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;

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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.

Key words: chlorophyll-A; membrane integrity; SOD activity; Linpor system; activated sludge; SBR

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} tons in 2013, among which domestic wastewater was nearly 4.85×10^{10} tons, accounting for nearly 70%. Total discharges of chemical oxygen demand (COD) and ammonia-nitrogen ($\text{NH}_3\text{-N}$) were 2.35×10^7 tons and 2.46×10^6 tons, respectively (MEP, 2014). Accordingly, nearly four thousand WWTPs with a capacity of 1.46×10^8 m³/d have been constructed in China by 2013 (MHURD, 2013). In these WWTPs, anaerobic-anoxic-oxic (A^2O), 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 bacteria, algae

and fish (Mendonca et al., 2013; Ma et al., 2013; Ma et al., 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., 2013). 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 A²/O treatment technology could successfully remove the acute toxicity of influent to zebrafish (Zhang et al., 2013; 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 (Li et al., 2005). 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; Mendonca et al., 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

80 responses of algae to some chemicals or toxicants, e.g. pesticides (Bisewska et al., 2012; Huang et
81 al., 2012), heavy metals (Zhang et al., 2013; Wei et al., 2014) and nanomaterials (Hazani et al.,
82 2013; Pereira et al., 2014). However, such intensive acute toxicity research has not been
83 conducted for the crude wastewater.

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

98 **2. Materials and methods**

99 **2.1 Wastewater sampling**

100 Three WWTPs (named **Plant 1**, **Plant 2** and **Plant 3**) under study are located in Liaoning
101 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), NH₃-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), NH₃-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).

2.3 Toxicity tests

The culture of *S. obliquus* was performed according to algal growth inhibition test protocol regulated in OECD 201 (**OECD, 2006**). *S. obliquus* used was obtained from the Institute of Hydrobiology of the Chinese Academy of Sciences (Wuhan, China). Non-axenic batch culture of *S. obliquus* was maintained in BG11 medium (**Yang et al., 2013**) at 24 ± 1 °C in a 12 : 12 h

light-dark cycle and a cool-white fluorescent light intensity of approximately 5000 lx. The cultures were shaken three times per day to ensure optimal growth. Toxicity test was conducted with 2×10^5 cell/mL of algal cells exposed to 80 mL wastewater samples in triplicate. Wastewater samples were filtered with 0.45 μ m membranes before algal toxicity test. Blank control with Milli-Q water was included for toxicity test of each wastewater sample while positive control test with potassium dichromate was performed once a month.

Cell density: Toxicity test on cell density or growth rate was performed according to OECD 201 (OECD, 2006). Optical density of algal culture at 690 nm was measured at 0 h and 72 h with spectrophotometer (V-560, Jasco, Japan). Cell density in the culture was determined according to liner equation based on direct cell counts (measured with hemocytometer, XB-K-25, China) and optical densities measured at 690nm. The following equation (2-1) was used to calculate the algal cell growth rate:

$$\mu = \frac{(\ln N_t - \ln N_0)}{t - t_0} \quad (2-1)$$

where μ is growth rate; t is the ending time of exposure; t_0 is the initial time of exposure; N_t is the cell density at t ; and N_0 is the cell density at t_0 .

Chlorophyll-A concentration: The chlorophyll-A concentration was determined based on ethanol extraction method (Yang et al., 2013). After algal cells were collected by centrifuging algal culture at $10,000 \times g$ for 10 min, the algal pellets were re-suspended by 10 mL of 95% ethanol and disrupted with a ultrasonic cell disruptor (VCX750, Sonics, USA) for 10 min at 750 W. The extracts were incubated in the dark at 4 °C for 24 h, and then centrifuged at $10,000 \times g$ for 10 min. Absorbance of the supernatant was measured at 649 nm and 665 nm against 95% ethanol. Following equations (2-2) and (2-3) were used to calculate the chlorophyll-A concentration and its

growth rate:

$$c = 13.95A_{665} - 6.88A_{649} \quad (2-2)$$

$$\mu = \frac{(\ln c_t - \ln c_0)}{t - t_0} \quad (2-3)$$

where c is chlorophyll-A concentration; A_{665} and A_{649} are optical densities at 665 and 649 nm, respectively. μ is chlorophyll-A growth rate; t is the ending time of exposure; t_0 is the initial time of exposure; c_t is chlorophyll-A concentration at t ; and c_0 is chlorophyll-A concentration at t_0 .

Enzyme extraction: The SOD activity of algal culture was determined after exposure to wastewater for 72 h. Algal cells were collected by centrifuging algal culture at $10,000 \times g$ for 10 min. After removal of the supernatant fluid, algal pellets were re-suspended with ice cold sodium phosphate buffer (50 mM, pH 7.8) and disrupted with ultrasonic cell disruptor (VCX750, Sonics, USA) for 20 min at 750 W. The homogenate was then centrifuged at $10,000 \times g$ for 10 min at 4 °C to obtain the supernatant for the assay of enzyme activity.

Activity of SOD was analyzed according to the method established by Beauchamp (Beauchamp and Fridovich, 1971) with some modification. The reaction mixture of sample group contained 5×10^{-3} mol/L sodium phosphate buffer, 1.3×10^{-2} mol/L D-Methionine, 7.5×10^{-5} mol/L nitroblue tetrazolium, 1×10^{-5} mol/L EDTA- Na_2 , 2×10^{-6} mol/L riboflavin and enzyme extract. In two control groups, sodium phosphate buffer was used instead of enzyme extract. After homogenizing, the mixtures, excluding one control group placed in total darkness, were illuminated by a fluorescent lamp (4000 lux) for 20 min, and then the absorbance was determined at 560 nm against the control from darkness. One unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of nitroblue tetrazolium reduction. The activity of SOD was calculated with the following equation (2-4):

$$SODA \text{ (U / mL)} = \frac{2(A_{CK} - A_S) \times V_T}{A_{CK} \times V_S \times V_r} \quad (2-4)$$

where *SODA* is SOD activity; A_{CK} and A_S are the optical densities of control and sample groups at 560 nm, respectively; V_T is the total volume of enzyme; V_r is the enzyme volume used in reaction; and V_S is the volume of algal culture.

Cell membrane integrity: Propidium iodide (PI) fluorescence staining and flow cytometric analysis were used for cell viability test as describe in (Wei et al., 2014). Algal cells were collected by centrifuging 300 mesh filtered cell culture at $5000 \times g$ for 5 min. After removal of the supernatant fluid, algal pellets were re-suspended with 200 μ L PI solution (50 μ g/mL) and incubated in the dark for 30 min before flow cytometric analysis (BD-FACSCanto, Becton-Dickinson Biosciences, USA). Cells were excited with an argon excitation laser (488 nm) and the fluorescence emission of 564 - 606 nm was detected. PI only enters cells with damaged membranes and intercalated with double-stranded nucleic acids to emit much stronger fluorescent. For each sample, 10,000 cells were collected and analyzed to obtain the percentage of cells with stronger fluorescence emission.

2.4 Statistical analysis

All statistical analyses were conducted using OriginPro 9.0 (OriginLab, USA) and SPSS 18.0 (IBM, USA). One-way ANOVA with Tukey's test ($p < 0.05$) was used for difference analysis and spearman rank correlation test ($p < 0.05$) was used for correlation analysis.

3. Results

3.1. Wastewater characteristics

Conventional chemical parameters of municipal wastewater samples were given in **Table 1**. The removal efficiencies of COD, TOC, $\text{NH}_3\text{-N}$ and TP in **Plant 1** with SBR treatment technology

were 86.1%, 27.7%, 14.9% and 84.4%, respectively. The concentrations of COD and TP in **Plant 1** effluent met the regulated standard, while NH₃-N was nearly four folds higher than the discharge limit. **Plant 2** with Linpor treatment technology removed the COD, TOC, NH₃-N and TP with 85.0%, 70.2%, 70.4% and 82.9%, respectively. As a result, the COD and TP of effluent met the discharge limits while NH₃-N exceeded the limit slightly. **Plant 3** with conventional activated sludge treatment technology performed the best in COD, TOC, NH₃-N and TP removal with the removal efficiencies of 93.0%, 70.2%, 89.5% and 84.0%, respectively, that COD and NH₃-N concentrations met the discharge limits while TP exceeded the limit slightly. The heavy metal concentrations of wastewater samples were given in **Table S2**. Only Cadmium (Cr) concentration of three effluents exceeded the discharge limit by 85.0% - 95.0%.

3.2. Acute toxicity test

3.2.1 Growth of cell density

After 72h exposure, the cell growth effect of municipal wastewater on *S. obliquus* was shown in **Fig.2**. Increased cell growth was observed after exposure to influents from three WWTPs. The promotion rates of wastewater on cell growth sampled from each treatment unit of **Plant 1** were 12.5%, 8.5%, 16.5% and 8.1%, respectively, which presented that SBR technique did not remove the promotion effect on cell growth successfully. In **Plant 2**, the promotion rates of influent, primary effluent and secondary effluent on algal growth were from 15.9% to 25.0% with no significant difference, while the effluent from NaClO disinfection unit strongly inhibited the cell growth by 131.8%. In **Plant 3**, samples of influent and primary treated effluent exhibited similar promotion effect with promotion rates of 13.1% - 15.4%, while secondary effluent presented no promotion effect on cell growth.

3.2.2 Chlorophyll-A growth

After 72 h exposure, the effect of municipal wastewaters on chlorophyll-A synthesis in *S. obliquus* was shown in **Fig.3**. All influents of three WWTPs accelerated the synthesis of chlorophyll-A in *S. obliquus*. The stimulation rates of wastewater samples of **Plant 1** along treatment process were 19.9%, 11.2%, 0.5% and 10.9%, respectively, which indicated a completely removal of stimulation effect on algal chlorophyll-A synthesis by the secondary treatment process. Influent of **Plant 2** promoted chlorophyll-A synthesis by 16.8%, which was not significantly reduced by primary treatment. In **Plant 2**, the secondary effluent exhibited not promotion but inhibition on algal chlorophyll-A synthesis by 34.5%, and the NaClO disinfection effluent showed extremely higher inhibition effect on chlorophyll-A synthesis by 173.0%. The promotion rates of wastewater samples of **Plant 3** on chlorophyll-A synthesis along treatment process were 25.5%, 24.0%, 1.1% and -13.3%, respectively. It can be seen that the promotion effect was completely removed by primary setting and then converted to be inhibition effect after activated sludge treatment unit.

After correlation analysis between toxicity effect and chemical parameters of wastewater, it was found that the chlorophyll-A promotion rate was related with TOC and TP significantly, the results were shown in **Fig.S1**. Chlorophyll-A promotion rates of 11 wastewater samples (influent, primary effluents and secondary effluents) showed significantly positive correlation with TP concentrations ($r = 0.793$, $p = 0.004$) and TOC concentrations ($r = 0.727$, $p = 0.011$), showed positive correlation with $\text{NH}_3\text{-N}$ concentrations ($r = 0.555$, $p = 0.077$) and COD concentrations ($r = 0.545$, $p = 0.083$).

3.2.3 SOD activity

After 72 h exposure, the influence of municipal wastewater on SOD activity in *S. obliquus* was shown in **Fig.4**. Algal SOD activities after exposure to three influent samples were all over 2.4-fold higher than that of control. Secondary treatments of **Plant 1** and **Plant 3** eliminated SOD activity promotion rates by 35.4% and 41.4% respectively, which were much higher than that of **Plant 2**. However, all the three secondary effluents still presented significantly promotion effects on SOD activity by 84.5% - 136.2%. UV disinfection process of **Plant 1** had no significant effect on wastewater toxicity to algal SOD activity, while NaClO disinfection effluent from **Plant 2** significantly inhibited the SOD activity by 81.2%.

3.2.4 Cell membrane integrity

After 72 h exposure, the influence of municipal wastewater on cell membrane integrity of *S. obliquus* was shown in **Fig.5**. Percentage of cell viability after exposure to influent of **Plant 1** decreased by 7.1% significantly, and this adverse effect was completely removed by SBR tank. Influent of **Plant 2** did not show adverse effect on cell membrane integrity, but NaClO disinfection effluent presented serious impact on cell membrane by 17.5%. **Plant 3** influent exerted small but significant adverse effect on algal cell membrane integrity by 0.33%, which was then successfully removed by the activated sludge treatment process.

4. Discussion

The performances of chemicals removal in three wastewater treatment processes were evaluated in our study. Chemical parameters of wastewaters including COD, NH₃-N, and TP were effectively reduced except for NH₃-N in **Plant 1**. Here, in contrast with conventional activated sludge and SBR, Linpor treatment process is a relatively new treatment technique used for municipal wastewater. Considering chemicals removal, all of these three treatment processes can

remove over 85% of COD, resulted that COD of all the effluent samples met the discharge limit regulated in China. As to the removal of nutrients, the removal ratio was 58% for ammonia nitrogen and 84% for phosphorous (on average), which was not satisfactory that $\text{NH}_3\text{-N}$ concentration of effluents from **Plant 1** and **Plant 2** exceeded the discharge limit and TP concentration of **Plant 3** effluent also exceeded the discharge limit. Our results were in accordance with the general treatment level of municipal WWTPs summarized in China in 2012, which showed the removal of COD and ammonia nitrogen were 85% and 37%, respectively. Accordingly, the residue nutrients (especially nitrogen), rather than COD, was becoming one of the concerns in China (Jin et al., 2014). In our study, **Plant 3** with conventional activated sludge treatment process was illustrated to be the best one in removal of COD, TOC, $\text{NH}_3\text{-N}$ and TP, which presented different results with other investigations on SBR (Qiu et al., 2010) and Linpor (Shen and Wang, 1999) processes. This may be contributed to the immature utilization experiences of relatively new treatment processes SBR and Linpor, compared with conventional activated sludge process, in industrial scale in China.

This study examined the toxicity targets of municipal wastewater in alga, *S. Obliquus*, at the biochemical (chlorophyll-A synthesis and SOD activity), structural (cell membrane integrity) and population (cell density) level. Chlorophyll-A is commonly used to express the light utilization ability of algae, which was confirmed to be related to chemical stresses on various types of plant (algae included) (Oguntimehin et al., 2008). SOD, one of the antioxidative enzymes to eliminate ROS (reactive oxygen species) or to reduce the damage effects, plays an important role in the cell defense against oxidative exposure. Membrane integrity is an important indicator of cell viability to express the toxicity on the cell level (Feng et al., 2010). Therefore, our research utilized these

four indicators as endpoints to present a comprehensive understanding of acute toxicity effect of municipal wastewater on *S. obliquus*, which was the first time to utilize so many algal endpoints to express the toxicity of municipal wastewater. Based on the quantitative analysis of various endpoints (promotion or inhibition) (Wei et al., 2014), the general sensitivity order of *S. obliquus* toxicity endpoints seemed to be in the order of: SOD activity > chlorophyll-A concentration > cell density > membrane integrity. It suggested that chlorophyll concentration and SOD were more sensitive than algal biomass for *S. obliquus*, which was consistent with the study result for *Microcystis aeruginosa* and *Selenastrum capricornutum* in toxicity response to chemicals (tetracycline) (Yang et al., 2013). Different acute toxic results using the four endpoints may due to their different responses to different kinds of substances in the wastewater. Cell growth and chlorophyll-A synthesis are more susceptible to nutrients, while SOD activity and cell membrane integrity are mainly influenced by some toxic substances that cause oxidative stress. Based on our results, the endpoints at biochemical level (e.g. chlorophyll-A synthesis and SOD activity) may be more sensitive than that of algal structure or its population level (e.g. cell membrane integrity and cell density).

Correlation analysis of acute algal toxicity and chemical parameters (COD, TOC, NH₃-N and TP) revealed that the chlorophyll-A synthesis in alga was significantly and positively correlated with the concentrations of TP and TOC in our study (**Fig.S1**). Our results were partly consistent with other published researches that chlorophyll-A synthesis was significantly related to both TP and total nitrogen (Bomchul and Kim, 2007; Lv et al., 2011; Chen et al., 2007), and partly consistent with that biomass were related to the concentration of TOC (Phillips et al., 2008). Our results suggested that the removal performance of TOC and TP should be highlighted in the

toxicity evaluation in municipal wastewater treatment, and further in the assessment of related eco-risk.

Our study aimed to present the whole toxicity of wastewater, instead of the analysis of specific pollutants. The whole toxicity test was used as an approach to comprehensively evaluate the combined toxicity of multiple pollutants or/and the toxic effects of unknown chemicals. Accordingly, we did not pay attention to much on the existence of heavy metals or synthetic micropollutants in wastewater. Here, in order to determine the relationship of individual heavy metal and the acute toxicity of wastewater, we determined the concentration of heavy metals. It should be noted that the heavy metals were existed in the wastewater as mixture, so that the relationship of individual metal concentration and the toxicity of wastewater did not illustrate their combined toxicity in wastewater. Our results showed that there was no significant correlation between individual heavy metal concentration and the algal acute toxicity. It can be seen that the whole toxicity test for wastewater is a better approach in comparison to the analysis of specific pollutants.

Disinfection is usually a chemical operation unit following secondary treatment in wastewater treatment process, whose objective is to inactivate the pathogenic microorganisms and minimize the related risks of waterborne diseases. UV, chlorination, NaClO and other advanced oxidation technique are commonly used in practice for disinfection of the effluents from WWTPs before discharging to the receiving water bodies (da Costa et al., 2014). In our study, NaClO disinfection used in **Plant 2** increased the toxicity of effluent on *S. obliquus* dramatically. This may due to the formation of disinfection byproducts (DBPs) during the disinfection process. First, the effluent after NaClO disinfection still contained NH₃-N (**Table 1**). Consequently, chloramines

(mainly monochloramine, which is formed from the reaction between NaClO and NH₃-N in the water) acted as disinfectant. It has been reported that chloramination can lead to the formation of DBPs, which are not all the same as those DBP species generated by chlorination, i.e., chloramination favors the formation of aromatic nitrogenous DBPs (Zhai et al., 2014). Some aromatic nitrogenous DBPs have been reported to form during chlorine disinfection of a saline primary wastewater effluent containing a high level of ammonia (Ding et al., 2013). Recent studies have shown that aromatic (nitrogenous) DBPs generally presented significantly higher developmental toxicity and growth inhibition than aliphatic DBPs (Yang and Zhang, 2013; Liu and Zhang, 2014). Compared with NaClO disinfection, UV radiation was demonstrated to be environmental friendly in our present study, which can be seen from that UV radiation did not increase the acute toxicity of wastewater on alga. This may attributed to that UV radiation was conducted with minimal production of DBPs (Hijnen et al., 2006; Li et al., 2009; USEPA, 1999). The toxic compounds produced by disinfectant treatment techniques may depend on the precursors occurring in the wastewater and the concentration of disinfectant used (Ferraris et al., 2005; Valenti et al., 2006; Petala et al., 2008). This research suggested that the choice of disinfection techniques for wastewater disinfection is also important considering the following eco-toxicity to the receiving water body.

5. Conclusions

The acute toxicity of municipal wastewater on chlorophyll-A synthesis in alga, *S. obliquus*, was significantly correlated to TP and TOC concentrations. Among four endpoints used in our study to characterize the acute toxicity to alga, *S. obliquus*, SOD activity and chlorophyll-A synthesis were found to be sensitive to municipal wastewater toxicity in evaluation of treatment

stage in WWTPs.

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Table 1 Characteristics of wastewater samples from different treatment stages of three WWTPs

Wastewater Plant	Wastewater samples	pH	DO (mg/L)	COD (mg/L)	TOC (mg/L)	NH ₃ -N (mg/L)	TP (mg/L)
Plant 1	IN	7.39±0.02	1.63±0.02	294±4	105±2.33	40.9±1.3	6.41±0.18
	AG	7.32±0.00	2.00±0.03	371±5	110±2.67	41.1±0.9	7.45±0.20
	SBR	7.60±0.03	0.74±0.02	41±2	69.3±1.79	37.1±0.7	0.97±0.03
	UV	7.33±0.01	0.45±0.01	41±3	76.1±2.01	34.8±0.5 ^a	1.00±0.02
Plant 2	IN	7.20±0.02	1.97±0.03	227±3	88.0±2.10	28.7±0.6	5.26±0.11
	AG	7.29±0.03	1.70±0.04	261±6	89.5±1.86	28.1±0.7	5.68±0.13
	PS	7.20±0.02	0.50±0.01	179±4	77.2±1.45	28.6±0.5	4.89±0.08
	Linpor	6.65±0.03	1.67±0.02	27±3	31.4±0.57	9.5±0.3	0.79±0.02
	NaClO	7.11±0.03	9.47±0.04	34±2	26.3±0.40	8.5±0.2 ^a	0.90±0.04
Plant 3	IN	8.00±0.02	0.09±0.00	158±3	203±3.10	38.9±1.0	7.96±0.14
	AG	8.05±0.02	0.09±0.01	140±2	209±4.02	40.0±0.8	5.68±0.24
	PS	8.84±0.03	3.10±0.04	67±3	158±2.08	33.0±0.6	3.11±0.04
	AS	7.43±0.01	2.70±0.05	11±2	60.4±1.21	4.1±0.1	1.27±0.02 ^a

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 activatedsludge unit. Values were expressed as mean ± standard deviation (n = 3). ^a: indicated that the effluent concentration exceeded the discharge limits according to Class1B in Chinese Discharge

Standard of Pollutants for Municipal Wastewater Treatment Plant (GB 18918-2002), where the pH values should be between 6 and 9, and the standard limits for COD, $\text{NH}_3\text{-N}$ and TP are 60, 8 and 1 mg/L, respectively.

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.

Fig.2. The effect of wastewater from different treatment stages of three WWTPs on algal cell growth (72h)

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. Different letters above each column indicate significant differences at $p < 0.05$. Values were expressed as mean $\pm$ standard deviation ($n = 3$).

Fig.3. The effect of wastewater from different treatment stages of three WWTPs on algal chlorophyll-A synthesis (72h)

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. Different letters above each column indicate significant differences at $p < 0.05$. Values were expressed as mean $\pm$ standard deviation ($n = 3$).

Fig.4. The effect of wastewater from different treatment stages of three WWTPs on algal SOD activity (72h)

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. Different letters above each column indicate significant differences at $p < 0.05$. Values were expressed as mean $\pm$ standard deviation ($n = 3$).

Fig.5. The effect of wastewater from different treatment stages of three WWTPs on algal cell membrane integrity (72h)

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. Different letters above each column indicate significant differences at $p < 0.05$. Values were expressed as mean $\pm$ standard deviation ($n = 3$).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5