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Reduction in toxicity of wastewater from three wastewater treatment plants to alga (*Scenedesmus obliquus*) in northeast China

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ABSTRACT

The toxicity of municipal wastewater to the receiving water bodies is still unknown, due to the lack of regulated toxicity based index for wastewater discharge in China. Our study aims at gaining insight into the acute toxic effects of local municipal wastewater on alga, *Scenedesmus obliquus*. Four endpoints, i.e. cell density, chlorophyll-A concentration, superoxide dismutase (SOD) activity and cell membrane integrity, of alga were analyzed to characterize the acute toxicity effects of wastewater from municipal wastewater treatment plants (WWTPs) with different treatment techniques: sequencing batch reactor (SBR), Linpor and conventional activated sludge. Influent and effluent from each treatment stage in these three WWTPs were sampled and evaluated for their acute toxicity. Our results showed that all three techniques can completely affect the algal chlorophyll-A synthesis stimulation effects of influent; the algal cell growth stimulation effect was only completely removed by the secondary treatment process in conventional activated sludge technique; toxic effects on cell membrane integrity of two influents from WWTPs with SBR and conventional activated sludge techniques were completely removed; the acute toxicity on SOD activity was partially reduced in SBR and conventional activated sludge techniques while not significantly reduced by Linpor system. As to the disinfection unit, NaClO disinfection enhanced wastewater toxicity dramatically while UV radiation had no remarkable influence on wastewater toxicity. Our results illustrated that SOD activity and chlorophyll-A synthesis were relatively sensitive to municipal wastewater toxicity. Our results would aid to understand the acute toxicity of municipal wastewater, as well as the toxicity removal by currently utilized treatment techniques in China.

1. Introduction

To protect the environment water body, wastewater treatment plants (WWTPs) are gradually constructed to treat the increasing volume of domestic and industrial wastewater. In China, the discharge of various kinds of wastewater reached 6.95×10^{10} t in 2013, among which domestic wastewater was nearly 4.85×10^{10} t, accounting for nearly 70%. Total discharges of chemical oxygen demand (COD) and ammonia–nitrogen (NH_3–N) were 2.35×10^7 t and 2.46×10^6 t, respectively (MEP, 2014). Accordingly, nearly four thousand WWTPs with a capacity of 1.46210^8 m^3/d have been constructed in China by 2013 (MHURD, 2013). In these WWTPs, anaerobic-anoxic-oxic (A^2/O), sequencing batch reactor (SBR) and conventional activated sludge are the major treatment techniques adopted, which account for 25.5%, 17.2% and 8.1%, respectively (CUWA, 2012). Even though these techniques in WWTPs can remove nearly 85% of COD and most of effluents could meet the national discharge standard, the removal of nutrients and emerging contaminants was not so satisfactory (Jin et al., 2014). Thus, whether the residues of contaminants in effluent can bring toxicity to the aquatic organisms in the receiving water body is still unknown since the toxicity related parameters are still not regulated for wastewater in Chinese national level. Accordingly, the toxicity evaluation of wastewater is drawing more and more attentions and needs more and more further studies.

Toxicity evaluation, especially acute toxicity, has already been adopted for wastewater management in many countries routinely (Power and Boumphrey, 2004), where various test organisms can be used to characterize the acute toxicity of wastewater (Manenti et al., 2015; Santos et al., 2014; El Hajjouji et al., 2014). Acute toxicity evaluation for municipal wastewater has also been conducted in recent years with aquatic organisms, such as luminous

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bacteria, algae and fish (Mendonca et al., 2013; Ma et al., 2013, 2005). Some investigations revealed that municipal wastewater could induce eutrophication of its receiving water body with the blooming of algae growth (Vargas-Gonzalez et al., 2014; Bayram et al., 2013). Although there are many researches on contaminants removal from wastewater during its treatment process, studies on the toxicity removal of wastewater are still limited (Mendonca et al., 2013; Ma et al., 2013; Zhao et al., 2014; Zhang et al., 2013a). It was illustrated that conventional activated sludge technology used in WWTPs can effectively reduce the toxicity of wastewater to *Vibrio fischeri* (Ma et al., 2013; Surujlal-Naicker et al., 2015), and A^2/O treatment technology could successfully remove the acute toxicity of influent to zebrafish (Zhang et al., 2013a; Vidal-Dorsch et al., 2013). However, there is still no study on the comparison of toxicity removal by different treatment techniques.

Alga is an important kind of test organism for toxicity evaluation because it is the primary food producer in aquatic ecosystem and can be easily cultivated. Any disturbance in algal dynamics may influence the balance of ecosystem it belongs to (Silva et al., 2009). Accordingly, algae has been commonly adopted in acute toxicity evaluation for industrial wastewaters such as coking wastewater (Zhao et al., 2014), pharmaceutical wastewater (Yu et al., 2014), kraft mill wastewater (Raptis et al., 2014), washing/cleaning wastewater (Laohaprapanon et al., 2012), as well as municipal wastewater (Mendonca et al., 2013, 2009).

Acute toxicity test with algae was employed in wastewater toxicity evaluation, e.g. algal growth inhibition test (Oral et al., 2007; Tigini et al., 2011; Abrantes et al., 2009; Kallqvist et al., 2008). In addition, other toxic endpoints, such as antioxidant enzyme activity of superoxide dismutase (SOD) and cell membrane integrity, were also adopted to understand the toxic responses of algae to some chemicals or toxicants, e.g. pesticides (Bisewska et al., 2012; Huang et al., 2012), heavy metals (Zhang et al., 2013b; Wei et al., 2014) and nanomaterials (Hazani et al., 2013; Pereira et al., 2014). However, such intensive acute toxicity research has not been conducted for the crude wastewater.

Our study aims to gain insight into the acute toxic effects of local municipal wastewater on alga, *Scenedesmus obliquus* (*S. obliquus*). Four endpoints (cell density, chlorophyll-A concentration, SOD activity and cell membrane integrity) are analyzed to characterize their biotoxicity effects. The toxicity of wastewater, here, was considered to "have significant difference with control". That is to say, if the effect of wastewater on alga does have significant difference with that of control, we called that kind of effect to be toxicity. Accordingly, the promotion effect was also regarded as toxic effect which may result in the bloom of algal growth and further cause eutrophication of its receiving water body. The wastewaters studied were sampled from three municipal wastewater treatment plants with different treatment techniques, i.e. SBR, Linpor and conventional activated sludge, in the northeast of China. Influent and each effluent from different treatment stage in these three WWTPs were sampled and measured their acute toxicity to alga. Our results would aid to comprehensive the toxicity situation of municipal wastewater, as well as the toxicity removal performance of current utilized treatment techniques in WWTPs in the northeast of China.

2. Materials and methods

2.1. Wastewater sampling

Three WWTPs (named Plant 1, Plant 2 and Plant 3) under study are located in Liaoning province, northeast of China. Treatment techniques applied in three WWTPs are SBR, Linpor and conventional activated sludge process, respectively, as shown in Fig. 1. Operation parameters of these WWTPs, such as influent flow and serving population, hydraulic retention time (HRT) and sludge retention time (SRT) were given in Table S1 in supplementary materials. Wastewater sampling was conducted during September to December in 2014 from each treatment stage (Fig. 1) based on the discrete sampling. Wastewater samples were stored at 4 °C until chemical analysis and toxicity test carried out within 48 h. It should be noted that there were disinfection units in Plant 1 and Plant 2 while there was no disinfection unit in Plant 3.

2.2. Chemical analysis

Conventional chemical parameters were measured, including pH, DO (dissolved oxygen), COD, TOC (total organic carbon), NH_3–N and TP (total phosphorous). The measurements were performed as described in the regulated standard methods (Chinese NEPA, 2002). The parameter of pH was determined using a pH meter (SG2, Mettler Toledo, China), DO was detected with a dissolved oxygen meter (YSI550A, USA), COD was measured with potassium dichromate method, TOC was measured with a total organic carbon analyzer (TOC-V_{CPH}, Shimadzu, Japan), NH_3–N was analyzed with sodium reagent method, and TP was measured using ammonium molybdate spectrophotometric method. Heavy metals were analyzed with ICP (inductively coupled plasma spectrometer, Optima 2000 DV, Perkin Elmer, USA).

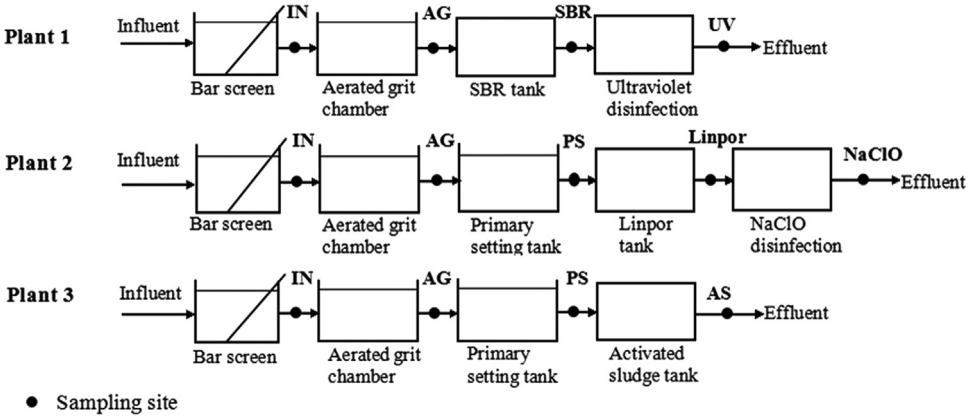

Fig. 1. Flowcharts of the three wastewater treatment plants and sampling scheme. Note: IN means influent, AG means aerated grit effluent, SBR means effluent from SBR tank, UV means ultraviolet disinfection effluent, PS means primary setting effluent, Linpor means effluent from Linpor unit, NaClO means NaClO disinfection effluent, AS means effluent from activated sludge unit.