The development of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal

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A B S T R A C T
The simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process was validated to potentially remove ammonium and COD from wastewater in a single, oxygen-limited, non-woven rotating biological contactor (NRBC) reactor. An ammonium conversion efficiency of 79%, TN removal efficiency of 70% and COD removal efficiency of 94% were obtained with the nitrogen and COD loading rate of 0.69 kg N/m³ d and 0.34 kg/m³ d, respectively. Scanning electron microscopy (SEM) observation and fluorescence in situ hybridizations (FISH) analysis revealed the existence of the dominant groups of bacteria. As a result, the aerobic ammonia-oxidizing bacteria (AOB), with a spot of aerobic heterotrophic bacteria were mainly distributed in the aerobic outer part of the biofilm. However, ANAMMOX bacteria with denitrifying bacteria were present and active in the anaerobic inner part of the SNAD biofilm. These bacteria were found to exist in a dynamic equilibrium to achieve simultaneous nitrogen and COD removal in NRBC system.

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1. Introduction

Ammonium pollution from sources such as food processing and agricultural industries is a growing concern to the water environment and as such has contributed to the increasing demand for more effective methods of nitrogen abatement (Hoa et al., 2006). Conventional biological removal of nitrogen from municipal and industrial wastewaters has been widely studied. It consists of two steps, aerobic nitrification and anaerobic denitrification, which are carried out in two separate reactors. But in many wastewaters, the low level of organic carbon is hardly sufficient for complete denitrification, and addition of an external organic matter source (methanol or acetic acid) is often necessary to achieve complete denitrification (Ahn, 2006). This increases the operating costs in the wastewater treatment plant due to the cost of the chemicals added and the treatment of the surplus sludge that is generated (Gong et al., 2008).

Recently, more attention has been focused on the novel and cost-effective biological nitrogen removal processes: such as anaerobic ammonium oxidation (ANAMMOX) (Jetten et al., 1999), single reactor high-activity ammonia removal over nitrite (SHARON) (Mulder and van Kempen, 1997), a combined process with two separate reactors in series of SHARON and ANAMMOX (SHARON/ANAMMOX) (van Dongen et al., 2001), completely autotrophic nitrogen removal over nitrite (CANON) (Sliekers et al., 2002), oxygen-limited autotrophic nitrification–denitrification (OLAND) (Pynaert et al., 2004) and single-stage nitrogen removal using ANAMMOX and partial nitratation (SNAP) (Lieu et al., 2006). ANAMMOX is based on energy conversion from anaerobic ammonium oxidation using nitrite as the electron acceptor (Eq. (2)) (Jetten et al., 1999). This process can remove ammonium from high-concentrated stream with addition of nitrite, but more substantial experiments showed that oxygen and low-organic carbon can completely inhibit the ANAMMOX activity when it is exposed to the enrichment culture (Strous et al., 1997; Waki et al., 2007). Thus, ANAMMOX process can be obtained under strictly anoxic and devoid of organic carbon source conditions. And then, a combination of ANAMMOX and denitrification process was studied to make ANAMMOX process be used extensively in the ammonium-rich wastewater (Dong and Ernest, 2003; Pathak et al., 2007; Chamchoi et al., 2008). It clearly demonstrated that ANAMMOX and denitrification processes could coexist in same environment. Another new principle for nitrogen removal process has been named CANON, an acronym for completely autotrophic nitrogen removal over nitrite. It only needs a single oxygen-limited step, the interaction of partial nitrification and ANAMMOX results in an almost complete conversion of ammonium to dinitrogen gas, along with small amounts of nitrate. It relies on the interaction of two groups of autotrophic bacteria (aerobic ammonia-oxidizing bacteria (AOB) and ANAMMOX bacteria) under oxygen-limiting conditions.
conditions that perform two sequential reactions, simultaneously. Under oxygen limitation, partial ammonium is oxidized to nitrite by aerobic ammonium oxidizers, such as *Nitrosomonas* and *Nitrobacter* (Eq. (1)) (Third et al., 2001), the ANAMMOX bacteria are protected from oxygen by this way. Following that the produced nitrite is utilized with the remainder of the ammonium by ANAMMOX bacteria and converted into dinitrogen gas (Eq. (2)) (Nielsen et al., 2005). The combination of the above two reactions (Eq. (3)) makes the removal of nitrogen successfully.

\[
\begin{align*}
2NH_4^+ + 3O_2 & \rightarrow 2NO_2^- + 4H^+ + 2H_2O \\
NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ & \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CHO_{0.3}N_{0.15} + 2.03H_2O \\
NH_4^+ + 0.85O_2 & \rightarrow 0.435N_2 + 0.13NO_3^- + 1.3H_2O + 1.4H^+ 
\end{align*}
\]

The CANON process can be obtained under the interaction of aerobic and anaerobic ammonium oxidizing bacteria with very low aeration in a single reactor, which is greatly reducing cost and energy requirements (consumes 63% less oxygen and 10% less reducing agent than traditional nitrogen removal systems). It is just suitable for the high-concentrated ammonium wastewater without COD. However, most of the ammonium-rich wastewater was produced with a certain concentration range of COD (such as, e.g., old landfill leachates). Thus, another new process was necessary to be studied. Denitrification was added into the CANON process to solve this problem. In this way, ammonium and COD can be removed in via of the partial nitrification, ANAMMOX and denitrification simultaneously in a single reactor (SNAD). However, as far as we know, the SNAD process has not yet been investigated in any previous experiments.

In this study, a novel non-woven rotating biological contactor (NRBC) reactor (Liu et al., 2008) was applied for the SNAD process. Non-woven rotating bio-disc has stronger adhesive ability than other fillers that allows for microorganisms to adhere and colonize throughout the material, thus providing favorable conditions for retention and cultivation of slowly growing anaerobic microorganisms (Rouse et al., 2005), making the microorganisms layered very easy, in addition. This is generally based on the principle that partial nitrification requires a certain aerobic condition for oxidation of ammonia, whereas denitrification (Pochana and Keller, 1999) and ANAMMOX occurs under anoxic condition in the presence of electron donors (Tchobanoglous et al., 2004). Under oxygen limitation, ammonium is oxidized to nitrite by AOB, the nitrite in the reactor can be used by ANAMMOX bacteria with ammonium, and finally to dinitrogen gas with small amounts of nitrate produced (Strous, 2000). Afterwards, COD as electron donor could oxidize nitrate to dinitrogen gas through denitrifying process for the completely nitrogen removal performance. The interaction of aerobic nitrifying, anaerobic ammonium oxidizing and anaerobic denitrifying bacteria under oxygen limitation has the potential to make an almost complete conversion of ammonium and organic carbon to dinitrogen gas and carbon dioxide.

The purpose of this study was to develop the SAND process for the simultaneous nitrogen and COD removal for the high-strength ammonium, but low-carbon wastewater treatment in the NRBC reactor. Some controlling strategies were optimized and the reactor performance was examined in the reactor experiment. The biological community and spatial distribution of AOB, ANAMMOX and denitrifying bacteria on the non-woven bio-disc was also investigated by scanning electron microscopy (SEM) observation and fluorescence in situ hybridizations (FISH), to yield valuable information toward understanding the biological foundation of this SNAD process.

### 2. Methods

#### 2.1. Synthetic wastewater

NH$_4^+$-N and NO$_2^-$-N were added into the influent synthetic wastewater in the form of (NH$_4$)$_2$SO$_4$ and NaNO$_2$, respectively. The composition of the mineral medium was: 1.25 g KHCO$_3$, 0.025 g KH$_2$PO$_4$, 0.3 g CaCl$_2$·2H$_2$O, 0.2 g MgSO$_4$·7H$_2$O, 0.00625 g FeSO$_4$, 0.00625 g EDTA per liter and 1 mL/L of trace elements solution. The trace element solution contained: 15 g EDTA, 0.43 g ZnSO$_4$·7H$_2$O, 0.24 g CoCl$_2$·6H$_2$O, 0.99 g MnCl$_2$·4H$_2$O, 0.25 g CuSO$_4$·5H$_2$O, 0.22 g NaMoO$_4$·2H$_2$O, 0.19 g NiCl$_2$·6H$_2$O, 0.21 g NaSeO$_4$·10H$_2$O, 0.014 g H$_2$BO$_4$, 0.05 g NaWO$_4$·2H$_2$O per liter. The compositions were based on the previous studies (Third et al., 2005).

#### 2.2. Inoculation sludge

The ANAMMOX biomass was taken from an ANAMMOX up-flow column reactor which packed with polyester non-woven biomass carrier (Fujii et al., 2002). The biomass was consisted of the planctomycete-like ANAMMOX bacteria. And then cultured in a sealed tank at 35 °C in an incubator (GHP-9160, China). The bacteria are chemolithoautotrophic, have a doubling time of 11 days. They have a very high affinity for the substrates ammonia and nitrite. As previously reported, they can be reversibly inhibited by oxygen and irreversibly by nitrite and phosphate (Strous et al., 1999).

The partial nitrification biomass was taken from an oxygen-limited nitrifying chemostat with temperature of 35 °C. pH of 8–8.2. The russet sludge concentration was 3.1 g VSS/L. It could oxidize NH$_4^+$-N to NO$_2^-$-N and NO$_3^-$-N at rate of 6.9 mg/g VSS h and 0.3 mg/g VSS h, respectively, which showed high-nitrosation activity.

#### 2.3. Reactor start-up and operational strategy

The small scale NRBC reactor used in the experiments was constructed of polymethyl methacrylate with a cover in the top. Each plate was mounted on a horizontal shaft by fixing with stainless steel and driven by a rotating contactor. The porous non-woven fabric (3 μm aperture) with a pyridinium-type polymer (Japan Vi-lene, US patent 5, 185, 415, 1993) was employed as a bacterial support. Temperature of liquid inside reactors was maintained at 35 °C by using a water jacket, which was referred to be the optimum temperature for AOB and ANAMMOX cultivation (Kim et al., 2006). A black-vinyl sheet enclosure was used to keep the bacteria away from the inhibition of light. The liquid was pumped into the bottom of the reactor and then recirculated by the rotation of the bio-disc in order to prevent toxic high-nitrate concentrations at the bottom of the reactor.

In the first phase, ANAMMOX process was started up strictly anaerobic conditions with the inoculation of ANAMMOX bacteria. The hydraulic retention time (HRT) was controlled by a peristaltic pump. The discs were rotated at 0.5 rpm to mix the substrate with 100% submergence of the disc surface area.

In the second phase, the cover was taken off for the entering of air, and then partial nitrification seed sludge was added into the NRBC reactor. The CANON process was conducted under controlling DO at the range of 0.5–0.7 mg/L by regulating the revolution of the bio-disc and the liquid level of the reactor. The influent medium was changed into an (NH$_4$)$_2$SO$_4$-based synthetic wastewater without NaNO$_2$ and other compositions were remained as above mentioned.

In the third phase, SNAD process was started up with addition of COD when the NH$_4^+$-N removal efficiencies were increased to 80%.
2.4. Chemical analysis

The concentrations of nitrogen compounds and volatile suspended solids (VSS) were measured according to standard methods (APHA, 1998). The NH$_4^+$-N and NO$_3^-$-N were measured by using the different colorimetric methods and NO$_2^-$-N was analyzed by using ultraviolet spectrophotometric method. Total nitrogen (TN) was measured by using the TOC analyzer equipped with a total nitrogen-measuring unit (TOC-VCPh, Shimadzu). The pH was determined potentiometrically with a digital, portable pH meter. The DO level was measured with a digital, portable DO meter (YSI, Model 55, USA).

2.5. SEM observation

Morphology characteristics of the biomass specimens were observed using SEM model JEOL JSM-5600LV. The samples for SEM were cut from the NRBC reactor by using a sterile blade. The biomass specimens were scraped from the inner and outer layers of the non-woven fabric carrier by a sterile blade, respectively, and then fixed by glutaraldehyde in paraformaldehyde solution for 3 h. Subsequently, dehydrated through a graded series of ethanol solutions: 25%, 50%, 75%, 90% and 100% (three times for each concentration), then gold-coated by a sputter.

2.6. FISH analysis

FISH and DAPI staining were performed according to the standard hybridization protocol (Amann, 1995). The following 16S rRNA-targeted oligonucleotide probes used for in situ detection the bacteria community composition in the SNAD process were purchased from TaKaRa Company (Dalian, China). The probes used in this study were fluorescein isothiocyanate (FITC) labeled EUB338 (Daims et al., 1999), CY5 labeled AMX820 (Schmid et al., 2005) and CY3 labeled NSO190 (Biesterfeld et al., 2001). Hybridizations were performed on 4% (w/v) paraformaldehyde-fixed biofilm samples for 2–4 h (Lee et al., 1999). For image acquisitions, an epifluorescence microscope (OlympusBX51, Japan) was used together with the standard software package delivered with the instrument (version 4.0). A Leica TCS-SP2 confocal scanning laser microscope (CSLM) (Leica, Germany) was used to observe more detailed localization.

3. Results and discussion

3.1. Nitrogen removal performances of the ANAMMOX stage

The NRBC reactor for the ANAMMOX process was started up in the experiment. Fig. 1 shows the results of the influent and effluent concentration of nitrogenous compounds. Under strictly anoxic condition, the process was operated with the same operational conditions of pH and temperature was kept at 8–8.2 and 35 °C, respectively. Influent NH$_4^+$-N and NO$_2^-$-N for the reactor were increased stepwise from 100 mg/L to 200 mg/L during this period. We gradually enhanced the influent concentration and shortened the HRT in order to increase the loading rate. Excellent treatment results were obtained with maximum NH$_4^+$-N and NO$_2^-$-N removal efficiencies of 90% and 94%, respectively.

From Fig. 1, we can see that there is a big fluctuation of the reactor performance during the first 21 days as the unsteady start-up period. On day 22, the removal efficiencies of NH$_4^+$-N and NO$_2^-$-N reached 94% and 96%, respectively, TN removal efficiency reached 86%, which fully indicated high activity of the ANAMMOX bacteria in the reactor. The removal efficiencies decreased when the influent concentrations of NH$_4^+$-N and NO$_2^-$-N were increased from 200 mg/L to 250 mg/L on the 28th day. Thus, influent NH$_4^+$-N and NO$_3^-$-N concentrations were both reduced to 200 mg/L to avoid the inhibition. Meanwhile, HRT was decreased from 13 h to 8 h in order to increase the loading of the reactor. The removal efficiencies of NH$_3^-$-N and NO$_3^-$-N increased regularly from 34% to 89% and 36% to 86% (days 52–81), respectively. TN removal efficiency reached 82% during the period. During the next 7 days (days 1550

H. Chen et al. / Bioresource Technology 100 (2009) 1548–1554

Fig. 1. Time courses of influent and effluent concentrations of nitrogen compounds during the operation of the ANAMMOX process with different HRTs.
82–88), the inflow was kept at 4.8 L/d (HRT of 6 h), and the removal efficiencies of NH$_4^+$–N and NO$_2^-$–N reached 95% and 93%, respectively. TN removal efficiency increased to 93%. The high-TN removal efficiencies obtained in this study showed that the ANAMMOX bacteria grew actively in the NRBC reactor. With an HRT of 6–5 h during the last 6 days, the removal efficiencies of NH$_4^+$–N and NO$_2^-$–N recovered to 90% and 94%, respectively. In addition, the TN loading rate was as high as about 2.1 kg N/m$^3$ reactor d. High-shock resistance had also been obtained by the ANAMMOX bacteria in this phase. Therefore, the ANAMMOX process in this study showed a high-ANAMMOX activity and shock resistance for high-strength ammonium wastewater, which provided good foundation for the following experiments.

3.2. Nitrogen removal performances during the stage of partial nitrification and ANAMMOX simultaneously

The CANON process was started up with taking off the cover, 1 L partial nitrification sludge was added into the NRBC reactor. Partial nitrification biomass was adsorbed on the polyester non-woven fabric carrier very well. DO in the reactor was controlled at about 0.5–0.7 mg/L by adjusting the liquid level in the region of three fifth of effective volume and changing the rotational speed of the plate at 2 rpm/min, while keeping the other operating parameters unchanged. The feed was changed to contain about 200 mg/L NH$_4^+$–N only. Fig. 2 shows the results of continuous operation stage of partial nitrification and ANAMMOX simultaneously.

Influent NH$_4^+$–N concentration was maintained at about 200 mg/L during this stage. During the first 11 days of this period, the effluent NH$_4^+$–N concentration decreased regularly from mg/L 122.8 to 79.2 mg/L. The NH$_4^+$–N removal efficiency was increased from 55% to 61%. However, the effluent NO$_2^-$–N concentration was lower than 4 mg/L which could be neglected. Under oxygen limitation, partial nitrification took place dominantly in the aerobic region of biofilm. The AOB oxidized ammonium to nitrite, consumed dissolved oxygen and so created an anaerobic microenvironment for the inner biofilm, where the produced nitrite was utilized with remainder of the ammonium by the ANAMMOX bacteria and converted into dinitrogen gas, as nitrite serves as electron donor and ammonium as electron acceptor (Strous, 2000). Thus, influent NH$_4^+$–N was removed with little NO$_3^-$–N produced. During the next 7 days (days 12–19), the NH$_4^+$–N removal efficiency maintained at about 61%. This was due to the competition of the substrate between AOB and ANAMMOX bacteria. As a substrate of the partial nitrification and ANAMMOX, NH$_4^+$–N was competed synchronously by AOB and ANAMMOX bacteria in the inner biofilm, and finally reached a new balance finally. From days 20 to 41, the NH$_4^+$–N removal efficiency increased from 61% to 81%, TN removal efficiency reached 72%. Nitrogen removal performances maintained at a stable stage in the last 12 days (days 30–41), the interspecific competition of AOB and ANAMMOX bacteria reached a new balance that could remove NH$_4^+$–N efficiently and stably. It was apparent that single-stage autotrophic nitrogen removal was achieved by the close cooperation between AOB and ANAMMOX bacteria.

3.3. Nitrogen removal performances during the stage of SNAD

To start-up the SNAD process under the same operating conditions, COD was introduced into the influent synthetic wastewater. After the addition of COD, DO in the reactor was slightly decreased to 0.4–0.6 mg/L. Fig. 3 shows the results of continuous operation of this stage. During the first period of 26 days, the concentration of COD was constant at 150 mg/L. We can see that there is a big fluctuation of the reactor performance in the first 11 days. The effluent concentration of NH$_4^+$–N and NO$_2^-$–N increased from 65.8 mg/L to 95.4 mg/L and 4.3 mg/L to 14.5 mg/L, respectively. However, the effluent concentration of COD decreased from 116.5 mg/L to 47.4 mg/L with the effluent concentration of NO$_2^-$–N levels gradually reached zero. From days 12 to 26, the effluent concentrations of NH$_4^+$–N and COD did not change obviously, however the effluent concentration of NO$_3^-$–N reduced to 1.4 mg/L. These results conjectured that partial nitrification took place
dominantly in the aerobic biofilm, ANAMMOX and denitrification took place dominantly in the anaerobic biofilm. The AOB oxidized ammonia to nitrite, consumed dissolved oxygen and so created an anaerobic microenvironment for the inner biofilm. The produced nitrite was utilized with the remainder of ammonium by the ANAMMOX bacteria and converted into dinitrogen gas, while denitrifying bacteria consumed COD as an electron donor to deoxidize nitrate to dinitrogen gas in the anaerobic zone of biofilm. Furthermore, the possible growth of some aerobic heterotrophic bacteria also has a potential to be a contributor for the COD consumption. From days 12 to 26, the removal efficiency of NH$_4^+$-N was lower than 52% when the ratio of carbon to nitrogen (C/N) source was kept at 3:4. It was apparent that the ANAMMOX activity became inhibited due to the high-COD concentration in this stage, thus the influent concentration of COD was reduced to 100 mg/L at day 27, namely the influent C/N was changed to 1:2. From days 27 to 35, the effluent NH$_4^+$-N and COD concentrations decreased from 68.2 mg/L to 55.7 mg/L, and 18.7 mg/L to 7.2 mg/L, respectively, with little nitrate and nitrite produced. The TN removal efficiency increased from 62% to 70%. During the last 11 days (days 36-46), the effluent NH$_4^+$-N and COD concentrations decreased to 6.8 mg/L and 1.7 mg/L, respectively, with nitrate and nitrite produced. The TN and COD removal efficiencies reached 70% and 94%, respectively. This could be attributed to the best C/N (1:2) under the conditions of this SNAD process that partial nitrification, ANAMMOX and denitrification, etc. nitrogen and COD removal courses had reached a new and stable balance that the reactor reached the best performance for the SNAD process in these conditions.

$0.345\text{C}_6\text{H}_{12}\text{O}_6 + \text{NO}_3^- \rightarrow 0.136\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.432\text{N}_2$ + 1.06H$_2$O + 1.35CO$_2$ + OH$^-$ \hspace{1cm} (4)

$\text{C}_6\text{H}_12\text{O}_6 + 8\text{NO}_2^- \rightarrow 4\text{N}_2 + 6\text{CO}_2 + 2\text{H}_2\text{O} + 8\text{OH}^-$ \hspace{1cm} (5)

$\text{C}_6\text{H}_12\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$ \hspace{1cm} (6)

According to the stoichiometric equations of partial nitrification (Eq. (1)), ANAMMOX (Eq. (2)) and denitrification (Eq. (4)) during the SNAD process, 271.0 mg d COD must be consumed by the denitrifying bacteria when the NH$_4^+$-N removal amount reached about 553.0 mg d at the end of the SNAD process. However, there was about 324.9 mg d COD consumed actually. This was due to the competition for NO$_2^-$-N by the denitrifying bacteria as the electron acceptor (Eq. (5)), which made the practical COD consumption was more than the theoretical value. The competition for oxygen by some aerobic heterotrophic bacteria in the aerobic biofilm also made the practical COD consumption was more than the theoretical value (Eq. (6)). In addition, the competition for oxygen by some aerobic heterotrophic bacteria made the DO was lower than the CANON stage as indicated by this experiment. In summary, partial nitrification, ANAMMOX and denitrification were dominantly during the SNAD process.

3.4. Observation of non-woven fabric biofilm

Visual indication shows that the color of the surface of non-woven fabric biofilm changed from brownish red to russet in the CANON and SNAD stages. Red is specific for ANAMMOX bacteria in the ANAMMOX stage. SEM was used to visualize the biofilm of the non-woven fabrics in the SNAD stage. The SEM images of the biofilm sampled from SNAD non-woven fabric biofilm on day 46 displays that the bacteria adhere densely on the non-woven fibrous material. Biofilm in the two regions (inner biofilm and outer biofilm) of the SNAD non-woven fabric appeared different because of the different situation of dissolved oxygen. In the outer layer of biofilm, biomass was mostly micrococcus, presumably AOB, with a spot of aerobic heterotrophic bacteria. In the inner layer of biofilm, biomass was typical cauliflower like aggregates, presumably ANAMMOX organisms. However, there was some other biomass in the inner layer of biofilm, presumably denitrifying organisms. The observation together with system performance results revealed the co-existence of partial nitrification, ANAMMOX and denitrification. The results of SEM observation were subject to confirmation by using FISH analysis.
3.5. Fluorescence in situ hybridizations analysis

Biofilm samples were taken from CANON stage on day 41 and SNAD stage on day 46, respectively, and then analyzed for the spatial distribution of bacteria by FISH with using EUB338 probe special to all the eubacterium, plus NSO190 specific to β-Proteobacteria-like bacteria and AMX820 specific to the ANAMMOX bacteria, respectively. The inner and outer layers of the non-woven fabric biofilm samples which were taken from the CANON and SNAD stages, respectively, were multi-hybridized with EUB338 (FITC) + AMX820 (CY5) + NSO190 (CY3). The fluorescence images of the samples which were taken from the CANON stage show that almost all bacteria hybridized with EUB338 were simultaneously hybridized with AMX820 or NSO190, and distributed throughout the biofilm. Most of bacteria in the anoxic biofilm were ANAMMOX bacteria dominantly and a spot of AOB. However, in the aerobic biofilm most of bacteria were consisted of AOB dominantly and a spot of ANAMMOX bacteria. The fluorescence images of the samples which were taken from the SNAD stage show that not all the bacteria hybridized with EUB338 simultaneously reacted to the probes AMX820 or NSO190. Namely, some other bacteria were hybridized with EUB338 only. According to the performances of the SNAD process, the bacteria which were hybridized with EUB338 only might be some new-growth bacteria for COD consumption during SNAP period, presumably some denitrifying bacteria. Fluorescence images clearly show that most of bacteria in the anoxic biofilm were ANAMMOX and denitrifying bacteria dominantly and a spot of AOB. However, in the aerobic biofilm most of bacteria were consisted of AOB dominantly, a spot of ANAMMOX and aerobic heterotrophic bacteria. These facts further demonstrated that the autotrophic and heterotrophic nitrogen removal in experimental non-woven fabric biofilm of the SNAD stage was catalyzed by three different groups, autotrophic ANAMMOX bacteria and autotrophic AOB, and heterotrophic COD consumption bacteria, mainly means denitrifying bacteria.

4. Conclusions

In this study, the SNAD process was demonstrated to be stable and effective for the high nitrogen and low-COD removal from wastewater, with temperature of 35 °C, pH of 8–8.2, DO of 0.4–0.6 mg/L and C/N of 1:2. After an operation of 46 days, the ammonium removal efficiency of 79%, TN removal efficiency of 70% and COD removal efficiency of 94% were achieved with nitrogen and COD removal via the simultaneous nitrification and partial nitritation (SNAP) process. However, in the aerobic biofilm most of bacteria were consisted of AOB dominantly, a spot of ANAMMOX and aerobic heterotrophic bacteria. These facts further demonstrated that the autotrophic and heterotrophic nitrogen removal in experimental non-woven fabric biofilm of the SNAD stage was catalyzed by three different groups, autotrophic ANAMMOX bacteria and autotrophic AOB, and heterotrophic COD consumption bacteria, mainly means denitrifying bacteria.

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