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Toxicity evaluation of wastewater collected at different treatment stages from a pharmaceutical industrial park wastewater treatment plant

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HIGHLIGHTS

- The toxicity testing methods of wastewater were established and compared.
- Wastewater toxicities had a significantly positive relation to TSS, TN, TP and COD.
- The toxicity limits of influent and effluent in a wastewater plant were calculated.
- The levels of toxicities and conventional indexes were reduced after most stages.

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ABSTRACT

The toxicity of water-receiving bodies, the effluent and other treatment stages in wastewater treatment plants has recently been of interest to the public due to the lack of a regulated toxicity-based index for wastewater discharge in China. This study aimed to evaluate the conventional pollution parameters and toxicities of wastewaters collected at different treatment stages from a pharmaceutical industrial park wastewater treatment plant through dehydrogenase activity (DHA) and bioluminescent bacteria (*Vibrio qinghaiensis*) tests. The results of an analysis of conventional parameters indicated that the total suspended solids (TSS), chemical oxygen demand (COD), total nitrogen (TN), ammonia nitrogen (NH_3–N), and total phosphorus (TP) were largely removed after various treatments. However, the TN, NH_3–N and COD still exceeded the regulated standards. The tested pharmaceutical park effluents were mainly polluted with organic pollutants and nitrogenous. The toxicity test results indicated that the toxicities could be markedly reduced after treatment, with the toxicities of two out of the six effluent samples at different treatment stages being greater than the influent toxicity. Spearman's rank correlation coefficients indicated a significantly positive correlation between the toxicity values obtained using the DHA and *Vibrio qinghaiensis* tests. Compared with the DHA measurement, the *Vibrio qinghaiensis* test was faster and more sensitive. Meanwhile, the toxicity indicators were significantly and positively correlated with the TSS, TN, TP and COD concentrations. These results may aid the understanding of the toxicity of pharmaceutical industrial park wastewaters and toxicity removal using the treatment techniques that are currently utilized in China.

1. Introduction

Rapid economic development and an increasing demand for health protection have promoted the development of pharmaceutical industry parks in China (Roschangar et al., 2015). As one of the world's largest pharmaceutical manufacturers and exporters, China produced more than 2.89 million tons of crude chemical drugs in 2011. Moreover, the pharmaceutical industry often generates high concentration wastewaters varying in character and quantity depending on the products and related manufacturing processes (Sitre and Satyanarayan, 2011). Pharmaceutical wastewaters often contain various toxic compounds, such as benzene, polycyclic aromatic hydrocarbons, and heterocyclic compounds (Sun et al.,

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2011). These chemical compounds are insufficiently biodegradable and cannot be completely removed from wastewaters, resulting in the generation of secondary pollutants and the continuous discharge of toxicants to wastewater treatment plants (WWTPs) (Yu et al., 2014). Toxicants contained in industrial wastewaters could destroy the normal operation of a pharmaceutical park WWTP (Zhao et al., 2014), inhibit the metabolic activity of microorganisms, reduce the activity of microorganisms, and even lead to poisoning or death, which resulted in the WWTP not meeting the stability standard (De Melo et al., 2013). Therefore, the evaluation of the toxicity of wastewaters from pharmaceutical industrial park WWTPs is drawing increasing attention and requires further studies.

Toxicity evaluations have been adopted for wastewater management in many WWTPs (Power and Boumphrey, 2004), where various test methods are used to characterize the wastewater toxicity (Manenti et al., 2015; Santos et al., 2014). Evaluations of the toxicity of pharmaceutical industrial wastewaters have also been conducted in recent years using organism test methods. Ji et al. used a luminescent bacteria method to test the toxicity of pharmaceutical wastewaters containing antibiotics through anaerobic digestion treatments (Ji et al., 2013). Carucci et al. used five biological toxicity determination methods to evaluate the toxicity of pharmaceutical wastewaters, such as a dehydrogenase activity determination method, a nitrification inhibition method, and a respiratory rate method (Carucci et al., 2006). Zhao et al. demonstrated that the pharmaceutical wastewater from a Chinese pharmaceutical wastewater treatment plant induced reproductive toxicity in male mice (Zhao et al., 2007).

Pharmaceutical industrial wastewaters exhibit complex characteristics. The analysis of toxic compounds in pharmaceutical industrial wastewaters is expensive and time consuming because wastewaters contains unidentified chemicals and compounds at low concentrations. Studies have shown that the analysis of conventional parameters is an important procedure for evaluating WWPTs, and additional processes and toxicity tests should be conducted (Forget et al., 2000). Bioassays can provide general information regarding the toxic effects caused by all of the components in wastewater, including unknown substances that elicit synergistic, antagonistic, or additive effects, and the use of bioassays has suggested the importance of investigating the adverse effects of wastewaters on water bodies (Bengtsson and Triet, 1994; Meriç et al., 2005).

Bioassays that employ different test methods can be used to directly characterize the toxicity levels of various industrial chemicals and wastewaters. Previous studies have demonstrated that the dehydrogenase activity (DHA) assay can be used to evaluate biological wastewater treatments and water toxicity because dehydrogenase is an essential enzyme for acquisition of the energy required for the microbial degradation of organic pollutants, and DHA largely reflects the activity state of an organism. The DHA assay as a bioassay method has high sensitivity, is simple to perform, has a low cost, and allows quantification (Barajas-Aceves et al., 2007; Zhang et al., 2011). The freshwater luminescent bacteria *Vibrio qinghaiensis* is one of the most widely used organisms in toxicity analyses, has been used to facilitate easy, rapid, and suitable freshwater bioassays, and provides reliable toxicity measurements of chemicals and wastewater (Ma et al., 1999; Ye et al., 2011). In this study, DHA and the luminescent bacteria *Vibrio qinghaiensis* were used as the test methods in the toxicity experiments.

In this study, samples were collected from the influent and final effluent and at six different treatment stages in a pharmaceutical industrial park wastewater treatment plant (PIP-WWTP). The following conventional parameters of the samples were analyzed: pH, total suspended solids (TSS), chemical oxygen demand (COD), total nitrogen (TN), ammonia (sum of NH_4^+ and NH_3 and subsequently referred to as NH_3–N), and total phosphorus (TP). The toxicity levels of the wastewaters were tested using measurements of DHA and the luminescent bacteria *Vibrio qinghaiensis*. This study aimed to determine the water quality and toxicity at every wastewater treatment stage, to evaluate the toxicity reduction efficiencies of the different treatment stages, to determine the correlations between conventional characteristics and toxic effects on wastewater and to predict the limit value of the influent and receiving water bodies in a PIP-WWTP.

2. Materials and methods

2.1. Sample collection and wastewater treatment process

The pharmaceutical wastewater used in this study was collected from the WWTP of an economic and technological development zone located in the east of Shijiazhuang, Hebei Province, China. The treatment techniques applied in this WWTP are a high-efficiency anaerobic filter (HAF), a biological Fe pool (BFP), a flow separate bed bio-reactor (FSBBR), a moving bed biofilm reactor (MBBR), oxidation ditch plus secondary sedimentation tank (OS) and a three-phase catalytic oxidation reaction pool (TPCORP), as shown in Fig. 1. Wastewater sampling at each treatment stage (Fig. 1) was conducted from September to December 2015 using discrete sampling. All of the samples were analyzed to assess conventional water quality parameters and toxicities to DHA and *Vibrio qinghaiensis*. Wastewater samples were stored at 4 °C until chemical analysis, and toxicity tests were carried out within 48 h (Zhang et al., 2015).

2.2. Analysis of conventional water quality parameters

Conventional chemical parameters, including pH, dissolved oxygen (DO), chemical oxygen demand (COD), total suspended solids (TSS), ammonia (NH_3–N), total nitrogen (TN) and total phosphorous (TP), were measured. The pH was determined using a pH meter (HQ30d, HACH, USA); the DO was detected with a dissolved oxygen meter (HQ30d, HACH, USA); the COD was measured using the potassium dichromate method; the TN was measured using the potassium persulfate digestion UV spectrophotometric method; the NH_3–N was analyzed using the sodium reagent method; and the TP was measured using the ammonium molybdate spectrophotometric method. The TN, NH_3–N and TP tests were performed using a UV spectrophotometer (2600UV-VIS, UNIC, China). All samples were tested in triplicate.

2.3. Bioluminescence inhibition assay using Vibrio qinghaiensis

The bioluminescence inhibition assay of the wastewater samples was conducted according to ASTM D5660-96 using a BHP9511 water quality toxicity analyzer (Beijing HAMAMATSU, China). The luminescent bacterium used was *Vibrio qinghaiensis* sp. *Nov*, which was provided by Beijing HAMAMATSU Photon Techniques Inc. A volume of 0.05 mL of a suspension of the freshwater luminescent bacterium was thoroughly mixed in a test tube with 2 mL of a water sample, and the relative light intensity was recorded after 15 min. All samples were tested in triplicate.

2.4. Enzyme activity inhibition assay using dehydrogenase in the activated sludge

Dehydrogenase activity was evaluated following a previously proposed method (Aragón et al., 2010). This method is based on the measurement of the color produced by the reduction of the original