe-Cu compared to that of AnSBR-C was


Influent

AnSBR-Fe-Cu

AnSBR-C

Fig. 8. 3D-EEM of influent and effluent from AnSBR-Fe-Cu (with the addition of catalytic iron) and AnSBR-C. The two reactors were operated under HRT 32 h.

Table 1
Summary of the results from UHPLC-QTOF

	Influent	AnSBR-Fe-Cu	AnSBR-C
Medium or weak polar species*	44 (8%)#	46 (8%)	54 (9%)
Strong polar species*	530 (92%)	530 (92%)	526 (91%)
Total species	574	576	580

*The species with retention time ≤ 2 min were regarded as strong polar species, while the species with retention time 2 min were regarded as medium or weak polar species.

#The number in the bracket was the ratio of the species to the total species.

Table 2
Summary of the results from GC-MS

Name of the compounds	Influent		AnSBR-Fe-Cu		AnSBR-C	
	PA*	RPA#	PA	RPA	PA	RPA
6,6′-Dimethyl-5,5′,8,8′-tetramethoxy-2,2′-binaphthylidene-1,1′-dione	196235	3.09	247679	6.39	201486	5.85
Acetic acid, ethyl ester	279478	4.41	431969	11.14	355725	10.34
Dodecane	196961	3.11	165811	4.28	155585	4.52
1-Dodecanamine, N,N-dimethyl-	2761717	43.56	790862	20.39	682179	19.82
Phenol, 2,4-bis(1,1-dimethylethyl)	22307	0.35	131233	3.38	98437	2.86
2-Propanone	519281	8.19	167684	4.32	157243	4.57
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	223301	3.52	357556	9.22	289873	8.42
n-Hexadecanoic acid	260061	4.1	148549	3.83	104911	3.05
6-Methylfuro[2,3-c]pyrid-5-one	156178	2.46	186489	4.81	147336	4.28
2-(2-N-Benzyl-N-methylaminoethyl)-4,5-dimethoxyphenylaceticacid, methyl ester	372461	5.87	90479	2.33	78300	2.28
Docosanoic acid	82071	1.29	91112	2.35	85055	2.47
Acetic acid, decyl ester	20003	0.32	41893	1.08	28224	0.82
Sulfurous acid, 2-propyl undecyl ester	49278	0.78	47407	1.22	33104	0.96
N-Methyl-N-benzyltetradecanamine	68133	1.07	ND	ND	ND	ND
Nonane, 4,5-dimethyl-	50038	0.79	51482	1.33	36710	1.07
1-Phenanthrenecarboxylic acid	ND	ND	291484	7.52	240252	6.98
3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl-	ND	ND	56123	1.45	40817	1.19
Octadecane (CAS) n-Octadecane	70196	1.11	45712	1.18	31604	0.92
1,1-Dibromo-2-(2,2-dimethylpropyl)cyclopropane	85848	1.35	ND	ND	ND	ND
Tetrasiloxane, decamethyl-	67302	1.06	44344	1.14	30393	0.88
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	101833	1.61	84055	2.17	65536	1.9
2-Bromo dodecane	49778	0.78	38878	1.00	27326	0.79
Promecarb 2,4-dinitrophenylether	ND	ND	ND	ND	ND	ND
3-(2-Methoxymethoxy-ethylidene)-2,2-Dimethyl-Bicyclo[2.2.1] Heptane	ND	ND	ND	ND	ND	ND
2-Bromotetradecane	41372	0.65	89211	2.30	70099	2.04
2,5-Dimethyl-4-methoxyphenol	75184	1.19	98961	2.55	78727	2.29
Eicosamethylcyclodecasiloxane	115403	1.82	49290	1.27	40080	1.16
Silikonfett SE30(GREVELS)	95459	1.51	67849	1.75	51194	1.49
1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	120771	1.9	61922	1.60	50374	1.46

*Peak area

#Relative peak area (%)

&Not detected

also consistent with the COD results, further indicating more organics were removed in AnSBR-C The peak areas of some organic species were decreased after treatment, including 1-dodecanamine, N,N-dimethyl-, 2-propanone, n-hexadecanoic acid and so on, while there were also organic species not degraded or even enriched such as phenol, 2,4-bis(1,1-dimethylethyl), docosanoic acid, and et al, which might be recalcitrant to biological degradation or produced by the transformation of other organic compounds.

4. Conclusions

The results from the present study clearly showed that catalytic iron could be used to improve the biological nitrogen removal from the biological and catalytic ozonation treated DFW, and the main conclusions were as follows:

1. The TN removal efficiency increased from 12% in the control reactor (AnSBR-C) to 30% in the reactor with catalytic iron (AnSBR-Fe-Cu), which was due to the additional electrons provided by catalytic iron for denitrification.
2. The residual COD in the effluent of AnSBR-Fe-Cu was higher than that in the effluent of AnSBR-C, and it could be due to the preferable utilization of catalytic iron instead of organics in the effluent for denitrification.
3. Both heterotrophic denitrification and autotrophic denitrification were the main pathways for nitrogen removal in AnSBR-Fe-Cu,since the microorganisms in the reactor can utilize organics for denitrification and at the same time the removed COD was not enough for denitrification, which had to be provided by catalyticiron.
4. Not all the organic species could be degraded during the biological denitrification process as revealed by 3DEEM, UHPLC-QTOF and GC-MS analysis.

Acknowledgements

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