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Advanced removal of organics and nitrogen in upgraded anaerobic-aerobic slaughterhouse wastewater treatment process

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ABSTRACT

Slaughterhouse wastewater (SWW) is one of the most harmful agriculture and food industrial wastewaters because of rich impurities, particularly organic matter, suspended solids, oil, grease, and nitrogen (N) nutrients. This study investigated organics and N nutrients removal performance in the developed upgraded anaerobic-aerobic slaughterhouse wastewater treatment process (U-AASWWTP). The supernatant reflux position, ratio and dissolved oxygen (DO) in the aerobic units play key roles, and the optimized solutions were 300% reflux to the second compartment at DO of 4.64–4.90 mg/L. Under such conditions, chemical oxygen demand (COD) removal efficiency only decreased from 98.14% to 95.94% when the loading rates increased from 500 to 2,000 g·COD/(m^3·d) gradually, whereas total nitrogen (TN) showed a different removal trend. Effluent TN was the lowest at 1,500 g·COD/(m^3·d) and the corresponding effluent COD, TN and NH_4^+–N all satisfied with the Chinese upcoming discharge standard. Therefore, U-AASWWTP can effectively remove COD and N from SWW and would become the main direction of traditional treatment processes upgrading to addressing the issue of upcoming stricter effluent standard. This study holds significant importance in ensuring the sustainable development of slaughtering industry in China. Additionally, this study enhances the understanding of the synergy relationship between anaerobic digestion and Anammox microorganisms, thereby promoting the intelligent application in SWW treatment processes.

Keywords: Organics and nitrogen removal; Microbial synergy; Anaerobic digestion (AD); Partial denitrification/partial nitrification-Anammox; Slaughterhouse wastewater (SWW) treatment

1. Introduction

The increase in population and living standards has led to a decline in water quality. Effluent wastewater from slaughterhouses is rich in impurities especially for organic matter, suspended solids, oil and grease, and N nutrients [1,2], which can cause significant damage to the marine environment [3] and have detrimental effects on both human beings and aquatic flora and fauna [4].

Accordingly, various world leading organisations, such as the United States Environmental Protection Agency (USEPA) and European Union (EU), have established effluent discharge standards for slaughterhouse wastewater (SWW). In China, the "Effluent Standard of Pollutants for Meat Processing Industry" (GB 13457-92) was first established

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in the last century. Recently, the second outline draft of the updated "Effluent Standard of Pollutants for Slaughter and Meat Processing Industry" has been made available for public input online (000014672/2018-01201). It could be found that the permitted maximum concentration of effluent total nitrogen (TN) has been added as 25 mg/L for existing enterprises, 20 mg/L for new enterprises and 15 mg/L for environmentally sensitive and economically developed regions, respectively. At the same time, effluent chemical oxygen demand (COD), total suspended solids, animal and vegetable oils and pH contamination level has been stricter to 80, 50, and 10 mg/L and 6–9, respectively.

In order to safely dispose of the effluent, proper treatment is required to meet these standards. For organic-rich wastewaters, such as SWW, biological treatment is usually preferred over other options like electrocoagulation, membrane separation, and advanced oxidation [4,5]. Among biological treatment methods, anaerobic processes are commonly used for efficient COD removal in high-concentration organic wastewater treatment due to their low sludge production and energy requirements [6]. However, simple anaerobic processes may struggle to meet the discharge standards for COD removal [7], and the high levels of organic nitrogen and ammonia (NH_4^+) present in SWW may not be effectively degraded. Therefore, combined anaerobic-aerobic processes has been developed and utilized to achieve high COD and NH_4^+ removal while reducing operation and maintenance costs [8]. However, effectively controlling TN removal remains challenging.

In this study, an upgraded anaerobic-aerobic slaughterhouse wastewater treatment process (U-AASWWTP) was developed specifically for SWW characteristics to achieve the anaerobic COD digestion and TN removal step by step. The developed U-AASWWTP incorporated the prepositional anaerobic COD digestion to address the inhibition of COD on TN removal through partial denitrification (PD)-Anammox and/or partial nitrification (PN)-Anammox. The introduction of supernatant reflux enhanced TN removal by providing optimal growth conditions for Anammox and denitrifying bacteria [9].

The Anammox conversion comprises the oxidation of ammonium with nitrite as electron acceptor to nitrogen gas under anoxic conditions as described in Eq. (1) [10]. The implementation of the Anammox process needs a preceding step in which (1) half of the ammonium in the wastewater is oxidized to nitrite by ammonia oxidizing bacteria (PN) [11,12] or (2) the PD is occurred until nitrite only by either autotrophic or heterotrophic microorganisms [13].

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 2.03H_2O + 0.066CH_2O_{1.5}N_{0.15} + 0.26NO_3^- \quad (1)$$

Regarding the presence of high organic matter compounds in many industrial wastewaters (such as SWW), Anammox bacteria are greatly affected because they were obligate chemolithoautotrophic [14]. On the other hand, some organotrophic Anammox species, such as *Candidatus* Jettenia asiatica, had the ability to co-metabolize volatile fatty acids (VFA) as electron donor with nitrite/nitrate as the electron acceptor, indicating more advantages than autotrophic Anammox process [15].

The Anammox process is suitable for treating wastewater with low C/N. The Anammox bacteria are no longer able to compete with heterotrophic denitrifying bacteria when C/N is higher than 1 [16]. When C/N ratio is 1.5–2.0, a complete TN removal is possible occurred [17]. However, SWW is rich in organics and its C/N is always between 8 and 15, which is unsuitable for Anammox bacteria survival. The multilevel structured reactor would give the opportunity for step-by-step anaerobic digestion and Anammox to simultaneously remove COD and TN effectively in the single system. Moreover, the denitrification-Anammox and dissimilatory nitrate reduction to ammonium (DNRA)-Anammox occurring in anoxic baffled reactor can improve TN removal in the mainline [18].

Therefore, the overall goal of this research was to enhance the step-by-step anaerobic digestion and Anammox for removal of organics and nutrients in the U-AASWWTP. The specific objectives were to: (1) investigate the effect of the supernatant reflux position, ratio and dissolved oxygen (DO) in biological contact oxidation (BCO) tank on COD and N species removal using Box–Behnken design (BBD) and response surface methodology (RSM); (2) summarize the optimum conditions for simultaneous COD and TN removal; (3) investigate the sustained SWW loading rate for U-AASWWTP; and (4) evaluate and summarize the mechanism of step-by-step anaerobic digestion and PD/PN-Anammox for simultaneous removal of organics and nutrients.

2. Materials and methods

2.1. Experimental set-up

The U-AASWWTP used in the current study is similar to the one from a previous study [9], but two stage aerobic units were used to enhance the nitrification and nitritation when the loading rate increased gradually. U-AASWWTP (Fig. 1) consists of regulating tank (RT), anaerobic-anoxic baffled reactor (A-ABR) with four anaerobic compartments (1A, 2A, 3A and 4A) and one anoxic compartment (5A), two-stage aerobic units (1O and 2O) and clarifier successively.

The acrylic bioreactor contained the 108 L anaerobic-anoxic baffled reactor (A-ABR) (23.4, 22.5, 21.6, 20.7 and 19.8 L for A1, A2, A3, A4 and A5, respectively) and 24.0 + 24.0 L aerobic units (O1 and O2). The design of each aerobic unit was referred to as the previous single biological contact oxidation reactor [9].

2.2. SWW composition

Synthetic SWW was prepared according to the real wastewater, which has been discussed in the previous study [19]. Synthetic SWW was prepared aimed to contain 2,000 ± 100 mg/L of COD and 180 ± 10 mg/L of TN by adding the fresh porcine blood and sodium citrate. Note that sodium citrate was not only used to adjust the C/N but also to avoid blood coagulation.

2.3. Experimental procedures

2.3.1. Experimental start-up

The inoculums were taken from the corresponding anaerobic, anoxic and aerobic tanks in the previous case

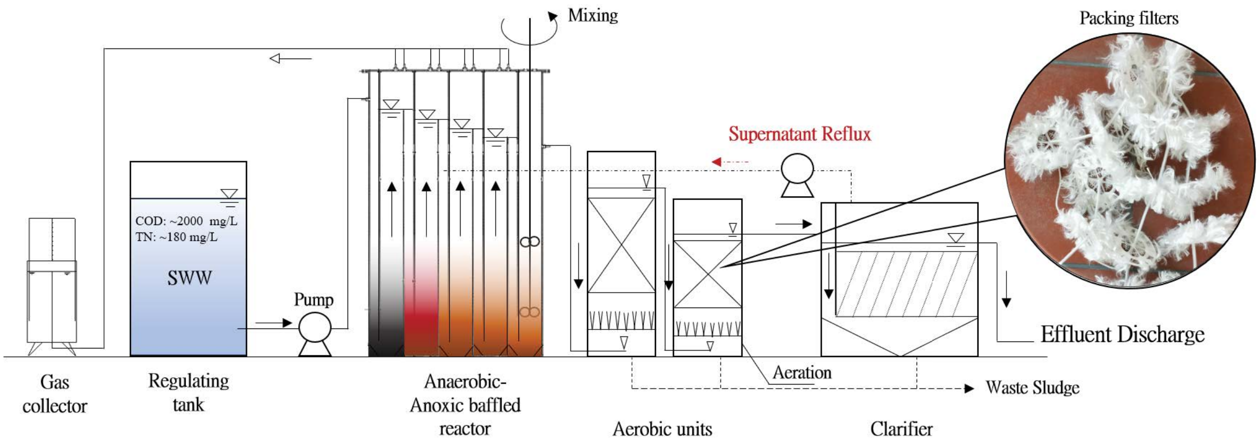

Fig. 1. Flowchart of upgraded anaerobic-aerobic slaughterhouse wastewater treatment process (U-AASWWTP).

study [19]. Seed sludge was acclimated in the developed U-AASWWTP bioreactor for 30 d in the continuous mode with the pollution load of 500 g·COD/(m^3·d) and run at room temperature of 25°C ± 2°C. The bioreactor was considered as start-up successfully when the effluent COD was kept less than 80 mg/L, which was the effluent limit in the online draft of "Effluent Standard of Pollutants for Slaughter and Meat Processing Industry" (000014672/2018-01201). Note that the Anammox microorganisms acclimated to the dominant bacterial community painfully slow due to the slow growth rate [20]. In this study, the whole period of BBD experiments was the process of forming the dominant Anammox bacterial community.

2.3.2. U-AASWWTP optimization

Applying the Design of Experiments (DoE) has the advantage that with fewer experiments needed information could be gained to estimate the interactions between the parameters and develop an empirical model [21]. BBDs is one of the DoE suitable for the simultaneous optimization of three independent parameters.

To determine the appropriate supernatant reflux position, ratio and DO in the aerobic unit for COD and TN removal in U-AASWWTP, the bioreactor was run according to the BBD in Tables 1 and 2 for 325 d together with the culture of Anammox microorganisms. The supernatant reflux position and ratio were chosen based on the previous study [9]. DO in each aerobic unit was run between 2–6 mg/L.

Two parallel samples for effluent were taken every 24 h. When the effluent COD and TN concentrations were stable (a *P*-value with a 95% confidence level) [22], two parallel samples for influent, effluent and at different compartments were taken and analyzed for COD, TN, NH_4^+–N, NO_2^-–N, NO_3^-–N and pH. Experiments were repeated if there was a sample analysis error greater than 5%.

2.3.3. Optimum operation conditions validation

The validation experiment was performed under the optimum supernatant reflux position, ratio and DO in the aerobic unit by RSM. The loading rate and temperature were both consistent with the BBD experiment. The samples taken and analysed were the same as described in section 2.3.2 – U-AASWWTP optimization. The experiment run for 15 d.

Table 1
Levels of variables chosen for the trials

Factors	Actual value (Levels)		
	–1	0	1
A: supernatant reflux position (compartment)	2	3	4
B: supernatant reflux ratio (%)	100	200	300
C: DO in BCO tank (mg/L)[a]	2	4	6

[a]DO fluctuated between 5% of the target concentration to meet the requirement.

2.3.4. U-AASWWTP loading investigation

U-AASWWTP was run over a period of 60 d to determine its limit value for organic loading rate. The organic loading rates gradually increased from 500 to 2,000 g·COD/(m^3·d) at an interval of 500 g·COD/(m^3·d). Note that the TN loading rates would synchronized-increase proportionally according to the characteristics of SWW. The process run 15 d for each organic loading rate. Profiles of COD, TN, NH_4^+–N, NO_2^-–N, and NO_3^-–N concentrations vs. waterflow from influent to effluent of U-AASWWTP were investigated when the corresponding removal efficiencies stable to evaluate and summarize the mechanism of step-by-step anaerobic digestion and Anammox for removal of organics and nutrients.

2.4. Experimental design and data analysis

Design–Expert (Version 8.0) software was used for the experimental design and data analysis [23,24]. BBD and RSM were applied to optimize the supernatant reflux position (*A*), ratio (*B*) and DO in BCO tanks (*C*) in a 15-run study as shown in Table 1. The low, middle, and high levels of each variable were designed as –1, 0, and +1, respectively. The detailed experimental design is shown in Table

Table 2
BBD for the study of three experimental variables, the supernatant reflux position, ratio and dissolved oxygen in BCO tank, and the achieved experimental and analytical results

Run no.	BBD codes	Experimental results (mg/L)[a]					
		COD	TN	TIN	NH_4^+–N	NO_3^-–N	NO_2^-–N
R1	+1, –1, 0	50.3 ± 5.3	91.0 ± 4.2	56.66 ± 0.39	0.45 ± 0.04	0.87 ± 0.10	55.34 ± 0.73
R2	–1, +1, 0	29.5 ± 2.1	48.4 ± 4.2	37.54 ± 1.76	0.79 ± 0.05	1.34 ± 0.13	35.41 ± 1.88
R3	0, 0, 0	23.5 ± 0.7	62.7 ± 16.2	51.21 ± 1.05	0.79 ± 0.97	0.61 ± 0.52	49.81 ± 1.47
R4	–1, –1, 0	19.5 ± 2.1	65.0 ± 0.8	56.31 ± 2.08	0.17 ± 0.18	1.74 ± 0.89	54.40 ± 2.24
R5	0, 0, 0	21.0 ± 0.7	60.8 ± 4.2	49.96 ± 1.29	0.69 ± 0.00	1.63 ± 0.05	48.33 ± 1.34
R6	+1, +1, 0	20.5 ± 3.5	52.5 ± 5.23	41.15 ± 0.84	0.45 ± 0.10	1.66 ± 0.06	39.04 ± 0.76
R7	0, 0, 0	20.5 ± 0.7	65.2 ± 2.69	54.74 ± 0.86	0.68 ± 0.17	1.28 ± 1.06	49.8 ± 0.45
R8	0, –1, –1	31.5 ± 6.4	129.5 ± 6.36	98.07 ± 1.68	76.59 ± 1.55	2.77 ± 0.55	18.72 ± 2.14
R9	+1, 0, –1	29.0 ± 0.0	110.0 ± 3.68	73.83 ± 1.50	46.77 ± 0.08	2.78 ± 0.20	24.28 ± 1.35
R10	–1, 0, –1	40.0 ± 3.6	91.3 ± 3.25	80.19 ± 3.02	5.00 ± 3.92	63.53 ± 0.16	11.66 ± 4.23
R11	0, +1, –1	41.5 ± 3.5	81.0 ± 0.21	70.71 ± 0.98	62.32 ± 0.63	2.71 ± 0.41	5.68 ± 1.46
R12	+1, 0, +1	72.0 ± 11.3	98.8 ± 5.30	61.35 ± 1.13	3.03 ± 1.40	BDL[b]	58.32 ± 1.72
R13	0, –1, +1	42.5 ± 2.1	101.8 ± 6.71	75.81 ± 0.78	0.69 ± 0.05	1.53 ± 0.45	73.59 ± 1.21
R14	0, +1, +1	37.5 ± 1.4	43.1 ± 1.70	33.38 ± 0.22	0.57 ± 0.17	0.41 ± 0.14	32.39 ± 0.84
R15	–1, 0, +1	39.0 ± 0.7	59.4 ± 1.84	44.4 ± 0.20	0.80 ± 0.06	1.26 ± 0.82	42.33 ± 0.64

[a]Values were average of four measurements/d and different between the measurements for each were less than 5%.
[b]BDL = Below detection limit (0.01 mg/L for NO_3^-–N).

2, including 12 factorial points and 1 center point with 2 additional experimental trials as the replicates of this point. The factorial points consisted of all possible combinations of the levels, except for the point of all high levels, which is one of the most important advantages of BBD [25]. Computation was carried out using the least squares method in multiple regression analysis [26]. The mathematical relationship of the response could be approximated by a quadratic polynomial equation in a system involving three significant variables *A*, *B*, *C* [Eq. (2)] [26]:

$$Y = C_0 + C_1A + C_2B + C_3C + C_{12}AB + C_{13}AC + C_{23}BC + C_{11}A^2 + C_{22}B^2 + C_{33}C^2 \quad (2)$$

where Y is the predicted yield, C_0 is the constant, C_1, C_2, and C_3 are the linear coefficients, C_{12}, C_{13}, and C_{23} are the cross-product coefficients, and C_{11}, C_{22}, and C_{33} are the quadratic coefficients.

Statistical analysis was carried out using analysis of variance (ANOVA) as described by Tong et al. [23]. P-values < 0.05 were considered statistically significant. The coefficient of determination, R^2, was used to express the goodness of fit of the polynomial model.

Moreover, one-way ANOVA was used to calculate the P-values (probability) for the effluent COD, TN, total inorganic nitrogen (TIN), NH_4^+–N, NO_2^-–N, and NO_3^-–N concentrations when U-AASWWTP run at different loading rates. A P-value with a 95% confidence level was considered to be significant and a 99% confidence level to be highly significant [22].

2.5. Analytical methods

Water samples were taken from each stage and then filtered by 0.45 μm membrane before detection except for COD and TN. NH_4^+, NO_2^-, and NO_3^- concentrations for effluent were measured by ion chromatography [27] using an ECO Compact IC pro system (Metrohm AG, Switzerland), and the method detection limits (MDLs) were 0.07, 0.04, and 0.01 mg/L, respectively. NH_4^+, NO_2^-, and NO_3^- concentrations from influent and U-AASWWTP were measured using a spectrophotometer (DR6000, HACH, USA) according to the Chinese NEPA Standard Methods [28], and the MDLs were 0.035, 0.003, and 0.052 mg/L, respectively.

COD was measured through Lovibond mid-range kits with the MDL of 0–1,500 mg/L according to standard methods [29]. TN concentration was measured using HACH TNT plus 827 test kit. pH was measured using a pH meter with calibrated electrode (FiveEasy Plus, Mettler Toledo, Switzerland; MDL: 0–14). DO was measured using a DO meter (Oxi 3310, WTW, Germany).

3. Results and discussion

Experiments designed by BBD (Table 2) were performed randomly at the set values of the supernatant reflux position, ratio and DO in the aerobic unit. Data collected for effluent COD and N species from U-AASWWTP were analysed. The relationship and effect among the supernatant reflux position, ratio and DO in each aerobic unit on anaerobic digestion and Anammox were mathematically modelled based on Eq. (2).

3.1. Profiles of COD and N species removal

Profiles of COD, TN, NH_4^+–N, NO_2^-–N, and NO_3^-–N concentrations vs. waterflow from influent to effluent of U-AASWWTP for three central points (0, 0, 0; Runs no. 3, 5, and 7) of BBD experiments are shown in Fig. 2. As shown in Fig. 2a, COD was removed successively along with the SWW flow. About 54.6% COD was removed at the supernatant reflux compartment. Both anaerobic digestion of COD and reflux of low concentration effluent contributed to this high COD removal efficiency at the supernatant reflux compartment, which was similar to the results observed at the previous study for traditional AASWWTP [9].

Fig. 2b–e shows the trends of TN and NH_4^+–N removal, and NO_2^-–N and NO_3^-–N accumulation, respectively. Similar to COD removal trend in A-ABR, TN decreased slowly before the supernatant reflux compartment together with organic digestion and then sharply because of reflux. And then TN slightly increased during the following processes, which was different to that of COD removal.

The organic N amination led to the NH_4^+ increased at A-ABR except for the supernatant reflux compartment. This might be due that the organic N was gradually mineralized to NH_4^+ along with the SWW flow. NH_4^+–N and TN contents decreased sharply at the supernatant reflux compartment mainly during the simultaneous occurrence of multiple N removal reactions, such as denitrification, partial denitrification and Anammox processes, which would be description and discussion with the later NO_2^-–N and NO_3^-–N description.

As shown in Fig. 2e, effluent NO_3^-–N concentration was approximately 51.99 ± 0.56 mg/L. This NO_3^-–N was drawn into the supernatant reflux compartment of A3 with a reflux ratio of 200%. However, both NO_3^-–N and NO_2^-–N contents in this compartment were very low, with concentrations of 0.69 ± 0.56 mg/L and below detection limit, respectively. This might be attributed to the NO_3^- introduced from both the supernatant reflux and upper compartment undergoing either (1) heterotrophic denitrification to N_2 or (2) PD to NO_2^-, which immediately reacted with NH_4^+ through the Anammox process [30]. The ratio of COD in A3 to NO_3^- from the reflux was only about 1.85, which was insufficient for complete heterotrophic denitrification. Therefore, PD was the main process responsible for NO_3^- removal, and PD-Anammox played a significant role in TN removal. Additionally, the presence of reddish-brown microbial granular sludge in the reflux compartment (Fig. 3a) confirmed the presence of Anammox bacteria. As the BBD experiment progresses, the reddish-brown microbial granular sludge containing Anammox bacteria gradually became the dominant community (Fig. 3b), which would be fully studied through the microorganisms' community analysis in the next step study. The reflux NO_3^- was not enough to consume all NH_4^+ in A3 compartment, and the remainder NH_4^+ was oxidized to effluent NO_3^- under aerobic conditions in aerobic units. Effluent NO_3^- was then reintroduced to A3 through reflux, resulting in a consistent concentration of 51.99 ± 0.56 mg/L. It could be also concluded that the optimal operating conditions could only appropriately reduce the concentration of TIN in effluent and alter the proportion of different N species (NH_4^+–N, NO_2^-–N or NO_3^-–N), but complete TIN removal cannot be achieved through supernatant reflux alone. To achieve complete TIN removal, the optimal operating conditions would to make the residual inorganic nitrogen all as NO_3^-, and then introduce the corresponding high-efficiency autotrophic denitrification to remove all the remained NO_3^- as previously developments [22,31]. This approach would be an effective advanced N removal method for SWW. Furthermore, strict discharge standard for NH_4^+–N in SWW must be met before discharge, which also necessitates minimizing the emission of NH_4^+ from U-AASWWTP. Hence, it is reasonable that NO_3^- is the main component of the residual TIN.

NO_2^-–N accumulation in the whole U-AASWWTP was lower than 0.7 mg/L (Fig. 2d), even at the supernatant reflux compartment. As the direct reactant of Anammox, the complete consumption of NO_2^- indicated that Anammox process

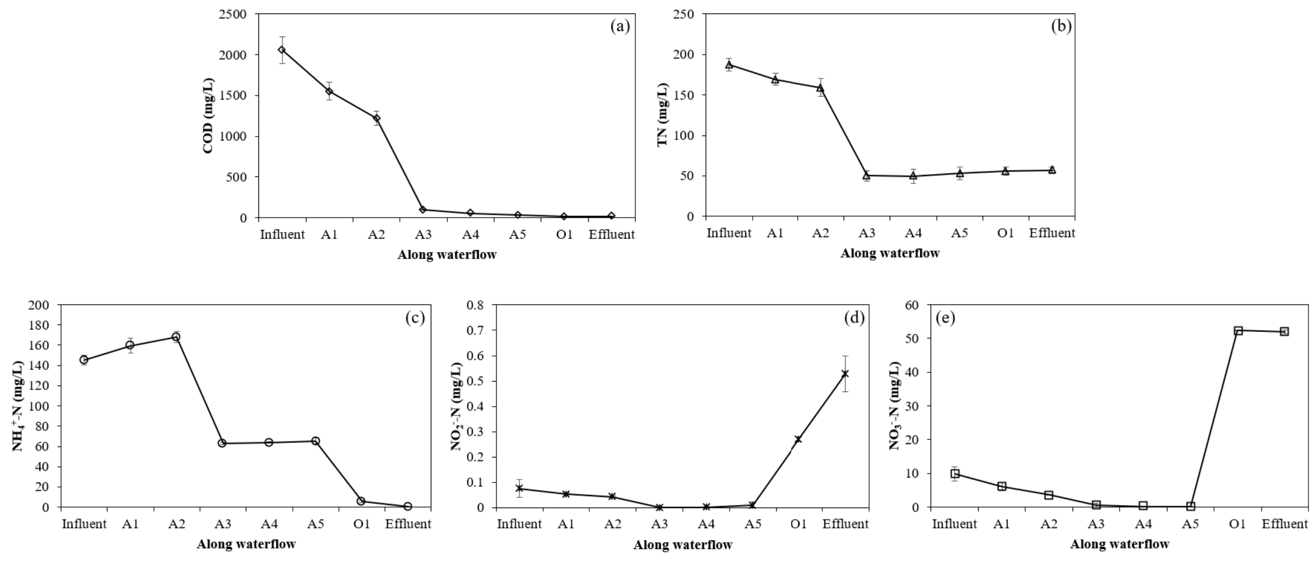

Fig. 2. Profiles of COD, TN, NH_4^+–N, NO_2^-–N, and NO_3^-–N concentrations vs. waterflow from influent to effluent in central points of BBD for U-AASWWTP.

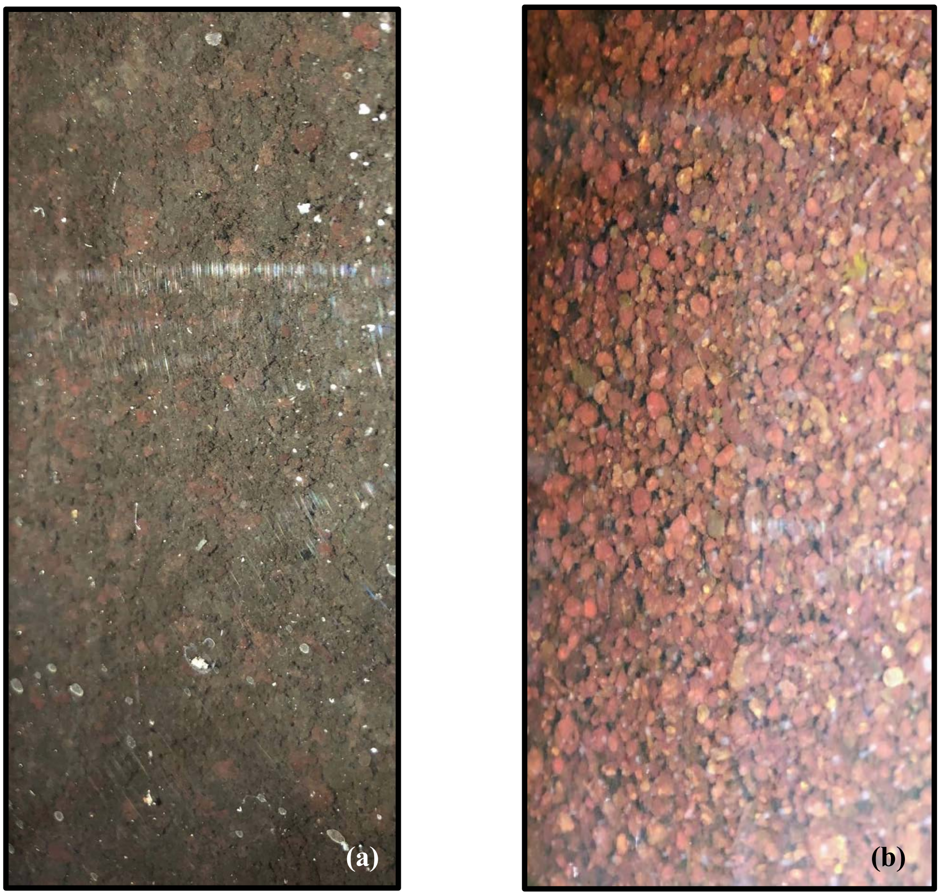

Fig. 3. Pictures of reddish-brown microbial granular sludge formation process in A-ABR. (a) Picture of 3A bottom on the 68th day of run and (b) picture of 2A bottom on the 300th day of run.

has been maximized [32]. Note that this low NO_2^-–N accumulation also led to NO_3^- becoming the absolute major component of TIN in effluent for further advanced treatment.

In summary, effluent N of U-AASWWTP was mainly residual NO_3^- at the three central points (0, 0, 0; Runs no. 3, 5, and 7). Appropriate reflux promoted the effective removal of TN and COD. Further acclimation of the reddish-brown microbial granular sludge and optimization of operating conditions through BBD were essential for more efficient TN removal.

3.2. Optimization of U-AASWWTP

3.2.1. Statistical analysis

Table 2 shows the three-factor, three-level BBD with observed values for the effluent COD, TN, TIN, NH_4^+–N, NO_3^-–N and NO_2^-–N concentrations from U-AASWWTP. The RSM was used for parameter estimation, specifying the correlation between the input variables and the output responses, as previously shown in Eq. (2). Consequently, in order to estimate the response functions for the effluent COD, TN and NH_4^+–N concentrations, the second-order polynomial Eqs. (3)–(5) were developed. Eqs. (3)–(5) represents the models in terms of coded values as a function of the supernatant reflux position (*A*), ratio (*B*), and DO in aerobic units (*C*) for effluent COD, TN and NH_4^+–N concentrations, respectively. Note that only effluent COD, TN and NH_4^+–N models were given in consideration to match the updating "Effluent Standard of Pollutants for Slaughter and Meat Processing Industry" in China (000014672/2018-01201).

$$Y(\text{COD}) = 21.67 + 5.47A - 1.84B + 6.13C - 9.94AB + 11.00AC - 3.75BC + 7.51A^2 + 0.76B^2 + 15.82C^2 \tag{3}$$

$$Y(\text{TN}) = 62.89 + 11.01A - 20.29B - 13.60C - 5.47AB + 5.16AC - 2.54BC + 1.18A^2 + 0.16B^2 + 25.79C^2 \tag{4}$$

$$Y(\text{NH}_4^+ - \text{N}) = 0.72 - 1.82A - 1.72B - 30.52C - 0.16AB + 4.75AC + 3.54BC - 3.38A^2 + 3.13B^2 + 31.19C^2 \tag{5}$$

Unfavorable effects are indicated by negative coefficients of the model components [8]. In the case of effluent COD [Eq. (3)], the unfavorable effects come from the model components *B*, *AB*, and *BC*. Similarly, *B*, *C*, *AB*, and A^2 have unfavorable effects on the effluent TN concentration [Eq. (4)]. Correspondingly, *A*, *B*, *C*, *AB*, and A^2 have unfavorable effects on the NH_4^+–N removal [Eq. (5)].

Besides, the adequate precision of the effluent COD, TN and NH_4^+–N concentrations were 9.153, 9.928, and 15.637, respectively. Thus, because all adequate precision values were greater than 4.00, the developed models can be used to navigate the BBD space [33]. Moreover, the model *F*-values were 6.09, 9.24, and 35.89 for effluent COD, TN and NH_4^+–N concentrations, respectively, implying the models are all significant. Correspondingly, there were only 3.03%, 1.24%, and 0.05% chance of this large "Model *F*-value" occurring due to noise, respectively.

3.2.2. Optimization of operating conditions

The effects of the supernatant reflux position, ratio and DO in aerobic units on the removal of COD and TN were studied to obtain the lowest effluent concentrations (Fig. 4). The optimization was accomplished at defined optimization conditions (Table 2) using built-in numerical optimization method of the statistical software Design–Expert 8.0. Eqs. (3) and (4) were defined as objective functions, whereas, the independent variables in their critical range were used as constraints. The cross-factor interaction effects between the optimum experimental conditions for effluent COD, TN and NH_4^+–N concentrations were shown in Fig. 2a–c, respectively. As shown in Fig. 4c, effluent NH_4^+–N increased sharply when DO in aerobic units was lower than 4 mg/L and was affected by the supernatant reflux position, ratio and DO in aerobic units simultaneously as DO range between 4 and 6 mg/L.

Numerical optimization was used to minimize effluent COD, TN and NH_4^+–N concentrations. The desirability value of 0.47 was determined to achieve the corresponding minimum concentrations of 26, 42.89, and 0.19 mg/L for effluent COD, TN and NH_4^+–N, respectively. The corresponding optimized supernatant reflux position, ratio and DO in aerobic units were refluxed to the second compartment with the ratio of 300% and 4.64–4.90 mg/L DO, respectively. Note that NO_2^- and NO_3^- had no corresponding discharge standard for SWW, hence, they were considered together with effluent TN concentrations without separate RSM.

3.2.3. U-AASWWTP performance at the optimum conditions

In section 3.3 – Analysis of organics and nutrients removal capacity, the optimum operating conditions of the supernatant reflux to the second compartment at the ratio of 300% and DO in aerobic units fixed at 4.64–4.90 mg/L were suggested by RSM. Fig. 5 shows effluent COD, TN and NH_4^+–N concentrations at the suggested optimum operating conditions at the same organic loading rate of 500 g·COD/(m^3·d). COD in effluent was ranged between 33 and 62 mg/L, which kept lower than the discharge standard of 80 mg/L.

During the first four days, U-AASWWTP was in the start-up stage, which was adjusted from the last BBD run. Effluent TN and NH_4^+–N showed the first increase and then decrease, which was mainly due to the adjustment of the reactants supply for Anammox bacteria. After that, effluent TN and NH_4^+–N concentrations were stable at 41.37–46.36 and 0.62–1.44 mg/L, respectively. Effluent TN was consistent with the RSM predictions of 42.89 mg/L, which was main consist of NO_3^-.

3.3. Analysis of organics and nutrients removal capacity

The organics and nutrients treatment capacity of U-AASWWTP was studied at different SWW loading rates. The organic loading rate was used to measure the overall pollution loads due to COD/TN was almost constant for SWW. TN loading rates would increase in proportion as the organic loading rates increase.

Fig. 5 shows the effluent COD, TN and NH_4^+–N concentrations from U-AASWWTP when the organic loading rates increased from 500 to 2,000 g·COD/(m^3·d) gradually. It could be found that effluent COD significantly increased when the organic loading rates increased from 500 to 1,000 g·COD/(m^3·d) (*P*-value = 0.004) and then remained basically stable as the loading rates increased further (*P*-value = 0.003). However, effluent COD was always lower than the discharge limit of 80 mg/L when the U-AASWWTP was running stably at each organic loading rate.

Effluent TN concentrations exhibited a different trend compared to COD as the loading rate increased. There was no significant difference in effluent TN concentrations at the loading rates of 500 and 1,000 g·COD/(m^3·d) (*P*-value = 0.250), but they were significantly higher than that at 1,500 g·COD/(m^3·d) (*P*-value = 0.000) and significantly lower than that at 2,000 g·COD/(m^3·d) (*P*-value = 0.000). Effluent TN remained stabled at 24.62 ± 0.91 mg/L at a loading rate of 1,500 g·COD/(m^3·d), which almost met the upcoming discharge standard of 25 mg/L (000014672/2018-01201). From this, it could be concluded that TN removal efficiency did not increase

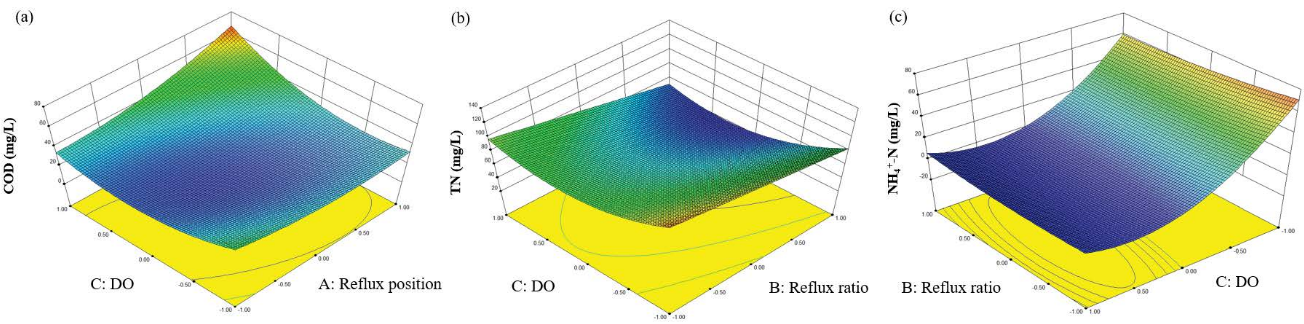

Fig. 4. Interaction between independent variables in U-AASWWTP shown on 3D surface plots. (a) Reflux position and dissolved oxygen of effluent COD, (b) reflux ratio and dissolved oxygen of effluent TN and (c) reflux ratio and dissolved oxygen of effluent NH_4^+–N.

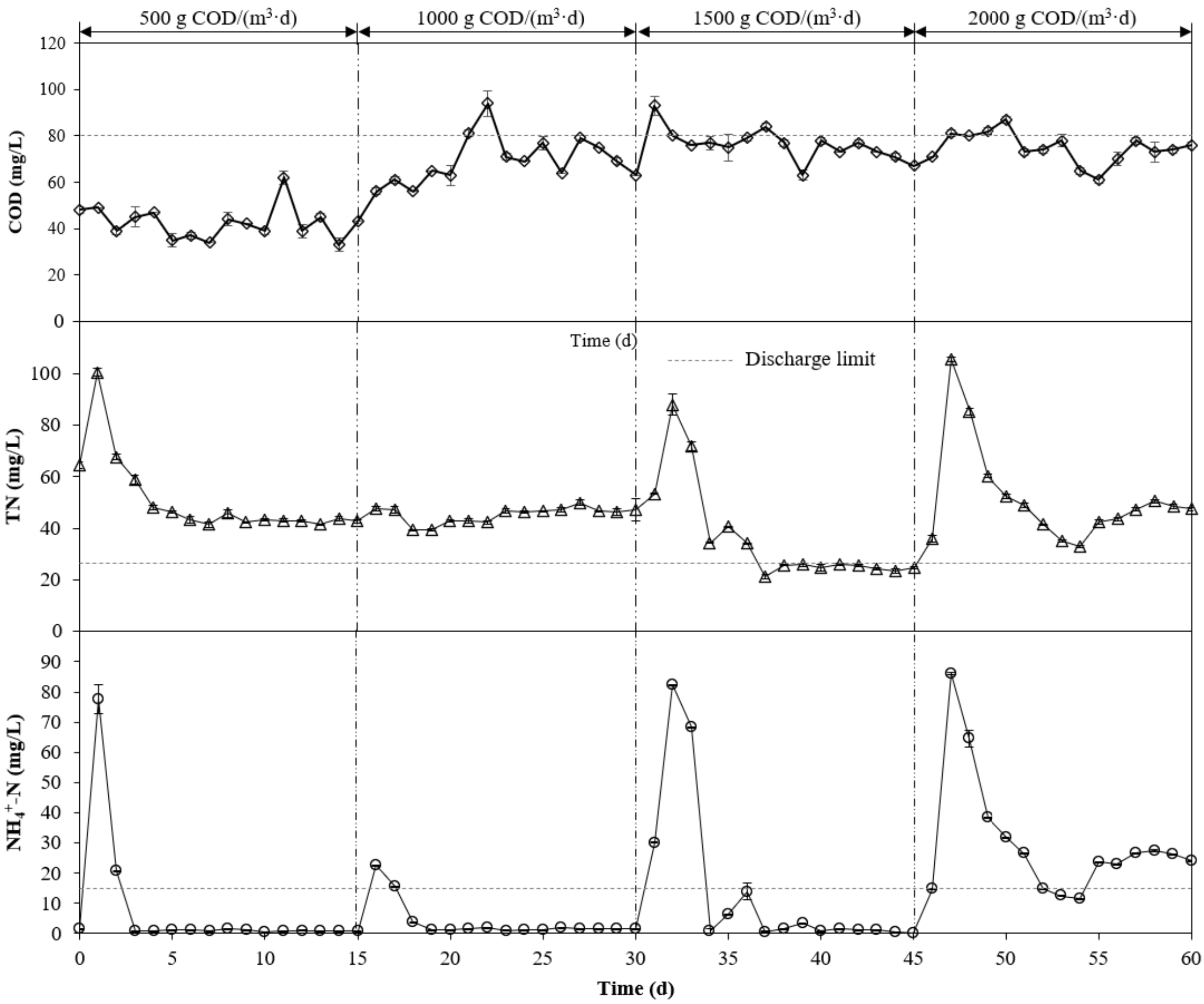

Fig. 5. Effluent COD, TN, and NH_4^+–N concentrations from U-AASWWTP at the different organic loading rates.

as the loading rate decreased, but rather had a significant relationship with the C/N and NH_4^+/NO_3^- ratio for fuller N removal from simultaneous PD and Anammox.

The design of U-AASWWTP in this study addressed the negative impact of high COD through prepositional anaerobic digestion. Both anaerobic digestion of COD and the reflux of low concentration effluent helped a low C/N ratio in the supernatant reflux compartment, as discussion in section 3.1 – Profiles of COD and N species removal. Increasing the loading rate appropriately promoted a decrease in the C/N ratio, thereby enhancing the simultaneous PD and Anammox process. The optimal synergy between anaerobic ammonia-oxidizing bacteria and denitrifying bacteria was achieved in an up-flow anaerobic sludge blanket when the C/N ratio was 0.6, and higher or lower C/N ratios would affect the TN removal efficiency [34]. In this study, the lowest effluent TN was observed when the organic loading rate was fixed at 1,500 g·COD/(m³·d) in the developed U-AASWWTP, indicating that the C/N ratio in the supernatant reflux compartment was most suitable for simultaneous Anammox and denitrification processes. The higher organic loading rate of 2,000 g·COD/(m³·d) leading to lower TN removal efficiency could further confirm the importance of prepositional anaerobic COD degradation to control the C/N ratio at the supernatant reflux compartment.

As shown in Fig. 5, effluent TN showed no significant difference in the loading rates of 500, 1,000 and 2,000 g·COD/(m³·d), but its composition was really different. When the loading rates were at 500 and/or 1,000 g·COD/(m³·d), there was almost no effluent NH_4^+ from U-AASWWTP. NO_3^- should be the main ingredients of TN, which would be shown in section 3.3.3. The residual NO_3^- during the U-AASWWTP was also in line with Eq. (1). Both the operating mode of U-AASWWTP and the mechanism of Anammox made it impossible for the system to completely remove inorganic nitrogen. The optimization or operating conditions could only reduce the concentration of effluent TIN as much as possible to ensure that the effluent TN could match the forthcoming discharge standards.

However, when the loading rate reached 2,000 g·COD/(m³·d), NH_4^+ became the predominant component in the effluent TN, and NO_2^- started to accumulate due to the PN process. The presence of residual NH_4^+ in the effluent indicated that the loading rate had exceeded the capacity of

U-AASWWTP to effectively remove TN. The nitrifying and Anammox bacteria in U-AASWWTP were unable to completely eliminate all NH_4^+ from SWW at such a high pollution load.

In summary, when the organic loading rate was fixed at 1,500 g·COD/(m^3·d), effluent TN was the lowest and effluent COD, TN and NH_4^+–N all satisfied with the Chinese upcoming discharge standard of 80, 25 and 15 mg/L, respectively. The developed U-AASWWTP would become the main direction of biological SWW treatment process upgrading for existing slaughtering and meat processing enterprises. This would be of great significance to ensure the sustainable development of the slaughtering industry in China.

The results of this study show that U-AASWWTP holds great potential as a technology for effectively removing COD and N species in SWW treatment. It is also suggested that the successful anaerobic degradation of COD ensures the appropriate COD/N ratio, thus facilitating efficient TN removal through PD/PN-Anammox processes. This research contributes to a better understanding of the synergy between anaerobic digestion and Anammox microorganisms, thereby promoting the intelligent application in SWW treatment processes. Further microbial investigations will be conducted to thoroughly analyze the mechanisms involved in N removal in the near future.

4. Conclusions

U-AASWWTP can effectively remove COD and N species from SWW via synergy anaerobic digestion and Anammox processes. The supernatant reflux position, ratio and DO in aerobic units play key roles in the system, and the optimized solutions were refluxed to the second compartment with a ratio of 300% at the DO in aerobic units of 4.64–4.90 mg/L. When the organic loading rate was fixed at 1,500 g·COD/(m^3·d), effluent TN was the lowest and effluent COD, TN and NH_4^+–N all satisfied with the Chinese upcoming discharge standard of 80, 25 and 15 mg/L, respectively. U-AASWWTP can effectively remove COD and N species from SWW and would become the main direction of traditional treatment processes upgrading to deal with the upcoming standard in China.

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