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Impacts of configurations of the up-flow anaerobic sludge blanket reactor and nitrogen forms on the nicotine degradation in tobacco sheet wastewater

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ABSTRACT

Tobacco sheet wastewater poses a high environmental risk due to its high nicotine concentration and complex composition. An up-flow anaerobic sludge blanket (UASB) reactor was selected to simulate the anaerobic biological treatment of tobacco sheet wastewater. The modification of the reactor's configurations, aiming at an enhanced nicotine removal efficiency (NRE), were studied. The effects of different forms of nitrogen, added into the tobacco leaching solutions, on the nicotine degradation rate (NDR) were also investigated. The results showed that NRE was at the highest when the UASB reactor was operated at a hydraulic retention time (HRT) of 18 h, pH of 6.5–7.0, temperature of 36–38°C, and granular up-flow velocity of 0.8 m/h. Results showed that NO_3–N enhanced nicotine degradation as well. The NDR observed over a period of 24 h negatively correlated with the rate of microbial production of CH_4, and positively correlated with the production of N_2O.

Keywords: UASB; Nicotine degradation rate; Reactor configurations; Forms of nitrogen

1. Introduction

Nicotine (1-methyl-2-(3-pyridyl)-pyrrolidine, $C_{10}H_{14}N_2$) is an amine compound consisting of a pyridine ring and a pyrrole ring. It quickly passes through most biological membranes, including the blood-brain barrier. Even at low concentrations, nicotine can cause diseases on the animal and human immune, respiratory and reproductive systems, while high doses may cause rapid death [1]. Due to its toxicity, nicotine has been classified as a chemical in the Toxic Release Inventory by the U.S. Environmental Protection Agency since 1994 [2]. Recent studies showed that nicotine and its metabolites may affect the apoptos is process in animal stem cells [3], and may relate to different types of cancer [4]. In addition, nicotine is one of the most harmful pollutants produced in the process of tobacco sheet production, because it has a high nitrogen content, slow degradation, and easy environmental releasing; it gets dissolved in water, contaminating surface water and groundwater, and threatening human health and ecological balance on a regional scale [5–7]. The efficient removal of nicotine and its toxic metabolic intermediates have become one of the key problems in treatment of tobacco sheet wastewater.

It has been reported that nicotine can be used and degraded by some microorganisms. Enders et al. found that yeast can degrade nicotine, however not completely [8]. In 1977, the Brown &Williamson tobacco company used *Cellulomonas* sp. to degrade nicotine and nitrate in tobacco in order to improve its quality [9]. Scholars began to study nicotine-degrading bacteria due to the increasing of tobacco production along with its pollution. The microorganisms, which can efficiently degrade nicotine, had been found, like *Pseudomonas putida* S16 [10,11], *Pseudomonas* sp. HZN6 [12,13] and *Ochrobactrumintermedium* DN2 [14]. Further studies have been conducted on degradation characteristics, degradation pathways of various bacteria, functional enzyme coding genes and their application in tobacco waste treatment. Preliminary research on the dynamic characteristics, pathways, and enzymatic characteristics of two nicotine degradation bacteria (*Pseudomonas* sp. HF-1 and *Arthrobacter* sp. HF-2) and one tobacco tar-degrading-

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bacteria (*Klebsiella* sp. MR4), which were detected in nicotine-contaminated soil, were performed by Ruan [15–17]. Most of these studies concerned the degradation of nicotine under single carbon or nitrogen source and aerobic culture condition, which laid a good foundation for the study of microbial degradation of nicotine in tobacco waste. Going forward, research must focus on the wastewater biological reaction system to understand the synergistic degradation mechanism of nicotine, and learn how to enhance the nicotine removal efficiency (NRE) of the functional microbes in a treatment system of tobacco sheet wastewater.

In this study, anup-flow anaerobic sludge blanket (UASB) reactor was constructed in the laboratory. Reactor configurations were modified to improve the NRE, furthermore nitrogen forms related to the nicotine degradation was analyzed in anaerobic system, which lays a foundation for exploring highly efficient nicotine-degrading bacteria and for the industrial application of treatment of tobacco sheet wastewater.

2. Materials and methods

2.1. Granular sludge and substrate

The granular sludge was obtained as seed material, diameters between 1.5–4.0 mm, density 1.04 ± 0.05 g/mL, from the Le-Qi sewage treatment plant (Yixing, China). The start-up period of a UASB reactor can be reduced substantially by using granular sludge from an operating UASB reactor as seed material. Such secondary start-up has been applied successfully in both mesophilic and thermophilic granules [18,19].

The tobacco sheet wastewater, using as substrate in the experiment, was extracted from tobacco waste (China Tobacco Jiangsu Industrial Co. Ltd., Nanjing, China) immersing in hot water, added with nutrients, trace elements, buffer solutions , and pure nicotine (in mg/g COD): $NaHCO_3$ (1000); NH_4Cl (260); $MgSO_4{\cdot}7H_2O$ (50); $K_2HPO_4{\cdot}3H_2O$ (30); $Na_3C_6H_5O_7{\cdot}2H_2O$ (30); $CaCl_2{\cdot}2H_2O$ (15); $NiSO_4{\cdot}6H_2O$ (8); $FeCl_3{\cdot}6H_2O$ (5); $ZnCl_2$ (0.6); $CoCl_2{\cdot}7H_2O$ (0.86); $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.4); $CuCl_2{\cdot}2H_2O$ (0.3) and nicotine (according acclimation phase).

2.2. Reactor design and operation

The UASB anaerobic reactor used in this study is made of plexiglass (effective volume 7.4 L, interior diameter 14 cm, height 60 cm) (Fig. 1).

The UASB was operated with continuous inflow. Granular sludge accounted for about 50% of the volume in the reaction zone. During the granular sludge acclimation phase, the COD and nicotine concentration were elevated in a stepwise manner (Table 1). The initial configurations [20,21] were: a hydraulic retention time (HRT) of 24 h, a pH of 7, a temperature of 35°C, and a up-flow velocity of 0.8 m/h. The acclimation lasted 52 d.

2.3. Modification of the UASB configuration

The reactor has been started at the initial configuration, and has been modified the HRT, pH, temperature, and up-flow velocity sequentially to find the optimal UASB configuration, when the COD and nicotine of the influent were 1000 mg/L and 100 mg/L respectively. The nicotine concentrations of the influent and effluent were tested every 12 h, at each time 3 samples were taken from each sampling port. Then the optimal parameter would replace the initial one, and become the starting parameters in next modification. The reactor was operated at the HRT of 48, 40, 30, 24, 18 and 12 h starting from 48 h. The HRT addition was conducted in a stepwise manner through 156, 132, 108, 96, 72 and 60 h, respectively, in order to reach a relatively steady-state condition. The reactor was operated at the pH of 4, 5, 6, 6.5, 7, 7.5, 8 and 9 starting from 4; at the temperature of 30, 32, 34, 36, 38 and 40°C starting from 30°C; at the up-flow velocity of 0.2, 0.4, 0.6, 0.8 and 1.0 m/h starting from 0.2 m/h. At

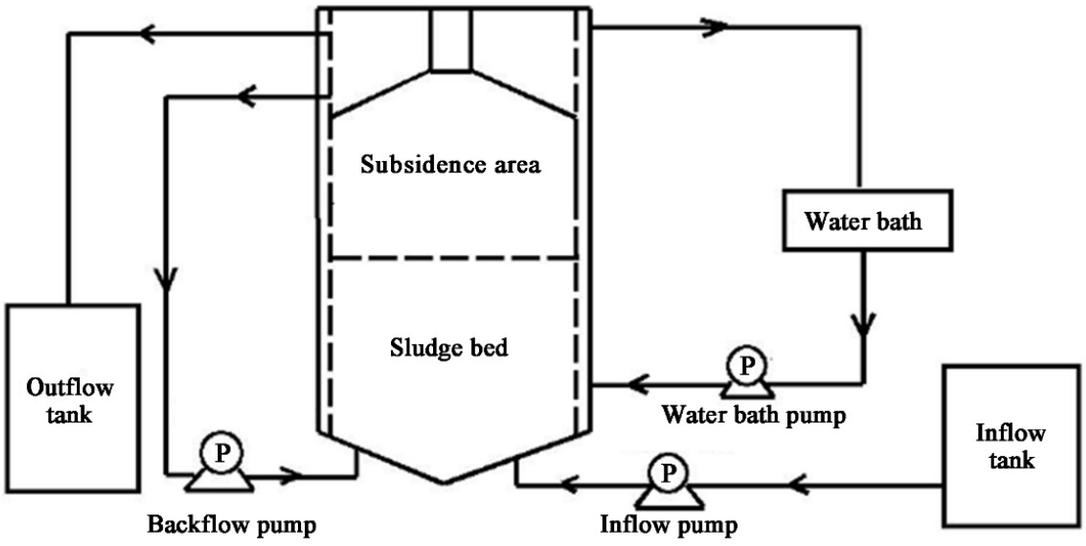

Fig. 1. UASB reactor constructed in the laboratory.

Table 1
COD and concentration of nicotine in each acclimation phase

Acclimation phase	Duration (d)	COD (mg/L)	COD removal efficiency (%)	Concentration of nicotine (mg/L)	NRE (%)
Phase I	6	400	84.0 ± 0.7	40	28.0 ± 3.2
Phase II	10	1000	67.7 ± 1.4	60	22.4 ± 2.4
Phase III	18	2000	58.3 ± 2.0	150	19.3 ± 2.9
Phase V	10	3000	49.9 ± 1.8	200	16.0 ± 3.0
Phase VI	8	5000	45.3 ± 1.6	400	15.8 ± 2.1

each parameter, the reactor was operated for about 60 to 108 h to reach the relatively steady-state condition. In three consecutive detections, when the variations of effluent nicotine concentrations were constant within 10%, steady-state conditions [22–24] were established and modified the next parameter.

2.4. Construction of the anaerobic micro-ecological system with different forms of nitrogen

The anaerobic micro-ecological system was constructed to study the effect of nitrogen forms on nicotine degradation. 10 g total solids (TS) of uniformly mixed granular sludge, weighed separately, was put into 200 mL serum bottle. Six groups (one was control, other five were experimental groups) of these systems were prepared. Each group contained three repeats used as parallel samples.

The experimental groups, numbered from 1 to 5, were added tobacco leaching solutions with different ratios of NO_3–N and NH_4–N, the NO_3–N to NH_4–N ratios were:100% to 0, 75% to 25%, 50% to 50%, 25% to 75%, and 0 to 100% (NO_3–N from $NaNO_3$, NH_4–N from NH_4Cl, ratios calculation based on the quantity of nitrogen). In addition, the control group, numbered as 6, added tobacco leaching solutions without nitrogen. After being injected 100 mL above solutions each, the serum bottles were subjected to N_2 (99.999%, Nanjing Special Gas) for 5 min and sealed with silica gel plugs to construct the anaerobic micro-ecological system. COD, nicotine, and total nitrogen were maintained at 1000 mg/L, 100 mg/L and 50 mg/L in each bottle, respectively. The serum bottles were sealed with rubber stoppers and placed in an incubator at a constant temperature of 35°C.

2.5. Analysis of gas and nicotine content

The serum bottles were statically cultured in a thermostatic cultivation box (DK-GJ003; Memmert) at 35°C for 24 h. Then 5 mL headspace gas was collected for testing the concentration of CO_2, CH_4, and N_2O. Nicotine concentration was tested after centrifugation and pumping filtration of remaining solutions.

2.6. Analysis methods

Samples' analysis included COD, pH, and TS, all according to Standard Methods [25]. The method was described by Ruan et al. [26], that measured the concentrations of CO_2, CH_4, and N_2O in the headspace of anaerobic micro-ecological systems by gas chromatography (7890A, Agilent). Nicotine was performed by high performance liquid chromatography (1260, Agilent) by injecting 5 µL treated sample solutions onto a C18 analytical column and eluted with 60 vol. 0.1% (volume to volume ratio) triethylamine water solution and 40 vol. of methanol. The mobile phase was pumped at a flow rate of 1.0 ml/min. The column temperature was set to 35°C. Nicotine factors were separated and detected at 254 nm, with retention times of 6.5 min. The integrated chromatogram was normalized and the relative percentage of each nicotine factor reported. Comparison of each nicotine sample chromatogram with that of a nicotine base reference standard chromatogram confirmed peak identity for quantification against a 3-point standard curve.

Data sets were analyzed and plotted by Origin Pro 9 and Microsoft Excel 2013 software. The calculation methods for CO_2, CH_4, and N_2O gas emission rate have been described by Ruan et al. [27]. NRE is calculated by $(N_{In}–N_{Out})/N_{In}$, where N_{In} (mg/L) is the nicotine concentrations in influent and N_{Out} (mg/L) is the nicotine concentrations in effluent. NDR is calculated by $\Delta N/(M\times T)$, where ΔN (ng) is the changed concentration of nicotine, M [g (TS)] is the quantity of granular sludge in each anaerobic micro-ecological system, T (h) is the culture time.

3. Results and discussion

3.1 Effects of the configuration of UASB on NRE

The nicotine removal efficiency responded to the changes of HRT (Fig. 2). The NRE was 39.5% when the HRT was 12 h, which may be too short a time for reactions to occur. The NRE increased with an increase of HRT, reaching a maximum of 66.2% at an HRT of 18 h. However, it decreased as the HRT increased from 24 h to 48 h, reaching a minimum of 38.4%.

The reduction of HRT, which provided less contact time for sludge granules to organic matters, would decrease the NRE. The Zhang's study, using pyrosequencing, also revealed the impacts of HRT on the microbial community structure and composition, even the dominant species varied in different HRT [28]. Moreover, the balance between HRT and the physical parameters of the reactor will effect the balance between granular sludge and flocculent sludge in the system, which would change the removal efficiency [29]. In addition, the decreased NRE was supposed to be reason of the accumulation of volatile fatty acids (VFA). The VFA producing bacteria were affected by the HRT, and VFA

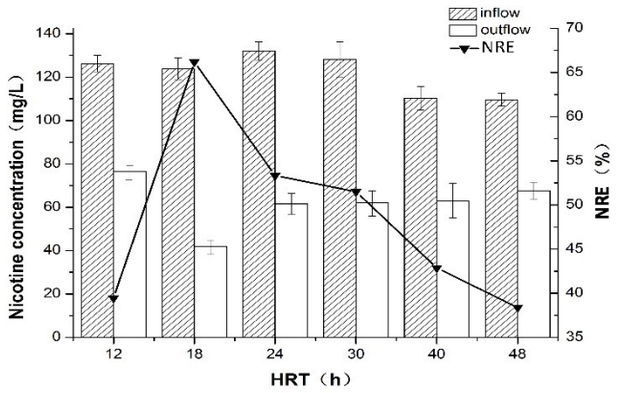

Fig. 2. NRE and nicotine concentration at different HRTs.

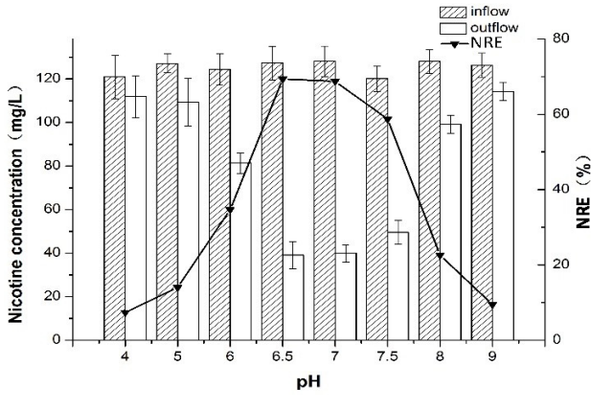

Fig. 3. NRE and nicotine concentration at different pH.

accumulates in long HRT [30], which can cause the degradation process fail [31], leading to acidification of the reactor and inactivation of degrading bacteria.

The pH affected the NRE (Fig. 3). A pH of 6.5–7.0 was found to be the most suitable, since it generated the highest NRE, i.e., 69.6%. The NRE decreased as pH decreased below 6.5 and as pH increased above 7.0. The NRE was 7.4% at a pH of 4.0, and 9.5% above a pH of 9.0.

Moreover, the pH affected not only the microbial community structure but also the microbial activities in the UASB. Denitrifying microbial function was effective within a pH range from 6.0 to 8.0, with the microbial activity being at an optimum around neutral [32]. Damaraju et al. explored the optimal pH from 6.5 to 7.5, in order to improve the potential of the denitrification process [33]. Consequently, due to the optimal pH in this study was from 6.7 to 7.0, denitrifying microbial function might play a major role in the degradation of nicotine in the UASB. In Jankowska's study, this optimal pH also had advantages on the microbial activities within the pH values between 6 and 9, which the lowest VFAs concentration was produced [34].

The optimal temperature for an efficient degradation of nicotine was 36–38°C (Fig. 4). The maximum NRE of 77.5% was achieved at 36°C. NRE decreased as temperature decreased below 36°C and increased above 38°C. The NRE was 52.5% and 57.6% at 30°C and 40°C, respectively.

The influence of temperature on the performance of an UASB reactor is very important because it affects significantly the hydrolysis process (because enzymes involved in the hydrolysis are very sensitive to temperature) [35–37], substrate utilization rate, settling of solids and gas transfer rates [38,39]. Although anaerobic degradation is feasible at psychrophilic, mesophilic, and the rmophilic temperature, low temperature generally results in a decrease in degrading bacteria growth rate [40]. Therefore, the optimal temperature in this study from 36 to 38°C maybe the best comprehensive feedback to every element. Furthermore, these temperatures benefit the anaerobic digestion and methanogenic activity [21,41], which closely associate with anaerobic degradation.

As shown in the histogram in Fig. 5, nicotine concentration in the outflow reached a minimum of 18.8 mg/L when the up-flow velocity was 0.8 m/h. However, the effect of up-flow velocity on nicotine degradation was

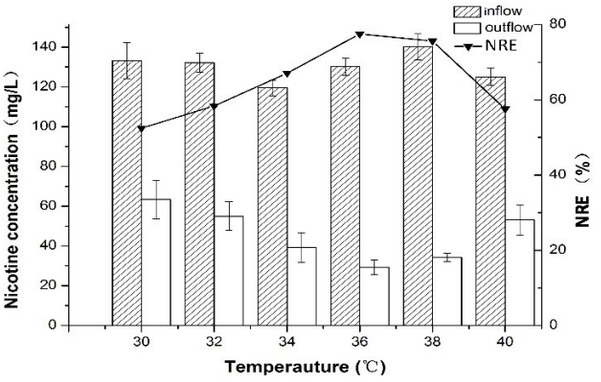

Fig. 4. NRE and nicotine concentration at different temperatures.

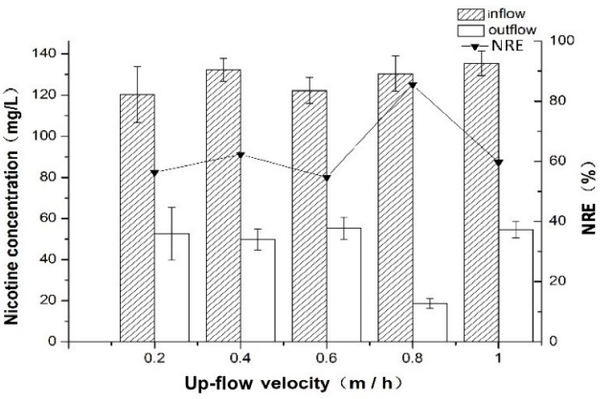

Fig. 5. NRE and nicotine concentration under different up-flow velocities.

irregular. When the velocity was 0.2, 0.4, 0.6, and 1.0 m/h, NREs were roughly the same (50%–55%). However, NRE significantly increased, reaching 85.3% when the velocity was set to 0.8 m/h. Suspended solids observed in the effluent increased significantly at a velocity of 1.0 m/h, indicating that the effect of the UASB decreased due to

the loss of sludge caused by hydraulic shear. Therefore, the optimal up-flow velocity of the granular sludge was inferred to be 0.8 m/h.

An appropriate up-flow velocity plays a major role in controlling the stable operational process performance of UASB reactors. Some microbes could not resist strong shear imposed on them. The increase of up-flow velocity could bring the strong shear to the sludge in the UASB reactor, which decreased the abundance of microbes, like *Firmicutes* [42], and deteriorated the removal efficiency due to detachment of the captured solids. In this study, with the increase of up-flow velocity, increased total suspended solids (TSS) has been observed, along with the turbidity both in the supernatant and effluent. This characteristic has been observed in Ozgun's research as well. A considerable increase of TSS and turbidity have been observed when up-flow velocity was increased from 0.6 m/h to 1.2 m/h [43].

3.2. Effect of nitrogen forms on nicotine degradation

The CH_4, CO_2, and N_2O emission rates and the nicotine degradation rate (NDR) listed in Table 2. The statistics showed the inhibition effect of NH_4–N and NO_3–N on CH_4 emission. The rate of inhibition of CH_4 emissions increased as the NO_3–N to NH_4–N ratio increased. As shown, NH_4–N and NO_3–N stimulated CO_2 production, and the maximum rate of CO_2 emission was obtained when the NH_4–N to NO_3–N ratio was 1 to 1. However, NO_3–N, which was used as reactant in denitrification, significantly stimulated the emission of N_2O, whose rates of emission increased as the concentration of NO_3–N.

As shown in Table 1, the NH_4–N to NO_3–N ratio played a role in nicotine degradation, which increased as the proportion of NO_3–N in the systems increased. The NDR increased with the proportion of NO_3–N and reached its maximum (23.71 ng/g h) in the groups to which only NO_3–N was added.

A correlation analysis was performed as part of this study. A strong negative correlation (R^2 = 0.9608) between the CH_4 emission rate and NDR was observed (Fig. 6), while a positive correlation (R^2 = 0.9775) between the N_2O emission rate and the NDR was found. However, little correlation (R^2 = 0.0448) existed between the CO_2 emission rate and the NDR, which indicates that nicotine degradation was not dependent on the activity of the entire anaerobic microbial community.

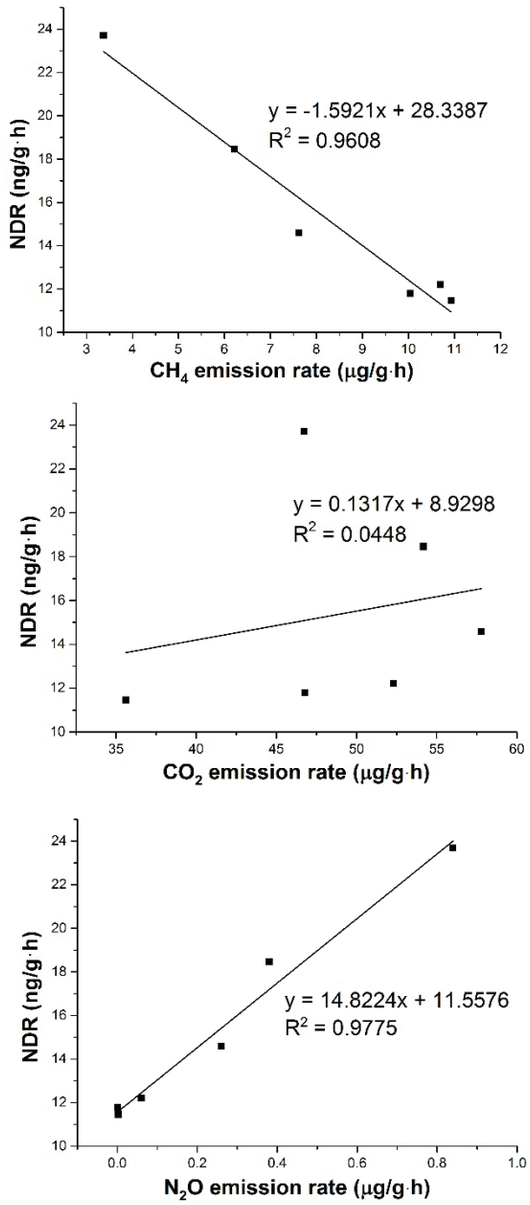

Fig. 5. NRE and nicotine concentration under different up-flow velocities.

Table 2
CH_4, CO_2, N_2O emission rates and the NDR with different forms of nitrogen

Forms of nitrogen	CH_4 emission rate (μg/g h)	CO_2 emission rate (μg/g h)	N_2O emission rate (μg/g h)	NDR (ng /g h)
NO_3–N: NH_4–N = 100%:0%	3.37 ± 0.21	46.73 ± 6.55	0.84 ± 0.21	23.71 ± 3.26
NO_3–N: NH_4–N = 75%:25%	6.22 ± 0.46	54.16 ± 3.70	0.38 ± 0.10	18.46 ± 2.47
NO_3–N: NH_4–N = 50%:50%	7.62 ± 0.24	57.78 ± 2.84	0.26 ± 0.03	14.58 ± 2.46
NO_3–N: NH_4–N = 25%:75%	10.70 ± 0.98	52.30 ± 8.42	0.06 ± 0.01	12.21 ± 1.79
NO_3–N: NH_4–N = 0%:100%	10.04 ± 1.28	46.77 ± 6.76	0.0004 ± 0.0002	11.79 ± 2.31
Control group	10.93 ± 0.42	35.60 ± 2.87	0.002 ± 0.001	11.46 ± 2.04

Forms of nitrogen significantly effect on the an ammox and denitrification, accompanying with methanogenesis in anaerobic degradation of nicotine. The slight change between control group and only NH_4–N added group may due to weak competitive ability of anammox bacteria under the co-occurrence of ammonium and high organic content [44,45], while methanogenesis may remain the majority. Moreover, methanol, one of the energy and carbon material in methanogenesis, was found to severely and irreversibly inhibit the activity of anammox bacteria [46,47]. The increasing ratio of NO_3–N in the forms of nitrogen, led to the reduction of CH_4 emission rate but increase N_2O emission rate and NDR. This phenomenon, which revealed the inhibition in methanogenesis but enhancement in denitrification, may be primarily generated from denitrification rather than methanogenesis became the preferred pathway [48]. Many researches indicated that the nitrate and nitrite could partially or completely inhibit the pure culture of methanogens, like *methanobacteriumomelianskii* [49], *methanobacteriumthermoautotrophicum*, *methanobacteriumformicicum* [50], *methanosarcinabarkeri* [51] and *methanosarcinamazei* [52]. Moreover, the improvement of denitrification seems to have positive effect on the nicotine degradation, and interaction mechanism needs further study.

4. Conclusion

For better degrading the nicotine in tobacco sheet wastewater, the optimal configuration of the lab-scale UASB reactor (effective volume 7.4 L, interior diameter 14 cm, height 60 cm) was found, as an HRT of 18 h, a pH of 6.5–7.0, a temperature of 36–38°C, and a granular up-flow velocity of 0.8 m/h. Nitrogen forms produced effects on nicotine degradation in anaerobic micro-ecological system, in such a way that nicotine degradation rate and N_2O emission rate were stimulated with the increasing ratios of NO_3–N, however, the CH_4 emission rate decreased. This might be reflected the enhancing microbial activity in denitrification, but inhibiting in methanogenesis. Further study of treatment technology of the tobacco sheet wastewater should be carried out in biodegradation mechanism.

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