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Preliminary performance and biofilm characteristics of ANAMMOX process in an improved FBR with mixture of honeycomb-like and non-woven carriers

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ABSTRACT

Honeycomb-like polypropylene carriers and ring non-woven carriers were filled in a fixed bed reactor (FBR) and mixed at the ratio of 1:1 to enrich ANAMMOX bacteria from the conventional activated sludge and start up ANAMMOX process. The DO concentration in the influent was below 0.05 mg/L and the temperature was maintained at 35°C inside the FBR. The reactor was operated for 87 d and the whole process was divided into start-up period and nitrogen loading enhancing period. In start-up period, influent NH_4^+-N and NO_2^--N concentrations were both 70 mg/L at HRT of 48 h. ANAMMOX activity first occurred on day 21. The removal efficiency of ammonium, nitrite and total nitrogen (TN) reached 90.24%, 100%, 88.8% respectively on day 53, indicating that ANAMMOX was successfully started up. In nitrogen loading enhancing period, influent NH_4^+-N and NO_2^--N concentrations both increased from 100 mg/L to 230 mg/L and subsequently HRT was shortened from 48 h to 36 h. The maximum TN loading rate and removal rate reached 0.31 kg $N\cdot m^{-3}\cdot d^{-1}$ and 0.25 kg $N\cdot m^{-3}\cdot d^{-1}$ with the average TN removal efficiency of 77.27%. Quantitative PCR indicated that ANAMMOX bacteria responsible for ANAMMOX reaction predominated in the mature biofilms from both the carriers. The scanning electron microscope (SEM) photograph showed that the mature biofilm scratched from the honeycomb-like carriers exhibited a morphology of self-aggregates while the mature biofilm attached on the non-woven carriers was bound or enwrapped by fine non-woven fibers. The mature biofilm in both carriers had the typical cauliflower appearance of ANAMMOX consortium. The FBR improved with mixture of two kinds of carriers can enhance ANAMMOX performance.

Keywords: ANAMMOX performance; Honeycomb-like polypropylene carriers; Ring non-woven carriers; Mature biofilm characteristics; FBR

1. Introduction

With the high pace of urbanization process, eutrophication and other nitrogen pollutions in the natural water bodies are increasingly serious. These phenomena are caused by the agricultural fertilizer and irrigation and industrial by-productions. However, conventional biological nitrogen removal process is limited for its high cost and complex procedure. Thus ANaerobic AMMonium OXidation (ANAMMOX) process has been put forward as a novel and promising biological nitrogen removal process by researchers in the field of wastewater treatment [1–3].

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

ANAMMOX is a biochemical reaction and , as expressed in Eq. (1), the functional bacteria called Anammox bacteria is a kind of anaerobic autotrophic bacteria, which can accomplish the oxidation of ammonia using nitrite as the

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electron acceptor and produce nitrogen gas [3]. Compared with the traditional nitrogen removal process ANAMMOX process is more cost-effective as it can save the demand for oxygen and organic carbon sources. Moreover, this process has an outstanding capacity of nitrogen removal from wastewater.

However, one major problem restricting the application of ANAMMOX process is the long start-up period resulting from the extremely low growing rate of ANAMMOX bacteria, whose generation time is usually at the range of 8~11 d [1–3]. The doubling time of ANAMMOX bacteria is evidently longer than that of other bacteria such as nitrifying bacteria and denitrifying bacteria. To overcome the problem, researchers has used the simple-controlling and stable-operating fixed bed reactor (FBR) filled with various kinds of supporting materials to achieve effective attachment of ANAMMOX bacteria. Acrylic fiber, polyethylene sponge strips and polyester non-woven cloth were chosen as carriers for enhancing the enrichment of ANAMMOX bacteria. Qiao et al. [4] argued that acrylic fiber biomass carriers had a high efficiency of retaining microorganisms and a good resistance of temperature decrease. NO_2^--N removal efficiency decreased from 94% to 89% when the temperature declined from 33.0°C to 26.2°C. Zhang et al. [5] chose FBR hoisted a polyethylene sponge with 8 strips and started up ANAMMOX within 56 days successfully. The reactor achieved a TN ($TN = NH_4^+\text{-}N + NO_2^-\text{-}N + NO_3^-\text{-}N$) removal rate of 3.6 kg $N\cdot m^{-3}\cdot d^{-1}$ with the max TN loading rate of 4±0.1 kg $N\cdot m^{-3}\cdot d^{-1}$ within 150 d, which might be attributed to the fact that a high porosity (96% in wet condition) of polyethylene sponge improved the enrichment of biomass. Phan et al. [6] filled an FBR with polyester non-woven carriers to study ANAMMOX process and the reactor attained a TN removal rate and efficiency of 1.54 ± 0.1 kg $N\cdot m^{-3}\cdot d^{-1}$ and 75%~78% on the condition of TN loading rate of 2.00 kg $N\cdot m^{-3}\cdot d^{-1}$. The polyester non-woven cloth carriers have high-efficiency attachment property to trap biomass. As a whole, those carriers as described processed excellent biomass capture property, however there still existed a serious problem. As the biofilm grew thicker, carriers had a difficulty in substrate diffusion and nitrogen discharge, which was unfavorable to the growth of microorganisms. Besides, all these studies focused one single kind of material as carriers, and few reports covered on mixed carriers based on some ratio. Mixture of some different kinds of carriers may benefit to enhance ANAMMOX performance in FBR. Here honeycomb-like polypropylene carriers and ring non-woven cloth carriers are chosen and mixed to improve FBR for ANAMMOX operation. Honeycomb polypropylene carrier has a great advantage of high porosity, erosion resistance and light weight so that it can enrich ANAMMOX bateria. High porosity allows timely escape of nitrogen bubbles, and slight scouring effects caused by gas can brush out the old biofilm to further improve diffusion of substrate [7]. Small size of ring non-woven carriers exhibited a more excellent capacity for enrichment of ANAMMOX bacteria [8]. The limited factors like substrate transfer and nitrogen discharge problem can be relieved by the honeycomb-like polypropylene carriers, while the biomass retention was enhanced by ring non-woven cloth carriers.

Honeycomb-like polypropylene carriers and ring non-woven carriers have their own advantages and shortcomings. Porous structure of the honey-like carriers can facilitate discharge of the produced biogas and realize net-capture of ANAMMOX bacteria while micro-porous fiber structure of the non-woven carriers can retain higher biomass of ANAMMOX bacteria in the FBR. But clogging of packed bed is prone to occur in the FBR with the non-woven carriers. The mixture of two kinds of the carriers are expected to combine their advantages and also overcome their shortcomings so as to improve retention capacity of ANAMMOX bacteria in the FBR and facilitate discard of the produced gas in ANAMMOX reaction, and thus it is expected to accelerate the start-up of ANAMMOX process and to elevate its operational performance. There are very limited researches on applying mixed carriers to improve FBR for ANAMMOX start-up and operation. This paper aims to investigate start-up and operation performance of the ANAMMOX process in the FBR filled with the mixture of honeycomb-like polypropylene carriers and ring non-woven cloth carriers at the ratio of 1:1. The mixed carriers have the advantages as follows: (1) the honeycomb-like polypropylene structure and micro-porous fiber structure could enhance the retention of Anammox bacteria; (2) the bio gas (mainly N_2) could be easily discharged from inter spaces among the honeycomb-like carriers and holes in them to avoid gas entrapment. ANAMMOX start-up and operational performances were discussed and the mature ANAMMOX biofilm characteristics were also explored in the FBR with the mixed carriers. This study is expected to offer some valuable data and promote industrial applications of ANAMMOX process.

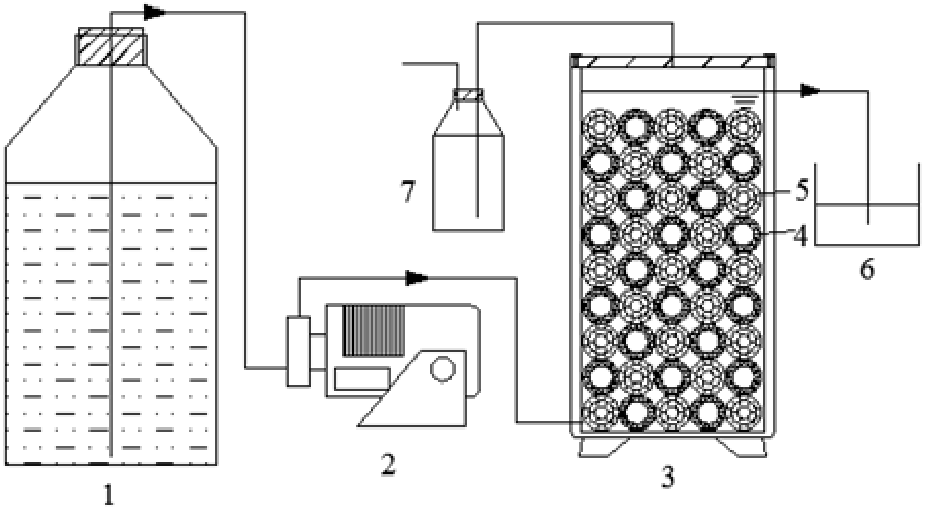
2. Materials and methods

2.1. Reactor

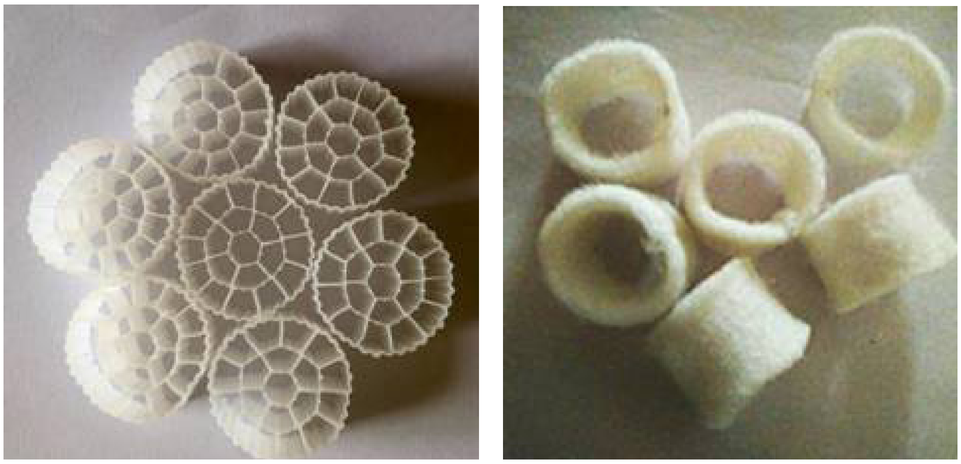
A columnar FBR made of plexi glass was applied in this study. As Fig. 1 depicts, the FBR has a height to diameter ratio of 5:2 with the efficient working volume of 2.6 L. FBR is sealed and wrapped around by a black cloth to protect the ANAMMOX bacteria from the inference caused by algae and light. The terminals of effluent vessel and exhaust pipe were submerged in water to cut off from the air. The reactor equipped with a thermostatic jacket was maintained at 35°C by circulation of thermostatic water. In the reactor, the pH was kept around 8.0 by adding 1 M Na_2CO_3 or HCl and the influent DO was controlled below 0.05 mg/L by flushing with N_2.

2.2. Carriers

The honeycomb-like polypropylene carriers and ring non-woven cloth carriers applied in this study were mixed at the volume ratio of 1:1. The characteristics of honeycomb-like polypropylene carriers and ring non-woven cloth carriers were described previously [7,8]. The appearance of the two carriers is presented as Fig. 1. Each honeycomb-like polypropylene carrier has an advantage of corrosion resistance and has a high specific surface area of 576 $m^2\,m^{-3}$. In the case of operating Anammox process, the produced N_2 can be easily discharged out of FBR filled with the honeycomb-like carriers and generated the slight scouring effect



(a) Schematic diagram of experimental device



(b) honeycomb-like polypropylene carriers (c) ring non-woven cloth carriers

Fig. 1. Schematic diagram of experimental device and carriers for ANAMMOX start-up and operation: (1) feed tank; (2) influent peristaltic pump; (3) FBR; (4) ring non-woven cloth carriers; (5) honeycomb-like polypropylene carriers; (6) effluent port; (7) water sealing.

on the cultivated biofilm attached on this kind of carriers. The scouring effect has several benefits: (1) it can reduce the biofilm thickness and facilitate the renewal of biofilm to improve substrate transfer efficiency; (2) it can further promotes the emission of N_2 and avoid the blockage of packed bed. The serrated structure of the honeycomb-like carriers can improve agglomeration growth of microorganisms. Each ring non-woven carrier has a thickness of 3 mm and a diameter of 2.5 cm. For the ring non-woven carrier, the fibrous structure ensures a large specific surface of 1338 m^2 m^{-3}, thus enhancing the enrichment of ANAMMOX bacteria.

2.3. Synthetic wastewater and seed sludge

The mineral medium and trace element in synthetic wastewater were prepared based on the ANAMMOX medium reported by Van de Graaf et al. and Strous et al. respectively [9,10]. It contains (g/L): $KHCO_3$ 1.25, KH_2PO_4 0.025, $CaCl_2{\cdot}2H_2O$ 0.3, $MgSO_4{\cdot}7H_2O$ 0.2, $FeSO_4$ 0.00625, EDTA 0.00625, and 1.25 mL/L of trace elements solution. The composition of the trace element solution was (g/L): EDTA 15, $ZnSO_4{\cdot}7H_2O$ 0.43, $CoCl_2{\cdot}6H_2O$ 0.24, $MnCl_2{\cdot}4H_2O$ 0.99, $CuSO_4{\cdot}5H_2O$ 0.25, $NaMoO_4{\cdot}2H_2O$ 0.22, $NiCl_2{\cdot}2H_2O$ 0.19, $NaSeO_4{\cdot}10H_2O$ 0.21, H_3BO_4 0.014, and $NaWO_4{\cdot}2H_2O$ 0.050. Ammonium and nitrite were added in the mineral medium as the form of $(NH_4)_2SO_4$ and $NaNO_2$ based on the required amount. Renew the wastewater every 2 d to exclude the obstructions caused by changes of water composition and other bacteria. Synthetic wastewater was flushed with N_2 gas to drive away DO and create an anaerobic environment every time it was prepared. The seed sludge was collected from the aerobic tank of Shengke Wastewater Treatment plant in Tianjin. The sludge characteristics are listed as followed: mixed liquor volatile suspended solids (MLVSS): 1833 mg/L; mixed liquor suspended solids (MLSS): 2328 mg/L; MLVSS/MLSS: 78.74%.

2.4. Analytical items and methods

Tests were conducted every other day to monitor the influent and effluent quality. According to the colorimetric method [11], Ammonia, nitrite and nitrate were determined spectrophotometrically. The pH was determined with a digital portable pH meter (PHS-3C, Rex, China) and the temperature was measure by a digital portable DO meter (YSI, Model 55, USA). The SEM observation was carried out according to the procedure as below: firstly, the samples were fixed with 3% glutaraldehyde for 1 h, and pH was 7.2 controlled by 0.1 M phosphate buffer solution; secondly, the fixed sample were dehydrated with 60%, 80% and 100% of ethanol, and each dehydration procedure lasted for 10 min; lastly, samples were dried at evaporation point of ethanol followed by mounting and coating. After all the preparations, SEM observation was conducted by the machine (JEOL JSM-5600LV, Tokyo, Japan). Quantitative PCR (qPCR) was conducted to investigate the percentage of ANAMMOX bacteria to the total bacteria in the mature biofilm in the improved FBR with the mixed carriers.

2.5. Operating strategy

The synthetic wastewater prepared was fed continuously by a peristaltic pump from the bottom of FBR to guarantee sufficient mixture and distribution of the wastewater. The temperature was controlled at 35°C and pH was approximately 8.0 in the FBR. During the start-up period (day 1~day 53), the concentration of NH_4^+-N and NO_2^--N in the influent were 70 mg/L and the inflow velocity was 0.90 ml/min. Correspondingly, HRT was maintained at 2 d and correspondingly TN loading was 0.07 kg $N{\cdot}m^{-3}{\cdot}d^{-1}$. After a successful ANAMMOX start-up, gradual enhancement of the influent nitrogen loading was realized by the way of increasing nitrogen concentration from 100 mg/L to 230 mg/L (day 55~day 71) and by increasing the inflow velocity from 0.90 ml/min to 1.20 ml/min (day 73~day 87). Correspondingly, HRT was from 2 days to 1.5 days and TN loading was from 0.10 kg $N{\cdot}m^{-3}{\cdot}d^{-1}$ to 0.31 kg $N{\cdot}m^{-3}{\cdot}d^{-1}$.

3. Results and discussion

3.1. Start-up period

Honeycomb-like polypropylene carriers and ring non-woven cloth carriers were mixed in equal proportion to improve the Anammox performance in a FBR. The mixed carriers have the advantages as follows: (1) the honeycomb-like polypropylene structure and micro-porous fiber structure could enhance the retention of Anammox bacteria; (2) the biogas (mainly N_2) could be easily discharged from inter spaces among the honeycomb-like carriers and holes in them to avoid gas entrapment. The temperature was maintained at 35°C and the pH was kept at 8.0 ± 0.1 in the reactor while the influent DO concentration was controlled below 0.05 mg/L. Variations of NH_4^+-N, NO_2^--N and NO_3^--N over time are shown as Fig. 2. The start-up period sustained for 53 d, including 3 phases: Phase 1 (sludge conversion phase, 1 ~ 19 d), Phase 2 (lagging phase, 19 ~ 29 d) and Phase 3 (activity increasing phase, 29 ~ 53 d), respectively. The HRT was controlled at 48 h and NH_4^+-N and NO_2^--N concentrations in the influent were maintained at 70 mg/L, corresponding that nitrogen loading rate (NLR) was 0.07 kg N m^3 d^{-1}.

In Phase 1, influent NH_4^+-N concentration was obviously higher than that of effluent, while NO_2^--N concentration showed a different change trend. The concentration of NO_2^--N in effluent dropped sharply. Nitrate production was nearly none. These phenomena could be observed in other reports [12,13]. Reasonable explanations were that, owing to environment changes from aerobic situation to anaerobic situation, most aerobic bacteria in the seeding sludge disintegrated. Both enough organic carbon source released by cell lyses and anaerobic condition were extremely beneficial to the growth of denitrifying bacteria. Thus proteins were metabolized to NH_4^+-N. Besides the ANAMMOX activity was very faint in the presence of organic carbon source so the consumption of NH_4^+-N was extremely limited and not observed. Consequently, a higher NH_4^+-N concentration in the effluent was than that in the influent. Nitrites were mainly converted to nitrogen gas through denitrification process so the nitrite amount declined greatly. On day 11, the maximum NH_4^+-N concentration in the effluent was 97.06 mg/L, 27.06 mg/L higher than that in the influent while NO_2^--N concentration fell down to almost zero. In this phase, there was no sign of ANAMMOX activity and endogenous denitrification was predominant.

During Phase 2, the influent NH_4^+-N concentration was close to the effluent NH_4^+-N and there was no ANAMMOX activity. Whereas, NO_2^--N concentration was on a slight rising trend, but still could be removed, implying weakening of the denitrifying activity. Due to the insufficient organic matters in the influent, denitrifying bacteria underwent an inferior position in the fierce competition for the limited organic source released from cell lysis of non-adapted bacteria. The denitrifying activity decreased, so that NO_2^--N consumption by denitrifying bacteria were declined. ANAMMOX bacteria could survive well in the organic-matter-less and anaerobic condition. However, the ANAMMOX activity was not observed due to the extremely growth of ANAMMOX bacteria. Denitrifying bacteria no longer took up dominant status but the amount of ANAMMOX bacteria was still limited.

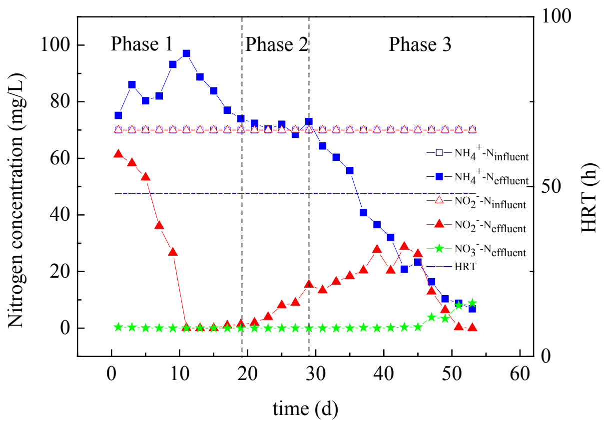


Fig. 2. Nitrogen removal effects of ANAMMOX during the start-up period.

In Phase 3, NH_4^+-N and NO_2^--N were removed simultaneously and continuously and the removed amounts of NH_4^+-N and NO_2^--N increased gradually, indicating the ANAMMOX activity gradually increased. On day 29, ANAMMOX activity occurred for the first time. The time for ANAMMOX occurrence was clearly shorter than that reported by the previous literatures [14–16]. Qin et al. [14] and Tang et al. [15] firstly observed ANAMMOX activity after about 2 months' operation. Nutchanat et al. [16] even required longer time (120 days) to observe ANAMMOX activity for the first time. In this phase, NO_3^--N production increased obviously. On day 53, NH_4^+-N and NO_2^--N concentration were decreased to 6.83 mg/L and 0 mg/L with NO_3^--N increasing to 8.85 mg/L. The max removal rate of NH_4^+-N and NO_2^--N reached 90.24% and 100% respectively, corresponding to TN removal rate of 6.22×10^{-2} kg N·m^{-3}·d^{-1} and TN removal efficiency of 88.8%. These results demonstrated ANAMMOX process was started up successfully in the mixed-carrier FBR, and the start-up period was significantly shorter than the period reported by Chen et al. [17] (longer than 85 d) and Li et al. [18] (91 d). FBR filled with the mixture of honeycomb-like polypropylene carriers and ring non-woven cloth carriers could accomplish ANAMMOX start-up process in a shorter time. The mixed carriers gave an advantage of high-efficient biomass attachment, easy gas discharge and adequate substrate transfer thus ANAMMOX bacteria could be effectively enriched and as a result the start-up period was shortened.

3.2. NL enhancing period

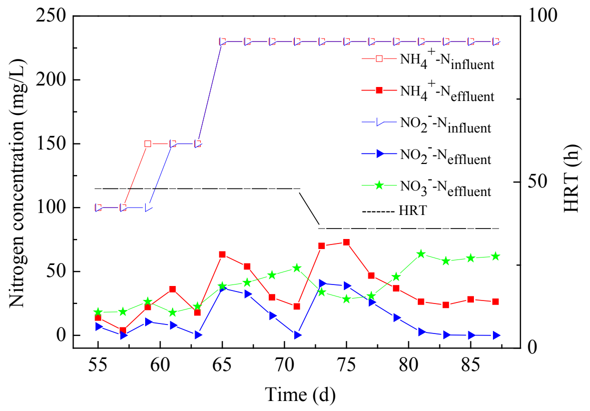
NL was stepwise enhanced from 55 d to 87 d. NL increase was accomplished by means of increasing the concentrations of substrates and reducing HRT. The calculations of TN loading rate and removal rate are shown in Eqs. (2) and (3). During 55 d ~ 71 d, the influent NH_4^+-N and NO_2^--N concentrations were elevated from 100 mg/L through 150 mg/L to 230 mg/L, while HRT was fixed at 48 h. From 73 d to 87 d, the influent NH_4^+-N and NO_2^--N concentrations were unchanged at 230 mg/L, whereas HRT was shortened from 48 h to 36 h.

$$\text{TN loading rate} = \text{influent TN}/\text{HRT} \tag{2}$$

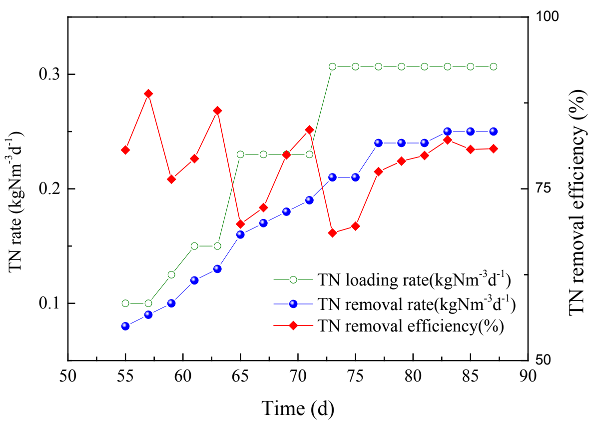
$$\text{TN removal rate} = (\text{influent TN} - \text{effluent TN})/\text{HRT} \tag{3}$$

3.2.1. Nitrogen removal effects

Nitrogen removal performance of the FBR with the mixed carriers during the NL enhancing period is shown in Fig. 3. In Fig. 3a, every time NL was raised, both NH_4^+-N concentration and NO_2^--N concentration in the effluent showed an immediate rise and after 4~8 d operation they decreased to a low level equivalent to the level before each time of NL increase. The nitrogen removal performance of the FBR filled with mixture of honeycomb-like polypropylene carriers and ring non-woven cloth carriers fluctuated and trended to be stable. During the NL enhancing period, the nitrogen removal performance of the FBR was not heavily inhibited by the accumulation of NO_2^--N. The reason was that the maximum value of NO_2^--N concentration in the effluent was 40.63 mg/L, which was below the NO_2^--N inhibit concentration of 100 mg/L reported by Strous et al. [19] and Jin et al. [20]. The results indicated that the FBR with the mixed carriers had a good resistance of nitrogen concentration shock and hydraulic shock. NO_3^--N concentration was slightly decreased firstly, increased subsequently, and finally tended to a steady constant of 60 mg/L. The final NH_4^+-N and NO_2^--N concentration trended to decrease to nearly 30 mg/L and 0 mg/L. According to Eq. (1), the increase of NO_3^--N production, the improvement of NH_4^+-N and NO_2^--N removal and NH_4^+-N and NO_2^--N in the effluent ranging in a low concentration suggested that nitrogen removal effects through ANAMMOX reaction were stable and enhanced gradually.



(a) Variations of nitrogen concentration in NL enhancement period



(b) Variations of TN removal with time in NL enhancement period

Fig. 3. The efficiency of nitrogen removal in NL enhancing period.

According to Fig. 3b, TN removal rate increased from 0.08 kg N·m^{-3}·d^{-1} to 0.25 kg N·m^{-3}·d^{-1} as TN loading rate was enhanced from 0.10 kg N·m^{-3}·d^{-1} to 0.31 kg N·m^{-3}·d^{-1}. The maximum TN loading rate (0.31 kg N·m^{-3}·d^{-1}) achieved in the improved FBR with mixed carriers was better than the maximum TN loading rate (0.27 kg N·m^{-3}·d^{-1}) achieved in another anaerobic up-flow FBR seeded with nitrifying and denitrifying sludge, while the maximum TN removal rates in the two FBRs both attained 0.25 kg N·m^{-3}·d^{-1} in the case of the maximum TN loading rate [21]. The results indicated that the improved FBR exhibited an better nitrogen removal performance compared with the FBR seeded with nitrifying and denitrifying sludge. In this study, honeycomb-like polypropylene carriers and ring non-woven carriers were filled into the FBR. Both of the carriers were beneficial to the enrichment of ANAMMOX bacteria. Simultaneously, honeycomb-like polypropylene carriers has a porous and corrugated structure, which could promote the nitrogen discharge. Thus the scouring effects in a small scale could renew the biofilm and enhance substrate transfer. The advantages of mixed carriers ensured FBR a good nitrogen removal performance at a high nitrogen loading.

In the NL enhancing period, TN loading rate was gradually enhanced 5 times as Fig. 4 depicted. In each condition of TN loading rate, an average value of TN removal rate was calculated. It can be seen from Fig. 4 that the average TN removal rate has a good correlation with TN loading rate in the range from 0.10 to 0.31 kg N·m^{-3}·d^{-1} during the NL enhancing period. The results indicates that the strong inhibition of ANAMMOX activity did not occur as the TN loading rate increased. Then a linear fit according to the scatters was conducted by Origin Pro 8.5.1. An equation was concluded as Eq. (4). The value of Square of R was larger than 0.997, indicating the equation has a higher fitting degree. P value is 3×10^{-5} so it is significant.

$$y = 0.0123 + 0.072183x \tag{4}$$

According to Fig. 4, the average of TN removal rate increased from 0.09 kg N·m^{-3}·d^{-1} to 0.24 kg N·m^{-3}·d^{-1} as TN loading rate was enhanced from 0.10 kg N·m^{-3}·d^{-1} to 0.31 kg N·m^{-3}·d^{-1}, suggesting a stable operation in FBR filled with mixture of honeycomb-like polypropylene carriers and ring non-woven cloth carriers. The mixed carriers processed the combined advantages of gas channels, substrate transfer improvement and biomass attachment reinforcement so that the reactor operation performance could be improved steadily as the nitrogen loading increased.

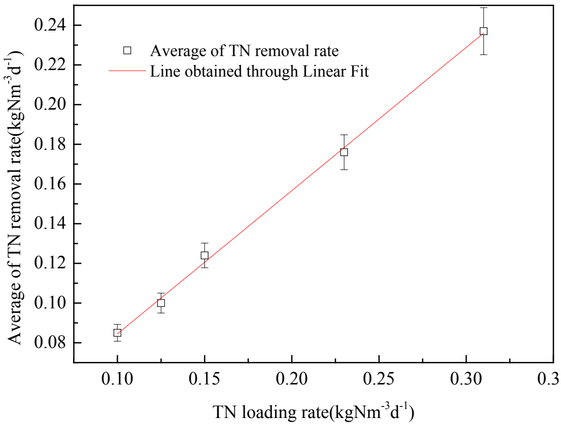


Fig. 4. Variations of average of TN removal rate profile in each TN loading rate in NL enhancing period.

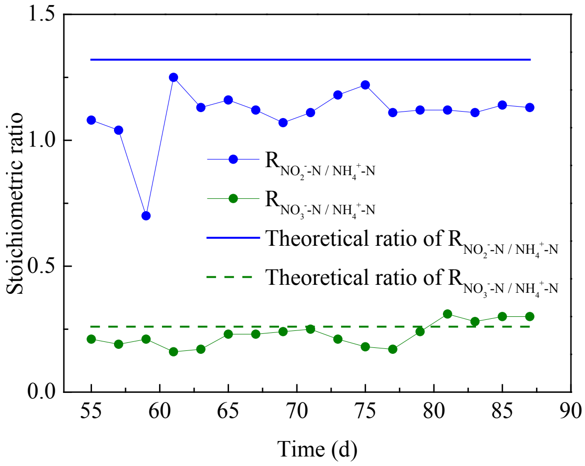


Fig. 5. Stoichiometric ratio changes with time in NL enhancing period.

3.2.2. Stoichiometric ratio changes

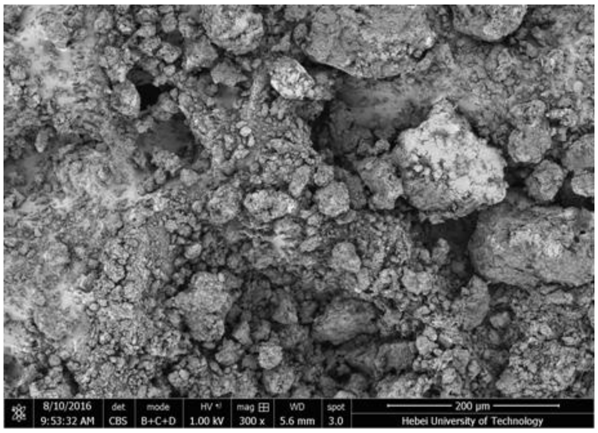
$$R_{NO2--N/NH4+-N} = (\text{influent } NO_2^-\text{-N} - \text{effluent } NO_2^-\text{-N})/(\text{influent } NH_4^+\text{-N} - \text{effluent } NH_4^+\text{-N}) \tag{5}$$

$$R_{NO2--N/NH4+-N} = \text{effluent } NO_3^-\text{-N}/(\text{influent } NH_4^+\text{-N} - \text{effluent } NH_4^+\text{-N}) \tag{6}$$

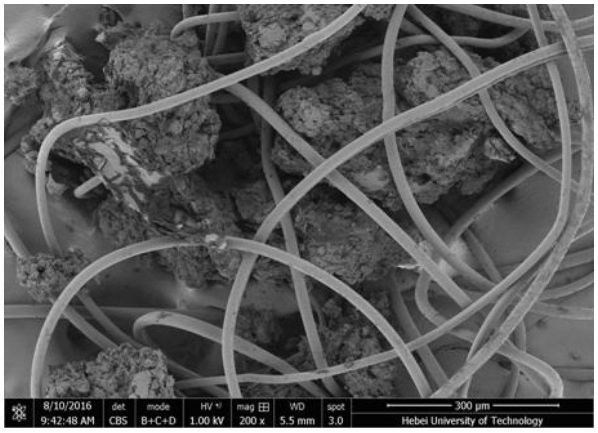
During the NL enhancing period, the stoichiometric ratios of ANAMMOX reaction were calculated based on Eqs. (5) and (6), which changed over time. As Fig. 5 illustrates, stoichiometric ratio of NO_2^--N removal to NH_4^+-N removal ($R_{NO2--N/NH4+-N}$) declined significantly when NL was raised on day 57 and after 2 d operation rebounded immediately. Later $R_{NO2--N/NH4+-N}$ verged to a certain value of 1.12, but slightly lower than theoretical 1.32 [22]. While the stoichiometric ratio of NO_3^--N production to NH_4^+-N removal ($R_{NO3--N/NH4+-N}$) was on a slight fluctuation and then verged to the value of 0.30, approaching to theoretical 0.26 [22] but slightly higher, indicating that nitrifying bacteria and ANAMMOX bacteria were attached to the mixed carriers. A small quantity of aerobic ammonium oxidizing bacteria (AOB) could coexist with ANAMMOX bacteria due to oxygen leakage of the FBR. Moreover, the pH of the FBR was controlled around 8 so NH_4^+-N stripping could occur. NH_4^+-N conversion by AOB and NH_4^+-N stripping could be two possible processes causing a decrease of $R_{NO2--N/NH4+-N}$. And also a small amount of NH_4^+-N was transformed into NO_2^--N and even into NO_3^--N by a small quantity of AOB and nitrite oxidizing bacteria (NOB), resulting in a slightly higher NO_3^--N production. So the $R_{NO2--N/NH4+-N}$ and $R_{NO3--N/NH4+-N}$ was displayed as demonstrated above. The timely consumption of oxygen by the nitrifying pathway could create an anaerobic condition and help ANAMMOX bacteria relieve the risk of oxygen stress. It is also the reason why nitrifying bacteria and ANAMMOX bacteria can co-exist in a single reactor [23] and bring benefits to each other in some respects.

3.3. Biofilm characterization

The morphology of bacteria in the mature biofilm was observed through SEM as Fig. 6 demonstrates. The mature



(a) biofilm on the honeycomb-like polypropylene carriers



(b) biofilm on the ring non-woven cloth carriers

Fig. 6. The SEM observation of the morphology of biofilm on the 87th day.

biofilm samples were collected from the honeycomb-like polypropylene carriers and ring non-woven cloth carriers on day 87. The results of qPCR demonstrated that the percentage of ANAMMOX bacteria to the total bacteria in the mature biofilm from the honeycomb-like carriers reached 63.8% and the percentage of ANAMMOX bacteria to the total bacteria in the mature biofilm from the non-woven cloth carriers reached 73.7%. In Fig. 6a the bacteria in the mature biofilm from the honeycomb-like polypropylene carriers displayed a morphology of self-aggregates in large or small size. While the bacteria in the mature biofilm from the ring non-woven cloth carriers agglomerated together and the aggregates were enwrapped by the non-woven fibers as described in Fig. 6b. The mature biofilms on both the two kinds of carriers had the cauliflower structure, which was proved to be the typical characteristic of mature ANAMMOX biofilm or sludge [24–26]. Besides, both of the two biofilms comprised spherical shaped bacteria and short rod-shaped bacteria based on the photograph. Similar bacterial composition was discovered by Wang et al. [25], Ni et al. [27] and Zeng et al. [26], who reported that spherical shaped bacteria were dominant in ANAMMOX biofilm microorganisms. On the 87th day, TN loading rate was up to 0.31 kg N·m^{-3}·d^{-1} and TN removal rate was 0.25 kg N·m^{-3}·d^{-1}. The FBR attained a higher enrichment of bacteria for the mixture of carriers. Honeycomb-like polypropylene carriers provided a gas passage, which could produce scouring effects and renew the biofilm to improve substrate transferring. The non-woven cloth carriers possessed the function of enwrapping the biomass up and promoting the biomass attachment. The combination could exploit the advantages of the two carriers to ensure the system to attain an expected nitrogen removal performance.

4. Conclusion

Successful start-up of ANAMMOX from the traditional activated sludge was achieved within 2 months in an FBR filled with mixture of honeycomb-like polypropylene carriers and ring non-woven carriers at ratio of 1:1. In the start-up process, ANAMMOX activity first appeared on day 21 and the NH_4^+-N, NO_2^--N and TN removal efficiency were up to 90.24%, 100%, 88.8%, respectively on day 53. During NL enhancement stage, the FBR exhibited a good nitrogen removal performance as the max TN loading rate and TN removal rate reached 0.31 kg N·m^{-3}·d^{-1} and 0.25 kg N·m^{-3}·d^{-1} respectively. The reactor could resist both substrates concentration loading shock and hydraulic shock. SEM photos showed that the mature biofilm taken from the honeycomb-like polypropylene carriers presented a form of self-aggregation while the mature biofilm in the ring non-woven cloth carriers were bounded by fine non-woven fibers. The mature biofilms in the two kinds of carriers possessed the typical ANAMMOX biofilm structure- cauliflower appearance. The results demonstrates that the FBR with the mixed carriers is suitable for ANAMMOX operation and can improve ANAMMOX performance.

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