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A combined evaluation of the characteristics and acute toxicity of antibiotic wastewater

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ABSTRACT

The conventional parameters and acute toxicities of antibiotic wastewater collected from each treatment unit of an antibiotic wastewater treatment plant have been investigated. The investigation of the conventional parameters indicated that the antibiotic wastewater treatment plant performed well under the significant fluctuation in influent water quality. The results of acute toxicity indicated that the toxicity of antibiotic wastewater could be reduced by 94.3 percent on average after treatment. However, treated antibiotic effluents were still toxic to *Vibrio fischeri*. The toxicity of antibiotic production wastewater could be attributed to the joint effects of toxic compound mixtures in wastewater. Moreover, aerobic biological treatment processes, including sequencing batch reactor (SBR) and aerobic biofilm reactor, played the most important role in reducing toxicity by 92.4 percent. Pearson's correlation coefficients revealed that toxicity had a strong and positive linear correlation with organic substances, nitrogenous compounds, S^{2-}, volatile phenol, cyanide, As, Zn, Cd, Ni and Fe. Ammonia nitrogen (NH_4^+) was the greatest contributor to toxicity according to the stepwise regression method. The multiple regression model was a good fit for [TU50-15 min] as a function of [NH_4^+] with the determination coefficient of 0.981.

1. Introduction

Antibiotic wastewater is characterized as a special kind of wastewater with extremely high concentrations of organic substances, antibiotic residues and other pollutants. Approximately 150–200 m^3 of antibiotic production wastewater is produced from 1 t of antibiotic products. Toxic components such as antibiotic residues, intermediates, sulfates and heavy metals cannot be completely removed by the existing wastewater treatment process, thus making the discharge of treated antibiotic wastewater a potential ecological threat to the receiving environment (Ji et al., 2013; Samaras et al., 2013).

China is one of the largest producer and exporter of antibiotics in the world. China had an annual production and export of 14.7 and 2.5 million t of antibiotics in 2009, respectively, compared with the global production of 20 million t during the same period (Guo et al., 2012). However, the discharge of antibiotic wastewater without adequate removal of pollutants has resulted in serious environmental pollution problems. Given the impact of wastewater effluent discharge into the environment, the concerns of people on conventional pollutants like COD and NH_4^+ are justified. Researchers found that conventional chemical analysis could only measure a few targeted parameters, and could not fully reflect the toxicity of the complex industrial wastewater because possible toxic compounds might be neglected or undetected (Magdeburg et al., 2012; Yi et al., 2009). Realistically, the detection of every components present in complex industrial stream is impossible. Thus, wastewater toxicity is a necessary parameter in the evaluation of possible environmental impact.

The toxicity of pharmaceutical wastewater has attracted increasing public attention (Mansour et al., 2012; Sitre and Satyanarayan, 2011; Zhao et al., 2007). Sitre et al. (2009) evaluated the acute toxicity of raw, neutralized, physic-chemically/biologically treated herbal pharmaceutical wastewater to *Ceriodaphnia dubia* in India. Among the toxicity test methods reported in literature, luminescent bacteria were the most widely used in monitoring the toxicity of environmental water samples; luminescent bacteria were proven effective in the toxicity evaluation of industrial wastewaters (Choi and Meier, 2001; Bengtsson and Triet, 1994). Toxicity tests with luminescent bacteria were performed by monitoring the luminescence inhibition rate after a certain exposure period to wastewater samples, and it could provide sensitive and reliable results in evaluating the toxicity of industrial wastewater (Yu et al., 2014). Mendonca et al. (2007) confirmed that the luminescent bacteria were more sensitive and accurate than *Daphnia magna* and *Lemna minor* for the toxicity

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analysis of cork-boiling wastewater. An integrated approach that combines chemical analyses and toxicity tests would display accurate toxicity assessment of the toxicity of complex industrial wastewater (Smital et al., 2011).

The discharge of antibiotic wastewater has resulted in serious environmental pollution problems in China as fish are dying and water is unsafe for drinking or irrigation in the receiving water. The pharmaceutical wastewater discharge standard (GB 21903-2008) published by Chinese EPA in 2010 added the parameter of acute toxicity (to luminescent bacteria) to guarantee the quality of discharged antibiotic wastewater. Existing studies have rarely reported the toxicity level of Chinese pharmaceutical wastewater. Besides, the published literatures were limited to the treated pharmaceutical effluents of specific types of pharmaceutical wastewaters, and only a few studies explored the toxic nature of antibiotic wastewater. In the current study, conventional physicochemical analysis and acute toxicity test using *Vibrio fischeri* (*V. fischeri*) were used to predict the potential impact of antibiotic wastewater discharge into the environment. This study aims to evaluate the toxicity of antibiotic wastewater throughout the entire treatment process for the follow up of detoxification processes and to detect sensitive parameters or substances that significantly correlated to wastewater to toxicity.

2. Materials and methods

2.1. Sample collection and wastewater treatment process

The antibiotic wastewater treatment plant is located in Shijiazhuang City, China, and receives wastewater from a drug manufacturer that has a daily production of 25 t of penicillin (P. G) and 10 t of oxytetracycline (OTC), with 10,000 m^3 of wastewater. The whole wastewater treatment process consist of hydrolysis/acidification processes, sequencing batch reactor (SBR) process and aerobic biofilm reactor process; these processes are typical combined processes for antibiotic wastewater treatment in China. A total of fifteen wastewater samples were collected at five sampling points (outlets of the equalization tank, hydrolysis/acidification tank, sequencing batch reactor tank, aerobic biofilm reactor and secondary clarifier) on August 1, 2011, December 17, 2011, and May 13, 2012. The final effluent is discharged into a river for landscape use after settling in the secondary clarifier.

Wastewater samples were collected in the form of composite samples from three individual samples collected at 8 h intervals. Each sample was divided into two subsamples. One subsample was stored at 4 °C for the physicochemical test, and the other sample was frozen and stored at −20 °C for the toxicity test. The wastewater samples were pretreated by membrane (0.45 μm) filtration before assaying. The samples were labeled according to sampling points and sampling batches. For example, Samples 2–3 represent the wastewater collected at Sampling Point 2 on May 13, 2012; the first number and second numbers refer to the sampling point and sampling batch, respectively.

2.2. Physicochemical analyses

Measuring of physicochemical parameters was conducted according to the standards methods (Chinese NEPA, 2002). The pH level was determined by using a pH probe (Delta320, Mettler Toledo, USA). COD was measured according to the potassium dichromate method. BOD_5 was determined by using the five-day biological oxygen demand (BOD) test method. Ammonia nitrogen (NH_4^+), total nitrogen (TN), volatile phenol, sulfates (SO_4^{2-}), total cyanide and Cr^{6+} concentrations were measured by a spectrophotometer (DR5000, Hach, USA): NH_4^+ was measured with salicylic acid–sodium hypochlorite; TN was spectrophotometrically measured by UV with hydrochloric acid after digestion by potassium persulfate; volatile phenol was measured with 4-aminoantipyrine bilin; SO_4^{2-} was measured with barium chromate; total cyanide was measured with isonicotinic–pyrazolone; Cr^{6+} was measured with diphenyl hydrazine. Total organic carbon (TOC) was analyzed by a TOC analyzer (TOC-V_{CPH}, Shimadzu, Japan). Cu, Zn, Ca, Cd, Pb, Fe, total Cd, and As concentrations were analyzed by an inductively coupled plasma-optical emission spectrometer (IRIS, Thermo, USA).

The presence of P. G and OTC were determined by using a triple-series four-pole ultra performance liquid chromatography-tandem mass spectrometer (1200-6410, Agilent, USA) after solid phase extraction. The antibiotic wastewater samples were firstly purified through protein precipitation processes with 30 percent zinc sulfate and twenty percent potassium ferrocyanide. Then the antibiotics were enriched and purified with Oasis HLB SPE, and separated through Agilent Plus C18 RRLC using 0.01 mol/L ammonium acetate solution (with 0.1 percent formic acid) and 0.1 percent acetonitrile as mobile phase. The UPLC–MS/MS parameters of P. G and OTC under optimized conditions and the parameters to evaluate the performance of the analytical method were shown in Table 1.

2.3. Acute toxicity measurement

Acute toxicity was analyzed by using the marine gram-negative bacterium *V. fischeri*. Freeze-dried *V. fischeri* (NRRL B-11177) was purchased from the Marine Culture Collection of China. The salinity of wastewater samples was adjusted to two percent NaCl equivalent concentration before contact with *V. fischeri*. The test was performed in the 96-well microplates according to the flash-assay protocol (International Organization for Standardization, 2010). The luminescent intensity was determined by using a multifunctional microplate reader in luminescence mode (MD SpectraMax M5, Molecular Devices, USA). The procedure was conducted according to the study of Mortimer et al. (2008), except for the calculation of the inhibition of luminescent intensity as the percentage of the unaffected control (two percent NaCl).

2.4. Data analysis and model development

The acute toxicity test results were expressed as the half effective concentration after 15-min of exposure (EC50-15 min). EC50-15 min (%) was responsible for the volume percentage of the wastewater sample diluted in the saline solution (V/V) that inhibits 50 percent of the luminescence output compared with the control. To explain the acute toxicity results and create graphical expressions conveniently, the acute toxicity values expressed as EC50-15 min were converted into toxicity units (TU50-15 min) by using the following formula:

$$[\mathrm{TU50-15\ min}] = 100[\mathrm{EC50-15\ min}]^{-1} \quad (1)$$

TU50-15 min is directly proportional to the toxicity and represents the amount of unknown toxic compound mixtures.

The relationships between the physicochemical parameters and the toxicological data were determined by using the SPSS 21.0 software: One-way ANOVA was used to test the differences in the water quality parameters between wastewater samples from different sampling points. Pearson's correlation analysis was used to test the significance of the correlations between the acute toxicities and the physicochemical parameters ($p < 0.05$). The multi variable regression model for the acute toxicities and the physicochemical parameters was established by using the stepwise linear regression method to identify the most significant factors.

3. Results and discussion

3.1. Water quality characteristics

The characteristics of the raw and processing antibiotic wastewater samples are summarized in Table 2. Previous studies reported that the characteristics of antibiotic wastewater varied

Table 1
UPLC–MS/MS parameters of P. G and OTC under optimized conditions and the parameters indicating the performance of the analytical method: limit of quantification (LOQ) and recoveries obtained in wastewater.

Compounds	Parent ion (*m*/*z*)	Daughter ion (*m*/*z*)	Collision energy (eV)	Working curve	Coefficients (R^2)	LOQ (ng/L)	Recoveries (%)
P. G	335	176	10	Y=461.74X	0.9997	20	74.6 ± 8.17
		160	15				
OTC	461	443	25	Y=3469.09X	0.9982	20	80.8 ± 9.01
		426	15				