Construction of the biosystem with low consumption and high efficiency of nitrogen and carbon removal and its application in teaching

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Abstract. Shortcut nitrification and denitrification coupled with anaerobic ammonia oxidation process is a new biological process for the removal of nitrogen and carbon in wastewater treatment, with the advantages of low energy consumption, low sludge production, which can greatly reduce the cost of wastewater treatment. Through the designed experiment of the system and the evaluation experiment of carbon and nitrogen removal effect, which were convenient for students to understand more clearly and provided hands-on activities for students. Experiment showed that the system with the removal of nitrogen and carbon functioned well, and enhanced students’ knowledge of the process, to motivate their interests in learning professional knowledge. In the period of experiment design and literature review, the abilities of independent thinking and problem solving were improved and the enthusiasm, initiative and creativity in scientific research also got enhanced.

1. Introduction

Traditional process of denitrification is by using ammonia oxidation to decompose nitrogenous organic compound into NH4+, which transformed to NO3- by nitrobacteria and finally restored NO3- into N2 through reverse nitrobacteria, and therefore the process realizes the removal of oxygen substance. However, the actual application of this method means high energy consumption and cost.

Recently, several new denitrification crafts come up to solve the problem so as to removal of nitrogen and carbon with low consumption and high efficiency. Normally, it concludes two ways: one is to reduce reaction time by shortcut nitrification and denitrification; the other one is to apply new-style nitrogen metabolic pathways to the removal of nitrogen and carbon in wastewater treatment as named anammox craft.

A new device on biosystem removal of nitrogen and carbon comes up to combine the two ways and an experimental facility was created to improve the experimental teaching which motivates the student’s interest and ability of practice.
2. Design of Device

2.1. Principle of design
Originally, nitrification changes NH$_4^+$ into NO$_2^-$, furthermore into NO$_3^-$, which process is called denitrification. Furthermore, shortcut nitrification denitrification means NH$_4^+$ into NO$_2^-$ and directly to N$_2$ with process of NO$_3^-$, which could be described as following: NH$_4^+$→NO$_2^-$→N$_2$. Delft University created the SHARON based on the shortcut nitrification denitrification craft. The SHARON take the different bacterial growth rate feature between ammonia oxidizing bacterial (AOB) and nitrite oxidizing bacterial(NO), in particular the AOB rate higher then NO when the high temperature is between 30$^\circ$C and 35$^\circ$C, could stabilize the accumulated NO$_2^-$-N. Currently, Netherland has already built two wastewater treatment factories by the biosystem carbon removal craft which proved the effectiveness of shortcut nitrification and denitrification.

Anaerobic ammonium oxidation (Anammox) was firstly brought up by Mr. Mudler under the supervisor of professor Kuenen at Delft in 1995. Anammox recommend NO$_2^-$-N as an electron acceptor and hereby made the NH$_4^+$-N oxidized into N$_2$. In 1990, Strous brought up a reaction formula of Anammox as indicated by Equation 1.

\[
\begin{align*}
\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.66\text{HCO}_3^- + 0.13\text{H}^+ &\rightarrow \\
1.02\text{N}_2 + 0.26\text{NO}_3^- + 2.03\text{H}_2\text{O} + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} &\quad (1)
\end{align*}
\]

In Equation 1, shortcut nitrification and denitrification could reduce the system total nitrogen load and degrade some organics by coupling two reactors to conduct shortcut nitrification and denitrification as well as Anammox. After reactor, the water fits the incoming water for Anammox reactor, which could further realize the denitrogenating as a precondition. The combination could reduce aeration rate alkali number and reactor time. The process described as Figure 1.

In Figure 1, the coupled system could reduce the system total nitrogen load and degrade some organics by coupling two reactors to conduct shortcut nitrification and denitrification as well as Anammox. After reactor, the water fits the incoming water for Anammox reactor, which could further realize the denitrogenating as a precondition. The combination could reduce aeration rate alkali number and reactor time. The process described as Figure 1.

2.2. Structure of Device
Both reactors of Shortcut nitrification denitrification and Anammox take the SBR as marked SBR1 and SBR2, which form the coupling device shown in Figure 2. The SBR 1 of Shortcut nitrification denitrification flow the water to SBR of Anammox through the mid inlet chest. The period of changing water is 12 hours and changing water ratio is 1:2. The coupling craft handling capacity is 6 L/d and inflow ratio is 500 mg/L with NH$_4^+$-N concentration.

The inflow system consists of concentrated solution microelement and alkali lye water tanks by setting up an automatic water distribution, which control the water turnover with the on/off of the relay.
procedure with timer setting. After inflow, blend and aerating system start to blow air so as to control ratio by rotor flow meter; then equably disperse air with micropore aeration disk. Experimental anaerobic and aerobic realize automatic control by timer.

![Diagram of two-stage Shortcut nitrification denitrification-Anammox Coupling Device](image)

**Figure 2.** Flow of two-stage Shortcut nitrification denitrification-Anammox Coupling Device

SBR1 reactor is made of polymethyl methacrylate binocular cylinder and inner diameter is 17 centimeters with a total volume at 9 liters while valid volume reaches 6 liters. Interlayer water bath is 3 centimeters and the reactor temperature constancy are $30 \pm 1 ^\circ C$. Electrical machinery set on the top of reactor can stir the sludge at 100 rpm. Meanwhile, sample connection of the reactor wall can be used to sampling and spoil disposal. Overflow set beside near the height of the flange plate prevent wastewater overflow from self-motion system chaos.

SBR2 reactor is the similar construction as SBR1 but the motor part is driven by magnetic force. The magnetic linkage structure ensures an airtight state of reactor and supply an anaerobic environment for Anammox reaction. The two cylinders is 3-centimeter interlayer of water batch, and water temperature is constancy set at $33 \pm 1 ^\circ C$ by heated from circulating pumping. An overflow hole is set on the top of flange plate.

3. Experimental result of device

3.1. Experiment water and inoculated sludge

Shortcut nitrification denitrification reactor take the prepared C/N organic wastewater as inflow and $\text{NH}_4^+$-N supplied by $\text{NH}_4\text{Cl}$ while COD supplied by CH$_3$COONa. Furthermore, some KH$_2$PO$_4$ is added to supply adequate phosphorus source and proper NaHCO$_3$ to adjust inflow water pH. Besides that, proper microelement is added into inflow water. Among inflow water concentration, $\text{NH}_4^+$-N ratio is 500 mg/L and COD/$\text{NH}_4^+$-N is about 1. The effluent of Shortcut nitrification denitrification reactor is subsequently as inflow water of the Anammox reactor. The advantage is less DO with low aeration rate and no need further deoxidization. The microelement distribution ratio is as followed: MgCl$_2$·2H$_2$O 25.07 g/L, CaCl$_2$·2H$_2$O 7.34 g/L; CuCl$_2$·2H$_2$O 0.112 g/L, ZnSO$_4$·7H$_2$O 0.015 g/L, MnCl$_2$·4H$_2$O 1.03 g/L, NaMO$_4$·2H$_2$O 0.0025 g/L.

At laboratory culture, nitration sludge and anaerobic ammonia oxidation granule sludge are inoculated separately in SBR1 and SBR2 reactors as illustrated in Figure 3(a,b).
a. nitration sludge  b. anaerobic ammonia oxidation granule sludge

Figure 3. Appearance of Two types sludge

3.2. Measure item and method

Main chemical index analysis method refers to water and wastewater monitor analysis method (4th edition) as retrieved. Ammonia concentration by Nessler’s reagent spectrophotometry, nitrite nitrogen concentration by N-(1-naphthyl)-quadrol spectrophotometry, nitrate nitrogen by phenol disulfonic acid spectrophotometry, total nitrogen by potassium peroxydisulfate spectrophotometry, CODCr by potassium dichromate rapid test method, DO and pH by potable determinator (American Hash).

3.3. Result and Discuss

3.3.1. Denitrify performance analysis of Device. Constancy operating period of Shortcut nitrification denitrification - Anammox coupling device performance is illustrated as Figure 4.

Figure 4. Denitrify performance of Device
Figure 4 shows that when inflow water NH$_4^+$_N ratio is about 500 mg/L, effluent of preparation reactor is between 159.81 and 178.09 mg/L, NO$_2^-$—N ratio is between 217.24 and 231.32 mg/L, NO$_3^-$—N ratio is between 15.48 and 23.15 mg/L, furthermore calculated to effluent water NO$_2^-$N/NH$_4^+$_N is about 1.24-1.42 (rate of NO$_2^-$_N accumulation is above 91%), therefore the effluent water is suitable for the inflow water of Anammox reactor.

After denitrified by Anammox reactor, the effluent water NH$_4^+$_N ratio is between 24.17 and 41.13 mg/L, NO$_2^-$—N ratio is between 16.71 and 25.74 mg/L, NO$_3^-$—N ratio is below 10 mg/L, while ∆m(NH$_4^+$_N): ∆m(NO$_2^-$—N): ∆m(NO$_3^-$—N) is 1:1.55:0.09, among them, NO$_2^-$—N/NH$_4^+$_N is bigger than theoretical value of Anammox reactor at 1.32, the reason is because NO$_2^-$—N is as same as matrix of denitrification and partly wiped off by denitrification process. Except that, the other matrix of denitrification is NO$_3^-$_N, therefore, partly NO$_3^-$_N is wiped off by Anammox effect, which makes the ratio of NO$_3^-$_N/NH$_4^+$_N is less than 0.26(compared with theoretical value). In the Coupling system, TN wipe off load is 0.44 kg N/(m$^3$.d) and removal rate is about 88%.

3.3.2.  Decarbonize performance analysis of Device. Decarbonize performance of Shortcut nitrification denitrification - Anammox coupling device is illustrated as Figure 5.

Figure 5 shows that inflow water COD of Shortcut nitrification denitrification reactor is about 500 mg/L and COD load is 0.5 mg COD/(mg ML SS·d), the effluent water after preparation treatment is between 214.39 and 246.38 mg/L, COD removal rate is about 53%; COD of effluent water in Anammox reactor is between 73.48 and 143.64 mg/L and AABO suitable for the organic environment. The effluent water of Preparation reactor contains DO, which seldom effect the AAOB activation because reactor runs at a low rate of DO. Besides that, the effluent water of preparation reactor pH is about 7.4, which is not suitable for AAOB active growth because of AAOB prefer alkaline condition.

In brief, the TN and COD in coupling device reach 88% and 80%, which ensure a stable operation.

4.  Teaching application and expansion

4.1.  Experimental facility innovation and application
The benefit to couple Shortcut nitrification denitrification and Anammox as discussed above could also shorten reaction time and energy saving in an environment-friendly practical teaching value. The device could run constantly and operate for several years, hereby supply annual experimental course to study new biosystem removal of nitrogen technique.

4.2.  Course appraisal mode explore
Routing practical teaching normally conducts from theory taught by teacher to hand operated by students. To apply new experimental device, practical course could link pre-class, in-class and after-class as a whole for appraisal mode. Pre-class request students to autonomously design practical process after
understanding the device function (mark as 30%). In-class request students to operate the device and monitor the sampling and data record (mark as 40%). After-class request students to analyze the data and problems faced during the experiment (mark as 30%). This new course appraisal could benefit both the new device and practical teaching.

Figure 6. Course Appraisal Mode

5. Conclusion
(1) During stable operation of coupling device, the inflow water NH$_4^+$-N is 500 mg/L and effluent water is 6 L/d, the denitrification effect is good after a 20-days operation. In coupling system, TN wiped off load is 0.44 kg N/ (m$^3$·d) while removal rate is 88%. In inflow water, the COD ratio is 500 mg/L (C/NH$_4^+$-N=1), inflow water COD load is about 0.5 mg COD/ (mg MLSS ·d). After pre-reactor treatment, the effluent water is between 214.39 and 246.38 mg/L, COD removal rate is about 53% and further wiped off after entering to Anammox reactor. Because Anammox reactor already fit the organic environment, so COD has less affection on AAOB. The coupling craft makes the COD removal rate to 80% and a better decarburization in a stable operation.

(2) Based on theory teaching, students have a preliminary understanding of Shortcut nitrification denitrification and Anammox. The device can help students to observe the process directly and new biosystem method. The new experimental device could combine the date monitoring and results analysis together, which improve the innovation and science literacy with a practical teaching reform.

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References
[1] Shu-qin KANG. Study on the treatment of high ammonia nitrogen wastewater by short-range nitrification - anaerobic ammonia oxidation process.[D]. Wuhan University of technology, 2008.
[2] Xia-hui ZHI, Hong-wu WANG, et al. Study on nitrification and denitrification of nitrification under normal temperature [J]. Environmental science research, 2006, 19(1), pp: 26-29.
[3] Ru-qi LI, Qiu-li HE, et al. The experiment teaching reform is promoted by homemade experiment teaching instrument. [J]. Higher science education, 2009(1), pp: 71-74.
[4] Huan-cheng, ZHOU Guo-hua, MA, et al. The experiment teaching instrument of self-made experiment improves the quality of experiment teaching. [J]. Laboratory science,2008,11 (1), pp: 153-155
[5] Loosdrecht M C M V, Jetten M S M. Microbiological conversions in nitrogen removal [J]. Water Science & Technology, 1998, 38(1), pp: 1-7.
[6] Helmer C, Kunst S, Juretschko S, et al. Nitrogen loss in a nitrifying biofilm system[J]. Water Science & Technology, 1999, 39(7), pp: 13-21.
[7] Xiao-di HAO. The Rotterdam DOKHAVEN sewage plant in the Netherlands. [J]. Water supply and drainage, 2003, 29(10), pp: 19-25.

[8] Mulder A, Van d G A A, Robertson L A, et al. Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor [J]. Fems Microbiology Ecology, 1995, 16(3), pp: 177–184.

[9] Graaf A A V D, Mulder A, Bruijn P D, et al. Anaerobic oxidation of ammonium is a biologically mediated process [J]. Applied & Environmental Microbiology, 1995, 61(4), pp: 1246-1251.

[10] Strous M, Kuenen J G, Jetten M S M. Key Physiology of Anaerobic Ammonium Oxidation[J].Applied & Environmental Microbiology, 1999, 65(7),pp: 3248-3250.

[11] Xu-liang CHEN, Study on treatment of monosodium glutamate wastewater by short-range nitrification - anaerobic ammonia oxidation process. [D]. Zhejiang university, 2006.

[12] Zheng-yong XU. Based on nitrification, anaerobic ammonia oxidation and denitrification coupling technology and its control strategy research. [D]. Hunan university, 2011.

[13] Water and waste water monitoring and analysis method. [M].4version.Beijing: China environmental science press, 2002