Feasibility of conventional and single-stage anaerobic ammonium oxidation processes for treating chlortetracycline wastewater

Miaomiao Liu, Hong Yao, Hao Liu, Jing Xu and Lushen Zuo

ABSTRACT

Conventional and single-stage anaerobic ammonium oxidation (ANAMMOX) was carried out in bench-scale reactors to treat chlortetracycline (CTC) wastewater. The total nitrogen (TN) removal efficiency and rate for conventional ANAMMOX was 66.6 ± 5.9% and 2.7 ± 0.2 kg N/(m^3·d), respectively, which was 58.6 ± 3.8% and 1.2 ± 0.1 kg N/(m^3·d) for single-stage ANAMMOX. Single-stage ANAMMOX showed higher tolerance to CTC than conventional ANAMMOX. The nitrogen removal of conventional and single-stage ANAMMOX began to deteriorate when CTC was added, to 40 and 80 mg/L, respectively, with the former totally inhibited at 120 mg/L CTC and the latter at 140 mg/L CTC. TN removal rates were recovered to 1.2 and 0.7 kg N/(m^3·d), respectively, when CTC concentration was reduced to 20 mg/L for 8 days. This study implied that ANAMMOX could be efficiently used to treat pharmaceutical wastewater, with single-stage implementation being more stable under antibiotic pressure.

Key words | anaerobic ammonium oxidation, antibiotic inhibition, nitrogen removal, pharmaceutical wastewater

Miaomiao Liu
Hong Yao (corresponding author)
Hao Liu
Jing Xu
Lushen Zuo
School of Civil Engineering,
Beijing Jiaotong University,
Beijing 100044,
China
E-mail: *yaohongts@163.com*

INTRODUCTION

In the past two decades, anaerobic ammonium oxidation (ANAMMOX) has been regarded as a promising and sustainable nitrogen removal strategy compared to the conventional nitrification/denitrification process because it is a completely autotrophic process with no carbon source demand and less oxygen consumption (Strous *et al.* 1999; Schmidt *et al.* 2003). An ANAMMOX process involves two steps: (i) ammonium is oxidized to nitrite by ammonium oxidation bacteria (AOB) with oxygen as the electron acceptor and then (ii) nitrite is reduced to nitrogen gas by anaerobic ammonium oxidation bacteria (AnAOB) using ammonium as the electron donor (Francis *et al.* 2007). The implementation of ANAMMOX processes included conventional ANAMMOX which segregated partial nitrification and ANAMMOX in two separated reactors (Van Dongen *et al.* 2001) and single-stage ANAMMOX which combined partial nitritation and ANAMMOX in one oxygen-limited reactor. The conventional ANAMMOX process was considered beneficial for the separated growth of AOB and AnAOB since oxygen inhibition was avoided (Francis *et al.* 2007). Single-stage ANAMMOX processes are easier to operate and have been successfully implemented with suspended activated sludge (Kuai & Verstraete 1998; Sliekers *et al.* 2002), biofilms (Vlaeminck *et al.* 2009), and granular sludge (Vlaeminck *et al.* 2010).

ANAMMOX processes are promising in nitrogen removal of various ammonium-rich wastewater, such as sludge digestion liquid (Strous *et al.* 1997; Furukawa *et al.* 2009), landfill leachate wastewater (Valencia *et al.* 2011), animal manure (Figueroa *et al.* 2012), and pharmaceutical wastewater (Tang *et al.* 2011). However, there are some difficulties hindering the application of ANAMMOX in pharmaceutical wastewater treatment, mainly the inhibitive effects of the matrix. Tang reported a performance collapse caused by toxic pollutants during 42 days' operation of a conventional ANAMMOX process (Tang *et al.* 2011). However, there have been few reports about the feasibility of single-stage ANAMMOX to treat pharmaceutical wastewater.

In this work, actual chlortetracycline (CTC) production wastewater with 160–244 mg/L of ammonium was treated with conventional and single-stage ANAMMOX processes in bench-scale reactors. The feasibility of ANAMMOX

doi: 10.2166/wst.2014.308

processes to treat actual CTC wastewater was evaluated. In addition, impacts of CTC on the nitrogen removal performance were investigated.

MATERIALS AND METHODS

Chemical analysis

Mixed liquids were collected and immediately filtered through 0.45 μm cellulose microfiber filters (Millipore, USA). Dissolved oxygen (DO) and pH were monitored on site four times every day by a potable analyzer (HACH, USA). Nitrogen compounds (ammonium, nitrite, and nitrate) and mixed liquor suspended solids (MLSS) were determined according to the US national standard methods (APHA 1998). Samples were filtered by 0.45 μm acetate cellulose membranes before analysis. Overall data about the nitrogen removal performance are shown in Table A.2 (available online at http://www.iwaponline.com/wst/070/308.pdf).

Wastewater and sludge sources

Synthetic wastewater was used as the influent to start up the ANAMMOX processes. Ammonium (200–300 mg/L) was the only nitrogen substrate for the single-stage ANAMMOX process, while ammonium and nitrite (both 200–300 mg/L) were provided simultaneously for the conventional ANAMMOX process. $KHCO_3$ (J&K Bailingwei Co., China) was added to provide alkalinity of 50–60 mM/L and maintain pH at 8.0. CTC production wastewater was collected from a full-scale wastewater treatment plant in a pharmaceutical production facility in the Inner Mongolia Autonomous Region, China. Anaerobic digestion (upflow anaerobic sludge blanket – UASB) and aerobic units (cyclic activated sludge system (CASS) and anoxic–oxic reactor) were employed to remove chemical oxygen demand (COD) and nitrogen compounds (Figure A.1, available online at http://www.iwaponline.com/wst/070/308.pdf). Effluent from the CASS reactor (CASS-eff), which contained 160.0–238.0 mg/L NH_4^+–N and 400–900 mg/L COD_{Cr}, was used as the influent for the single-stage ANAMMOX process. A pilot-scale short-cut nitrification process was operated on-site at the pharmaceutical production facility to treat CTC production wastewater. Its effluent (SHARON-eff) contained 199–244 mg/L NH_4^+–N, 198–232 mg/L NO_2^-–N, and 300–500 mg/L COD_{Cr} and was used as the influent for the conventional ANAMMOX process (Table A.1, available online at http://www.iwaponline.com/wst/070/308.pdf).

ANAMMOX sludge inoculated were collected from conventional and single-stage ANAMMOX processes of Gaobeidian wastewater treatment plant located in Beijing, China. The single-stage ANAMMOX sludge was red biofilm on Raschig rings from a CSTR (continuous stirred-tank reactor) ANAMMOX reactor, while the conventional ANAMMOX sludge was red granules with a diameter of approximately 3 mm from a UASB ANAMMOX reactor (Figure A.2, available online at http://www.iwaponline.com/wst/070/308.pdf).

Operation of bench-scale ANAMMOX process (conventional and single-stage)

To benefit the operation, conventional and single-stage ANAMMOX processes were carried out in a UASB and CSTR, respectively, with a total volume of 0.8 L (Figure 1). For conventional ANAMMOX, inoculated sludge was added until MLSS reached 4,600 mg/L. For single-stage

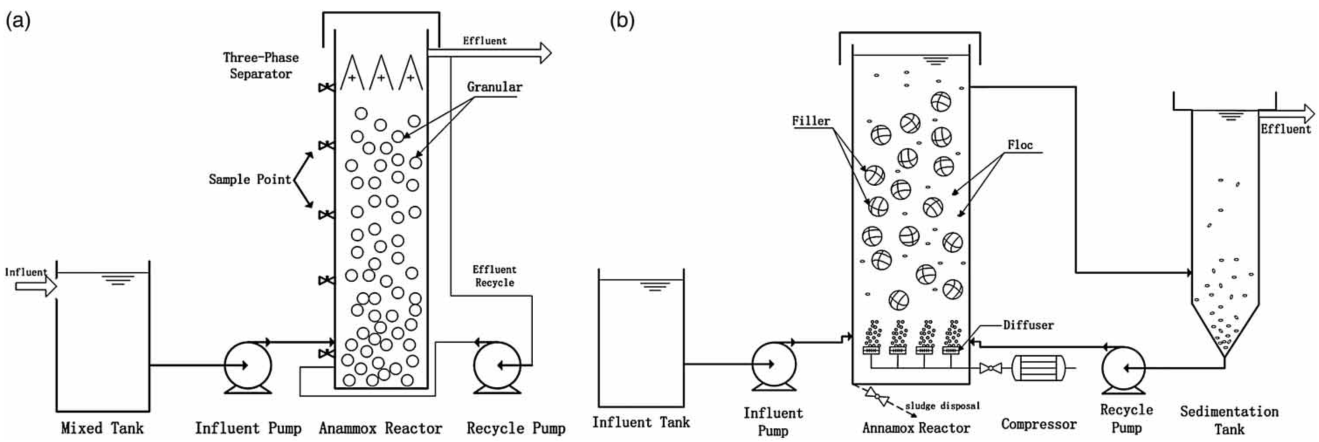

Figure 1 | Flow chart of (a) conventional ANAMMOX and (b) single-stage ANAMMOX.

ANAMMOX, inoculated Raschig rings were added to a compaction rate of 20%. Each experimental operation lasted for 86 days, with the main procedures shown in Table A.3 (available online at http://www.iwaponline.com/wst/070/308.pdf). When the ANAMMOX processes were successfully started up with synthetic wastewater, the influent was gradually replaced with actual CTC production wastewater. Temperature was maintained at 32–35 °C using electronic heating rods. pH was kept in the range of 8.0–8.3 by addition of $KHCO_3$. For the conventional ANAMMOX process, no aeration was employed, to guarantee the anaerobic condition. For the single-stage ANAMMOX process, DO was maintained at 0.8–1.0 mg/L by adjusting aeration rate according to the DO monitoring results.

CTC inhibition experiments

For the inhibition experiments, another two UASB and CSTR reactors were operated in parallel with the above conventional and single-stage ANAMMOX systems. The processes were initiated with synthetic wastewater as described above. When stable performance was achieved, CTC was added into both reactors with gradually increasing concentrations (5–140 mg/L). The nitrogen removal of both ANAMMOX processes was determined under each CTC level to evaluated the inhibition effect of CTC.

RESULTS AND DISCUSSION

Concentration of CTC in the wastewater

According to the results from ultra-performance liquid chromatography tandem mass spectrometry, CTC was detected in the range of 20–100 mg/L in the raw influent of the full-scale CTC production wastewater. However, CTC was always below the detection limit (5 μg/L) in the CASS-eff and SHARON-eff, possibly due to its keen hydrolysis and sludge adsorption (Loftin *et al.* 2008). Similarly, CTC was undetectable in the effluent from both conventional and single-stage ANAMMOX processes during the inhibition experiments.

Nitrogen removal performance of the conventional ANAMMOX system treating actual pharmaceutical wastewater

Synthetic wastewater with ammonium and nitrite at 198.0–225.0 mg/L was initially fed to the ANAMMOX system. After 33 days of this start-up procedure, the effluent ammonium and nitrite were reduced to about 60 mg/L (Figure 2(a)) and the total nitrogen (TN) removal rate gradually increased from 1.5 kg N/(m^3·d) to 2.6 ± 0.1 kg N/(m^3·d) along with an increase of influent TN load (Figure 2(b)). From day 34 onward, the influent was replaced with partially nitritated CTC wastewater (SHARON-eff, Table A.1). The nitrogen removal performance was well maintained, with the TN removal rate of 2.2–2.3 kg N/(m^3·d). This was further improved with the increase of influent TN load (from 3.0 to 3.9 kg N/(m^3·d)) after the system adjusted to the matrix of the actual wastewater. The final TN removal rate was maintained at 2.7 ± 0.2 kg N/(m^3·d) (Figure 2(b)). However, there was a performance fluctuation when the influent TN load was raised to 4.2–4.7 kg N/(m^3·d), which resulted in a sudden increase of the effluent ammonium and nitrite from 40 mg/L to 80–100 mg/L. When the influent TN load gradually decreased to below 4.2 kg N/(m^3·d), the effluent ammonium and nitrite decreased to 50 mg/L. At this point, the TN removal efficiency was 66.6 ± 5.9% and the TN removal rate was 2.7 ± 0.2 kg N/(m^3·d) (Figure 2).

The concentrations of COD_{Cr} during the conventional ANAMMOX process were slightly reduced by about 100 mg/L during the whole operation period (Figure A.3, available online at http://www.iwaponline.com/wst/070/308.pdf). This further assured that autotrophic ANAMMOX was the main nitrogen removal process other than heterotrophic denitrification. It also indicated that organics present in the pharmaceutical wastewater had limited effects on ANAMMOX bacteria.

Tang *et al.* (2011) reported a reduced efficiency of conventional ANAMMOX in treating nitritated pharmaceutical wastewater containing colistin sulfate and kitasamycin, where ammonium removal decreased from 85% to zero and nitrite removal decreased from 97 to 25–57%. Fernandez *et al.* (2009) found that TN removal of conventional ANAMMOX decreased from 100 to 75% when exposed to 20 mg/L chloramphenicol or 50 mg/L tetracycline. Our work suggested the feasibility of the conventional ANAMMOX process in treating CTC wastewater.

Inhibition of CTC on conventional ANAMMOX processes

The effect of CTC on the conventional ANAMMOX process was investigated using synthetic wastewater added with different levels of influent CTC (Figure 3). The conventional ANAMMOX process was stabilized using synthetic wastewater containing 200–225 mg/L ammonium and nitrite. With no CTC addition, the TN removal efficiency reached 70%, while the TN removal rate increased to 2.4 kg N/(m^3·d).

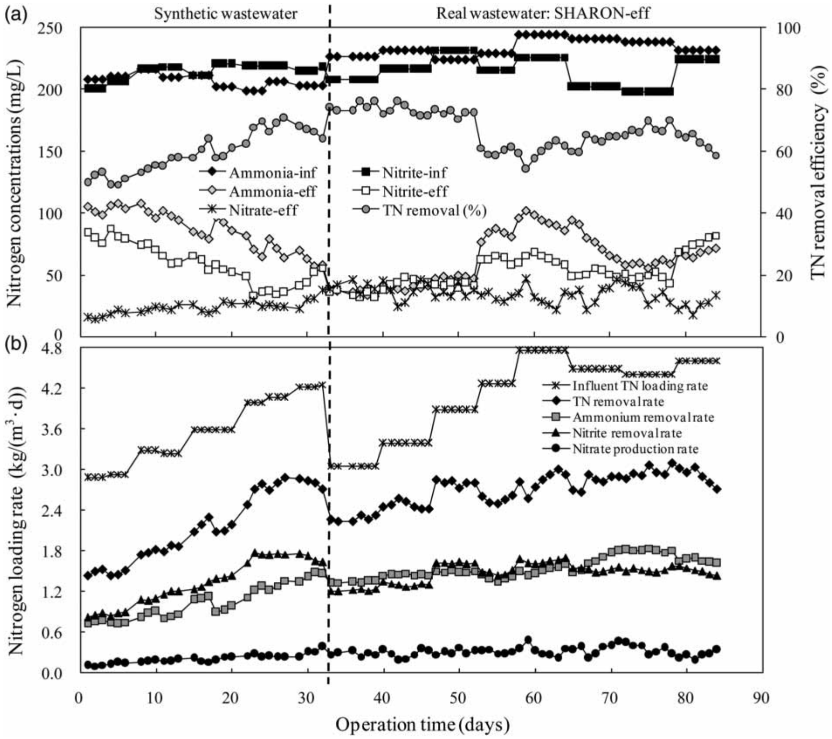

Figure 2 | (a) Concentrations of nitrogen compounds in the conventional ANAMMOX system treating synthetic and real wastewater. (b) Removal and production rates of nitrogen compounds in ANAMMOX process treating synthetic and real wastewater.

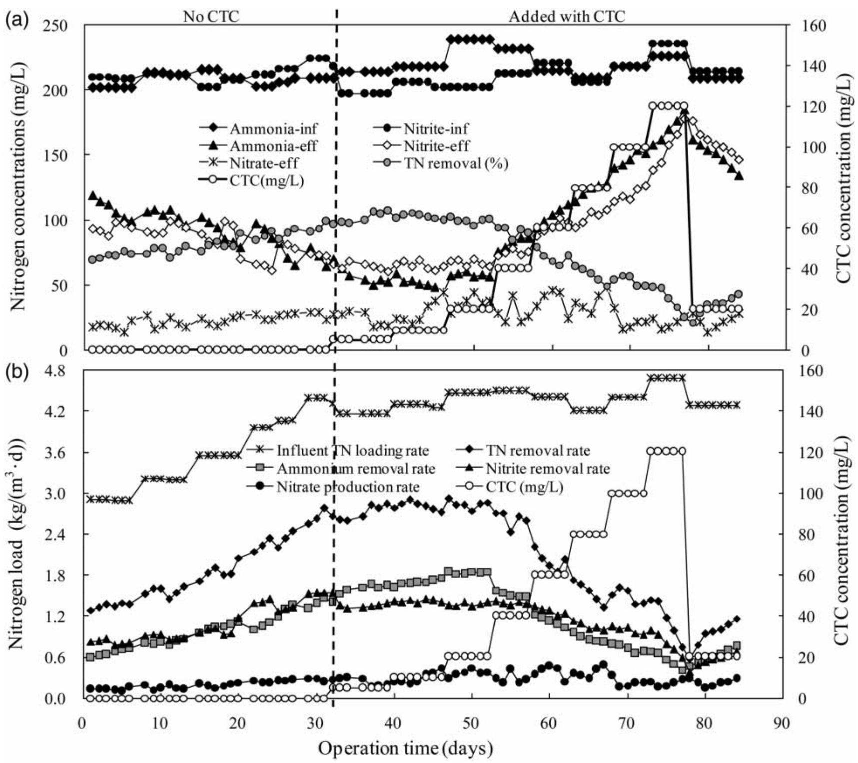

Figure 3 | (a) Concentrations of nitrogen compounds during the conventional ANAMMOX process under different CTC concentrations. (b) Removal and production rates of nitrogen compounds during the conventional ANAMMOX process under different CTC concentrations.

Influent CTC was added from 5 mg/L to 120 mg/L, with the dosing time for each CTC concentration at 5–7 days. As shown in Figure 3, the effluent ammonium and nitrite were 44–63 and 32–48 mg/L when the system was exposed to 5–20 mg/L CTC. The TN removal efficiency was 63.3–64.2% and the TN removal rate was 2.6–2.9 kg N/(m^3·d), indicating that the conventional ANAMMOX process could function normally under relatively low CTC levels. However, the nitrogen removal performance significantly decreased when CTC level was increased to 40–120 mg/L. The effluent ammonium and nitrite gradually increased to 185 and 178 mg/L, respectively. The TN removal efficiency and TN removal rate stepped down to 15.8% and 0.7 kg N/(m^3·d). When CTC was reduced to 20 mg/L, the nitrogen removal performance partially recovered, with TN removal efficiency and rate increasing to 27.2% and 1.2 kg N/(m^3·d) after 7 days.

There have been several reports about antibiotic inhibition on the ANAMMOX process. In general, short-term inhibition studies showed high inhibition thresholds of antibiotics for ANAMMOX processes. For example, Fernandez *et al*. found that 200 mg/L tetracycline and 400 mg/L chloramphenicol only caused 50% inhibition to the specific ANAMMOX activity (Fernandez *et al*. 2009). Lotti *et al*. found that the 50% inhibition concentrations to specific ANAMMOX activity were as high as 650 and 1,100 mg/L for sulfathiazole and tetracycline (Lotti *et al*. 2012). However, inhibitive effects during long-term operations were quite different. It was reported that the cumulative toxicity from toxic pollutants in pharmaceutical wastewater resulted in a performance collapse of conventional ANAMMOX during long-term operation (42 days), although no inhibition was found during short-term operation (Tang *et al*. 2011). Noophan *et al*. found that 5 ± 3.5 mg/L oxytetracycline could completely inhibit ANAMMOX activities in 5 weeks (Noophan *et al*. 2012). The discrepancy in results of CTC inhibitive effects was possibly due to various operation conditions such as biomass and influent nitrogen load (Liu & Horn 2012). The inhibition effect of 20 mg/L CTC was not significant in this work, indicating that the conventional ANAMMOX process could be used to treat pharmaceutical wastewater under the operational conditions tested.

Nitrogen removal performance of single-stage ANAMMOX system treating actual CTC pharmaceutical wastewater

The nitrogen removal performance during single-stage ANAMMOX treatment is shown in Figure 4. The single-stage reactor was fed with synthetic wastewater with 200–225 mg/L ammonium during the start-up period. The influent ammonium load gradually increased from 1.5 to 2.2 kg N/(m^3·d). Ammonium was reduced to 80–100 mg/L, and the TN removal rate was around 0.9 kg N/(m^3·d) once Raschig rings with red biofilm were inoculated. With the increase of influent ammonium load, the TN removal rate increased to 1.4 kg N/(m^3·d) at the end of the start-up period. The effluent ammonium was reduced to approximately 50 mg/L. Also, nitrite was between 4.8 and 24.8 mg/L and nitrate was in the range of 16.0–26.5 mg/L.

From day 34, influent of the single-stage ANAMMOX was replaced with effluent from the CASS reactor (CASS-eff, Table A.1). In order to avoid operation shock, the influent ammonium load was kept at 1.4 kg N/(m^3·d) when the influent was shifted from synthetic wastewater to CASS-eff. During the initial 22 days of treating CASS-eff, the TN removal rate showed a slight decrease from 1.2 to 1.0 kg N/(m^3·d), possibly because of the inhibitive matrix compounds in CASS-eff. The TN removal rate gradually increased to 1.3 kg N/(m^3·d), nearly the same level as when treating synthetic wastewater. The nitrite and nitrate production loads were 0.05–0.24 and 0.13–0.26 kg N/(m^3·d), indicating little nitrite accumulation or nitrification.

As shown in Table 1, the nitrogen removal rate was in the range of 0.06–1.5 kg N/(m^3·d) for single-stage ANAMMOX implemented with different reactor types. Sliekers *et al*. obtained a TN removal rate of 0.06 kg N/(m^3·d) in a CANON (completely autotrophic nitrogen removal over nitrite) reactor in which simultaneous partial nitrification and ANAMMOX were first found to happen (Sliekers *et al*. 2002). Their group reported a TN removal efficiency of 1.5 kg N/(m^3·d) in a gas-lift CANON system treating high ammonium wastewater (Sliekers *et al*. 2003). De Clippeleir *et al.* reported a TN removal efficiency of 1.1 kg N/(m^3·d) in a sequential batch reactor (SBR) with low volumetric exchange (De Clippeleir *et al*. 2009). Cho *et al*. found a TN removal efficiency of 0.35 kg N/(m^3·d) in an up-flow biofilm ANAMMOX reactor (Cho *et al*. 2011). The high nitrogen removal efficiency in this study implied that single-stage ANAMMOX is an efficient process to treat pharmaceutical wastewater.

Inhibition of CTC on single-stage ANAMMOX system

To examine the effects of CTC on the single-stage ANAMMOX, additional CTC solution (final concentrations of 20–140 mg/L, 6–8 days at each concentration) was added to the system. As shown in Figure 5, TN removal

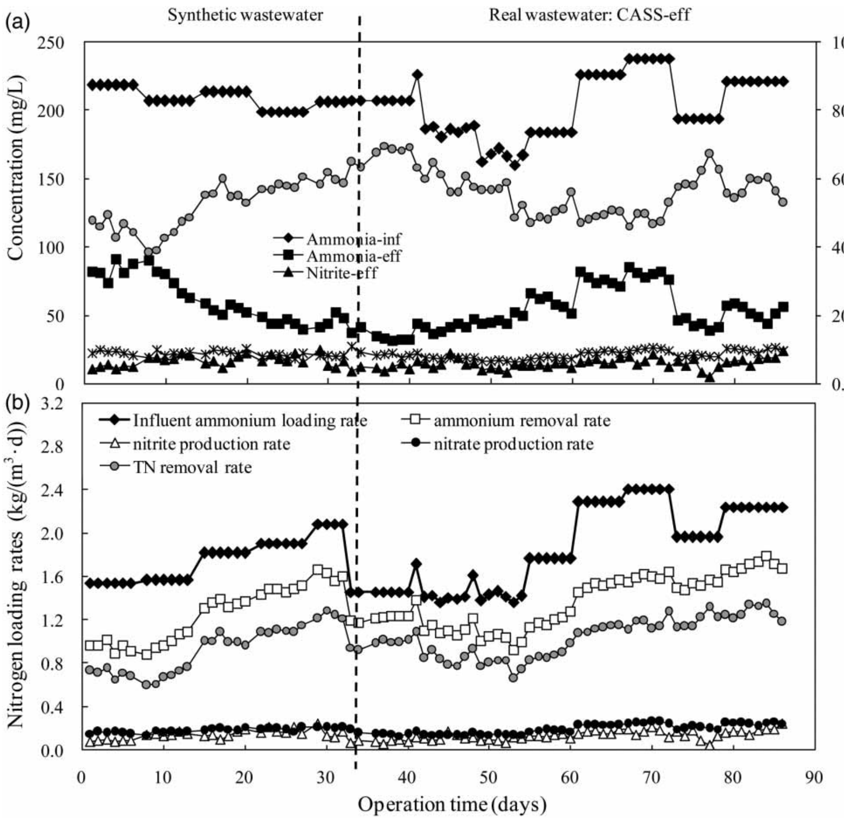

Figure 4 | (a) Concentrations of nitrogen compounds during the single-stage ANAMMOX process treating synthetic and real wastewater. (b) Removal and production rates of nitrogen compounds in the single-stage ANAMMOX process treating synthetic and real wastewater.

Table 1 | Overview of nitrogen removal rates in single-stage ANAMMOX reactors

Single-stage ANAMMOX	Reactor type	Type of influent	Influent ammonia (mg/L)	TN removal rate (kg N/(m^3·d))	Reference
CANON	SBR	Synthetic wastewater	131	0.06	Sliekers *et al.* (2002)
CANON	Gas-lift		1545 ± 62	1.5	Sliekers *et al.* (2003)
OLAND	SBR		100–300	1.1	De Clippeleir *et al.* (2009)
OLAND	Up-flow biofilm		40–300	0.35	Cho *et al.* (2011)
CANON	CSTR	Pharmaceutical wastewater	160–238	1.2	This study

CANON: completely autotrophic nitrogen removal over nitrite; OLAND: oxidation and oxygen-limited autotrophic nitrification–denitrification; SBR: sequential batch reactor; CSTR: continuous stirred-tank reactor.

efficiency and rate were 62.5% and 1.2–1.3 kg N/(m^3·d) when no CTC was added into the system. When CTC was added up to 20–60 mg/L, the nitrogen removal performance was maintained with the TN removal efficiency and rate reaching 53.1–63.8% and 1.2 ± 0.1 kg N/(m^3·d). However, the effluent ammonium sharply increased to as high as 190 mg/L when the CTC level was further raised to 60–140 mg/L. Meanwhile, the TN removal efficiency and rate gradually decreased to 2.4% and 0.1 kg N/(m^3·d). When the CTC level was reduced to 20 mg/L, the effluent ammonium gradually decreased to 90 mg/L and TN removal rate recovered to 0.7 kg N/(m^3·d) within 10 days. The above results showed that CTC could strongly inhibit the functional bacteria responsible for nitrogen removal

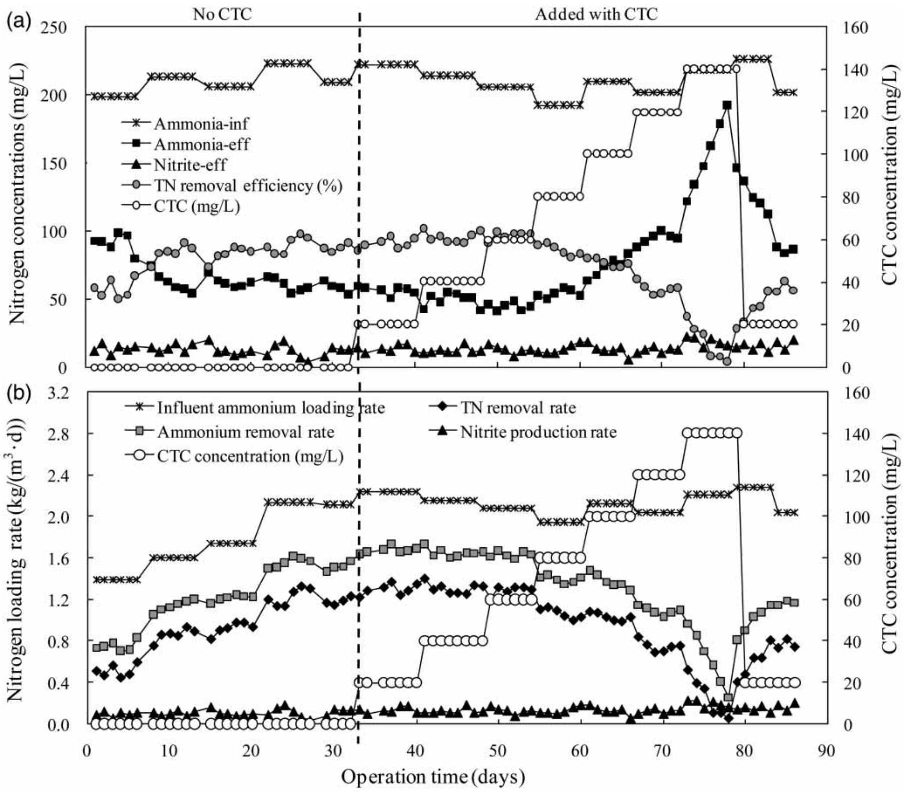

Figure 5 | (a) Concentrations of nitrogen compounds during the single-stage ANAMMOX process under different CTC concentrations. (b) Removal and production rates of nitrogen compounds during the single-stage ANAMMOX process under different CTC concentrations.

in the single-stage ANAMMOX process at relatively high CTC levels (60–140 mg/L), which could be reversed to some degree if the CTC exposure is reduced to a lower level (20 mg/L).

The single-stage ANAMMOX could withstand higher CTC exposure levels (60 mg/L) compared to the conventional ANAMMOX process (20 mg/L) according to our inhibition experiments. Based on this result, single-stage ANAMMOX may be superior in treating ammonium-rich pharmaceutical wastewater containing antibiotics and other toxic materials. One possible reason is the layer distribution of the biofilm in single-stage ANAMMOX, which provides a differentiated micro-environment for AOB and AnAOB, the functional microbes of ANAMMOX. AOB tend to grow in the external aerobic layer while AnAOB tend to grow in the inner anaerobic layer (Vlaeminck *et al*. 2010; Liu & Horn 2012). Due to the substrate transfer resistance, the CTC level was lower in the inner layer than that in the external layer. Correspondingly, the inhibitive pressure of CTC on AnAOB was less than that on AOB. The attenuated adverse effect on AnAOB was favorable to the single-stage ANAMMOX, considering that AnAOB are more vulnerable to CTC due to their slower growth rate (doubling time: 11–14 days) (Strous *et al*. 1998). More research is needed to better understand the stability of single-stage ANAMMOX under the stress of antibiotics and other inhibitive factors.

CONCLUSIONS

In this work, bench-scale conventional ANAMMOX and single-stage ANAMMOX processes were found efficient for nitrogen removal from actual pharmaceutical wastewater under proper conditions. The single-stage process was found to be more tolerant to CTC than conventional ANAMMOX, implying that single-stage ANAMMOX possesses a higher potential for nitrogen removal from pharmaceutical wastewater. Both processes showed a tendency of recovery when CTC was reduced to 20 mg/L, suggesting that bacteria present in both systems can regain the nitrogen removal ability possibly due to their resistance to antibiotics.

ACKNOWLEDGEMENTS

This research was financially supported by the Fundamental Research Funds for the Central Universities (2013JBM012) and the National Natural Scientific Foundation of China (NSFC–51078023).

REFERENCES

APHA 1998 *Standard Methods for the Examination of Water and Wastewater*. 20th edn. American Public Health Association/ American Water Works Association/Water Environment Federation, Washington, DC, USA.

Cho, S., Fujii, N., Lee, T. & Okabe, S. 2011 Development of a simultaneous partial nitrification and anaerobic ammonia oxidation process in a single reactor. *Bioresource Technology* **102** (2), 652–659.

De Clippeleir, H., Vlaeminck, S. E., Carballa, M. & Verstraete, W. 2009 A low volumetric exchange ratio allows high autotrophic nitrogen removal in a sequencing batch reactor. *Bioresource Technology* **100** (21), 5010–5015.

Fernandez, I., Mosquera-Corral, A., Campos, J. L. & Mendez, R. 2009 Operation of an Anammox SBR in the presence of two broad-spectrum antibiotics. *Process Biochemistry* **44** (4), 494–498.

Figueroa, M., Vázquez-Padín, J. R., Mosquera-Corral, A., Campos, J. L. & Méndez, R. 2012 Is the CANON reactor an alternative for nitrogen removal from pre-treated swine slurry? *Biochemical Engineering Journal* **65**, 23–29.

Francis, C. A., Beman, J. M. & Kuypers, M. M. M. 2007 New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *The ISME Journal* **1** (1), 19–27.

Furukawa, K., Inatomi, Y., Qiao, S., Quan, L., Yamamoto, T., Isaka, K. & Sumino, T. 2009 Innovative treatment system for digester liquor using anammox process. *Bioresource Technology* **100** (22), 5437–5443.

Kuai, L. P. & Verstraete, W. 1998 Ammonium removal by the oxygen-limited autotrophic nitrification-denitrification system. *Applied and Environmental Microbiology* **64** (11), 4500–4506.

Liu, S. & Horn, H. 2012 Effects of biofilm geometry on deammonification biofilm performance: a simulation study. *Bioresource Technology* **116**, 252–258.

Loftin, K. A., Adams, C. D., Meyer, M. T. & Surampalli, R. 2008 Effects of ionic strength, temperature, and pH on degradation of selected antibiotics. *Journal of Environmental Quality* **37**, 378–386.

Lotti, T., Cordola, M., Kleerebezem, R., Caffaz, S., Lubello, C. & van Loosdrecht, M. C. M. 2012 Inhibition effect of swine wastewater heavy metals and antibiotics on anammox activity. *Water Science and Technology* **66** (7), 1519–1526.

Noophan, P., Narinhongtong, P., Wantawin, C. & Munakata-Marr, J. 2012 Effects of oxytetracycline on anammox activity. *Journal of Environmental Science and Health Part A – Toxic/ Hazardous Substances & Environmental Engineering* **47** (6), 873–877.

Schmidt, I., Sliekers, O., Schmid, M., Bock, E., Fuerst, J., Kuenen, J. G., Jetten, M. S. M. & Strous, M. 2003 New concepts of microbial treatment processes for the nitrogen removal in wastewater. *FEMS Microbiology Reviews* **27** (4), 481–492.

Sliekers, A. O., Derwort, N., Gomez, J. L. C., Strous, M., Kuenen, J. G. & Jetten, M. S. M. 2002 Completely autotrophic nitrogen removal over nitrite in one single reactor. *Water Research* **36** (10), 2475–2482.

Sliekers, A. O., Third, K. A., Abma, W., Kuenen, J. G. & Jetten, M. S. M. 2003 CANON and Anammox in a gas-lift reactor. *FEMS Microbiology Letters* **218** (2), 339–344.

Strous, M., VanGerven, E., Zheng, P., Kuenen, J. G. & Jetten, M. S. M. 1997 Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (anammox) process in different reactor configurations. *Water Research* **31** (8), 1955–1962.

Strous, M., Heijnen, J. J., Kuenen, J. G. & Jetten, M. S. M. 1998 The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology* **50** (5), 589–596.

Strous, M., Fuerst, J. A., Kramer, E. H. M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K. T., Webb, R., Kuenen, J. G. & Jetten, M. S. M. 1999 Missing lithotroph identified as new planctomycete. *Nature* **400** (6743), 446–449.

Tang, C. J., Zheng, P., Wang, C. H., Mahmood, Q., Zhang, J. Q., Chen, X. G., Zhang, L. & Chen, J.-W. 2011 Performance of high-loaded ANAMMOX UASB reactors containing granular sludge. *Water Research* **45** (1), 135–144.

Valencia, R., Zon, W. v. d., Woelders, H., Lubberding, H. J. & Gijzen, H. J. 2011 Anammox: An option for ammonium removal in bioreactor landfills. *Waste Management* **31** (11), 2287–2293.

Van Dongen, U., Jetten, M. S. M. & Van Loosdrecht, M. C. M. 2001 The SHARON-Anammox process for treatment of ammonium rich wastewater. *Water Science and Technology* **44** (1), 153–160.

Vlaeminck, S. E., Terada, A., Smets, B. F., Van der Linden, D., Boon, N., Verstraete, W. & Carballa, M. 2009 Nitrogen removal from digested black water by one-stage partial nitritation and Anammox. *Environmental Science & Technology* **43** (13), 5035–5041.

Vlaeminck, S. E., Terada, A., Smets, B. F., De Clippeleir, H., Schaubroeck, T., Bolca, S., Demeestere, L., Mast, J., Boon, N., Carballa, M. & Verstraete, W. 2010 Aggregate size and architecture determine microbial activity balance for one-stage partial nitritation and Anammox. *Applied and Environmental Microbiology* **76** (3), 900–909.

First received 27 March 2014; accepted in revised form 25 June 2014. Available online 5 July 2014