Journal of Hazardous Materials 191 (2011) 103–109

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Sorption and biodegradation of tetracycline by nitrifying granules and the toxicity of tetracycline on granules

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ARTICLE INFO

Article history:
Received 11 January 2011
Received in revised form 11 April 2011
Accepted 12 April 2011
Available online 19 April 2011

Keywords:
Tetracycline
Nitrifying granular sludge
Sorption
Biodegradation
Toxicity

ABSTRACT

This paper examines the simultaneous sorption and biodegradation performance of tetracycline (TC) by the nitrifying granular sludge as well as the short-term exposure toxicity of TC. The removal of TC was characterized by a quick sorption and a slow process of biodegradation. The adsorption process fits pseudo-second-order kinetic model, with a complex mechanism of surface adsorption and intra-particle diffusion. Both temperature and mixed liquor suspended solid (MLSS) influenced TC sorption to the granules. TC biodegradation was enhanced with the increase of COD and NH_4^+–N concentrations, with except of the NH_4^+–N concentrations higher than 150 mg/L. With the ATU addition, TC degradation was weakened remarkably, indicating a synergistic effect of multiple microbes. Results of the short-term exposure (12 h) effects showed that the respirometric activities of the microbes decreased greatly. The addition of TC also decreased the rate of NH_4^+–N utilization considerably, with the half saturation constant (K_s) increasing from 297.7 to 347.2 mg/L.

1. Introduction

Antibiotics, a group of pharmaceutically active compounds (PhACs), are widely used in human and veterinary therapy and as growth promoters in livestock-farming [1]. In 1999, the antibiotics used in Europe as human and veterinary medicines were about 8500 and 4700 metric tons, respectively, with tetracycline (TC) being the second most widely used antibiotics [2]. TC, constituting a family of antibiotics, exhibits broad-spectrum antimicrobial activities [3]. Most of the TC administered to the treated species is excreted via feces and urine as unmodified parent compounds, with only small fractions being metabolized or absorbed in the body [4]. It is reported that non-negligible concentrations of TC were found in pig slurry (up to 5 mg/L) [5]. Residues of TC have been detected in many surface water resources that receive discharges from manufacturing facilities, municipal wastewater treatment plants and agricultural runoff [6,7]. These bioactive compounds may promote the transfer and spread of antibiotic-resistance genes among microorganisms [8].

TC is known to be highly sorbed to clay materials, soil and sediments [9,10]. However, the occurrence of TC in surface waters indicates that its sorption to solids is reversible and nonbiodegraded TC may become bioavailable when localized conditions change and favor their mobility in the environment. Several authors have studied the interaction between activated sludge processes and PhACs and show that many of the pharmaceutical pollutants are mainly eliminated by sorption to sludge rather than biodegradation [3,11]. Compared to the conventional activated sludge, aerobic granules yield high biomass concentrations and sludge retention time (SRT), which is beneficial in the removal of pollutants persistent to biodegradation. Moreover, due to the higher biomass concentrations as well as the higher tolerance to toxicity, aerobic granules are less sensitive to fluctuation than activated sludge [12,13].

There is still a lack of data describing the degradation mechanisms of PhACs but it is likely that the biodegradation of PhACs in wastewater is due to the co-metabolic activity [14]. Currently, several studies have reported that ammonia oxidizing bacteria (AOB) are capable of co-metabolizing a variety of PhACs that typically resist biodegradation and AMO (ammonium monooxygenase), a catalyst in the process of nitrification, may be important in the co-metabolism of PhACs [15,16]. Nitrifying granular sludge (NGS), accumulated with nitrifying bacteria, was adopted in this study to investigate the fate of TC in the granular sludge system. In TC-containing wastewater, the microbial activity can usually be inhibited due to the toxicity resulting from its own substrate [12]. This toxicity can reduce both chemical oxygen demand (COD) removal and nitrification, which are key processes in the wastewa-

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doi:10.1016/j.jhazmat.2011.04.048

ter treatment system. Thus, the toxicity of TC on the granules should also be studied, since the bacteria are responsible for pollutant degradation [17].

This paper was conducted to investigate the sorption and biodegradation performance of TC by NGS at lab-scale. The effects of various factors including temperature, mixed liquor suspended solid (MLSS) concentration, initial concentrations of COD and ammonium as well as the addition of AMO inhibitor were evaluated to obtain quantitative information of the behavior of TC in the granular sludge system. The toxicity of TC on the granules was also studied to consider its potential impact on this process. It is expected that this work will provide an alternative way of removing TC using biological methods.

2. Materials and methods

2.1. Chemicals

Tetracycline (≥98%) was of analytical grade and purchased from Sigma (St. Louis, MO, USA). Stock TC solutions (100 mg/L) were prepared in purified water. Other chemicals were analytically graded and commercially available, obtained from Beijing Chemical Reagent Factory, China.

2.2. Nitrifying granular sludge and synthetic wastewater

Nitrifying granular sludge was cultivated in a sequencing batch reactor (SBR) fed with synthetic wastewater (COD 500 mg/L, NH_4^+–N 350 mg/L) as described by Shi et al. [18]. The composition of mineral-salts medium is shown in Table S1. This medium was used together with other supplements such as ammonium, organic substrates and TC according to the experimental purposes. Mature granules were about 2 mm (Fig. S1) and morphology characteristics showed that the granules had a compact and round-shaped structure with large porosity. Both nitrifying bacteria and heterotrophic bacteria had high activities, with above 98% of NH_4^+–N (350 mg/L) and 90% of COD (500 mg/L) removed. This granular sludge was used for the following experiments.

2.3. TC sorption and degradation experiments

Control experiments, processed without the addition of granules, confirmed that photodegration and the adsorption on containers of TC could be neglected, which is also confirmed by Wang et al. [19]. The sorption and degradation kinetic tests using nitrifying activated sludge (NAS) were also done as contrast experiments. NAS was cultivated using the synthetic wastewater (COD 500 mg/L, NH_4^+–N 350 mg/L), which is similar to that above.

Preliminary experiments were conducted to determine the equilibrium time. Tetracycline concentrations in the aqueous phase were analyzed at 0.5, 1, 2, 4, 8, 16, 24, 36 and 48 h. Results showed that the removal of TC almost reached the maximum after 36 h and rarely changed then. Based on the results, 36 h was chosen as the equilibration time to ensure adequate time was provided to reach equilibrium in this study. The initial pH of the mixed liquor was adjusted to 7.5–8.0 by $NaHCO_3$ and each test was conducted in duplicates.

Sterilized granules was used for the sorption tests to avoid the influence of TC degradation and the inactivation of granules was carried out with the addition of sodium azide (NaN_3) at 0.3% weight to volume (w/v) [14]. Sorption kinetic experiments were conducted in 1 L glass beakers with TC solution at various concentrations (10, 20, and 30 mg/L). An MLSS of 2000 mg/L was used at the beginning of the tests. Under the condition of continuous stirring with a magnetic stirrer, samples were collected at predetermined time intervals. The concentrations of residual TC were measured immediately after it was filtered with a 0.22 μm filter membrane. The effects of temperature and MLSS were investigated during the equilibrium adsorption tests with temperature and MLSS varying in the ranges of 25–45 °C and 1000–5000 mg/L, respectively.The degradation kinetic tests were done in the similar way used for the sorption kinetics experiments except for the presence of sludge activities. Besides, 500 mg/L COD, 100 mg/L NH_4^+–N and mineral-salts medium were added in order to ensure the activities of microbes. Experiments of COD and NH_4^+–N effects were carried out in 250 mL flasks with COD and NH_4^+–N concentrations varying in the ranges of 0–1000 mg/L and 0–200 mg/L, respectively. To investigate the role of AOB, 10 mg/L of allylthiourea (ATU) was added as the AMO inhibitor [20]; meanwhile, tests without ATU were also included for control.

2.4. Short-term exposure effects of TC on nitrifying granules

The specific oxygen utilization rate (SOUR) and the nitrogen utilization rates were determined for granules with and without TC exposure. TC-treated granules were exposed to 20 mg/L of TC for about 12 h and named TC-granules; while control-granules without TC treatment were used for control. The specific oxygen utilization rates of heterotrophic bacteria $(SOUR)_H$, AOB $(SOUR)_{NH_4}$ and nitrite oxidizing bacteria (NOB) $(SOUR)_{NO_2}$ were determined using methods described by Liu et al. [21]. The respective substrates used were glucose and sodium acetate, NH_4Cl and $NaNO_2$. The nitrogen-source utilization kinetics were determined in 250 mL flasks with about 10 g/L MLSS (similar to that in the SBR) and 150 mL NH_4^+–N solution ranging from 50 to 800 mg/L. Aeration was provided in order to maintain DO above 3 mg/L. After reacting for 2.5 h, samples were collected and NH_4^+–N concentrations were determined immediately.

2.5. Analytical methods

Differences among the treatments were considered significant if significance level α (the level of confidence of the *t*-test) was less than 0.05. The analysis of TC in the filtered solutions was performed using a UV/vis spectroscopy (UV-2000, Ruili Inc., China) at 360 nm [22]. NH_4^+–N, NO_2^-–N, pH and MLSS were analyzed according to the standard methods [23]. The DO levels were measured with a DO meter (YSI Model 85, USA). TC uptake was calculated by performing a mass balance using the following equation:

$$q_t = \frac{C_0 - C_t}{\mathrm{MLSS}} \quad (1)$$

where q_t is the amount of adsorbed TC at time t (mg/g); C_0 (mg/L) and C_t (mg/L) are the initial and residual TC concentrations at time t, respectively.

3. Results and discussion

3.1. Sorption and biodegradation performance of TC on the granular sludge

The relationship between the contact time and the TC adsorption and biodegradation by NGS is shown in Fig. 1. The non-sterilized granules showed an excellent performance of adsorption and biodegradation. The TC removal rates were 3.01, 5.17 and 7.30 mg/g SS when the initial TC concentrations were 10, 20 and 30 mg/L, respectively. In this study, it is assumed that the elimination of TC in the non-sterilized granules system is achieved by sorption and biodegradation, and the degraded TC can be calculated by the following equation [24]:

$$C_d = C_0 - C_e - q \cdot X \quad (2)$$

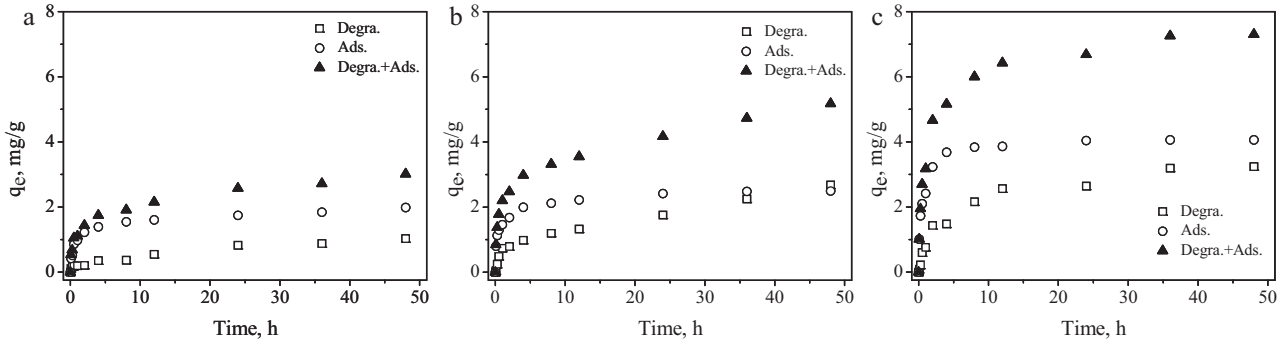

Fig. 1. Effects of contact time on TC biosorption and degradation at various initial concentrations (Degra. and Ads. represent degradation and adsorption of TC respectively). (a) 10 mg/L; (b) 20 mg/L; (c) 30 mg/L.

where C_d is the degraded TC concentration (mg/L), C_0 the initial TC concentration in the wastewater (mg/L), C_e the TC concentration remaining in the water phase (mg/L), q the amount of TC sorbed on the sludge (mg/g) and X is the MLSS concentration (g/L).

The respective sorption and biodegradation of TC is also shown in Fig. 1, from which it is vividly observed that the sorption occurred quickly and almost achieved the maximum sorptive capacity after 4 h. One possible explanation is that TC was initially adsorbed by the exterior surface of the granules and then entered the interior via pores to realize internal adsorption. However, TC is bulky organic molecule and the molecular sieving effect leads to a lower sorption rate [25]. As the diffusion resistance increased, the diffusion processes reached equilibrium. In comparison, TC biodegradation proceeded gradually and the degradation process did not stop after 24 h. It appears that the removal of TC in the NGS system is characterized by a quick sorption on the granules and a slow process of biodegradation. Previous study demonstrated that no evidence of biodegradation for TC was observed in the activated sludge and the sorption was found to be the principal removal mechanism [3]. However, in the present study NGS showed outstanding biodegradation ability and the percents of degradation accounted for 34.14%, 51.76% and 44.38% at initial TC concentrations of 10, 20 and 30 mg/L, respectively. The ability of TC biodegradation may be improved for further if the granules are acclimated with the TC-contained wastewater. Contrast experiments in the NAS system showed similar removal performance of TC compared to NGS, with TC removal rate of 2.49, 5.01 and 7.48 mg/g SS (Fig. S2). However, the proportion of degradation in this process was below 35%, with a main removal pathway of adsorption. In addition, activated sludge has poorer settling performance [26]. So the nitrifying granular system behaved more superiorly compared with nitrifying granular system.

Michaelis–Menten equation is usually used to describe the rate of an enzyme reaction for a chemical compound degradation in sludge as shown in Eq. (3) [27]:

$$\frac{dC}{dt} = -\frac{V_{max}C}{K_m + C} \tag{3}$$

The integrated form of Eq. (3) becomes:

$$\frac{C_0 - C}{t} + K_m\frac{\ln(C_0/C)}{t} = V_m \tag{4}$$

where V_m is the maximum reaction rate, changing with the concentrations of extra- and intra-cellular enzymes in a sludge system; K_m is a Michaelis constant. A lower K_m indicates a greater extent of binding. The ratio of V_m/K_m is considered to indicate the competitiveness of a microorganism. This model is introduced to describe the degradation rates of TC at different initial concentrations, and the data fits the model quite well ($R^2 > 0.99$). Therefore, it is clear that there was enzyme induction during the removal of TC. As shown in Table 1, values of K_m and V_m increased with the initial TC concentration, indicating a greater extent of binding between TC and the granular sludge at lower initial concentrations. This is because less TC would have more chance to bind to the constant biomass concentration [28].

3.2. Sorption of TC on the granular sludge

3.2.1. TC sorption affected by temperature and granular sludge concentrations

The effect of temperature on TC removal is shown in Fig. S3, which reveals that the TC sorption on NGS increased with the temperature, indicating an endothermic reaction. These results are in substantial agreement with those of Wang and Yates [29]. In order to describe the adsorption progress and investigate the mechanisms of adsorption, adsorption isotherm models (viz. Langmuir and Freundlich) are used. Table 2 summarizes the test results and sorption isotherms for TC resembled Freundlich curve ($R^2 > 0.95$) significantly, indicating that the surface of granules is heterogeneous and the exponential distribution of the sites and their energies [30]. This may be contributed to the large number of various functional groups on the granules surface. Gao et al. [31] reported that the granules have kinds of functional groups such as amine, carboxyl, lipid and phosphatemay, which provide binding sites for dye biosorption.

The sorption behavior of TC was apparently influenced by the amount of NGS in the system (Fig. S4). The relation between the amount of TC adsorbed and the initial granule concentrations fits the following equation:

$$q_e = 2.4752 \times \mathrm{MLSS}^{-0.2167} \tag{5}$$

With the increase of granules concentrations, the removal efficiency of TC increased while the amount of sorbed TC on a unit of sludge decreased. This phenomenon may be ascribed to the increase of suspended solid in the system, which raised the number of reactive sites available to adsorb TC from the solution. Besides, the decrease of q_e may be contributed to the splitting effect of flux (concentration gradient) between sorbate and sorbent with the increasing MLSS, which caused a decrease TC amount adsorbed onto the unit weight of granules [32]. Nevertheless, the overall amount of sorbed TC on the granules increased. It is vividly concluded that the increase of the granules concentrations enhanced the total sorption of TC, and benefited the removal of TC from the water. Compared to the conventional activated sludge, aerobic granules yield a high biomass and thus could enhance the sorption process.

3.2.2. Adsorption kinetics

The adsorption kinetics that describes the solute uptake rate governing the residence time of the sorption reaction is an important characteristic that defines the efficiency of sorption. The

Table 1
Michealis-Menten kinetic values of TC biodegradation.

Initial concentrations, mg/L	K_m, mg/L	V_m, mg/(L h)	V_m/K_m, h^{-1}	R^2
10	10.24	0.11	0.0103	0.9997
20	19.13	0.18	0.0092	0.9996
30	29.36	0.35	0.0118	0.9990

Table 2
Freundlich and Langmuir isotherm constants for adsorption of TC onto granular sludge (initial TC = 20 mg/L, pH 7.5–8.0).

Temperature, K	Langmuir $\frac{Ce}{qe} = \frac{1}{qm}Ce + \frac{1}{Kaqm}$			Freundlich $\ln\ qe = \ln KF + \frac{1}{n}\ln Ce$		
	q_m,mg/g	k_a, 1/mg	R^2	n	K_f, mg/g	R^2
298	9.5057	0.0365	0.6777	1.6437	0.6075	0.9575
308	15.2207	0.0312	0.7950	1.4065	0.6645	0.9894
318	6.4935	0.050	0.4150	0.5187	0.1085	0.9518

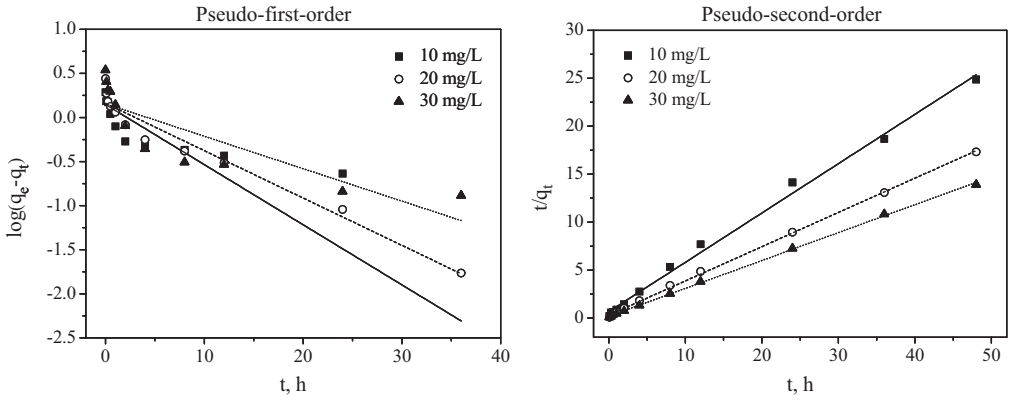

Fig. 2. Pseudo-first-order and pseudo-second-order adsorption kinetic plots of TC onto NGS (MLSS = 2000 mg/L; temperature = 25 ± 1 °C; pH 7.5–8.0).

experimental data complied very well with the pseudo-second order kinetic model, with the correlation coefficients R^2 higher than 0.99 (Fig. 2 and Table S2). These results suggest that the sorption rate is controlled by chemical sorption involving valence forces or covalent forces between TC and the granules.

The intra-particle diffusion model was also proposed to determine whether intra-particle diffusion is rate limiting [33].

$$q_t = k_p t^{1/2} + C \quad (6)$$

where the intercept C represents the thickness of the boundary layer. A larger intercept means a greater effect of the boundary layer.

According to Eq. (6), a plot of q_t vs. $t^{1/2}$ showed multilinearities in Fig. S5, indicating that three steps took place in the adsorption process. In the first step, the sharper linear portion is due to the boundary layer diffusion. While the second linear portion, describing the gradual adsorption stage, represents that intra-particle diffusion is rate-controlled. The third portion is attributed to the final equilibrium stage and the intra-particle diffusion began to slow down due to the low adsorbate concentrations left in the solutions [33]. The second and third portion fail to pass through the origin, clearly demonstrating that besides of intra-particle diffusion, boundary layer diffusion controlled the adsorption to some degree. The large porosity was likely beneficial in the transmission of substrates through the granule, but molecular sieving effect made intra-particle diffusion a rate-determining step due to the bulky organic molecule structure of TC. It is possible that the mechanism of TC removal on NGS was complex and both surface adsorption and intra-particle diffusion contributed to the rate-determining step.

3.3. TC biodegradation affected by COD, ammonium and AMO inhibitors

As mentioned above, NGS showed outstanding biodegradation ability compared to the conventional activated sludge. To investigate the effect of COD and ammonium on the TC removal efficiency, constant initial TC concentration of 20 mg/L but different COD or NH_4^+–N concentration were used. As can be seen from Fig. 3a, the higher initial COD condition gave higher TC removal efficiency. At a COD concentration of 500 mg/L, the TC elimination efficiency after 36 h was highest and achieved to 5.98 mg/g SS. At 1000 mg/L of COD, the value declined but changed little compared to that at 500 mg/L. In view of the results above, conclusions were drawn that the removal of TC could be promoted in the presence of easily biodegradable substrates. It is supposed that high initial COD concentrations increased the microbial co-metabolism of TC and thus improved the biodegradation of TC. However, for bisphenol A, a weakly estrogenic chemical, the presence of easily biodegradable organic substrates contrarily slowed down its degradation [24]. Higher degradation rates of pharmaceuticals were found in the case of lower initial TOC operation [34]. The comparison above showed that microorganisms degraded various organic compounds via different approaches, and additional carbon sources may be necessary to improve the biodegradation of TC.

The effect of NH_4^+–N showed similar changes to that of COD (Fig. 3b). With data analysis it was found that the amount of TC removal and the initial NH_4^+–N concentration (≤150 mg/L) were linearly dependent, and the relation could be expressed as follows:

$$q_e = 4.7453 \times c_{NH_4^+-N} + 0.0096 \quad (7)$$

TC removal efficiencies after 36 h was enhanced in the presence of NH_4^+–N and rose with the increase of NH_4^+–N concentrations,

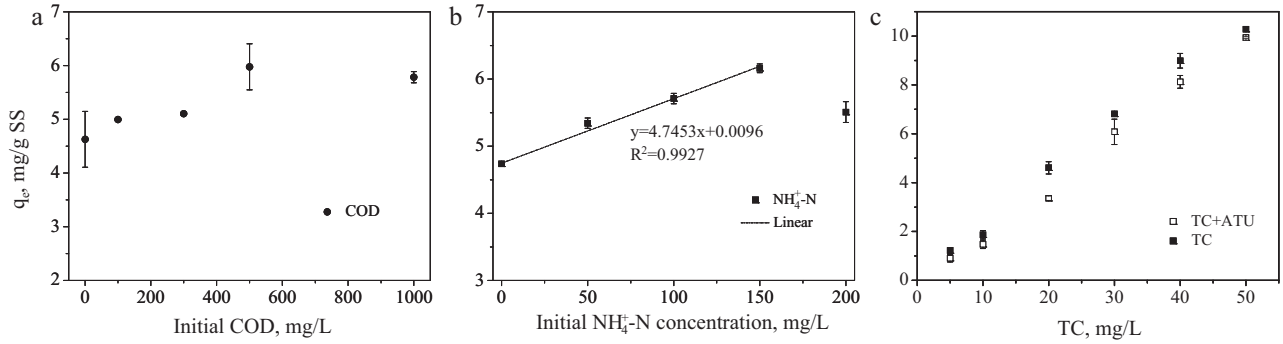

Fig. 3. Changes of TC removal efficiencies influenced by different substrates: (a) COD; (b) NH_4^+–N; (c) ATU.

reaching the highest value of 6.16 mg/g SS at 150 mg NH_4^+–N/L. The results are in substantial agreement with those of Tran et al. [14], who studied the degradation of some persistent pharmaceuticals (such as clofibric acid, diclofenac, etc.). One reason could be that higher initial NH_4^+–N concentrations enhanced the microbial co-metabolic activity and thus elevated the TC degradation. When NH_4^+–N concentration reached 200 mg/L, the removal efficiency of TC decreased on the contrary. At this level of NH_4^+–N concentration, free ammonia (FA) in the aqueous solution reached 10.5 mg/L and the nitrifying bacteria (both AOB and NOB) were inhibited when FA concentrations were higher than 10 mg/L according to previous study [35]. It is possible that higher initial NH_4^+–N concentrations produced higher concentrations of FA, which conversely inhibited the activities of microorganisms and reduced the degradation of TC. Therefore, for purpose of increasing TC removal, a proper concentration of NH_4^+–N was essential.

As shown above, the ammonium concentrations obviously influenced the degradation of TC. Both AOB and NOB existed extensively compared to the conventional sludge system and produced abundant AMO in the NGS system. Thus, the rate of the degradation process would be dependent on the amount of AMO induced by means of co-metabolism [15,16]. As proved by Tran et al. [14], with ATU addition the nitrification could be neglected and then ATU was chosen as AMO inhibitor to investigate the contributive role of nitrification process in TC degradation. Results indicate that the efficiency of TC degradation decreased in the case of ATU addition, and changes between samples with and without ATU addition were remarkably different in statistics ($P < 0.05$) (Fig. 3c). We originally assumed that the process of TC biodegradation was probably due to the synergistic effects of the multiple microbes, such as AOB and heterotrophic bacteria, and nitrification process may play a role in the degradation process. This is consistent with the results above. However, more research is necessary in order to confirm this hypothetical explanation.

3.4. Short-term exposure effects of TC on the granules

As a pharmaceutically active compound, it is essential to study the toxic effects of TC on the microbial activities, and thus to evaluate its potential impact on the biochemical process. SOUR and the nitrogen utilization rates were selected to visually characterize the short-term exposure effects of TC on the microbial activities.

With 12 h of TC exposure, the respirometric activities of heterotrophic bacteria, AOB and NOB in the granules all decreased greatly and dropped from 3.99, 2.70 and 1.72 mg O_2/g SS/h to 3.29, 2.30 and 1.32 mg O_2/g SS/h, respectively (Fig. 4a). The correlation coefficient was found to be significant at the 95% confidence level ($P = 0.039$), indicating that TC treatment significantly influenced the respirometric activities of the microbes. Concerning the control granules, $(SOUR)_{NH_4}/(SOUR)_{NO_2}$ was 1.57. After TC exposure, both $(SOUR)_{NH_4}$ and $(SOUR)_{NO_2}$ decreased and the ratio increased to 1.74 instead, showing that NOB were more sensitive to the TC stimulation. Most granules have a layered structure that protects bacteria [36]. In our previous study, we investigated the distribution of microbes within granules and found that AOB occupied the outside of the granule and NOB was in the deeper layers [18]. This structure theoretically protected NOB from toxicity more efficiently, which is contrary to the conclusion above. A possible reason for the difference may be that for NOB the toxic effect of TC was much higher than the sheltering effect of the granules. And thus we conclude that different kinds of microbes responded diversely to external stimuli. The result was also confirmed in a previous study, which showed that chloramphenicol was utilized ($C > 100$ mg/L) to inhibit denitrifying organisms in batch cultures and enrich Anammox biomass [37].

Batch experiments of the nitrogen utilization rates were designed to investigate the toxic effects of TC for further. In the reaction systems, the substrate utilization rate directly depends on the concentration of microorganisms in the granules and the concentration of the substrate surrounding microorganisms [38]. The Monod's equation is used to describe the relationship [39], which is shown as follows:

$$V = k\frac{S}{K_s + S} \tag{8}$$

where k = maximum specific substrate utilization rate; K_s = saturation constant for substrate; and S = substrate concentration in the reactor.

By measuring the initial and final concentrations of NH_4^+–N (i.e. S_0 and S_e), and the MLSS of the sludge (X) in flasks, the specific substrate utilization rate (V) was calculated. In double reciprocal form, Eq. (8) can be changed to the following form:

$$\frac{1}{V} = \frac{K_s}{k}\frac{1}{S} + \frac{1}{k} \tag{9}$$

A plot of $1/V$ versus $1/S$ results in a line (Fig. 4b) and then K_s and k can be calculated according to the slope and intercept of the best-fit line. For the control-granules, $k = 0.12\ d^{-1}$ and $K_s = 297.7$ mg/L; while for TC-treated granules, $k = 0.10\ d^{-1}$ and $K_s = 347.2$ mg/L. Comparing data of the two groups, conclusions were drawn that the TC exposure decreased the rate of NH_4^+–N utilization. T-test indicated that the results were significant compared with the control group ($P < 0.05$) and 20 mg/L TC indeed had a toxic effect on the microbial activities of the nitrogen utilization. In conclusion, short-term exposure of TC indicated potential toxic effects on the granular system. However, more work about the SBR performance under the condition of long-term exposure of TC should be carried out in order to complete the present results. It is also expected that with TC acclimation a steady and effective granular sludge sys-

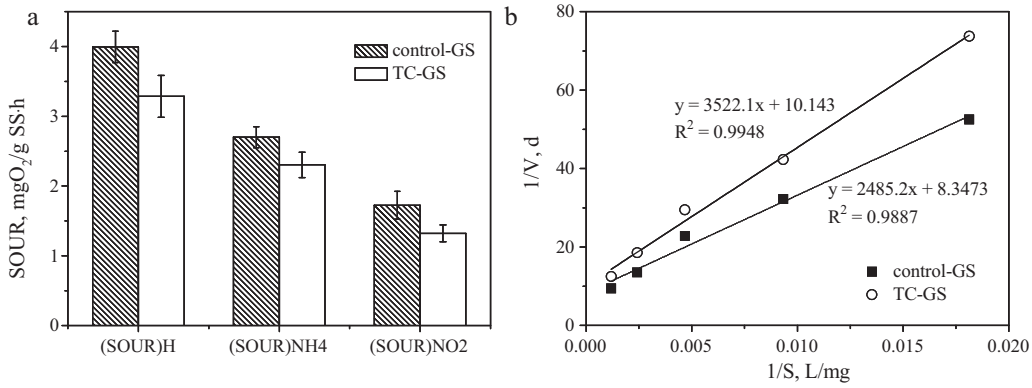

Fig. 4. Effects of TC addition on nitrifying granular sludge: (a) SOUR; (b) Nitrogen utilization rates.

tem can be obtained, thus further improving the removal of TC in wastewater.

4. Conclusions

The removal process of TC in the nitrifying granules system was characterized by a process of quick sorption and slow biodegradation. TC sorption was an endothermic chemical process, with a complex mechanism of surface adsorption and intra-particle diffusion. NGS showed outstanding degradation abilities and the process was likely attributed to the synergistic effects of multiple microbes. The presence of COD and NH_4^+–N enhanced the removal of TC, except at NH_4^+–N concentrations higher than 150 mg/L. ATU inhibition greatly decreased TC degradation. Short-term exposure of TC obviously decreased the respirometric activities of microbes in the granules as well as the rates of NH_4^+–N utilization.

Supporting information

The Supporting information includes data for the composition of mineral-salts medium; kinetic parameters for the adsorption of TC onto NGS; the observation of the nitrifying granules; adsorption and degradation of TC as well as the percent of degradation after 36 h; effects of temperature and aerobic granule concentrations on TC removal; plots of the intra-particle diffusion kinetics equation for adsorption of TC.

Acknowledgements

This work was supported by the Program for New Century Excellent Talents in University (NCET-10-0528), the National Natural Science Foundation of China (50978157, 21007033), the Excellent Young Scientist Foundation of Shandong Province (BS2010HZ009) and the Independent Innovation Foundation of Shandong University (2009TS022, 2009GN048).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.04.048.

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