

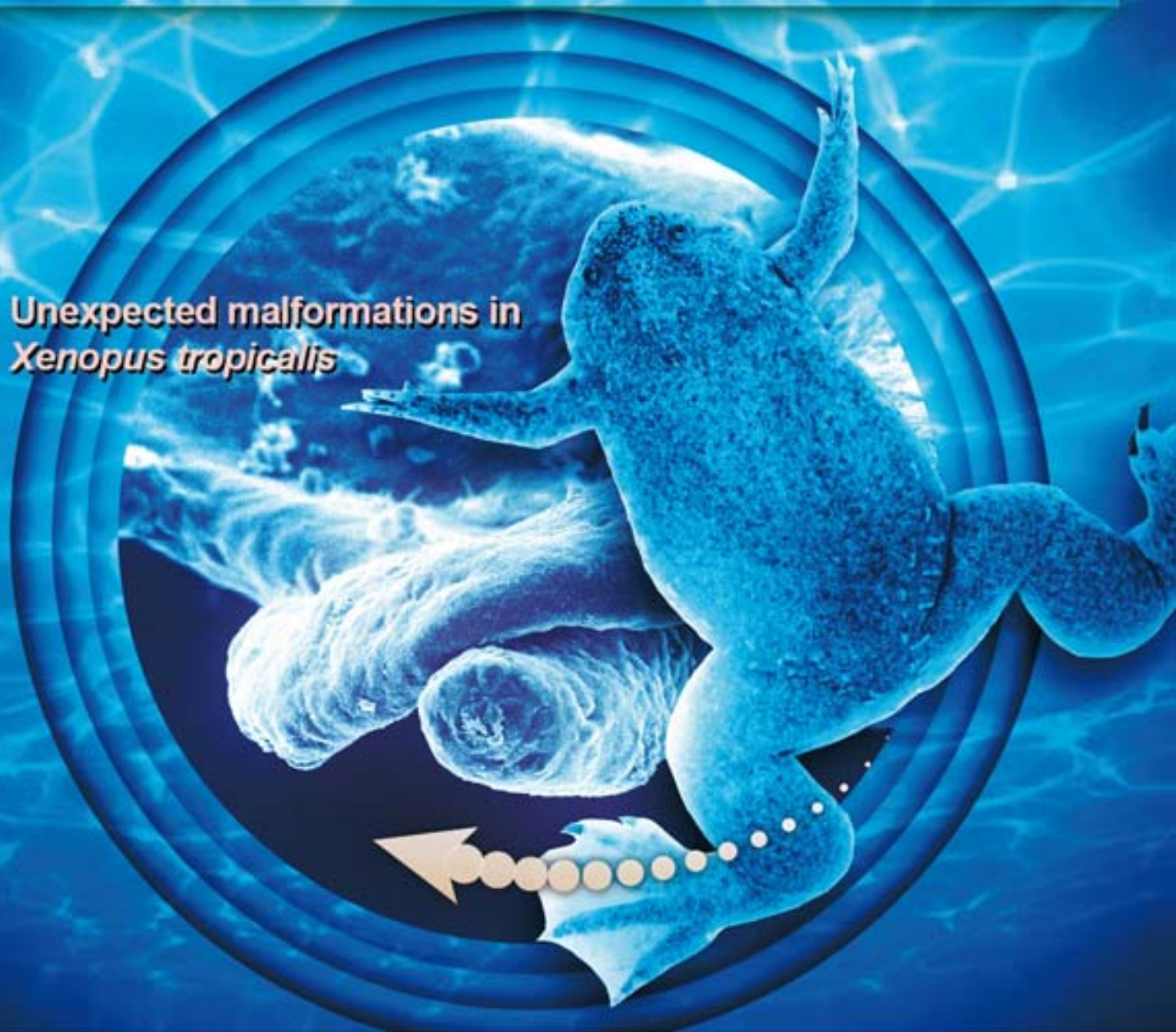
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Unexpected malformations in  
*Xenopus tropicalis*



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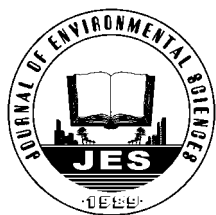
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## Elucidating the removal mechanism of *N,N*-dimethyldithiocarbamate in an anaerobic-anoxic-oxic activated sludge system

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### ABSTRACT

*N,N*-Dimethyldithiocarbamate (DMDTC) is a typical precursor of *N*-nitrosodimethylamine (NDMA). Based on separate hydrolysis, sorption and biodegradation studies of DMDTC, a laboratory-scale anaerobic-anoxic-oxic (AAO) system was established to investigate the removal mechanism of DMDTC in this nutrient removal biological treatment system. DMDTC hydrolyzed easily in water solution under either acidic conditions or strong alkaline conditions, and dimethylamine (DMA) was the main hydrolysate. Under anaerobic, anoxic or oxic conditions, DMDTC was biodegraded and completely mineralized. Furthermore, DMA was the main intermediate in DMDTC biodegradation. In the AAO system, the optimal conditions for both nutrient and DMDTC removal were hydraulic retention time 8 hr, sludge retention time 20 day, mixed-liquor return ratio 3:1 and sludge return ratio 1:1. Under these conditions, the removal efficiency of DMDTC reached 99.5%; the removal efficiencies of chemical organic demand, ammonium nitrogen, total nitrogen and total phosphorus were 90%, 98%, 81% and 93%, respectively. Biodegradation is the dominant mechanism for DMDTC removal in the AAO system, which was elucidated as consisting of two steps: first, DMDTC is transformed to DMA in the anaerobic and anoxic units, and then DMA is mineralized to CO<sub>2</sub> and NH<sub>3</sub> in the anoxic and oxic units. The mineralization of DMDTC in the biological treatment system can effectively avoid the formation of NDMA during subsequent disinfection processes.

## Introduction

*N*-Nitrosodimethylamine (NDMA) has been known as a carcinogenic and mutagenic compound since the 1960s (Patal, 1982). In recent years, NDMA has been studied widely as a novel nitrogenous disinfection by-product (Choi and Valentine, 2002; Mitch and Sedlak, 2002; Hatt et al., 2013). Research has shown that NDMA can be produced in disinfection processes using chlorine or chloramines in water or wastewater treatment plants (Sedlak et al., 2005; Yoon et al., 2011). An investigation conducted in the USA showed that the effluent of a municipal wastewater treatment plant disinfected by chlorine con-

tained 200–400 ng/L of NDMA (Pehlivanoglu-Mantas et al., 2006). In 1998 NDMA was included in the US EPA's list of priority pollutants found in drinking water (US EPA, 1998). The California State Government has mandated that NDMA concentrations in drinking water should not exceed 10 ng/L (DHS, 2002), while the Environment and Energy Department of Ontario, Canada set a NDMA limit of 9 ng/L (MOE, 2003).

*N,N*-Dimethyldithiocarbamate (DMDTC) is a dimethylamide and is considered an important precursor of NDMA (Mitch and Sedlak, 2004; Padhye et al., 2013). It has been reported that the NDMA molar conversion yield from DMDTC was about 3% ((mol/L)/(mol/L)) after reaction with chloramines (Selbes et al., 2013). DMDTC is an important chemical material and organic synthetic intermediate. It is widely used in the production of rubber,

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pesticides and bactericides (Goldman et al., 2008). It is commonly used as a rubber vulcanization accelerator and as a styrene-butadiene rubber polymerization terminator. As an important bactericide, DMDTC not only is used in industrial circulating water cooling systems, but also is the intermediate species of the synthetic pesticides thiram and ziram. In addition, DMDTC is used to prepare chelating resins and mineral flotation collectors. Nearly 1400 metric tons of DMDTC is applied in the USA annually (Weissmahr et al., 1998). The US EPA (2001) estimates a peak concentration of 860  $\mu\text{g/L}$  in surface water and average concentrations of 19  $\mu\text{g/L}$  for ziram alone.

Wastewater treatment plants (WWTPs) are a potential source of DMDTC pollution in the environment (Mitch and Sedlak, 2004). A previous survey demonstrated that DMDTC concentrations were between 277–1358  $\mu\text{g/L}$  in the influent of a typical municipal WWTP in Shanghai, China (Cao and Li, 2012). That WWTP did not process any industrial wastewater, indicating that the concentration of DMDTC may be even higher in WWTPs accepting industrial wastewater. Research has shown that NDMA-contaminated DMDTC-containing treatment chemicals and fungicides contribute to pulses of high concentrations of NDMA in raw sewage (Mowbray, 2002). Therefore, it is of interest to study the removal of DMDTC in WWTP. To date, reports investigating the removal of DMDTC in WWTP are limited. Mitch and Sedlak (2004) investigated the fate of NDMA precursors in municipal WWTP, indicating that although secondary biological treatment effectively removed dimethylamine (DMA), secondary treatment was less effective at removing other NDMA precursors, including dimethylamides. Therefore, elucidating the fate of DMDTC in secondary biological treatment processes is beneficial for better understanding the removal of dimethylamides as NDMA precursors.

The main purpose of this study was to investigate the removal of DMDTC in biological wastewater treatment processes. First, the hydrolysis of DMDTC at different pH values was investigated. Then sorption experiments were performed with sterilized sludge, as was DMDTC biodegradation under anaerobic, anoxic and oxic conditions. Finally, a laboratory-scale anaerobic-anoxic-oxic (AAO) system was constructed to investigate the removal of DMDTC in WWTP with nutrient removal. The effects of operational parameters such as hydraulic retention time (HRT), sludge retention time (SRT), mixed-liquor return ratio (MLRR) and sludge return ratio (SRR) were evaluated. Based on the above investigation, the removal mechanism of DMDTC during activated sludge processes was elucidated.

## 1 Materials and methods

### 1.1 Reagents

DMDTC (purum, 40% in  $\text{H}_2\text{O}$ ), acetonitrile and methanol (HPLC grade,  $\geq 99.9\%$ ) as high performance liquid chromatography (HPLC) mobile phase, sodium bicarbonate (analytical reagent,  $\geq 99.5\%$ ), and phenyl isothiocyanate (GC grade,  $\geq 99.0\%$ ) as derivative reagents were purchased from Sigma-Aldrich Company (USA). DMA (37%, W/W, in  $\text{H}_2\text{O}$ ) was purchased from Chem Service, Inc. (West Chester, USA). Sodium carbonate (analytical reagent,  $\geq 99.8\%$ ), iodine (analytical reagent,  $\geq 99.8\%$ ) and potassium iodide (analytical reagent,  $\geq 99.0\%$ ) as derivative reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (China).

### 1.2 Seed sludge and synthetic wastewater

The seed sludge was obtained from the return activated sludge tanks at the Quyang Municipal Wastewater Treatment Plant, Shanghai, China. It was washed three times, using tap water, to remove unnecessary organic substrate residues. After settling and drawing the supernatant, the final mixed liquor suspended solids (MLSS) of the seed sludge was in the range of 8000–10000 mg/L.

Synthetic wastewater was used for all the experiments. The synthetic wastewater was prepared using tap water and supplemented with nutrients, trace elements, and buffering compounds. The synthetic wastewater composition is listed as follows (mg/L):  $\text{C}_6\text{H}_{12}\text{O}_6$  150, peptone 150, NaAc 80,  $\text{NH}_4\text{Cl}$  80,  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  26.3,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  20,  $\text{CaCl}_2$  10.6,  $\text{NaHCO}_3$  80,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  0.45,  $\text{H}_3\text{BO}_3$  0.045,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  0.009, KI 0.054,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  0.036,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.036, EDTA 3. The main characteristics of the synthetic wastewater were: chemical organic demand (COD) = 300–350 mg/L,  $\text{NH}_3\text{-N}$  = 20–30 mg/L,  $\text{PO}_4^{3-}\text{-P}$  = 5–6 mg/L. The initial concentration of DMDTC introduced into the synthetic wastewater was 5000  $\mu\text{g/L}$ .

### 1.3 Effects of pH on DMDTC hydrolysis

Some research indicates that substances containing dithio groups are not stable in aqueous solution, and would hydrolyze under both acidic and alkaline conditions (Watanabe et al., 2004; Brunner, 2009). The hydrolysis is faster when the carbon atom is connected to a nitrogen atom or oxygen atom (Duan et al., 2010). To investigate the effect of pH on DMDTC hydrolysis, experiments were carried out at initial pH values ranging from 2.0 to 13.0. In total 12 sets of 500 mL brown glass bottles with stoppers were prepared. Each set was performed in triplicate, for which a solution (250 mL) containing 11 mg/L DMDTC was dosed, and pH was adjusted by NaOH or  $\text{H}_2\text{SO}_4$  as appropriate. All the bottles were kept in an incubator at  $30 \pm 1^\circ\text{C}$  in the dark. At a predetermined time each set was sampled and analyzed for DMDTC and DMA contents by

HPLC. The hydrolysis rate was determined by monitoring the decrease rate of DMDTC in the solutions.

#### 1.4 Sorption experiments

The sorption experiments were performed with sterilized sludge to avoid negative influence from DMDTC biodegradation. The sterilization procedure was modified according to the method reported by Wang and Grady (1994). They found that sorption isotherms of di-n-butyl phthalate on live and sterilized biomass were the same, when the loss of biomass during autoclaving was considered. Therefore, it is reasonable to use sterilized sludge to evaluate the sorption performance of activated sludge. The sludge sample was taken from the incubation reactor and sterilized by autoclaving at 121°C for 30 min. The sludge was then centrifuged and washed with milli-Q water three times. After the last centrifugation, the sludge was dissolved in the synthetic wastewater to a MLSS level of 6000 mg/L, and stored in a refrigerator at 4°C for no more than 24 hr. Immediately before the sorption experiment, the sludge was diluted to the required MLSS level with the synthetic wastewater, and the initial pH was adjusted to 7.5.

The sorption experiments were conducted in 500 mL Erlenmeyer flasks. The flasks were filled with 350 mL sterilized sludge solution and dosed with a DMDTC standard working solution. The initial concentration of DMDTC was around 10 mg/L and the MLSS concentration was 3000 or 5000 mg/L. The flasks were immediately sealed with butyl rubber stoppers and shaken on a thermostatic rotary shaker at 125 r/min and 20°C. Samples were collected from the flasks at 4, 8, 12, 16, 20 and 24 hr. All samples were centrifuged at 6000 r/min for 10 min. The supernatant was filtered through a glass fiber filter and the filtrate was analyzed by HPLC. All sorption experiments were carried out in triplicate.

#### 1.5 Biodegradation experiments

The DMDTC biodegradation experiments were also conducted in 500 mL Erlenmeyer flasks. The seed sludge, synthetic wastewater, and DMDTC were added to the flasks. The initial concentrations of MLSS and DMDTC were 3000 mg/L and 10 mg/L, respectively. All the flasks were spiked with resazurin (final concentration 1 mg/L). For the oxic experiments, the flasks were loosely capped to ensure oxygenation of the flasks. For the anaerobic experiments, the flasks were sealed with butyl rubber stoppers, and their headspace was purged with nitrogen for 20 min. Finally, for the anoxic experiments, the flasks were prepared as the anaerobic microcosms, but nitrate was added (final concentration 30 mg/L). Controls were prepared by autoclaving the flask medium for 30 min, at 121°C, and sodium azide was added to a final concentration of 0.1% (W/W). All flasks were shaken on a thermostatic rotary shaker at 125 r/min, in the dark.

Unless otherwise noted, biodegradation experiments were conducted under the following conditions: temperature = 25°C, initial pH = 8.0, MLSS = 3000 mg/L and initial COD = 350 mg/L. Samples were collected from flasks after 4, 8, 12, 16, 20 and 24 hr. All samples were centrifuged at 6000 r/min for 10 min. The supernatant was then passed through a glass fiber filter and the filtrate was analyzed by HPLC. All the degradation experiments were carried out in triplicate.

#### 1.6 Laboratory-scale anaerobic-anoxic-oxic activated sludge system

The laboratory-scale AAO system consisted of a 2.4 L anaerobic unit, a 2.4 L anoxic unit, a 7.2 L aerobic unit and a settling unit (Fig. 1). Motor-driven mixers were employed in the anaerobic and anoxic units. An air pump and an electric agitator were employed in the oxic unit.

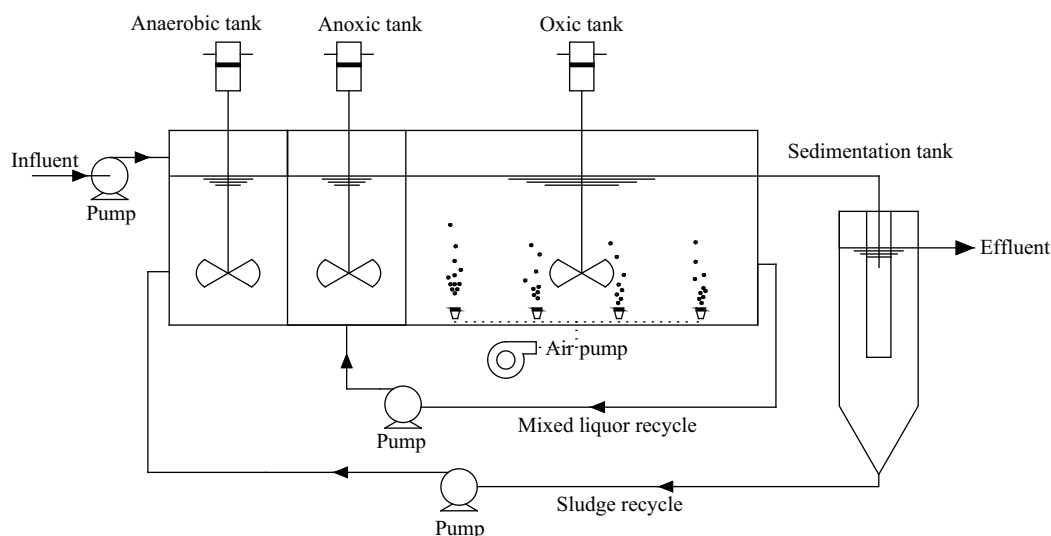


Fig. 1 Schematic diagram of the anaerobic-anoxic-oxic (AAO) activated sludge system.

The sludge was returned from the bottom of the settling unit to the anaerobic unit. The mixed liquor was recycled from the aerobic unit to the anoxic unit. The whole system was placed in a temperature-controlled room at 25°C. The MLSS in the system was in the range of 3000–3500 mg/L.

To study the effect of HRT on DMDTC removal in the AAO system, SRT, MLRR and SRR were fixed at 20 day, 2:1 and 1:1, respectively. The HRT was carried out for the following durations: 6, 8, 10, and 12 hr. To investigate the effect of SRT, 10, 15, 20, and 25 day were used, while HRT, MLRR and SRR were fixed at 8 hr, 2:1 and 1:1, respectively. To evaluate the effects of MLRR, 1:1, 2:1, 3:1 and 4:1 were used, while HRT, SRT and SRR were fixed at 8 hr, 20 day and 1:1, respectively. The influence of SRR was investigated using ratios of 1:2, 1:1, 3:2 and 2:1, while HRT, SRT and MLRR were fixed at 8 hr, 20 day and 3:1, respectively.

The seed sludge was acclimated in the AAO activated sludge system with synthetic wastewater. During the acclimation period, the operational parameters were as follows: SRT = 20 day, HRT = 8 hr, SRR = 1:1; MLRR = 3:1. The AAO system was placed in a temperature controlled room at 25°C. The sludge was acclimated for 2 months. After the acclimation process, the MLSS was stabilized at 3000–3500 mg/L; the ratio of mixed liquor volatile suspended solids (MLVSS) to MLSS was stabilized in the range of 0.82–0.85; the sludge settling velocity was 30%.

### 1.7 Analytical methods

DMDTC content was determined by HPLC (Agilent Technologies 1200 Series, USA) after pre-column derivatization (Cao and Li, 2012). Water samples (25.0 mL) were contained in a 50 mL colorimetric tube. The pH was adjusted to 8.0–8.5 using 0.1 mol/L NaOH and 0.1 mol/L HCl solutions. The derivatization reagent ( $I_2/KI$  solution) was added dropwise, until the sample was oxidized to a light yellow color. Next, the samples were filtered with a 0.45  $\mu$ m membrane and derivatized samples (50  $\mu$ L) were injected into the HPLC system. Final chromatographic conditions were an isocratic elution with acetonitrile: water = 80:20 (V/V). The flow rate was 1 mL/min. UV detection was performed at 254 nm.

DMA was also determined by pre-column derivatization and HPLC method (Sahasrabudhhey et al., 1999). After the water sample (15.0 mL) was collected in a 25 mL volumetric flask, 1.25 mL of 4% phenyl isothiocyanate and 1.25 mL of 5%  $NaHCO_3$  were added and heated in a 40°C water bath for 10 min. Next 1.25 mL of 5%  $Na_2CO_3$  was added and samples were heated for 5 min. After cooling samples to room temperature, they were brought to a constant volume using acetonitrile/water (V/V = 30/70). Next the samples filtered using a 0.45  $\mu$ m membrane then subjected to HPLC. Final chromatographic conditions were a gradient elution using solvent A (milli-Q water) and solvent B (acetonitrile). At  $t = 0$  the mobile phase consisted

of 70% A and 30% B and held for 2 min. A linear gradient to 100% B was performed over the next 5 min. At  $t = 12$  min the initial conditions (70% A and 30% B) were restored and held for 3 min. The flow rate was 1 mL/min and the injection volume was 50  $\mu$ L. UV detection was performed at 240 nm.

Total organic carbon (TOC) was determined using a Shimadzu TOC-VCPN analyzer (Kyoto, Japan). COD, volatile suspended solid (VSS), MLSS, total nitrogen (TN),  $NH_3-N$  and total phosphorus (TP) were measured following APHA standard methods (APHA et al., 1998).

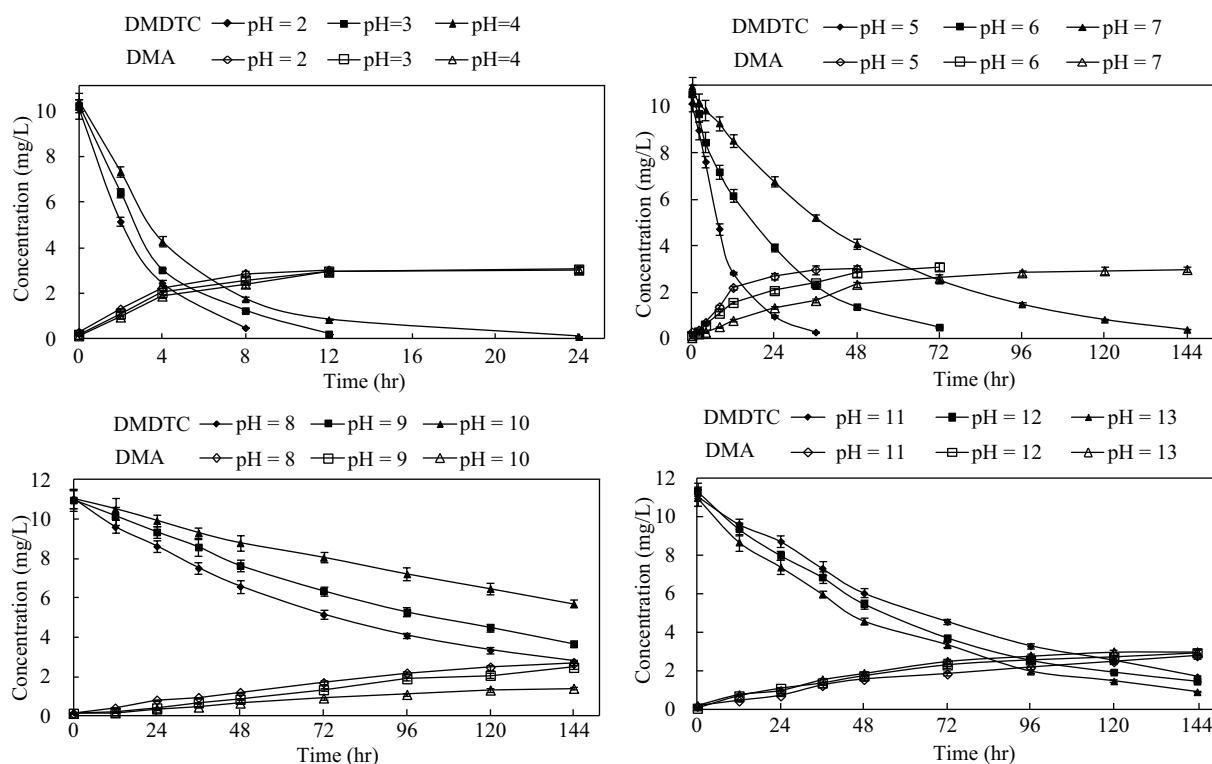
## 2 Results and discussion

### 2.1 Hydrolysis of DMDTC

The hydrolytic trend of DMDTC over the pH range of 2–13 is shown in **Fig. 2**. Under acidic conditions (pH = 2.0–6.0), the hydrolysis rate increased rapidly as the pH decreased. In the samples of pH = 2.0–3.0, DMDTC was almost completely hydrolyzed in 8–12 hr. Conversely, when the pH increased to 6.0–7.0, the hydrolysis process was prolonged to 3–7 days. Under neutral or weakly alkaline conditions (pH = 8.0–10.0), DMDTC was decomposed more slowly. The hydrolysis efficiencies of the samples at pH of 10.0 on day 3 and day 7 were only 26.8% and 48.3%, respectively. The hydrolysis rate, however, increased rapidly under strongly alkaline conditions (pH = 11.0–13.0). When the pH increased to 11.0–13.0, the DMDTC concentration was decreased by 84.8%–91.8% day 7.

Since the DMDTC molecule contains a DMA group, and DMA was detected during the hydrolysis of DMDTC, DMA might be one of the hydrolysates of DMDTC. Here, DMA concentrations increased with the reduction of DMDTC concentrations (**Fig. 2**). Some reports have indicated that DMA was stable in solution, even under strong ultraviolet radiation conditions (Sedlak et al., 2005; Duan et al., 2010; Sakai et al., 2012; Soltermann et al., 2013). Therefore, the concentration of DMA should not further change with the hydrolysis of DMDTC. If 1 mol DMDTC produces 1 mol DMA during hydrolysis, the theoretical yield of DMA is 31.5% on a mass basis. This means that 1 g/L DMDTC hydrolyzed completely generates 0.315 g/L DMA. Mass balance in the present work shows that the yield of DMA was 26.5%–34.5%. These experimental results are close to the theoretical value. Therefore, it was concluded that DMA was the main product of DMDTC during its hydrolysis.

Substances containing  $-CSS^-$  groups are unstable in aqueous solutions and easily hydrolyzed under both acidic and alkaline conditions (Watanabe et al., 2004; Brunner, 2009; Duan et al., 2010). The present work suggests that the hydrolysis rate of DMDTC in aqueous solution is pH dependent. First-order kinetics was used to describe



**Fig. 2** Concentration profiles of *N,N*-dimethyldithiocarbamate (DMDTC) and dimethylamine (DMA) during the hydrolysis of DMDTC in the pH range of 2 to 13.

the hydrolysis of DMDTC at different pH, and the rate constants and half-lives are shown in **Table 1**. The data suggest that the hydrolysis reaction occurs by both an acid-catalyzed mechanism and base-catalyzed mechanism. At  $\text{pH} < 7$ , the hydrolysis rate increased rapidly as pH decreased. The reaction followed an acid-catalyzed path, in which a hydrogen ion performed an electrophilic attack on the nitrogen atom, and the decomposition products were DMA and  $\text{CS}_2$  (**Fig. 3**). Under alkaline conditions, when  $\text{pH} > 10$ , the rate constant increased rapidly with increasing pH. The reaction followed a base-catalyzed path, in which a hydroxide ion performed a nucleophilic attack on the carbonyl carbon atom next to an amide bond. Here, the products were DMA,  $\text{CS}_2$  and  $\text{H}_2\text{O}$ . Under weakly alkaline conditions ( $8 < \text{pH} < 10$ ), however, the rate constant was not significantly affected by pH.

## 2.2 Sorption of DMDTC on activated sludge

DMDTC concentration profiles during the sorption experiments are shown in **Fig. S1**. These results indicated that the sorption efficiencies of DMDTC on sludge were in the meager range of 3%–5%. DMDTC is a hydrophilic chemical ( $\text{p}K_{\text{ow}} = 1.17$ ) and tends to remain in the aqueous phase, rather than transferring to the sludge phase. Therefore, sorption of DMDTC on the sludge can be regarded as negligible during its removal with the activated sludge.

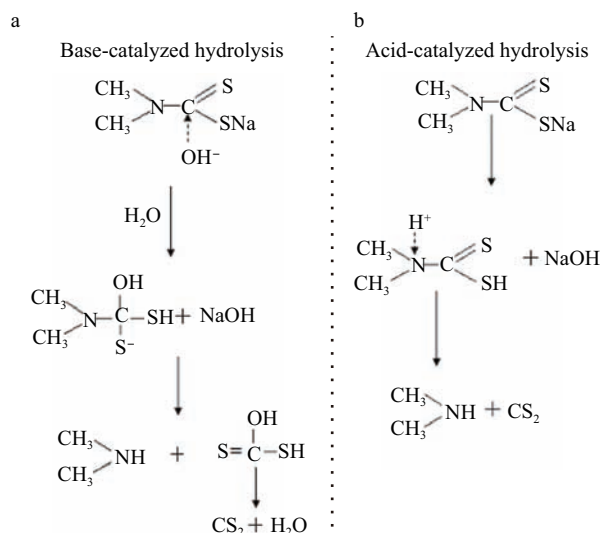
**Table 1** DMDTC hydrolysis rate at different pH

pH	Hydrolysis rate constant (mg/(L·hr))	Correlation coefficient ( $R^2$ )	Half-life (hr)
2.0	0.370	0.9976	1.88
3.0	0.299	0.9838	2.32
4.0	0.195	0.9918	3.56
5.0	0.099	0.9974	6.99
6.0	0.0424	0.9993	16.34
7.0	0.0217	0.9951	31.94
8.0	0.0100	0.9952	69.30
9.0	0.0075	0.9986	92.40
10.0	0.0045	0.9986	154.00
11.0	0.0127	0.9966	54.57
12.0	0.0148	0.9965	46.82
13.0	0.0173	0.9977	40.06

## 2.3 Biodegradation of DMDTC under different redox conditions

The DMDTC concentration profiles under anaerobic, anoxic and oxic conditions are shown in **Fig. 4**. These results indicate that the DMDTC removal efficiencies were above 95%, in the 20 hr samples, under all the above redox conditions. The control test reactions showed that the DMDTC hydrolysis rate was much lower than its biodegradation rate, and the total hydrolysis efficiency of DMDTC was only 15% in the 20 hr samples. As sorption





**Fig. 3** Proposed hydrolysis mechanism of DMDTC. (a) base-catalyzed hydrolysis; (b) acid-catalyzed hydrolysis.

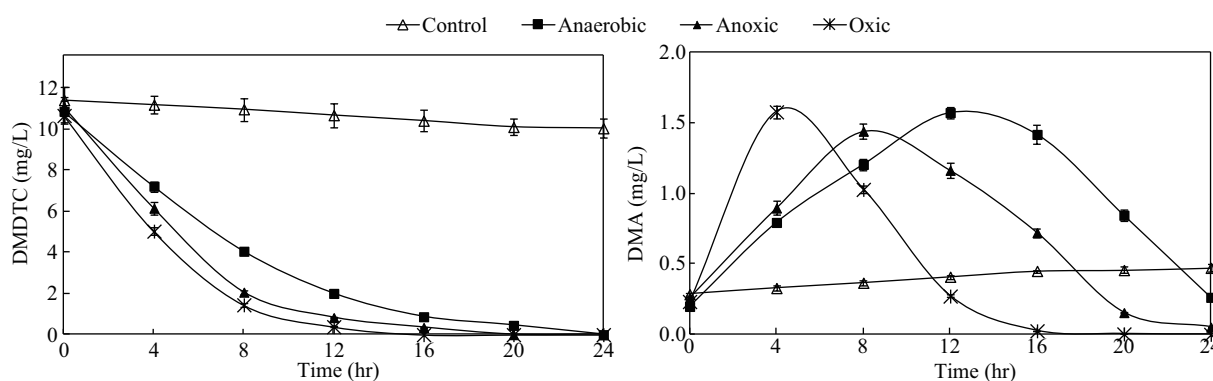
of DMDTC on the sludge was also limited, the dominant removal mechanism of DMDTC in the activated sludge system is projected to be biodegradation.

The biodegradation kinetics of DMDTC under the anaerobic, anoxic and oxic conditions is well described by first-order kinetics (**Table 2**). The degradation rates follow the order of oxic > anoxic > anaerobic.

DMA was also monitored in biodegradation tests. Under anaerobic, anoxic, and oxic conditions, the concentrations of DMA first increased and then decreased. The DMA concentration produced by hydrolysis in the control test was below 0.5 mg/L; conversely, the highest concentrations of DMA, in the activated sludge experiments, were up to 1.5

**Table 2** DMDTC biodegradation kinetics

	Kinetic equation	Correlation coefficient ( $R^2$ )
Anaerobic	$C_t = 10.87e^{-0.1436t}$	0.9830
Anoxic	$C_t = 11.10e^{-0.2130t}$	0.9908
Oxic	$C_t = 10.67e^{-0.2621t}$	0.9798



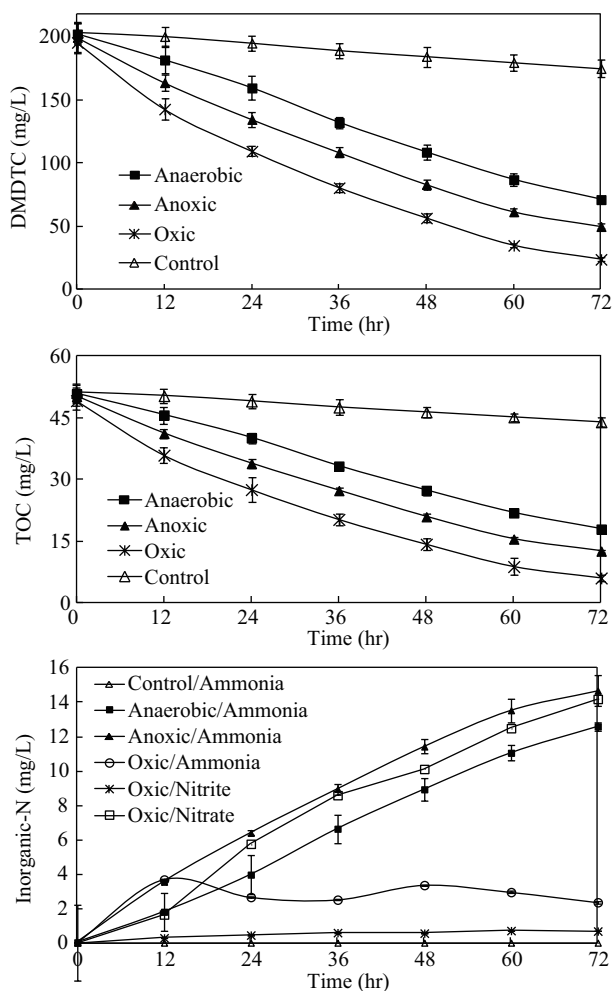
**Fig. 4** Concentration profiles of DMDTC and DMA during biodegradation of DMDTC under anaerobic, anoxic and oxic conditions.

mg/L. These results demonstrate that DMA is an important intermediate during the biodegradation of DMDTC and can be further degraded by the activated sludge.

In order to further investigate the mineralization of DMDTC, levels of  $\text{NH}_3\text{-N}$  and TOC during the biodegradation process were assessed. Since nitrification can occur under oxic conditions, nitrate and nitrite concentrations were also measured. The initial concentration of DMDTC was 200 mg/L (**Fig. 5**). During the biodegradation of DMDTC, the concentrations of inorganic nitrogen increased while TOC showed a downward trend. However, no  $\text{NH}_3\text{-N}$  was detected in the control test, and TOC only decreased slightly. Theoretically, 1 mol DMDTC is equal to 3 mol of TOC and when 1 mol of DMDTC is mineralized completely, 1 mol  $\text{NH}_3\text{-N}$  can be produced. In the present study, under anaerobic, anoxic, and oxic conditions, the removal amounts of DMDTC in these conditions were 131.0, 150.4 and 176.1 mg/L, respectively. The residual concentrations of DMDTC were 69.0, 49.6 and 23.9 mg/L, respectively. The concentrations of produced  $\text{NH}_3\text{-N}$  were 12.6, 14.6 mg/L and 16.8 mg/L, respectively (nitrite and nitrate are included in this data for the oxic experiment group). The residual TOC concentrations were 17.9 mg/L, 12.6 mg/L, 6.0 mg/L, respectively, and the calculated TOC levels based on the residual DMDTC were 19.6 mg/L, 12.5 mg/L and 6.0 mg/L, respectively. These data indicated that approximately 98% of the residual TOC caused by DMDTC and nearly all the removed DMDTC was eventually mineralized. The residual DMDTC can be degraded as the reaction proceeds.

#### 2.4 Removal of DMDTC via the AAO system

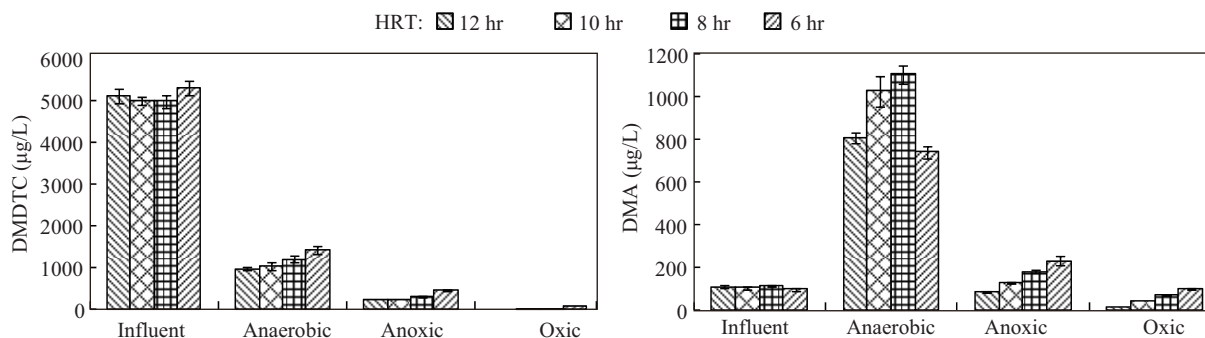
The changes of DMDTC and DMA concentrations in various units of the AAO treatment system at different HRT (6–12 hr) are shown in **Fig. 6**. Since DMDTC can be easily hydrolyzed to DMA, here a small amount of DMA was detected in the influent. In the anaerobic unit, most DMDTC was transformed to DMA, which resulted in a high concentration of DMA. In the anoxic unit, DMA was degraded and DMDTC continued to be transformed to DMA. In the oxic unit, DMA was degraded to a low



**Fig. 5** Concentration profiles of DMDTC, total organic carbon (TOC) and inorganic nitrogen during the biodegradation of DMDTC under anaerobic, anoxic and oxic conditions.

concentration. Less residual DMA and DMDTC were detected at longer HRT.

The changes of DMDTC and DMA in various units of the AAO treatment system at different SRTs for the whole treatment process (10–25 days) are shown in Fig. 7. When SRT was decreased from 25 to 20 day, there were no obvious differences in DMDTC or DMA concentrations.



**Fig. 6** Changes in DMDTC and DMA in the various units of the AAO treatment system at different HRTs.

The DMDTC concentration was lower than 25 µg/L and the DMA concentration was below 20 µg/L. The total removal efficiency of DMDTC was 99.6%. However, when SRT was decreased to 10 day, DMDTC and DMA in the final effluent increased significantly (50 and 40 µg/L, respectively).

Compared with HRT and SRT, the effects of MLRR and the SRR on the change in DMDTC and DMA concentrations in the AAO treatment system were not significant (Fig. 8). With the increase of MLRR and the SRR, the concentrations of DMDTC and DMA in the anaerobic and anoxic units decreased gradually. There was no significant difference observed in the oxic effluent.

According to the above test results, biodegradation of DMDTC in the AAO system was elucidated as follows. First, DMDTC was degraded to DMA, with this step mainly occurring in the anaerobic and anoxic units. Second, DMA was further degraded to inorganic substances, i.e.,  $\text{NH}_3\text{-N}$  and  $\text{CO}_2$ , with this step occurring primarily in the anoxic and oxic units. The mineralization of DMDTC in this biological treatment system effectively avoided the formation of NDMA during subsequent disinfection processes.

The removal of conventional parameters such as COD and nutrients was also investigated (Figs. S2–S5). Based on the removal of both nutrients and DMDTC, the following operational parameters were recommended: HRT = 8 hr, SRT = 20 day, MLRR = 3:1, and SRR = 1:1. Under the above recommended conditions, the removal efficiency of DMDTC reached 99.5%, and the concentrations of DMDTC and DMA, in the final effluent, were both below 20 µg/L. The removal efficiencies of COD,  $\text{NH}_3\text{-N}$ , TN and TP were 90%, 98%, 81%, and 93%, respectively. The average concentrations of COD,  $\text{NH}_3\text{-N}$ , TN and TP in the effluent were 34.7 mg/L, 0.38 mg/L, 7.12 mg/L, and 0.45 mg/L, respectively. Therefore, the existing wastewater treatment process can achieve removal of trace organic pollutants by adjusting operational parameters.

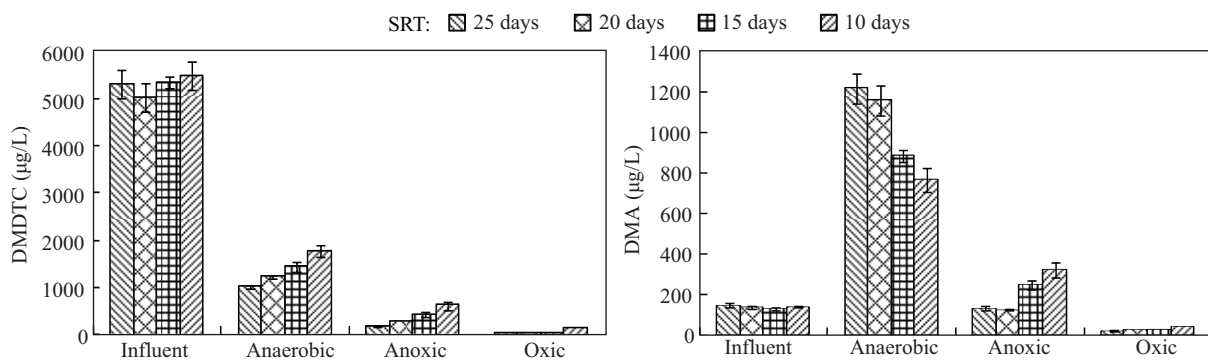


Fig. 7 Changes in DMDTC and DMA in the various units of the AAO treatment system at different SRTs.

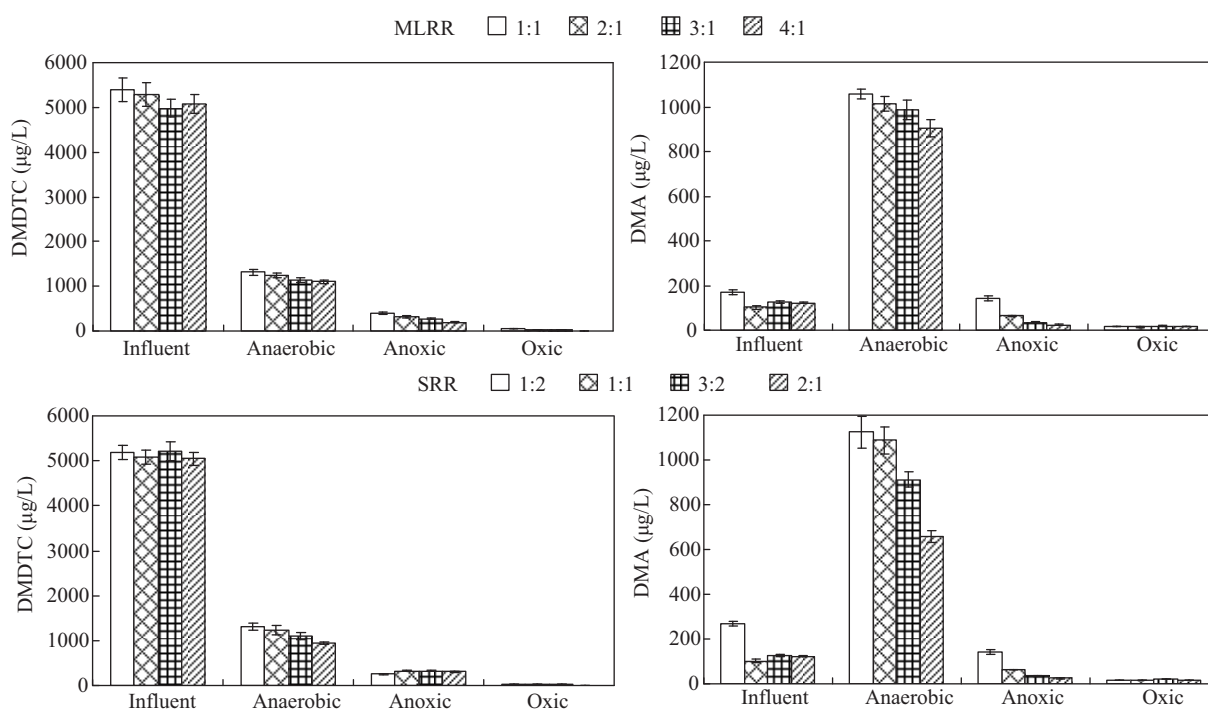


Fig. 8 Changes in DMDTC and DMA concentrations in the various units of the AAO treatment system at different MLRRs, and SRRs.

### 3 Conclusions

DMDTC hydrolyzed easily in a water solution and DMA was the dominant hydrolysate. The hydrolysis occurred by an acid-catalyzed mechanism, in acidic conditions, and by a base-catalyzed mechanism, in strongly alkaline conditions. DMDTC was degraded and mineralized under anaerobic, anoxic, and oxidic conditions. The biodegradation is best described by first-order kinetics and the degradation rate followed the order of oxidic > anoxic > anaerobic. DMDTC is first transformed to DMA and is further mineralized completely under anaerobic, anoxic, and oxidic conditions. The optimal conditions for both nutrient and DMDTC removal in the AAO system were as follows: HRT = 8 hr, SRT = 20 day, MLRR = 3:1, and SRR = 1:1. Under these conditions, the removal efficiency of DMDTC reached 99.5%; the removal efficiencies of COD, NH<sub>3</sub>-N, TN and TP were 90%, 98%, 81% and 93%,

respectively. Biodegradation is the dominant mechanism for DMDTC removal in the AAO system and it can be described in two steps. First, DMDTC was transformed to DMA with this step mainly occurring in the anaerobic and anoxic units. Second, DMA was further mineralized to inorganic substances, such as NH<sub>3</sub>-N and CO<sub>2</sub> with this process mainly occurring in the anoxic and oxidic units. The mineralization of DMDTC in the biological treatment system can effectively avoid the formation of NDMA during subsequent disinfection processes.

### Acknowledgments

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### Supporting materials

Supplementary data associated with this article can be found in the online version.

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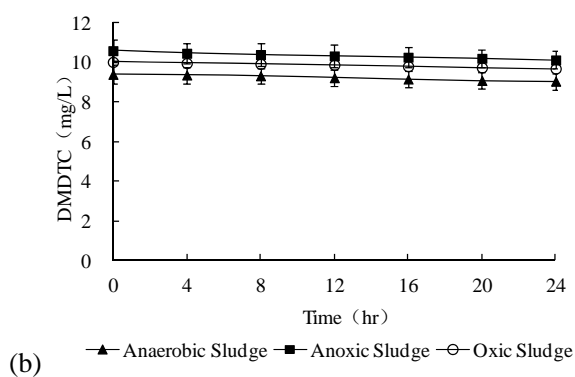
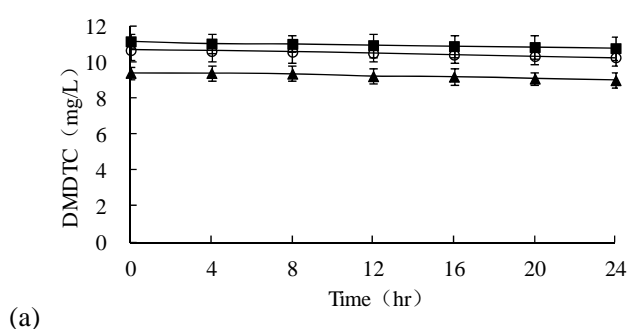
## Supporting materials

### Elucidating the removal mechanism of N,N-dimethyldithiocarbamate in an anaerobic-anoxic-oxic activated sludge system

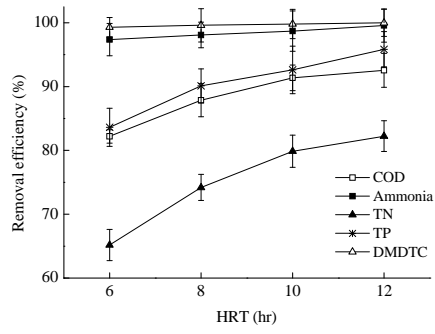
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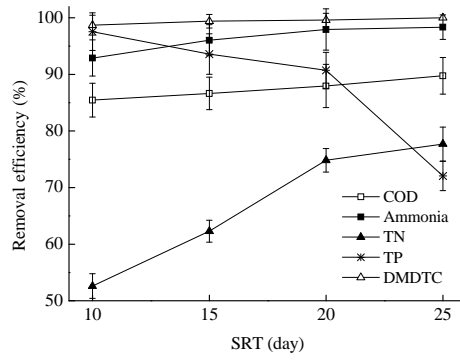
2. Henan Institute of Metallurgy, Henan Academy of Science, Zhengzhou 450053, China



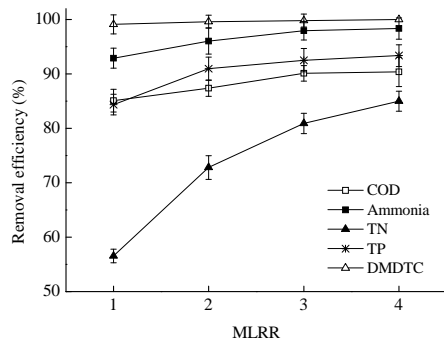
**Fig. S1** DMDTC concentration profiles during sorption experiments with the sterilized sludge: (a) MLSS=3000 mg/L; (b) MLSS=5000 mg/L.



**Fig. S2** Removal efficiencies of both DMDTC and conventional parameters in the AAO system at different HRTs.

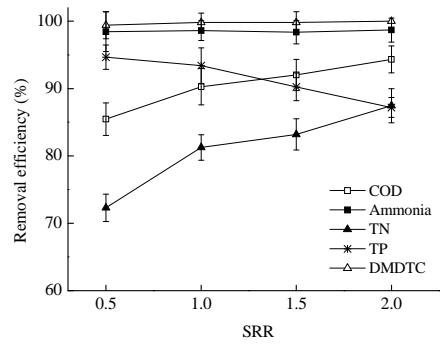


**Fig. S3** Removal efficiencies of both DMDTC and conventional parameters in the AAO system at different SRTs.



**Fig. S4** Removal efficiencies of both DMDTC and conventional parameters in the AAO system at different MLRRs.





**Fig. S5** Removal efficiencies of both DMDTC and conventional parameters in the AAO system at different SRRs.



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