Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Degradation of kanamycin from production wastewater with high-concentration organic matrices by hydrothermal treatment

Mei Tang[1], *Fan Li*[2], *Min Yang*[1,2], *Yu Zhang*[1,3,*]

[1] *State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, University of Chinese Academy of Sciences, Chinese Academy of Science, Beijing 100085, China*
[2] *Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, University of Chinese Academy of Sciences, Chinese Academy of Science, Beijing 100085, China*
[3] *National Engineering Laboratory for Industrial Wastewater Treatment, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

ARTICLE INFO

Article history:
Received 16 February 2020
Revised 22 April 2020
Accepted 22 April 2020
Available online 31 May 2020

Keywords:
Pharmaceutical wastewater
Hydrothermal process
Antibiotic antibacterial activity
Hydrolysis
Refractory organic wastewater

ABSTRACT

It is known that many kinds of fermentative antibiotics can be removed by temperature-enhanced hydrolysis from production wastewater based on their easy-to-hydrolyze characteristics. However, a few aminoglycosides are hard to hydrolyze below 100°C because of their stability expressed by high molecular energy gap (ΔE). Herein, removal of hard-to-hydrolyze kanamycin residue from production wastewater by hydrothermal treatment at subcritical temperatures was investigated. The results showed the reaction temperature had a significant impact on kanamycin degradation. The degradation half-life ($t_{1/2}$) was shortened by 87.17-fold when the hydrothermal treatment temperature was increased from 100°C to 180°C. The $t_{1/2}$ of kanamycin in the N_2 process was extended by 1.08-1.34-fold compared to that of the corresponding air process at reaction temperatures of 140-180°C, indicating that the reactions during hydrothermal treatment process mainly include oxidation and hydrolysis. However, the contribution of hydrolysis was calculated as 75%-98%, which showed hydrolysis played a major role during the process, providing possibilities for the removal of kanamycin from production wastewaters with high-concentration organic matrices. Five transformation products with lower antibacterial activity than kanamycin were identified using UPLC-QTOF-MS analysis. More importantly, hydrothermal treatment could remove 97.9% of antibacterial activity (kanamycin EQ, 1,109 mg/L) from actual production wastewater with COD_{Cr} around 100,000 mg/L. Furthermore, the methane production yield in anaerobic inhibition tests could be increased about 2.3 times by adopting the hydrothermal pretreatment. Therefore, it is concluded that hydrothermal treatment as a pretreatment technology is an efficient method for removing high-concentration hard-to-hydrolyze antibiotic residues from wastewater with high-concentration organic matrices.

* Corresponding author.
E-mail: zhangyu@rcees.ac.cn (Y. Zhang).

https://doi.org/10.1016/j.jes.2020.04.032
1001-0742/

Introduction

Fermentative antibiotic production wastewater has recently been focused because of the presence of high concentration of antibiotics (Larsson, 2014; Rehman et al., 2015; Tang et al., 2019a; Tang et al., 2019b; Yi et al., 2017). Antibiotics with a concentration of several hundred to thousands of mg/L in production wastewater, would severely disturb the stability of the biological wastewater treatment systems and greatly promote the proliferation of antibiotic resistance genes during the treatment processes (Aydin et al., 2014; Chelliapan et al., 2006; Liu et al., 2014; Ma et al., 2009). So, it is essential to remove antibiotics and their antibacterial activities from production wastewater before biological treatment. Enhanced hydrolysis has recently been focused as an effective approach for selective removal of antibiotics because many antibiotics are susceptible to hydrolysis (Mitchell et al., 2014, 2015; Shen et al., 2017). Our previous study based on frontier molecular orbital theory has shown that many kinds of antibiotics could be hydrolyzed below 100°C (Tang et al., 2019b; Yi et al., 2016). However, a few aminoglycosides with a relatively high energy gap (7.1562-7.6161 eV calculated by density functional theory (DFT)) were found difficult to hydrolyze under a mild condition (Tang et al., 2019b).

It is known that hydrothermal treatment is conducted under the subcritical conditions and can accelerate acid-base-catalyzed organic reactions by generating abundant hydronium and hydrated hydroxyl ions (Tekin et al., 2014). It was employed to convert biomass into valuable products or biofuels (Fang et al., 2018; Munir et al., 2018; Tekin et al., 2014) and also tried for the removal of some refractory substances like 4,4'-dichlorodiphenyltrichloro-ethane, diclofenac and lincomycin from aqueous solutions (Nose et al., 2007; Wang et al., 2018b; Weiner et al., 2013) (summarized in Appendix A Table S1). In addition, hydrothermal method as the pretreatment for anaerobic digestion feedstock were used to enhance the methane yield, shorten digestion time and reduce the required energy and size of the digester (Yousefifar et al., 2017). On the basis of the studies, there are some commercial applications of this technology (Yousefifar et al., 2017). Therefore, hydrothermal treatment as pretreatment process of antibiotic production wastewater to degrade high-concentration hard-to-hydrolyze antibiotic residues may be feasible. However, there is limited study regarding removal of hard-to-hydrolyze antibiotics from production wastewater by hydrothermal treatment, and it is not clear if the removal of antibiotics will be adversely affected by the presence of abundant organic matrices in antibiotic production wastewater.

Kanamycin, a hard-to-hydrolyze aminoglycoside, has strong antibacterial activity against Gram-negative and positive bacteria. It has been widely used as a veterinary medicine and a certain concentration of kanamycin can promote plant regeneration (Naidu, 2011; Wei et al., 2019). Kanamycin is produced by microbial fermentation. China is the largest producer of fermentative antibiotics including kanamycin, and a large amount of antibiotic production wastewater is discharged during the production process. Kanamycin production wastewater containing high-concentration of residual kanamycin may lead to generation and prevalence antibiotic resistance during the biological treatment. However, to our knowledge, little information was reported about the kanamycin treated by thermal process or anaerobic digestion. Therefore, in this study, the effectiveness of thermal treatment was investigated for the pretreatment of fermentative kanamycin production wastewater. The degradation kinetics of kanamycin in aqueous solution were firstly studied under different pH and temperature conditions to optimize the hydrothermal process. Then, the synergism of oxidation and hydrolysis were investigated under different atmospheric conditions (air and nitrogen (N_2)) to determine the degradation mechanism. The transformation pathway was explored by identifying the hydrolyzing products (TPs) using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) analysis. Lastly, the hydrothermal treatment was applied to the pretreatment of actual kanamycin production wastewater with abundant organic matrices (COD_{Cr}, around 100,000 mg/L). The effectiveness of the thermal pretreatment in the removal of antibiotic activity was evaluated by conducting anaerobic inhibition tests. The results of this study will provide an alternative approach for the removal of hard-to-hydrolyze antibiotics from production wastewaters with abundant organic matrices.

1. Materials and methods

1.1. Chemicals and materials

Kanamycin (98.0%, analytical standard) was purchased from J&K Chemical Ltd. (China). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were of analytical grade and supplied from Sinopharm Chemical Reagent Co. Ammonium formate (AF) and formic acid (FA) were of HPLC grade and obtained from J&K Chemical Ltd (Beijing, China). HPLC-MS grade methanol (MeOH) and acetonitrile (ACN) were purchased from Fisher Chemicals (New Jersey, USA). Antibiotic assay medium no.3 and nutrient agar were purchased from Beijing Aoboxing Biotech Co. Ltd (China). Ultrapure water was purified to 18.2 MΩ•cm using a Milli-Q Synthesis (Milli-Q System, Millipore, Billerica, MA, USA). The solution pH was measured using a pH meter (Mettler-Toledo, Switzerland).

1.2. Hydrothermal treatment experiments

Firstly, kanamycin aqueous solution (100 mg/L, 50 mL) with pH adjusted to the desired level by dilute HCl (0.1 mol/L) or NaOH (0.1 mol/L) was introduced into a Teflon-lined stainless-steel autoclave (100 mL) (Wang et al. 2018a, 2018b; Weiner et al. , 2013). Then, the reactor was tightly sealed and heated in an electric oven for 5 hr or 14 hr at 85-180°C. After designated time intervals, the autoclave was taken out from the oven and cooled naturally to lower the inner reactor pressure. After that, the autoclave was opened to collect samples, which were transferred to a refrigerator at 4°C immediately, then analyzed the antibacterial activity and concentration of kanamycin as soon as possible. The internal hydrothermal reaction of the autoclave was carried out at autogenously saturated water pressures corresponding to different temperatures, and it is considered that reaction started when the oven reached the preset temperature (Wang et al., 2018b). For investigating the role of oxygen in the reaction, the solution was purged with N_2 for 60 min and then sealed quickly and heated in an electric oven.

In order to demonstrate the application potential, the hydrothermal treatment process was also applied to the treatment of actual kanamycin production wastewater from a pharmaceutical company in Fujian Province, China. The concentrations of kanamycin and COD_{Cr} were 1,109 ± 26 mg/L and 98,133 ± 2,203 mg/L, respectively. The pH value of the production wastewater was around 7.4, and pH was not adjusted during the treatment process to reduce the cost in practical applications. All the experiments were carried out in triplicate.

1.3. Anaerobic inhibition test with and without hydrothermal treatment

Anaerobic inhibition tests were performed as batch studies in a methanogenic automatic monitoring device (Bioprocess Control, Sweden) according to our previous studies (Tang et al., 2019b; Yi et al., 2016). The anaerobic granular sludge (mixed liquor volatile suspended solids (MLVSS) of 100,000 mg/L) used in this experiment was from a sewage treatment plant in Beijing, China. The final MLVSS was about twice of the COD_{Cr} concentration according to previous reports (Cetecioglu et al., 2013; Cetecioglu et al., 2012). So 80 mL wet granular sludge was added into each serum bottle and then 40 mL kanamycin production wastewater and 280 mL tap water for dilution were added to make the final MLVSS at 20,000 mg/L, respectively. Inhibition tests were performed in 500 mL glass serum bottles at 37 ± 1°C, which were filled with wet granular sludge (MLVSS = 20,000 mg/L) and kanamycin production wastewater with or without hydrothermal treatment or sodium acetate (10,000 mg/L COD_{Cr}, control test). Before sealing, the system was adjusted to pH 7.0 ± 0.5 and then purged with N_2 for 5 min. The COD_{Cr} of raw wastewater was diluted to 10,000 mg/L for the tests. Cumulative methane production was automatically monitored during anaerobic batch tests. All conditions were tested in parallel.

1.4. Analytical methods

1.4.1. Chemical analysis

COD_{Cr} was measured on a spectrophotometer (Merck, Germany) following digestion and determined according to the National Standard Methods of China. Total organic carbon (TOC) was determined using a TOC-VCPH analyzer (Shimadzu, Japan).

1.4.2. Antibacterial activity assay

The microbial assay (real-time quantitative turbidimetry) for determining residual antibacterial activity of antibiotics in wastewater has been used in many studies (Tang et al., 2019a; Yi et al., 2016, 2017; Zhang et al., 2015). The residual antibacterial activity of the water samples was evaluated by comparing the inhibition effect of the target antibiotic and related substances on a sensitive and representative Gram-positive bacterial strain (*Staphylococcus aureus* (CMCC (B) 26003), purchased from National Institutes for Food and Drug Control) with that of kanamycin standard sample. The linear response of different concentrations of kanamycin and the calibration curve were generated using linear regression analysis. The linear range was 4-18 mg/L. Antibiotic equivalent quantity (kanamycin EQ, mg/L) was used to express residual antibacterial activity. The detailed procedures for determining the antibacterial activity of antibiotic solutions are provided in Appendix A.

1.4.3. UPLC-ESI-MS/MS analysis

The analysis of kanamycin is based on our previous article (Tang et al., 2019b). The mobile phase flow rate was 0.5 mL/min, with a gradient from 100% B (0.2% FA in 10/30/60 methanol /100 mmol/L AF aqueous solution/ acetonitrile) for 0.2 min to 35% A (0.4% FA in 200 mmol/L AF aqueous solution) from 0.5 to 1.5 min, where it was held until 4.5 min. The mobile phase was returned to 100% B after 4.5 min, with 3 min for re-equilibration. The sample volume of 20 μL was injected into the column (CORTECTS HILIC, 90 Å, 1.6 μm, 2.1 × 100 mm, Waters) thermostatted at 40°C. Mass spectrometry was performed using a Waters Micromass XEVO TQ MS (triple-quadrupole) detector operated with an electrospray ionization source (Micromass, Manchester, UK) in positive ion mode. The precursor ions, product ions and the optimized parameters were the same as our previous study (Tang et al., 2019b).

1.4.4. UPLC-QTOF-MS analysis

The identification of the hydrolysis products of kanamycin was conducted on a Waters Acquity UPLC system (Milford, MA, USA) equipped with a Waters XevoTM G2 QTOF-MS and ESI source (Tang et al., 2019a). The initial concentration of 1,000 mg/L of kanamycin was employed to generate sufficient product signals for measurement. The chromatographic conditions were the same as those for kanamycin detection by UPLC-ESI-MS/MS. The ion source was operated in positive mode. MS parameters of kanamycin were set as follows: capillary voltage 1.0 kV, cone voltage 48 V, source temperature 150°C, desolvation temperature 900°C, desolvation gas flow rate 1,000 L/hr, and MS/MS collision energy 20 - 40 eV. Accurate mass spectra were recorded across the range of 50 - 600 *m/z* for kanamycin. Data acquisition and analysis were performed using Masslynx with Mass Fragment and Metabolynx software (Waters, Milford, MA, U.S.).

1.5. Degradation kinetic analysis

The mechanisms of hydrothermal reactions are very complicated due to the formation of many unidentified products during severe reaction conditions. To simplify the kinetic equation, many studies have reported that the hydrothermal processes of tannin (Braghiroli et al., 2014), furfural derivatives (Reza et al., 2014), glycerol (Qadariyah et al., 2011), and 1-ethyl-4-[(4-methoxyphenyl)ethynyl]benzene (Yu et al., 2017) follow pseudo-first-order kinetics. The overall hydrothermal reaction includes two reactants in the following forms, and the amount of water is in excess, so it can be considered to be constant.

Chemical compound + Water ⇒ Products

$$-\frac{d[C_R]}{dt} = k[C_R]^a[C_W]^b$$

$$-\frac{d[C_R]}{dt} = k_h[C_R]^a$$

$$\text{When} a = 1, \text{ then } \ln\frac{C_R}{C_{R_0}} = k_h t \Rightarrow t_{1/2} = \frac{\ln_2}{k_h} \quad (1)$$

in which a is reaction order of chemical compound, b is reaction order of water, k is the kinetic constant of chemical compound degradation, k_h (min^{-1}) is $k[C_w]^b$, and C_R0, C_R, and C_W are the concentrations of chemical compound and water at times 0 and t, respectively.

The activation energy (Ea, J/mol) can be calculated according to the Arrhenius equation as follows:

$$\ln k_h = -\frac{E_a}{RT} + \ln A \quad (2)$$

where A is the pre-exponential factor, R is the universal gas constant (8.314 J/mol K) and T is the absolute reaction temperature (K).

It has been reported that the reactions that occur during the hydrothermal treatment process mainly include oxidation and hydrolysis (Tekin et al., 2014; Yousefifar et al., 2017). Therefore, the contributions of oxidation (C_o) and hydrolysis (C_h) to the degradation of kanamycin during hydrothermal treatment according to the $t_{1/2}$ in air and N_2 conditions are shown as follows:

$$C_o = \frac{t_{1/2}(N_2) - t_{1/2}(Air)}{t_{1/2}(N_2)} \times 100\% \quad (3)$$

$$C_h = 1 - C_o \quad (4)$$

Table 1 – The degradation rates and half-lives of different reaction conditions according to pseudo-first-order kinetics.

Reaction conditions	k (min^{-1})	$t_{1/2}$ (min)	R^2
100°C, air	0.0007	990.21	0.9633
110°C, air	0.0013	533.19	0.9415
120°C, air	0.0024	288.81	0.9758
140°C, air	0.0071	97.63	0.9700
150°C, air	0.0142	48.81	0.9213
160°C, air	0.0193	35.91	0.9587
180°C, air	0.0610	11.36	0.9553
140°C, N_2	0.0053	130.78	0.9609
150°C, N_2	0.0130	53.32	0.9587
160°C, N_2	0.0179	38.72	0.9482
180°C, N_2	0.0498	13.92	0.9546

2. Results and discussion

2.1. *Effects of pH and temperature on hydrothermal treatment process*

The hard-hydrolyzed lincomycin could be almost completely removed at 160°C for 180 min at pH 2.0 (Wang et al., 2018b). In the preliminary experiment, kanamycin could also be removed by 70.2% at 160°C for 180 min without adjusting pH. Therefore, to optimize the pH condition, the effects of different initial pH (3.0-11.0) on kanamycin removal were investigated at 160°C for 180 min, as shown in Appendix A Fig. S1. Kanamycin was removed by 75.5%, 67.5%, 68.3%, 96.4% and 21.6%, respectively at an initial pH of 3.0, 5.0, 7.0, 9.0 and 11.0. Except for the initial pH 3.0 treatment, descending of pH from 5.0-11.0 to 3.7-9.5 was observed for the other treatments, indicating acidic transformation products may be generated during hydrothermal treatment (Appendix A Fig. S1). In general, the optimal pH for kanamycin degradation during hydrothermal treatment process was around 9.0.

Fig. 1 shows the degradation kinetics of kanamycin over a temperature from 85°C to 180°C in terms of antibacterial activity (kanamycin EQ). It is clear that a temperature of 100°C could overcome the energy gap of kanamycin (ΔE=7.6759 eV calculated by DFT), and kanamycin degradation was significantly influenced by reaction temperature. The higher the reaction temperature is, the faster the degradation rate will be. It could be explained that raising temperature will lead to increase of the effective collision between reactive molecules (Wang et al., 2018b). Similar observations were reported that higher temperature accelerate the degradation rate of lincomycin during hydrothermal treatment process (Wang et al., 2018b).The degradation half-life calculated was shortened by 87.17-fold, from 990.21 min to 11.36 min with increasing temperature from 100 to 180°C (Table 1). In addition, the *Ea* of kanamycin was calculated as 77.74 kJ/mol (R^2 = 0.9978) by Eq. (2), which is higher than that of hard-to-hydrolyze lincomycin (68.87 kJ/mol) (Wang et al., 2018b), indicating that the structure of kanamycin is more stable than lincomycin.

2.2. *Relationship between antibacterial activity and antibiotic concentration*

The correlation between the kanamycin concentration determined by UPLC-ESI-MS/MS and the antibacterial activity (kanamycin EQ) of the reaction solution is shown in Appendix A Fig. S2. The correlation factor was 0.993, indicating that the parent kanamycin contributed the majority of antibacterial activity. Thus as long as kanamycin was completely removed during the hydrothermal process, the solutions after reaction would lose most of their antibacterial activity.

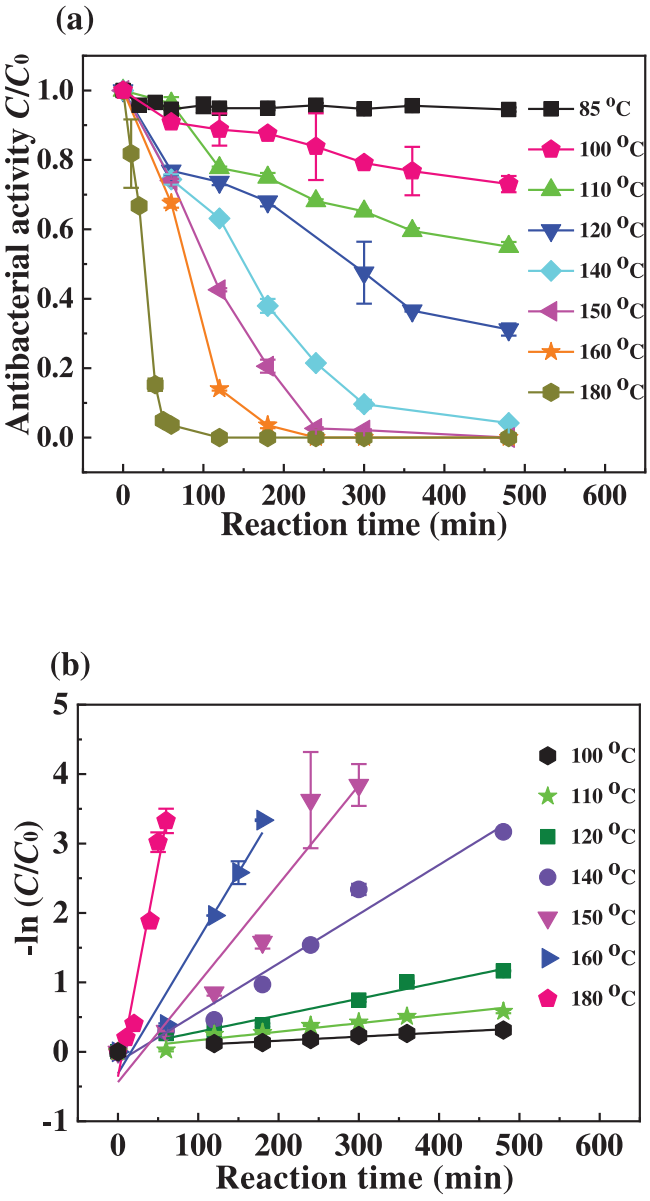

Fig. 1 – Effect of reaction temperature of hydrothermal treatment on (a) degradations of kanamycin, (b) degradation pseudo-first-order kinetics. Experimental conditions: initial kanamycin concentration 100 mg/L, pH 9.0.

2.3. *Contributions of oxidation and hydrolysis to the removal of kanamycin*

It has been reported that the reactions that occur during the hydrothermal treatment process mainly include oxidation and hydrolysis (Tekin et al., 2014; Yousefifar et al., 2017). The water-soluble portions of reactants disperse into water and hydrolysis takes place firstly, during which nucleophiles (H_2O or OH^-) attack chemical compounds (Tang et al., 2019b).

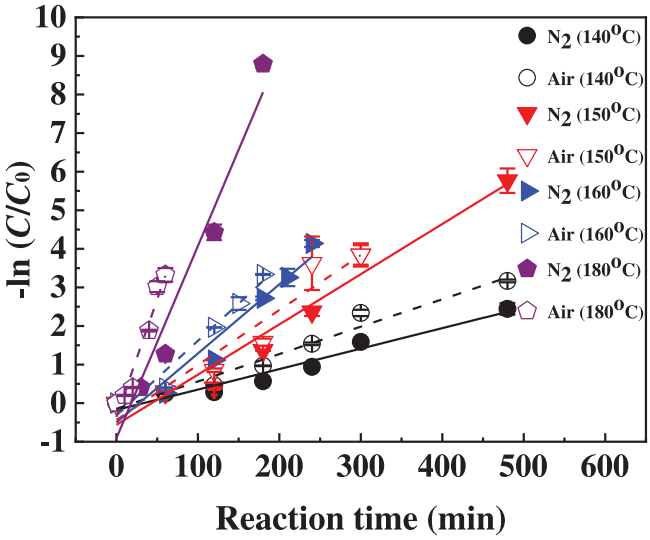

Fig. 2 – Effect of gas in the solution on degradation pseudo-first-order kinetics. Experimental conditions: initial kanamycin concentration 100 mg/L, pH 9.0.

Table 2 – Accurate mass measurements of possible degradation products by UPLC-QTOF-MS.

Product	Retention time (min)	Molecular formula	Measured mass (*m/z*)	Calculated mass (*m/z*)	ΔError (mDa)
TP1	0.85	$C_6O_2NH_7$	126.0553	126.0555	0.7
TP2	1.24	$C_{13}O_7N_3H_{23}$	334.1610	334.1614	-0.2
TP3	1.46	$C_{14}O_7N_3H_{23}$	346.1575	346.1614	-3.5
TP4	1.10	$C_{15}O_8N_3H_{27}$	378.1860	378.1876	-1.5
TP5	1.89	$C_{12}O_9N_5H_{27}$	386.1895	386.1887	1.4

Meanwhile, macromolecules such as biomass polymers depolymerize into monomeric chains, which further produce various intermediates containing free radicals via oxidation by a number of parallel and consecutive reactions (Tekin et al., 2014; Yousefifar et al., 2017).

To investigate the role of oxidation and hydrolysis during the process, N_2 was bubbled into the solution for 60 min prior to the reaction, and the reactor was sealed after inflation with a lid immediately to ensure removal of dissolved oxygen before the reaction. Kinetic parameters were calculated and compared for the degradation of kanamycin under different atmospheric conditions (air and N_2) (Table 1, Fig. 2, Appendix A Fig. S3). The half-lives in paired air and N_2 treatments at 140, 150, 160 and 180°C were 97.63, 130.78 min; 48.81, 53.32 min; 35.91, 38.72 min; and 11.36, 13.92 min, respectively. Thus, the degradation half-life of kanamycin antibacterial activity of the reaction in the N_2 process was extended by 1.08-1.34-fold compared to that in the air process at reaction temperatures of 140-180°C.

It is clear that the removal of dissolved oxygen will reduce the removal of kanamycin, which might be attributed to the decrease in free radical production. Dissolved oxygen in the solutions could attack the weak carbon-hydrogen bonds of the target compounds during the hydrothermal treatment process, resulting in the production of hydroperoxyl and alkyl radicals (Li et al., 1991). Therefore, the free radicals have played a role in hydrothermal treatment processes (Padoley et al., 2012; Ricq et al., 2000; Yousefifar et al., 2017). And the in-situ sampling device was needed to further determine the dominant radicals during reactions.

To further determine the existence and role of oxidation, the TOC was measured during the reaction process. As shown in Appendix A Fig. S4a, the TOC removal efficiency was 21.9% and 32.1%, respectively, at 150°C and 160°C in an air atmosphere, indicating that mineralization of kanamycin partly occurred. Thus, in the hydrothermal treatment process, synergism between oxidation and hydrolysis could be the main degradation mechanisms. Oxidation and hydrolysis processes also occurred during the hydrothermal treatment of lincomycin (Wang et al., 2018b). At the same time, the contribution of oxidation (C_o) and hydrolysis (C_h) to the degradation of kanamycin during hydrothermal treatment were evaluated by using Eqs. (3) and ((4). The contributions of hydrolysis were 75%, 92%, 93% and 98% in the air atmosphere at 140, 150, 160 and 180°C, respectively, revealing that the hydrolysis contributed more to the removal of kanamycin under higher temperature.

2.4. *Changes of transformation products over the process*

The extracted chromatograms of five possible products all exhibited good chromatographic peak shapes, and the response of the peaks also showed a general change from no signal to gradually increasing during the reaction time (0-5 hr) (Fig. 3), indicating the transformation process of the products. According to the mass spectra, including MS and MS/MS spectra (Appendix A Fig. S5), the elemental compositions of these five possible degradation products (TP1-TP5) were assigned by a TOF analyzer with high accuracy (< 5 mDa), as shown in Table 2. Thus, TP1, 2, 3, 4 and 5 were speculated to be $C_6O_2NH_7$ (*m/z*, 126.0555), $C_{13}O_7N_3H_{23}$ (334.1614), $C_{14}O_7N_3H_{23}$ (346.1614), $C_{15}O_8N_3H_{27}$ (378.1876) and $C_{12}O_9N_5H_{27}$ (386.1887), respectively. During the degradation of lincomycin in solution with hydrothermal treatment, a total of seven major intermediates were speculated (Wang et al., 2018b). Diclofenac could be removed completely at 200°C and three chlorinated metabolites including 1-(2,6-dichlorophenyl)indol-2-one, *N*-phenyl-2,6-dichloroaniline, and 2,6-dichloroaniline were identified (Weiner et al., 2013). As the peak area of kanamycin decreased, the peak areas of TP1, 2, 4 and 5 increased during 5 hr, however, the peak area of TP3 increased and then decreased a little at 4 hr, indicating TP3 was degraded to transformation products with smaller molecular weight such as TP1 or TP2 (Fig. 4, Appendix A Fig. S6). In order to reveal the degradation pathway of kanamycin, however, further work is required to identify structures of these transformation products.

2.5. *Application of hydrothermal treatment to kanamycin production wastewater*

2.5.1. *Enhanced degradation of kanamycin from production wastewater*

The COD_{Cr} of raw kanamycin production wastewater was 100,000 mg/L. The effect of organic matrices in kanamycin production wastewater on the degradation of kanamycin during the hydrothermal process was firstly assessed by diluting raw wastewater to give COD_{Cr} of 10,000 and 40,000 mg/L, respectively. The final concentration of kanamycin was adjusted to 1000 mg/L by adding the standard solution. It was found that at 160°C for 5 hr , a kanamycin removal of 99.87% was achieved in treating the solution without complicated matrices, while only 62.3% and 40.1% were achieved in treating solutions with COD_{Cr} of 10,000 and 40,000 mg/L, respectively (Appendix A Fig. S7). It was clear that the organic matrices in raw production wastewater had some adverse impacts on the removal of kanamycin during the thermal treatment process. There may be two reasons. On the one hand, the or-

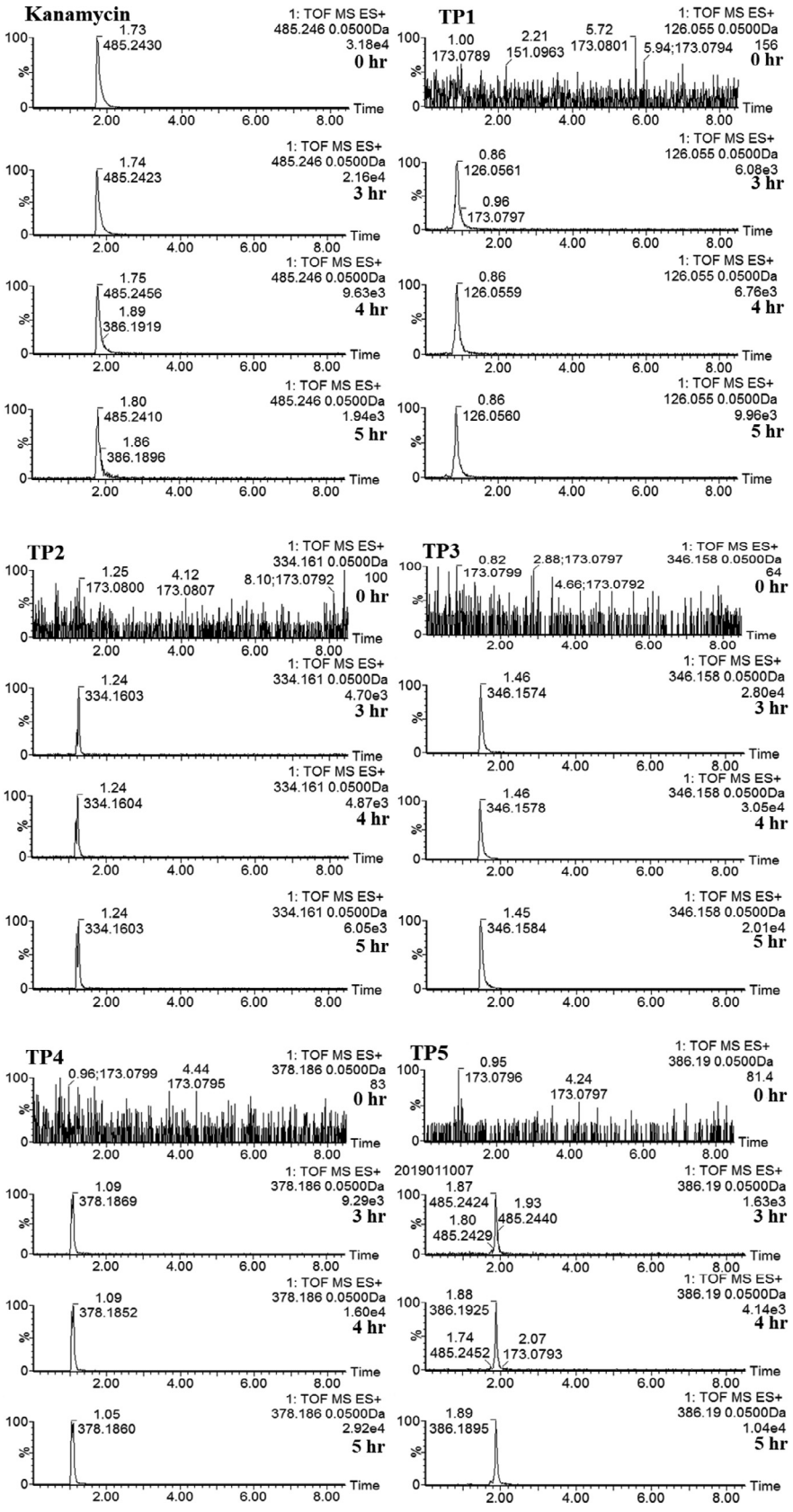

Fig. 3 – Extracted ion chromatograms of kanamycin and possible degradation products (TP1, TP2, TP3, TP4 and TP5) during reaction time 0 to 5 h. Experimental conditions: initial kanamycin concentration 1,000 mg/L, reaction temperature 160°C, pH 9.0.

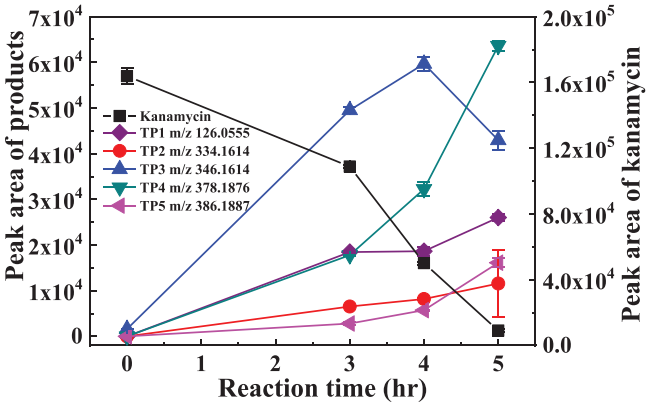

Fig. 4 – The evolution process of possible degradation products by hydrothermal treatment using UPLC-QTOF-MS. Experimental conditions: initial kanamycin concentration 1,000 mg/L, reaction temperature 160°C, pH 9.0.

ganic matrices will consume free radicals generated during hydrothermal process to prevent oxidation of kanamycin. On the other hand, some organic matrices may adsorb or complicate kanamycin to affect the hydrolysis of kanamycin during the reaction.

Residual antibacterial activity might not all come from parent kanamycin in the actual wastewater, so the residual antibacterial activity (kanamycin EQ mg/L) removal efficiencies were evaluated for raw wastewater in a temperature range between 160°C and 200°C. The residual antibacterial activity could be degraded more quickly at 200°C than that at 180°C (Fig. 5a). Kanamycin removal of 97.9% and 96.4% was achieved in terms of antibacterial activity when the thermal treatment was performed at 200°C and 180°C for 8 hr, respectively. Meanwhile, the COD_{Cr} concentration kept to be an almost constant value (Fig. 5b), showing that mineralization by oxidation was negligible during the treatment process. Thus, though there are some effects from the organic matrices, kanamycin in the production wastewater with a COD_{Cr} as high as 100,000 mg/L could be effectively removed by elevating the temperature to 200°C. Since many pharmaceutical factories can provide steam, hydrothermal treatment has operational feasibility. In addition, the cost of hydrothermal treatment is lower than wet oxidation which has been applied in the industrial wastewater treatment, because it occurs at lower temperatures and pressures in the absence of oxidants (Yousefifar et al., 2017).

2.5.2. *Comparison of anaerobic inhibition with/without hydrothermal pretreatment*

Since the anaerobic inhibition tests is commonly employed for the treatment of high concentration organic wastewater (Chelliapan et al., 2006; Rehman et al., 2015), anaerobic digestion tests were performed for kanamycin production wastewater before and after hydrothermal pretreatment. The methane production yields during the anaerobic inhibition tests were shown in Fig. 5c. Without pretreatment of the actual kanamycin production wastewater, the total methane production was 320.3 mL. Whereas, after hydrothermal pretreatment (200°C, 8 hr), the total methane production was increased to 727.6 mL, indicating that the hydrothermal pretreatment could decrease the inhibition of anaerobic processes and increase the methane production by kanamycin production wastewater. The total of methane production of control assay was 1,283.6 mL. The carbon source of control was sodium acetate, which can be degraded easily by anaerobic sludge, while the complex substrates with high COD_{Cr}

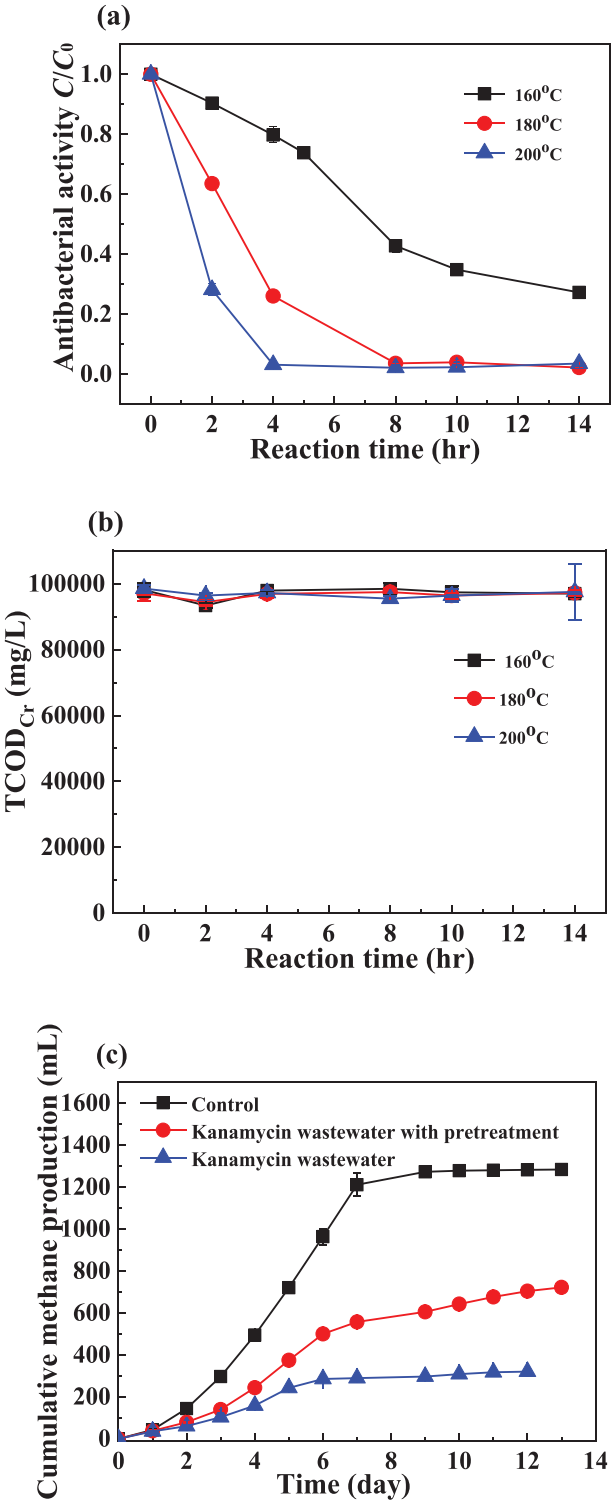

Fig. 5 – (a) Effect of reaction temperature on actual kanamycin production wastewater by hydrothermal treatment. (b) The changes of $TCOD_{Cr}$ in reaction solution during hydrothermal process. (c) Results of anaerobic inhibition test about cumulative methane production profiles for kanamycin production wastewater with hydrothermal treatment (200°C, pH 7.4, 8 h), without pretreatment and control inhibition assay without antibiotic addition. Error bars represent standard deviations.

concentration in actual wastewater was difficult to be fully utilized by microorganism, leading to the lower methane production than that in control test. The antibacterial activity and anaerobic inhibition by antibiotics were decreased indicating the lower biological effects of kanamycin degradation products. Besides antibacterial activity, the acute toxicity and biodegradability of the degradation products will be investigated in the further study.

3. Conclusion

In this study, the degradation process and mechanisms of the hard-to-hydrolyze antibiotic kanamycin during hydrothermal treatment process were investigated. Kanamycin and its antibacterial activity could be removed efficiently by a hydrothermal treatment process. Synergistic oxidation and hydrolysis may be the main degradation mechanisms, but hydrolysis played a major role during the hydrothermal treatment process. Meanwhile, five transformation products with lower antibacterial activity were identified using UPLC-QTOF-MS analysis. More importantly, kanamycin antibacterial activity could be eliminated 97.9% from actual production wastewater with high-concentration organic matrices. Furthermore, the methane production yield in anaerobic inhibition tests could be increased about 2.3 times with the hydrothermal pretreatment than that of without pretreatment, which verified the reliability of the hydrothermal treatment. This study provides a comprehensive understanding of kanamycin degradation in the hydrothermal treatment process, and offers an alternative pretreatment technology for high-concentration hard-to-hydrolyze antibiotics removal from refractory production wastewater with high-concentration organic matrices.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Nos. 21590814 and 81861138051). We thank Mr. Daoxin Zhang and Mr. Yunpeng Shen for their help in sample collection.

Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.04.032.

REFERENCES

Aydin, S., Ince, B., Cetecioglu, Z., Ozbayram, E.G., Shahi, A., Okay, O., et al., 2014. Performance of anaerobic sequencing batch reactor in the treatment of pharmaceutical wastewater containing erythromycin and sulfamethoxazole mixture. Water Sci. Technol. 70 (10), 1625–1632.

Braghiroli, F.L., Fierro, V., Izquierdo, M.T., Parmentier, J., Pizzi, A., Celzard, A., 2014. Kinetics of the hydrothermal treatment of tannin for producing carbonaceous microspheres. Bioresource Technol. 151, 271–277.

Cetecioglu, Z., Ince, B., Gros, M., Rodriguez-Mozaz, S., Barcelo, D., Orhon, D., Ince, O., 2013. Chronic impact of tetracycline on the biodegradation of an organic substrate mixture under anaerobic conditions. Water Res. 47 (9), 2959–2969.

Cetecioglu, Z., Ince, B., Orhon, D., Ince, O., 2012. Acute inhibitory impact of antimicrobials on acetoclastic methanogenic activity. Bioresour. Technol. 114, 109–116.

Chelliapan, S., Wilby, T., Sallis, P.J., 2006. Performance of an up-flow anaerobic stage reactor (UASR) in the treatment of pharmaceutical wastewater containing macrolide antibiotics. Water Res. 40 (3), 507–516.

Fang, J., Zhan, L., Ok, Y.S., Gao, B., 2018. Minireview of potential applications of hydrochar derived from hydrothermal carbonization of biomass. J. Ind. Eng. Chem. 57, 15–21.

Larsson, D.G.J., 2014. Pollution from drug manufacturing: review and perspectives. Philos. Trans. R. Soc. B-Biol. Sci. 369 (1656), 7.

Li, L., Chen, P., Gloyna, E.F., 1991. Generalized kinetic model for wet oxidation of organic compounds. Aiche J. 37 (11), 1687–1697.

Liu, M., Ding, R., Zhang, Y., Gao, Y., Tian, Z., Zhang, T., et al., 2014. Abundance and distribution of Macrolide-Lincosamide-Streptogramin resistance genes in an anaerobic-aerobic system treating spiramycin production wastewater. Water Res. 63, 33–41.

Ma, W.L., Qi, R., Zhang, Y., Wang, J., Liang, C.Z., Yang, M., 2009. Performance of a successive hydrolysis, denitrification and nitrification system for simultaneous removal of COD and nitrogen from terramycin production wastewater. Biochem. Eng. J. 45 (1), 30–34.

Mitchell, S.M., Ullman, J.L., Teel, A.L., Watts, R.J., 2014. pH and temperature effects on the hydrolysis of three beta-lactam antibiotics: ampicillin, cefalotin and cefoxitin. Sci. Total Environ. 466-467, 547–555.

Mitchell, S.M., Ullman, J.L., Teel, A.L., Watts, R.J., 2015. Hydrolysis of amphenicol and macrolide antibiotics: chloramphenicol, florfenicol, spiramycin, and tylosin. Chemosphere 134, 504–511.

Munir, M.T., Mansouri, S.S., Udugama, I.A., Baroutian, S., Gernaey, K.V., Young, B.R., 2018. Resource recovery from organic solid waste using hydrothermal processing: Opportunities and challenges. Renew. Sust. Energ. Rev. 96, 64–75.

Naidu, P.S.V., 2011. Influence of bavistin, cefotaxime, kanamycin and silver thiosulphate on plant regeneration of mentha piperita (L.)? An important multipurpose medicinal plant. J. Phycol..

Nose, K., Hashimoto, S., Takahashi, S., Noma, Y., Sakai, S., 2007. Degradation pathways of decabromodiphenyl ether during hydrothermal treatment. Chemosphere 68 (1), 120–125.

Padoley, K.V., Tembhekar, P.D., Saratchandra, T., Pandit, A.B., Pandey, R.A., Mudliar, S.N., 2012. Wet air oxidation as a pretreatment option for selective biodegradability enhancement and biogas generation potential from complex effluent. Bioresource Technol. 120 (3), 157–164.

Qadariyah, L., Mahfud, S., Machmudah, S., Wahyudiono, Sasaki, M., Goto, M., 2011. Degradation of glycerol using hydrothermal process. Bioresour. Technol. 102 (19), 9267–9271.

Rehman, M.S.U., Rashid, N., Ashfaq, M., Saif, A., Ahmad, N., Han, J.I., 2015. Global risk of pharmaceutical contamination from highly populated developing countries. Chemosphere 138, 1045–1055.

Reza, M.T., Wirth, B., Luder, U., Werner, M., 2014. Behavior of selected hydrolyzed and dehydrated products during hydrothermal carbonization of biomass. Bioresour. Technol. 169, 352–361.

Ricq, N., Barbati, S., Ambrosio, M., 2000. Optimization of the degradation of sewage sludge by wet air oxidation. Study of the reaction mechanism on a cellulose model compound. Analusis 28 (9), 872–877.

Shen, Y., Zhao, W., Zhang, C., Shan, Y., Shi, J., 2017. Degradation of streptomycin in aquatic environment: kinetics, pathway, and antibacterial activity analysis. Environ. Sci. Pollut. R. 24 (16), 14337–14345.

Tang, M., Dou, X., Tian, Z., Yang, M., Zhang, Y., 2019a. Enhanced hydrolysis of streptomycin from production wastewater using CaO/MgO solid base catalysts. Chem. Eng. J. 355, 586–593.

Tang, M., Gu, Y., Wei, D., Tian, Z., Tian, Y., Yang, M., et al., 2019b. Enhanced hydrolysis of fermentative antibiotics in production wastewater: hydrolysis potential prediction and engineering application. Chem. Eng. J., 123626.

Tekin, K., Karagöz, S., Bektaş, S., 2014. A review of hydrothermal biomass processing. Renew. Sust. Energ. Rev. 40, 673–687.

Wang, M., Zhang, B., Cai, C., Xin, Y., Liu, H., 2018a. Acidic hydrothermal treatment: characteristics of organic, nitrogen and phosphorus releasing and process optimization on lincomycin removal from lincomycin mycelial residues. Chem. Eng. J. 336, 436–444.

Wang, M., Zhang, B., Wang, J., Cai, C., Xin, Y., Liu, H., 2018b. Degradation of lincomycin in aqueous solution with hydrothermal treatment: Kinetics, pathway, and toxicity evaluation. Chem. Eng. J. 343, 138–145.

Wei, D., Meng, H., Zeng, K., Huang, Z., 2019. Visual dual dot immunoassay for the simultaneous detection of kanamycin and streptomycin in milk. Anal. Methods.

Weiner, B., Baskyr, I., Poerschmann, J., Kopinke, F.D., 2013. Potential of the hydrothermal carbonization process for the degradation of organic pollutants. Chemosphere 92 (6), 674–680.

Yi, Q., Gao, Y., Zhang, H., Zhang, H., Zhang, Y., Yang, M., 2016. Establishment of a pretreatment method for tetracycline production wastewater using enhanced hydrolysis. Chem. Eng. J. 300, 139–145.

Yi, Q., Zhang, Y., Gao, Y., Tian, Z., Yang, M., 2017. Anaerobic treatment of antibiotic production wastewater pretreated with enhanced hydrolysis: simultaneous reduction of COD and ARGs. Water Res. 110, 211–217.

Yousefifar, A., Baroutian, S., Farid, M.M., Gapes, D.J., Young, B.R., 2017. Fundamental mechanisms and reactions in non-catalytic subcritical hydrothermal processes: a review. Water Res. 123, 607–622.

Yu, L., Cao, Y., He, W., Li, G., Huang, J., Zhu, H., 2017. The decomposition process and kinetic analysis of benzene-based liquid crystal in hydrothermal system. Chem. Eng. J. 326, 1177–1185.

Zhang, H., Zhang, Y., Yang, M., Liu, M., 2015. Evaluation of residual antibacterial potency in antibiotic production wastewater using a real-time quantitative method. Environ. Sci. Processes Impacts. 17 (11), 1923–1929.